

# Pre- and post-copulatory sexual selection in a sexually cannibalistic praying mantid

Anuradhi Jayaweera

Bachelor of Science (Hons)

Department of Biological Sciences

Macquarie University

This thesis is presented for the degree of Doctor of  
Philosophy

January 2018

## **Declaration**

I declare the work presented in this thesis is my own and was undertaken during my Ph.D. candidature. Wherever sources of information or the work of others have 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.

Anuradhi Jayaweera

January 2018

## Table of Contents

List of Original Publications	iv
Acknowledgements	v
Abstract	viii

---

<b>Introduction</b>	<b>1</b>
<b>Chapter outline</b>	<b>19</b>
<b>Chapter 1</b>	<b>22</b>
<b>Chapter 2</b>	<b>32</b>
<b>Chapter 3</b>	<b>40</b>
<b>Chapter 4</b>	<b>63</b>
<b>Chapter 5</b>	<b>77</b>
<b>Summary and Conclusions</b>	<b>103</b>

---

## List of Original Publications

- 1) **JAYAWEERA, A. & BARRY, K. L.** 2017. Male antenna morphology and its effect on scramble competition in false garden mantids. *The Science of Nature*, 104 (9-10), 75. DOI: 10.1007/s00114-017-1494-0
- 2) **JAYAWEERA, A., RATHNAYAKE, D. N., DAVIS, K. S. & BARRY, K. L.** 2015. The risk of sexual cannibalism and its effect on male approach and mating behaviour in a praying mantid. *Animal Behaviour*, 110, 113-119.  
<http://dx.doi.org/10.1016/j.anbehav.2015.09.021>
- 3) **JAYAWEERA, A. HERBERSTEIN, M. E. & BARRY, K. L.** 2017. Effect of sexual cannibalism on male ejaculatory expenditure in a praying mantid. In preparation.
- 4) **JAYAWEERA, A. & BARRY, K. L.** 2015. The Effect of Female Quality on Male Ejaculatory Expenditure and Reproductive Success in a Praying Mantid. *Plos One*, 10, e0124209. DOI:10.1371/journal.pone.0124209
- 5) **JAYAWEERA, A., RATHNAYAKE, D. N., DEAN, B. & BARRY, K. L.** 2017. Chemical signalling and context dependent polyandry in false garden mantids *Pseudomantis albofimbriata* (Mantodea, Mantidae). *Austral Entomology* (under review)

## Acknowledgements

Firstly, I am very much grateful to my supervisors Dr. Katherine L. Barry and Prof. Mariella Herberstein, for their valuable guidance, supervision and encouragement given to me throughout my research project. They always encourage me to open up and reveal myself and my research to the international research community and thereby to enhance my interpersonal skills. My postgraduate experience, in a totally new environment away from home, would not have been this awesome without your support, kindness and great understanding.

I would like to extend my gratitude to the postgraduate research panel members at the Department of Biological Sciences, Macquarie University; Dr. Martin Whiting, Prof. Michael Gillings and Dr. Ajay Narendra, for their continued feedback on my research progress. Their valuable suggestions and appreciation enabled me to complete this thesis on time.

The Behavioural Ecology lab group at Macquarie became a home away from home for me. Thank you to the senior members of my lab group—Matthew Bulbert, James O’Hanlon, Raelene Giffney, Scott Fabricant, Patricio Lagos, Michael Strack, Thomas White, and Giselle Muschett for your love and great company which made a stress free friendly environment. I would also like to thank MD Mahmudunnabi, Mohammad Ameri, Kawsar Khan, Nicole O’Donnell and Marlis Dumke for their great company throughout my PhD career. Thank you to all the undergraduate volunteers for supporting my research – Kaytlyn Davis, Christopher Slattery, Claudia Clammarata, Zachary Sheehan, Andrew Ly, Kelton Jarvis, Barbara Dean, Alexia Dalley. Finally, I would like to thank all the academic members of the Department of Biological Sciences for providing a nice and friendly research community.

My special thanks go to Debra Birch, Nicole Vella and Nadia Saurez-Bosche at the Microscopy unit, Macquarie University for their wonderful assistance with fluorescence- and scanning electron microscopy. Thank you for being so nice to me; the days spent at microscopy were really amazing. I would also like to extend my gratitude to all the technical and administrative staff in the Department of Biological Sciences, Macquarie University, for providing me with an exceptional environment to conduct my research. Thank you to the higher degree research office members, Macquarie University for your kind assistance in all higher degree related issues. I would like to express my gratitude to Macquarie University for granting me a postgraduate scholarship and PhD candidature, and for providing me with an exceptionally great student life.

During my PhD I collaborated with Dr. Gregory Holwell at the University of Auckland, New Zealand on praying mantid genitalia morphology (although the project results were not included in this thesis). Thank you Gregory Holwell, for all of your support and encouragement. Also, my special thanks go to Chrissie Painting, University of Auckland, New Zealand for reviewing some of my manuscripts. Your suggestions really helped me to produce good publications from my research. Thank you Kasun Rathnayake for your assistance in statistical analyses and Jim McLean for proofreading some of my thesis chapters.

I would like to express my gratitude to the University of Peradeniya, Sri Lanka for granting me study leave to conduct my PhD. Thank you all academic staff members in the Department of Zoology, University of Peradeniya for your support given to me throughout my postgraduate career. Special thanks go to my undergraduate research supervisor Dr. Inoka Karunarathne for building up my interest in animal behaviour and guiding me on the path towards scientific research.

It was not easy to do a postgraduate degree thousands of miles away from my loved ones. Thank you so much to my mother, father, brother, sisters and all other family members for your love, understanding and appreciation which always encouraged me to follow my dreams. Special thanks go to my husband Darshana Rathnayake for all your love and support; you have been a great strength of mine during the past ten years. Without you I would not have been able to accomplish this. My heartiest love goes to the cute little fellow who joined our lives a few months ago, my darling son Nethuka; thank you for giving me the world's best feeling—motherhood. You are the best thing that has happened in my life over these difficult postgraduate years. Finally, thank you all my dear Sri Lankan friends in Australia for all the great days we spent together. Your company wiped away all the stresses during postgraduate time. Thanks for making an amazing four years of memories in Australia.

Anuradhi Jayaweera

January 2018

## Abstract

Animals have evolved intriguing mating strategies, through sexual selection, that presumably maximize their lifetime reproductive success. Sexually cannibalistic systems are a fascinating mating system in which to study the evolution of reproductive behaviour. This allows us to understand how male and female mating strategies evolve through the inter-relationship of sexual selection and sexual cannibalism. Even though sexually cannibalistic mating systems are widely used in describing female mating strategies, evidence for male mating strategies in such systems is sparse. Therefore, this thesis investigates how sexual selection and sexual cannibalism shape pre- and post-mating strategies in the false garden mantid *Pseudomantis albofimbriata* with a focus on male mating behaviour. More specifically, this research provides evidence for; 1) the selection of male traits that aid in effective and efficient mate location where males with more trichoid sensilla on their antennae locate females more quickly, 2) context dependent strategic male mating resource investment in response to sexual cannibalism (but not female quality), 3) potential post-copulatory paternity assurance mechanisms through a prolonged female refractory period. Finally, my thesis provides evidence and explanations for why males do not reject highly risky females in a sexually cannibalistic mating system. My thesis makes contributions to behavioural ecology and evolution, as it explores and describes this intriguing mating system from the male perspective.



## **Introduction**

Darwin's (1859) theory of natural selection failed to describe some conspicuous male traits that are problematic for their survival (Andersson and Iwasa, 1996). As a solution to explain these male traits, Darwin (1871) introduced the theory of sexual selection, which is defined as selection on traits for competition to access a mating partner rather than for survival. Secondary sexual traits which often appear at maturity of an animal (e.g. peacock tails and lion's manes), are known to play a major role in competition over mates (Andersson, 1994). Sexual selection is composed of two major evolutionary forces; mate competition and mate choice (Andersson and Iwasa, 1996). As female reproductive investment (i.e. gamete production, bearing and rearing young) generally outweighs male investment, males are predicted to be the sex that competes over access to females (male competition). Conversely, females select a mate based on various phenotypic and behavioural male traits (female choice) (Bateman, 1948, Parker et al., 1972). However, sex role reversal is not uncommon among animals where males invest more in reproduction than females and females compete over males (reviewed in Bonduriansky, 2001, Edward and Chapman, 2011).

Contests are the most commonly reported type of mate competition, and are responsible for the evolution of male traits, such as weapons. However, there are many other forms of mate competition such as scramble competition and endurance rivalry (Andersson and Iwasa, 1996). Although Darwin (1871) introduced the concept of female choice (which progresses through to female preference for conspicuous male traits), he failed to suggest an underlying mechanism. Instead, subsequently developed genetic models explained why females prefer these traits (Kirkpatrick, 1982, Andersson and Simmons, 2006).

Darwin's theory mostly considered sexual selection (competition or choice) as it leads up to mating but not beyond (pre-copulatory sexual selection). It was not until the 1970s (e.g. Parker, 1970a, Parker, 1970c) that our understanding of selection on traits and mechanisms that occur after copulation developed (post-copulatory sexual selection).

### **Pre-copulatory sexual selection**

Sexual selection that occurs prior to copulation is composed of two major mechanisms; male-male competition and mate choice. At this stage males are competing with each other in order to obtain a female for mating and females select males based on various male traits.

#### *Pre-copulatory male - male competition*

Pre-copulatory male competition is thought to be responsible for the evolution of most conspicuous male traits that increase a male's likelihood of obtaining a mate by successfully competing with other males. Male contests often involve males directly fighting with each other where stronger/dominant males gain an advantage over their weaker/sub-ordinate counterparts, and hence gain access to females (Le Boeuf, 1974, Ligon et al., 1990, Jormalainen et al., 1994). Some animals have evolved different types of weaponry to fight with conspecifics to obtain mates. These weapons are mainly used to defend critical resources (e.g. female feeding or egg laying sites) that control the mating opportunities for males (see Emlen, 2008). Endurance rivalry is another form of pre-copulatory male-male competition where males that are sexually active for longer periods than others gain the advantage of having more mates (Andersson, 1994, Judge and Brooks, 2001, Lidgard et al., 2005, Higham et al., 2011).

Male mating success does not always rely on physical strength to win direct contests with rivals. Some males, especially invertebrates, are in a race to find a mate and their mating success is determined by how quickly and effectively they can locate a female, which is known as scramble competition (Andersson, 1994, Herberstein et al., 2017). In these systems, the male that first locates and approaches the female will often gain an advantage in mating first with that female. Scramble competition is responsible for the evolution of male traits which aid in quickly locating females, such as increased mobility (small body size), early maturation (protandry), and effective sensory systems (Vencel, 2004, Kasumovic and Andrade, 2009, Moya-Larano et al., 2009, Barry, 2013, Herberstein et al. 2017).

#### *Pre-copulatory mate choice*

##### *a) Female choice*

Pre-copulatory female choice is the well-known form of mate choice where females select males based on their physical and behavioural traits (Kirkpatrick, 1982). Female choice is thought to be responsible for the evolution of sexually dimorphic traits in animals, such as bird tail length (Balmford et al., 1994). This process is the result of two major mechanisms:

- 1) Run-away sexual selection: females prefer an arbitrary trait in males and both the female preference and the male trait are heritable (Kirkpatrick, 1982). Therefore, females bear daughters who prefer this arbitrary trait and sons that exhibit the trait (see Hedrick, 1988). Often, the male trait becomes more conspicuous. Evolution of multiple ornaments (black throat and belly feather, yellow eye markers and spiked tail feathers) in male sage grouse is a good example of run-away sexual selection in animals (Vehrencamp et al., 1989, Pomiankowski and Iwasa, 1993).

2) Good genes hypothesis: this hypothesis predicts that females select male traits that confer good genes and hence a probable increase in the viability of their offspring (Hamilton and Zuk, 1982). Therefore, it is not an arbitrary trait or random preference as in run-away selection, instead females select males for their genetic quality. A somewhat related mechanism is the handicap principle where females select males with handicaps, such as costly elongated male bird tails (Jennions, 1993), as these males are signalling the possession of good genes that enable them to survive in spite of the handicap trait (Zahavi and Zahavi, 1999).

#### *b) Male choice*

Pre-copulatory male mate choice is not widely reported in animals. However, male mate choice can evolve if reproduction is costly to males and if females vary greatly in reproductive quality (Trivers, 1972, Dewsbury, 1982). For example, male two spotted gobies (*Gobiusculus flavescens*) prefer to mate with brightly coloured females and display intense courtship behaviours towards these females (Amundsen and Forsgren, 2001). Males may reject or accept females of varying reproductive qualities due to pre-copulatory mate choice (Berglund and Rosenqvist, 2001, Barry et al., 2010) or they may change their courtship and/or copulatory behaviours towards preferred females (Amundsen, 2000, Amundsen and Forsgren, 2003, Tudor and Morris, 2009).

### **Post-copulatory sexual selection**

The existence of post-copulatory sexual selection was only recognised recently (Birkhead and Pizzari, 2002). Both male-male competition and mate choice can continue after copulation in the forms of sperm competition (Parker, 1970a, Parker, 1984) and cryptic mate choice (Eberhard, 1996, Bonduriansky, 2001).

### *Sperm competition*

If females mate with more than one male (polyandry), sperm from two or more males compete within the female reproductive tract for the fertilization of the ova (Parker, 1970a). Polyandry is more common than previously thought (Arnqvist and Nilsson, 2000, Simmons, 2001a, Griffith et al., 2002), consequently sperm competition is also widespread among animals (see Simmons, 2001b). In polyandrous systems, males are under the continuous threat of sperm competition and have evolved different defensive and offensive strategies in response (Simmons, 2001b).

Mate guarding and mating plugs are the most common types of male defensive strategies to avoid sperm competition (Parker, 1970a, Parker, 1970b, Simmons, 2001b, Snow et al., 2006). For example, male sugarcane rootstalk borer weevils *Diaprepes abbreviatus* remain mounted on the female in copula for more than 16 hours as a mate guarding strategy, as prolonged mate guarding reduces female remating (Harari et al., 2003). Some male spiders (e.g. *Argiope keyserlingi*) break off their genital parts within the female reproductive opening to serve as mating plugs and prevent the female from remating (Uhl et al., 2010, Herberstein et al., 2012). Further, ejaculatory components (accessory gland products and/or sperm by itself) of some males, especially insects, are known to function as inhibitors of female receptivity for subsequent males (Scott, 1986, Simmons, 2001b, Radhakrishnan and Taylor, 2007).

Offensive strategies include the removal of rival sperm from the female reproductive tract using specialized genitalia (Waage, 1979, Waage, 1986), influence of sperm usage by females through seminal fluid components (den Boer et al., 2008, den Boer et al., 2010) and strategic ejaculation (Kelly and Jennions, 2011). For example, male flour beetles *Tribolium castaneum* are capable of removing rival-male sperm from the female reproductive tract using their genital structures (Haubruge et al., 1999). Strategic ejaculation is a widespread male behaviour shown mainly by insects where males allocate

their sperm depending on the risk and intensity of sperm competition; when the risk of sperm competition is high, males ejaculate more sperm and when the intensity of sperm competition is high they transfer less sperm to their partners (Parker and Pizzari, 2010, Kelly and Jennions, 2011). For example, Mediterranean fruit fly *Ceratitidis capitata* transfer more sperm to their mates in the presence of a rival male during copulation due to increased risk of sperm competition (Gage, 1991) and male bush crickets transfer less sperm when they mate with highly fecund large females as the intensity of sperm competition is high (Simmons and Kvarnemo, 1997).

### *Cryptic female choice*

Female choice can continue even after copulation in the form of cryptic female choice, where polyandrous females bias fertilisation by favouring specific males (Eberhard, 1996). Cryptic female choice is difficult to distinguish from other post-copulatory sexual selection mechanisms, such as sperm competition, as both of these mechanisms occur within the female reproductive tract and result in variation in fertilisation success for males. However, some experimental studies have been able to use novel methods in order to distinguish cryptic female choice from other post-copulatory mechanisms (Edvardsson and Arnqvist, 2000, Pizzari and Birkhead, 2000, Holwell, 2007, Schneider and Lesmono, 2009). For example, female jungle fowl (*Gallus gallus domesticus*) selectively prefer to fertilize their eggs with the sperm of dominant males and eject subordinate male sperm after mating (Pizzari and Birkhead, 2000). Edvardsson and Arnqvist (2000) found that male red flour beetles that rub the female abdomen faster during copulation gain higher fertilisation success. Initially it was not clear whether fast leg rubbers are better sperm competitors or whether females preferentially fertilize their eggs with sperm of fast leg rubbers. To distinguish between these two, they conducted mating trials by manipulating the female perception of leg rubbing rate by shortening male legs and found increased

fertilization for fast leg rubbers is a result of cryptic female choice, but not of sperm competition (Edvardsson and Arnqvist, 2000). Observations of internal female reproductive anatomy also provide indirect evidence for the possibility of cryptic female choice in animals. For example multiple sperm storage structures in the Australian redback spider *Letrodectus hasselti* facilitates female control of paternity when they mate with multiple partners (Snow and Andrade, 2005).

### *Cryptic male choice*

Male mate choice can also continue after mating in the form of cryptic male choice (reviewed in Bonduriansky, 2001, Edward and Chapman, 2011). Cryptic male choice is predicted to evolve under similar conditions as male mate choice: when females vary greatly in their reproductive quality and consequently result in varying male reproductive success (Edward and Chapman, 2011). Rather than overt mate choice, males vary investment into the ejaculate strategically depending on female quality (Engqvist and Sauer, 2001, Reinhold et al., 2002). For example male scorpion flies *Panorpa cognata* produce bigger nuptial gifts when they copulate with highly fecund females and may copulate for longer, transferring more sperm (Engqvist and Sauer, 2001). However, as with cryptic female choice, it is difficult to distinguish cryptic male choice from sperm competition as both mechanisms may cause deferential reproductive investment in males.

## Introduction to study species

Praying mantids are a group of predatory insects varying from cursorial hunters to sit-and-wait predators (Svenson and Whiting, 2004). Many of them exhibit scramble competition polygynous mating systems where males do not engage in any combat but scramble to locate females quickly (Maxwell 1999). Mantids use both chemical (long distance) and visual (short distance) cues in locating females (Roeder, 1935, Edmunds, 1975, Robinson and Robinson, 1979, Maxwell 1999, Hurd et al., 2004, Perez, 2005, Barry et al., 2010). Female praying mantids are capable of multiple mating, and multiple paternity has been recorded for some species (Barry et al., 2011, Umbers et al., 2011, Watanabe et al., 2011), setting the stage for sperm competition (Prokop and Vaclav, 2005, Allen et al., 2011). They are also well known for their sexually cannibalistic behaviour in which females kill and consume their partners before, during, or immediately after copulation (Maxwell, 1999, Prokop and Vaclav, 2005, Lelito and Brown, 2006, Barry et al., 2008, Lelito and Brown, 2008, Prokop and Vaclav, 2008, Maxwell et al., 2010, Brown et al., 2012).

This thesis examines pre- and post-copulatory sexual selection in the sexually cannibalistic false garden mantid *Pseudomantis albofimbriata*. It is a common mantid species native to Australia and distributed throughout eastern Australia (Holwell et al., 2007). These are seasonal mantids that adults can only find in summer months from December – March. Males and females are present in two colour morphs - green and brown - in the wild. They are sexually size dimorphic; females are approximately double the size of males (Barry et al., 2008) and have short wings compared to the fully developed wings of males. Therefore, female *P. albofimbriata* are less mobile. They emit sex pheromones to attract males from a long distance (Holwell et al., 2007, Barry et al., 2010). Male *P. albofimbriata* are scramble competitors with well-developed antennae possessing numerous sensory hairs (i.e. sensilla) that detect female emitted sex pheromones



effectively (Holwell et al., 2007). Juvenile nutrition affects male body size, and hence their scramble competition ability as, in this species, large males are more successful in locating females compared to smaller males (Barry, 2013). Even though it is predicted that male antenna morphology may play a crucial role in scramble competition in this system (Barry, 2013), experimental evidence is still lacking.

Female false garden mantids often attract multiple males simultaneously via the initial pheromone plume (Barry, 2010) and can mate with multiple partners (Barry et al., 2011). Soon after mating, female *P. albofimbriata* become chemically unattractive (Barry et al., 2011). However, rates of polyandry, the period of chemical unattractiveness of mated females, and how females react to the presence of multiple males in close visual range is yet to be determined. Further, if female *P. albofimbriata* mate multiple times, male strategies against post-copulatory sexual selection mechanisms such as sperm competition and cryptic mate choice are also under-documented (but see Allen et al., 2011).

Female false garden mantids are well known for their cannibalistic tendencies and females in poor condition cannibalise their partners as a foraging strategy to increase their nutritional status and reproductive output (i.e. pre-copulatory cannibalism) (Barry et al., 2008). However, nearly half of the males that are attacked by females can still initiate copulation and transfer sperm successfully whilst being eaten (Barry et al., 2009, Jayaweera et al., 2015). Although pre-copulatory cannibalism is considered an extreme form of sexual conflict, studies focusing on the risk factors of cannibalism and its effect on pre-and post-copulatory male behaviour are relatively scarce (but see Barry et al., 2008, Barry et al., 2009, Barry, 2010).

## References

- ALLEN, L. E., BARRY, K. L., HOLWELL, G. I. & HERBERSTEIN, M. E. 2011. Perceived risk of sperm competition affects juvenile development and ejaculate expenditure in male praying mantids. *Animal Behaviour*, 82, 1201-1206.
- AMUNDSEN, T. 2000. Why are female birds ornamented? *Trends in Ecology & Evolution*, 15, 149-155.
- AMUNDSEN, T. & FORSGREN, E. 2001. Male mate choice selects for female coloration in a fish. *Proceedings of the National Academy of Sciences*, 98, 13155-13160.
- AMUNDSEN, T. & FORSGREN, E. 2003. Male preference for colourful females affected by male size in a marine fish. *Behavioral Ecology and Sociobiology*, 54, 55-64.
- ANDERSSON, M. 1994. *Sexual Selection*, Princeton, New Jersey, Princeton University Press.
- ANDERSSON, M. & IWASA, Y. 1996. Sexual selection. *Trends in Ecology & Evolution*, 11, 53-58.
- ANDERSSON, M. & SIMMONS, L. W. 2006. Sexual selection and mate choice. *Trends in Ecology & Evolution*, 21, 296-302.
- ARNQVIST, G. & NILSSON, T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behaviour*, 60, 145-164.
- BALMFORD, A., JONES, I. L. & THOMAS, A. L. 1994. How to compensate for costly sexually selected tails: the origin of sexually dimorphic wings in long-tailed birds. *Evolution*, 1062-1070.
- BARRY, K. L. 2010. Influence of female nutritional status on mating dynamics in a sexually cannibalistic praying mantid. *Animal Behaviour*, 80, 405-411.
- BARRY, K. L. 2013. You are what you eat: food limitation affects reproductive fitness in a sexually cannibalistic praying mantid. *PLoS One*, 8, e78164.

- BARRY, K. L., HOLWELL, G. I. & HERBERSTEIN, M. E. 2008. Female praying mantids use sexual cannibalism as a foraging strategy to increase fecundity. *Behavioral Ecology*, 19, 710-715.
- BARRY, K. L., HOLWELL, G. I. & HERBERSTEIN, M. E. 2009. Male mating behaviour reduces the risk of sexual cannibalism in an Australian praying mantid. *Journal of Ethology*, 27, 377-383.
- BARRY, K. L., HOLWELL, G. I. & HERBERSTEIN, M. E. 2010. Multimodal mate assessment by male praying mantids in a sexually cannibalistic mating system. *Animal Behaviour*, 79, 1165-1172.
- BARRY, K. L., HOLWELL, G. I. & HERBERSTEIN, M. E. 2011. A paternity advantage for speedy males? Sperm precedence patterns and female re-mating frequencies in a sexually cannibalistic praying mantid. *Evolutionary Ecology*, 25, 107-119.
- BATEMAN, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity*, 2, 349-368.
- BERGLUND, A. & ROSENQVIST, G. 2001. Male pipefish prefer ornamented females. *Animal Behaviour*, 61, 345-350.
- BIRKHEAD, T. R. & PIZZARI, T. 2002. Postcopulatory sexual selection. *Nature Reviews Genetics*, 3, 262-273.
- BONDURIANSKY, R. 2001. The evolution of male mate choice in insects: A synthesis of ideas and evidence. *Biological Reviews*, 76, 305-339.
- BROWN, W. D., MUNTZ, G. A. & LADOWSKI, A. J. 2012. Low mate encounter rate increases male risk taking in a sexually cannibalistic praying mantis. *Plos One*, 7, e35377.
- DARWIN, C. 1871. *The Descent of Man and Selection in Relation to Sex*. London: Murray.
- DEN BOER, S. P., BAER, B. & BOOMSMA, J. J. 2010. Seminal fluid mediates ejaculate competition in social insects. *Science*, 327, 1506-1509.
- DEN BOER, S. P., BOOMSMA, J. J. & BAER, B. 2008. Seminal fluid enhances sperm viability in the leafcutter ant *Atta colombica*. *Behavioral Ecology and Sociobiology*, 62, 1843-1849.

- DEWSBURY, D. A. 1982. Ejaculate cost and male choice. *American Naturalist*, 119, 601-610.
- EBERHARD, W. G. 1996. *Female control: sexual selection by cryptic female choice*, Princeton, New Jersey, Princeton University Press.
- EDMUNDS, M. 1975. Courtship, mating and possible sex pheromones in three species of Mantodea [Ghana]. *Entomologist's Monthly Magazine*, 111, 53-57.
- EDVARDSSON, M. & ARNQVIST, G. 2000. Copulatory courtship and cryptic female choice in red flour beetles *Tribolium castaneum*. *Proceedings of the Royal Society B-Biological Sciences*, 267, 559-563.
- EDWARD, D. A. & CHAPMAN, T. 2011. The evolution and significance of male mate choice. *Trends in Ecology and Evolution*, 26, 647-654.
- EMLEN, D. J. 2008. The evolution of animal weapons. *Annual Review of Ecology, Evolution, and Systematics*, 387-413.
- ENGQVIST, L. & SAUER, K. P. 2001. Strategic male mating effort and cryptic male choice in a scorpionfly. *Proceedings of the Royal Society B-Biological Sciences*, 268, 729-735.
- GAGE, M. J. G. 1991. Risk of sperm competition directly affects ejaculate size in the Mediterranean fruit fly. *Animal Behaviour*, 42, 1036-1037.
- GRIFFITH, S. C., OWENS, I. P. F. & THUMAN, K. A. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, 11, 2195-2212.
- HAMILTON, W. D. & ZUK, M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science*, 218, 384-387.
- HARARI, A. R., LANDOLT, P. J., O'BRIEN, C. W. & BROCKMANN, H. J. 2003. Prolonged mate guarding and sperm competition in the weevil *Diaprepes abbreviatus* (L.). *Behavioral Ecology*, 14, 89-96.

- HAUBRUGE, E., ARNAUD, L., MIGNON, J. & GAGE, M. G. 1999. Fertilization by proxy: rival sperm removal and translocation in a beetle. *Proceedings of the Royal Society of London B: Biological Sciences*, 266, 1183-1187.
- HEDRICK, A. V. 1988. Female choice and the heritability of attractive male traits: an empirical study. *American Naturalist*, 267-276.
- HERBERSTEIN, M., WIGNALL, A., NESSLER, S., HARMER, A. & SCHNEIDER, J. 2012. How effective and persistent are fragments of male genitalia as mating plugs? *Behavioral Ecology*, 23, 1140-1145.
- HERBERSTEIN, M. E., PAINTING, C. J., HOLWELL, G. I. 2017. Scramble competition polygyny in terrestrial arthropods. *Advances in the Study of Behavior*. Cambridge, Massachusetts : Elsevier.
- HIGHAM, J. P., HEISTERMANN, M. & MAESTRIPIERI, D. 2011. The energetics of male-male endurance rivalry in free-ranging rhesus macaques, *Macaca mulatta*. *Animal Behaviour*, 81, 1001-1007.
- HOLWELL, G. 2007. Spermatophore feeding and mating behaviour in praying mantids (Mantodea: Liturgusidae). *Journal of Zoology*, 271, 255-260.
- HOLWELL, G. I., BARRY, K. L. & HERBERSTEIN, M. E. 2007. Mate location, antennal morphology, and ecology in two praying mantids (Insecta : Mantodea). *Biological Journal of the Linnean Society*, 91, 307-313.
- HURD, L. E., PRETE, F. R., JONES, T. H., SINGH, T. B. & PORTMAN, R. T. 2004. First identification of a putative sex pheromone in a praying mantid. *Journal of Chemical Ecology*, 30, 155-166.
- JAYAWEERA, A., RATHNAYAKE, D. N., DAVIS, K. S. & BARRY, K. L. 2015. The risk of sexual cannibalism and its effect on male approach and mating behaviour in a praying mantid. *Animal Behaviour*, 110, 113-119.
- JENNIONS, M. D. 1993. Female choice in birds and the cost of long tails. *Trends in Ecology & Evolution*, 8, 230-232.

- JORMALAINEN, V., MERILAITA, S. & TUOMI, J. 1994. Male choice and male-male competition in *Idotea baitica* (Crustacea, Isopoda). *Ethology*, 96, 46-57.
- JUDGE, K. A. & BROOKS, R. J. 2001. Chorus participation by male bullfrogs, *Rana catesbeiana*: a test of the energetic constraint hypothesis. *Animal Behaviour*, 62, 849-861.
- KASUMOVIC, M. & ANDRADE, M. 2009. A change in competitive context reverses sexual selection on male size. *Journal of Evolutionary Biology*, 22, 324-333.
- KELLY, C. D. & JENNIONS, M. D. 2011. Sexual selection and sperm quantity: meta-analyses of strategic ejaculation. *Biological Reviews*, 86, 863-884.
- KIRKPATRICK, M. 1982. Sexual selection and the evolution of female choice. *Evolution*, 36, 1-12.
- LE BOEUF, B. J. 1974. Male-male competition and reproductive success in elephant seals. *American Zoologist*, 14, 163-176.
- LELITO, J. P. & BROWN, W. D. 2006. Complicity or conflict over sexual cannibalism? Male risk taking in the praying mantis *Tenodera aridifolia sinensis*. *American Naturalist*, 168, 263-269.
- LELITO, J. P. & BROWN, W. D. 2008. Mate attraction by females in a sexually cannibalistic praying mantis. *Behavioral Ecology and Sociobiology*, 63, 313-320.
- LIDGARD, D. C., BONESS, D. J., BOWEN, W. D. & MCMILLAN, J. I. 2005. State-dependent male mating tactics in the grey seal: the importance of body size. *Behavioral Ecology*, 16, 541-549.
- LIGON, J. D., THORNHILL, R., ZUK, M. & JOHNSON, K. 1990. Male-male competition, ornamentation and the role of testosterone in sexual selection in red jungle fowl. *Animal Behaviour*, 40, 367-373.
- MAXWELL, M. R. 1999. The risk of cannibalism and male mating behavior in the Mediterranean praying Mantis, *Iris oratoria*. *Behaviour*, 136, 205-219.

- MAXWELL, M. R., GALLEG0, K. M. & BARRY, K. L. 2010. Effects of female feeding regime in a sexually cannibalistic mantid: fecundity, cannibalism, and male response in *Stagmomantis limbata* (Mantodea). *Ecological Entomology*, 35, 775-787.
- MOYA-LARAÑO, J., VINKOVIĆ, D., ALLARD, C. & FOELLMER, M. 2009. Optimal climbing speed explains the evolution of extreme sexual size dimorphism in spiders. *Journal of Evolutionary Biology*, 22, 954-963.
- PARKER, G. 1984. Sperm competition and the evolution of animal mating strategies. *Sperm Competition and the Evolution of Animal Mating Systems*, 1-60.
- PARKER, G. A. 1970a. Sperm competition and its evolutionary consequences in the insects. *Biological Reviews*, 45, 525-567.
- PARKER, G. A. 1970b. Sperm competition and its evolutionary effect on copula duration in the fly *Scatophaga stercoraria*. *Journal of Insect Physiology*, 16, 1301-1328.
- PARKER, G. A. 1970c. Sperm competition and its evolutionary effect on copula duration in the fly *Scatophaga stercoraria*. *Journal of Insect Physiology*, 16, 1301-1328.
- PARKER, G. A., BAKER, R. R. & SMITH, V. G. F. 1972. The origin and evolution of gamete dimorphism and the ample female phenomenon. *Journal of Theoretical Biology*, 36, 529-553.
- PARKER, G. A. & PIZZARI, T. 2010. Sperm competition and ejaculate economics. *Biological Reviews*, 85, 897-934.
- PEREZ, B. 2005. Calling behaviour in the female praying mantis, *Hierodula patellifera*. *Physiological Entomology*, 30, 42-47.
- PIZZARI, T. & BIRKHEAD, T. R. 2000. Female feral fowl eject sperm of subdominant males. *Nature*, 405, 787-789.
- POMIANKOWSKI, A. & IWASA, Y. 1993. Evolution of multiple sexual preferences by Fisher's runaway process of sexual selection. *Proceedings of the Royal Society of London B: Biological Sciences*, 253, 173-181.

- 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.
- PROKOP, P. & VACLAV, R. 2008. Seasonal aspects of sexual cannibalism in the praying mantis (*Mantis religiosa*). *Journal of Ethology*, 26, 213-218.
- RADHAKRISHNAN, P. & TAYLOR, P. W. 2007. Seminal fluids mediate sexual inhibition and short copula duration in mated female Queensland fruit flies. *Journal of Insect Physiology*, 53, 741-745.
- REINHOLD, K., KURTZ, J. & ENGQVIST, L. 2002. Cryptic male choice: sperm allocation strategies when female quality varies. *Journal of Evolutionary Biology*, 15, 201-209.
- ROBINSON, M. H. & ROBINSON, B. 1979. By dawn's early light: matutinal 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.). *The Biological Bulletin*, 69, 203-220.
- SCHNEIDER, J. M. & LESMONO, K. 2009. Courtship raises male fertilization success through post-mating sexual selection in a spider. *Proceedings of the Royal Society B-Biological Sciences*, 276, 3105-3111.
- SCOTT, D. 1986. Inhibition of female *Drosophila melanogaster* remating by a seminal fluid protein (esterase-6). *Evolution*, 40, 1084-1091.
- SIMMONS, L. W. 2001a. The evolution of polyandry: an examination of the genetic incompatibility and good-sperm hypotheses. *Journal of Evolutionary Biology*, 14, 585-594.
- SIMMONS, L. W. 2001b. *Sperm Competition and its Evolutionary Consequences in the Insects*, Princeton, New Jersey: Princeton University Press.
- SIMMONS, L. W. & KVARNEMO, C. 1997. Ejaculate expenditure by male bushcrickets decreases with sperm competition intensity. *Proceedings of the Royal Society B-Biological Sciences*, 264, 1203-1208.



- SNOW, L. S., ABDEL-MESIH, A. & ANDRADE, M. C. 2006. Broken copulatory organs are low-cost adaptations to sperm competition in redback spiders. *Ethology*, 112, 379-389.
- SNOW, L. S. & ANDRADE, M. C. 2005. Multiple sperm storage organs facilitate female control of paternity. *Proceedings of the Royal Society of London B: Biological Sciences*, 272, 1139-1144.
- SVENSON, G. J. & WHITING, M. F. 2004. Phylogeny of Mantodea based on molecular data: evolution of a charismatic predator. *Systematic Entomology*, 29, 359-370.
- TRIVERS, R. 1972. *Parental Investment and Sexual Selection*, Biological Laboratories, Harvard University Cambridge, MA.
- TUDOR, S. M. & MORRIS, M. R. 2009. Variation in male mate preference for female size in the swordtail *Xiphophorus malinche*. *Behaviour*, 146, 727-740.
- UHL, G., NESSLER, S. H. & SCHNEIDER, J. M. 2010. Securing paternity in spiders? A review on occurrence and effects of mating plugs and male genital mutilation. *Genetica*, 138, 75-104.
- UMBERS, K. D. L., HOLWELL, G. I., STOW, A. J. & HERBERSTEIN, M. E. 2011. Molecular evidence for variation in polyandry among praying mantids (Mantodea: Ciulfina). *Journal of Zoology*, 284, 40-45.
- VEHRENCAMP, S. L., BRADBURY, J. W. & GIBSON, R. M. 1989. The energetic cost of display in male sage grouse. *Animal Behaviour*, 38, 885-896.
- VENCL, F. V. 2004. Allometry and proximate mechanisms of sexual selection in *Photinus* fireflies, and some other beetles. *Integrative and Comparative Biology*, 44, 242-249.
- WAAGE, J. K. 1979. Dual function of the Damselfly penis - sperm removal and transfer. *Science*, 203, 916-918.
- WAAGE, J. K. 1986. Evidence for widespread sperm displacement ability among Zygoptera (Odonata) and the means for predicting its presence. *Biological Journal of the Linnean Society*, 28, 285-300.

- WATANABE, E., ADACHI-HAGIMORI, T., MIURA, K., MAXWELL, M. R., ANDO, Y. & TAKEMATSU, Y. 2011. Multiple paternity within field-collected egg cases of the praying mantid *Tenodera aridifolia*. *Annals of the Entomological Society of America*, 104, 348-352.
- ZAHAVI, A. & ZAHAVI, A. 1999. *The Handicap Principle: A Missing Piece of Darwin's Puzzle*, Oxford University Press.

## Chapter outline

Animals have evolved intriguing mating strategies that to maximize their lifetime reproductive success. However, little is known about the underlying forces responsible for the evolution of such strategies. My Ph.D. research investigates how sexual selection and sexual cannibalism shape pre and post-mating strategies in praying mantids, with special focus on male mating behaviour using both behavioural and microscopic approaches.

*Chapter 1* describes male antenna morphology and its effect on scramble competition in *Pseudomantis albobimbrata*. This study describes different types of sensillae on male false garden mantid antennae using scanning electron microscope images and is the first study to show the effect of male antenna morphology on scramble competition. I identified six different types of antennal sensilla on male *P. albobimbrata* antennae and three of them (basiconic, coelacapitular and campaniform) have never been described before for male false garden mantids. I introduced new nomenclature for grooved basiconic sensillae by comparison with major antennal sensilla nomenclature systems and with sensillae on other praying mantid species. This chapter is published in *The Science of Nature*. The co-author Katherine L. Barry provided support in designing and conducting the experiment and also provided helpful feedback and suggestions regarding the manuscript.

*Chapter 2* investigates the risk of sexual cannibalism and its effect on male approach and mating behaviour in false garden mantids. This is the first study to test and show that female mating status has an effect on sexual cannibalism in a praying mantid mating system. Behavioural experiments showed that females in poor body condition and mated females are more likely to cannibalise their partners. However, males did not show any

risk avoidance behaviours and approached females regardless of the risk of sexual cannibalism. This chapter is published in *Animal Behaviour* and co-authored by Darshana N. Rathnayake and Kaytlyn S. Davis, who provided support in conducting experiments and Katherine L. Barry who provided help in designing and conducting experiments, and in manuscript preparation.

*Chapter 3* explores the effect of sexual cannibalism on male ejaculatory expenditure in false garden mantids. This is the first study to show an increase in sperm transfer in response to sexual cannibalism in a praying mantid mating system. Sperm transfer trials revealed that cannibalised males transfer significantly more sperm to their partners, thereby maximizing their reproductive success from the only/last mating in their lifetime. This study showed that removal of the head might act as the main cue that triggers the increased sperm transfer by cannibalised males. The co-author Katherine L. Barry provided helpful suggestions on experimental design, data interpretation and the manuscript preparation and Marie E. Herberstein provided helpful suggestions for manuscript preparation.

*Chapter 4* investigates the effect of female quality on male ejaculatory expenditure and reproductive success in false garden mantids. Even though male *P. albofimbriata* prefer females in good body condition at the pre-copulatory stage, the results of this chapter showed that male preference does not continue during/after mating and males transfer their sperm regardless of female quality. In a single mating scenario, reproductive success depends only on the number of eggs that a female carries and not on the number of sperm transferred, which explains the lack of strategic ejaculation in response to female quality in the present study. This chapter is published in *Plos One* and co-authored by Katherine

L. Barry, who provided support in designing and conducting experiments, interpretation of results, and in manuscript preparation.

*Chapter 5* examines the unreceptivity of mated females and female multiple mating tendencies in the *P. albofimbriata* mating system. Results of field enclosure studies confirmed that mated female *P. albofimbriata* become chemically unattractive to other males for an average of eight days. The unreceptivity of mated females is likely a male-induced strategy as it might secure single paternity of all eggs laid in the first clutch. However, if the female attracts multiple males, the males stay near the female or mating pair until their chance for mating. In this scenario, females mate with multiple partners. This chapter is submitted to *Austral Entomology* and currently under review. Co-authors Darshana N. Rathnayake, Barbara Dean and Katherine L. Barry were involved in data collection and provided helpful suggestions on experimental design and the manuscript.

## **Chapter 1**

### **Male antenna morphology and its effect on scramble competition in false garden mantids**

Anuradhi Jayaweera & Katherine L. Barry

Published in *The Science of Nature*

---

Pages 23-31 of this thesis have been removed as they contain published material. Please refer to the following citation for details of the article contained in these pages.

Jayaweera, A., & Barry, K. L. (2017). Male antenna morphology and its effects on scramble competition in false garden mantids. *The Science of Nature*, 104:75.

DOI: [10.1007/s00114-017-1494-0](https://doi.org/10.1007/s00114-017-1494-0)

### **The risk of sexual cannibalism and its effect on male approach and mating behaviour in a praying mantid**

Anuradhi Jayaweera, Darshana N. Rathnayake, Kaytlyn S. Davis & Katherine L. Barry

Published in *Animal Behaviour*

---



Pages 33-39 of this thesis have been removed as they contain published material. Please refer to the following citation for details of the article contained in these pages.

Jayaweera, A., Rathnayake, D. N., Davis, K. S., & Barry, K. L. (2015). The risk of sexual cannibalism and its effect on male approach and mating behaviour in a praying mantid. *Animal Behaviour*, 110, p. 113-119.

DOI: [10.1016/j.anbehav.2015.09.021](https://doi.org/10.1016/j.anbehav.2015.09.021)

**Effect of sexual cannibalism on male ejaculatory expenditure  
in a praying mantid**

Anuradhi Jayaweera, Marie E. Herberstein & Katherine L. Barry

---

## Abstract

Strategic ejaculation by males, especially insects, is predicted under several scenarios such as under the risk of sperm competition and as part of cryptic male choice. However, limited future mating opportunities may also cause strategic ejaculation in animals. In precopulatory cannibalistic systems, females gain a reproductive advantage to the detriment of males. Theory predicts strong selection on males to counteract female aggression in a way to maximize their reproductive success from the final mating opportunity in their life time, but direct evidence of such strategies is sparse. We used the sexually cannibalistic false garden mantid *Pseudomantis albofimbriata* to test the effect of precopulatory cannibalism on male sperm allocation. We predicted cannibalised males would transfer more sperm to their mates as they do not have any future mating opportunities. If cannibalised males do transfer more sperm to their mates, we were also interested in determining the cue that triggers these males to increase their sperm transfer (i.e. whether the loss of the head causes increased sperm allocation). To test these hypotheses, we compared the number of sperm transferred by males in non-cannibalistic matings, in cannibalistic matings, and in experimentally manipulated headless matings. Cannibalised and headless males behaved similarly and transferred significantly more sperm than uncannibalised males. These results suggest cannibalised males invest more/all of their sperm in the current mating and the removal of the head (and therefore the primary ganglion) might play a role in triggering this increased sperm allocation.

Key words: cannibalism, headless males, *Pseudomantis albofimbriata*, strategic ejaculation

## Introduction

Males of variety of animal taxa have evolved optimal sperm allocation strategies with respect to mating context (e.g. depending on the risk and intensity of sperm competition) which maximize their lifetime reproductive success (Gage, 1991, Gage and Baker, 1991, Yusa, 1994, Marconato and Shapiro, 1996, Fuller, 1998, Allen et al., 2011, Gress and Kelly, 2011, Lupold et al., 2011). It is selectively beneficial for males to allocate their ejaculate strategically depending on the mating context, as sperm production can impose significant costs (Dewsbury, 1982). Post-copulatory sexual selection mechanisms such as cryptic male choice and sperm competition have been identified as the major causes of strategic ejaculation in animals (Bonduriansky, 2001, Parker and Pizzari, 2010). Theoretical and empirical evidence predicts and shows that males invest more mating resources in high quality/fecund females, resulting in greater reproductive success (Bonduriansky, 2001, Engqvist and Sauer, 2001, Reinhold et al., 2002). Similarly, in support of predictions, males adjust their ejaculatory components relative to the risk and intensity of sperm competition (Shapiro et al., 1994, Gage and Barnard, 1996, Simmons and Kvarnemo, 1997, Simmons, 2001, Mallard and Barnard, 2003, Simmons et al., 2007).

In addition to female quality and the risk of sperm competition, the availability/probability of future mating opportunities is also likely to result in strategic ejaculation. Life history theory (Stearns, 1976) predicts that older males should invest more mating resources in the current mating as the probability of having another mating is low. Accordingly, it has been shown that male mating investment in *Drosophila pseudoobscura* increases with age (Dhole and Pfennig, 2014). Similarly, a model developed by Galvani and Johnstone (1998) predicts that males should allocate less sperm early in the breeding season as there will be future matings, and that they should invest more sperm towards the end of the breeding season as the number of available mates reduces dramatically.

In sexually cannibalistic mating systems, the probability of future mating opportunities depends solely on whether the male is attacked and killed by the female. For cannibalised males who are capable of transferring sperm to females while being eaten (copulatory cannibalism), the current mating will be their last and/or only mating opportunity (Elgar and Nash, 1988, Barry et al., 2009, Wu et al., 2013, Jayaweera et al., 2015). Therefore, it is likely that cannibalised males will invest more in the current mating than uncannibalised males (who may have future matings), in order to maximize their reproductive success. Even though there have been many studies conducted on pre-copulatory mating strategies in sexually cannibalistic systems (Maxwell, 1999, Herberstein et al., 2002, Schneider and Elgar, 2002, Fromhage and Schneider, 2005, Lelito and Brown, 2006, Barry et al., 2008, Lelito and Brown, 2008, Barry et al., 2009, Barry, 2010, Barry et al., 2010, Maxwell et al., 2010, Roggenbuck et al., 2011, Brown et al., 2012, Kralj-Fiser et al., 2012, Barry, 2013, Barry, 2015) studies that investigate the effect of cannibalism on post-copulatory mating strategies are not very common (but see, Andrade, 1996, Schneider and Elgar, 2001, Fromhage et al., 2003, Nessler et al., 2009). For example, cannibalised male spiders generally invest more in the current mating in terms of increased copulation duration compared to uncannibalised males (Andrade, 1996, Schneider and Elgar, 2001, Fromhage et al., 2003). By increasing the length of the copulation, cannibalised male spiders may transfer more sperm to their partners. Further, cannibalised male Orb-web spiders, *Argiope lobata*, break off their genitalia within the female reproductive tract significantly more often than uncannibalised males, as it functions as a mating plug and guarantees their paternity (Nessler et al., 2009). Due to genital damage (e.g. Herberstein et al., 2012, Zimmer et al., 2014) and their peculiar reproductive morphology (Herberstein et al., 2011), male modern spiders generally have only two mating opportunities in their life time. By contrast, other sexually cannibalistic insects such as praying mantids are capable of multiple matings in nature (Barry et al., 2011, Umbers et al., 2011, Watanabe et al., 2011). Therefore, it is likely that praying

mantids have evolved much stronger ejaculatory strategies in response to sexual cannibalism than spiders.

In the current study we used the sexually cannibalistic false garden mantid *Pseudomantis albofimbriata* to determine the effects of sexual cannibalism on male sperm allocation. False garden mantids show precopulatory cannibalism and only half of cannibalised males succeed in transferring sperm to females (Barry et al., 2009, Jayaweera et al., 2015). For those males that are successful in mating, the current opportunity will be the final mating, as opposed to males that escape cannibalism and may mate in the future. We predicted that cannibalised males would transfer more sperm to their partners than non-cannibalised males, thereby maximizing their lifetime reproductive success. If cannibalised males do transfer more sperm, we also investigate the cue that triggers increased sperm transfer and we predicted decapitation as such a cue.

## Methods

### *Study species and study sites*

The false garden mantid *Pseudomantis albofimbriata* is a common mantid species distributed throughout Eastern Australia. Juvenile mantids ( $N = 150$ ) were collected in December 2014 -January 2015 from Ku-ring-gai Bicentennial Park, West Pymble ( $33^{\circ} 45' 37.76''\text{S}$ ,  $151^{\circ} 08' 20.88''\text{E}$ ) and Yamba Reserve, Ryde ( $33^{\circ} 49' 0''\text{S}$   $151^{\circ} 6' 0''\text{E}$ ), Australia. All individuals were found on the leaves and flowers of *Lomandra longifolia* bushes.

### *Ethics statement*

No permits were required for the described field collections/studies by New South Wales state law for the collection of invertebrates from locations outside of a national park. The study did not involve endangered or protected species.

### *Rearing, measuring and sexing mantids*

Mantids were reared individually within inverted transparent plastic cups (425 ml) of which the bottom end was replaced by mesh to facilitate better airflow. They were reared at a controlled temperature (25 - 26°C), light (14h) and humidity (55%) under laboratory conditions. Juvenile mantids were fed with two small crickets *Acheta domestica* (body mass =  $0.040 \pm 0.001$  g,  $N = 20$ ) three times per week until maturity, and watered daily. Once they became adults, the pronotum length (i.e. fixed size) was measured using electronic callipers and mass was recorded to three decimal places using a PB303-S DeltaRange scale. Individuals were sexed using differences in wing morphology. After adult eclosion, both males and females continued with the usual diet of two small crickets three times per week.

### *Mating trials*

Mating trials were conducted during the morning hours (0830 - 1130) in February - March 2015. Mating trials were conducted on logs fixed to Perspex frames (30 cm × 50 cm). First the female was introduced onto a log and she was given five minutes to settle into the novel environment. Then a male was introduced 5 cm away from the female. There were three different types of mating trials; 1) Normal matings without sexual cannibalism ( $N = 20$ ), where females were fed normally and mating trials were conducted just after feeding the female. In these trials the male was introduced 5 cm behind the

female to prevent cannibalism. 2) Cannibalised matings ( $N = 20$ ), where females were used at least one day after feeding and males were introduced from the front of the female, maximising the probability of sexual cannibalism (see Barry et al. 2009). 3) Headless mating ( $N = 15$ ), where trials were conducted in a similar way to normal mating, however, once a male mounted and intromitted, his head was cut off using sharp scissors. In this treatment male heads were cut  $\sim 5 - 10$  minutes after a successful intromission as generally the male head is fully separate from the body 5 - 10 minutes after intromission in normal cannibalism trials (A. Jayaweera, personal observations). All mating pairs were observed and copulation duration was recorded. The age of individuals was controlled so that on the day of the trial, females were  $24.0 \pm 0.57$  ( $N = 55$ ) days post-adult emergence and males were  $21.69 \pm 0.79$  ( $N = 55$ ) days post-adult emergence. Consequently, there was no significant difference in female and male age among treatments (Kruskal-Wallis test; [female age]:  $\chi^2 = 3.60$ ,  $P = 0.165$ ,  $df = 2$ ; [Male age]:  $\chi^2 = 4.666$ ,  $P = 0.097$ ,  $df = 2$ , Table 1).

### *Sperm counting methods*

After the mating terminated, the female was immobilized with CO<sub>2</sub> gas and dissected along the mid-dorsal line from posterior to anterior without damaging the interior organs. Her abdomen was fully opened by pinning the body wall to a wax tray. Next, the spermatophore was isolated (if present) and placed into a 1.5 mL Eppendorf tube containing 30  $\mu$ L of Grace's Insect Medium (Grace's Insect Medium, Supplemented ( $\times 1$ ), liquid, Life Technologies, Carlsbad, CA 92008). The spermatheca was then located and separated using fine forceps and placed onto a wax board containing 30  $\mu$ L of Grace's Insect Medium. The spermatheca was macerated very gently with small pins (for 90 seconds) under a dissecting microscope. After maceration, the contents of the spermatheca were pipetted out of the wax board into a 1.5 mL Eppendorf tube with



Grace's Insect Medium. An additional 70  $\mu\text{L}$  of Grace's Insect Medium was added to the spermatheca to make the total dilution 100  $\mu\text{L}$ . The spermatheca was soaked for 10 minutes in Grace's Insect Medium, allowing the majority of sperm to come into solution (see Gress and Kelly, 2011). Twenty  $\mu\text{L}$  of the spermatheca sample was pipetted into a new 1.5 mL Eppendorf tube, taking care not to transfer large clumps into the new tube. The solution was then stained with 5  $\mu\text{L}$  of Synergy Brands (SYBR) Green 1 nucleic acid gel stain (1  $\mu\text{L}$  SYBR Green 1 (10,000 $\times$  concentrate in DMSO) + 49  $\mu\text{L}$  of Grace's Insect medium) for 10 mins. After that, 10  $\mu\text{L}$  of the sample was pipetted out into a haemocytometer (Neubauer improved double net ruling SVZ2NIOU) and the number of sperm were counted in all grid squares under a fluorescence microscope. Sperm were identified easily, as sperm heads were dark green in colour under the FITC fluorescence channel. The same procedure was repeated to count the number of sperm within the spermatophore, except it was macerated by three quick ruptures with fine forceps. The total numbers of sperm found in the spermatheca and the spermatophore were calculated separately by dividing the sum of sperm by grid volume (0.9  $\mu\text{L}$ ) and then multiplying by the dilution factor (100  $\mu\text{L}$ ). Finally, the total number of sperm transferred by each male was calculated by adding the number of sperm found in the spermatheca to the number of sperm in the spermatophore.

#### *Data analyses*

Data were analysed using SPSS 21.0 for Windows and were checked for normal distribution (Kolmogorov-Smirnov test) before analysis. Unless otherwise stated, all values are mean  $\pm$  standard error and all statistical tests are two tailed. We performed a Kruskal-Wallis test to compare female age, male age and copulation duration between the three different treatments. A one way ANOVA test was used to determine the difference in total number of sperm transferred between the three different treatments. Post hoc

tests (Tukey HSD) were performed to determine the difference in total sperm number between the treatment groups. We performed general linear models to determine the relationships between total sperm number and copulation duration, male size, male condition, female size and female condition for each treatment.

## Results

In all normal mating without cannibalism ( $N = 20$ ), cannibalised mating ( $N = 20$ ) and headless mating ( $N = 15$ ) trials, males successfully intromitted and transferred sperm to their females. We found a significant difference in the total number of sperm transferred between the three different treatments (one way ANOVA;  $F = 5.764$ ,  $df = 2$ ,  $P = 0.005$ , Table 1, Figure 1). Post hoc tests confirmed that cannibalised males and headless males transferred significantly higher numbers of sperm than normally mated males (Tukey HSD; [cannibalised matings vs. normal matings]:  $P = 0.033$ ; [headless matings vs. normal matings]:  $P = 0.008$ ). However, there was no significant difference in total sperm number between cannibalised mating and headless matings (Tukey HSD;  $P = 0.729$ ). We found no significant difference in copulation duration between the different treatments (Kruskal-Wallis test;  $\chi^2 = 3.708$ ,  $df = 2$ ,  $P = 0.157$ , Table 1, Figure 2). The total number of sperm transferred was independent of copulation duration, male size, male condition, female size and female condition (Table 2).

## Discussion

As predicted, cannibalised males transferred a significantly higher number of sperm to their mates than non-cannibalised males. Specifically, cannibalised males transferred twice as much sperm as those males that were not cannibalised. These data suggest that cannibalised males terminally invest their mating resources in the current mating as predicted by life history theory (Stearns, 1976). Sexual cannibalism is an extreme form of sexual conflict in this mating system (Barry et al., 2008, Barry et al., 2009, Jayaweera et al., 2015) and increased sperm transfer by cannibalised males may counteract the costs imposed on males by the females. It is possible that the normally mated males partitioned ejaculatory components between current and future mating opportunities. Female praying mantids do not appear to be sperm limited and receive sufficient sperm from a single mating to fertilise all eggs (Jayaweera and Barry, 2015). Therefore, non-cannibalised males may partition their ejaculate for future matings. However, sperm number may have an effect on the reproductive success if females mate with multiple males (Parker, 1990, Parker et al., 1990, Parker and Pizzari, 2010). Unmated female *P. albobimbrata* attract multiple males simultaneously to their initial pheromone plume (Barry, 2010, Barry, 2013, Barry and Wilder, 2013, Barry, 2015), raising the probability of multiple matings. However, polyandry is likely to be a relatively rare phenomenon in this mating system as a single mating is the most common scenario compared to multiple matings in both natural and semi-natural environments (K. Barry & A. Jayaweera, unpublished data).

Similar to previous findings, we found that sperm number is independent of male size, male condition, female size and female condition (Jayaweera and Barry, 2015). This result may be due to the narrow range of variability in males used in the current study (i.e. all males were on the same feeding treatment throughout their development). We did not find any difference in the copulation duration between cannibalised and non-

cannibalised males. Copulation duration is often linked to the number of sperm transferred, but only in those animals that transfer free flowing sperm during copulation (reviewed in Andrade, 1996, Schneider and Elgar, 2001, Simmons, 2001, Engqvist and Sauer, 2003, Fromhage et al., 2003). As praying mantids transfer sperm in units called spermatophores, the number of sperm transferred does not relate to copulation duration.

Interestingly, we found that males from the headless treatment behaved very similarly to cannibalised males, transferring a significantly higher number of sperm compared to non-cannibalised males. In *P. albofimbriata*, females usually attack the thoracic region of the male (Barry et al 2009) and eventually the male head is separated from the rest of the body (this generally occurs ~10 minutes after cannibalism begins; K. Barry & A. Jayaweera, personal observations). As separation of the head from the rest of the body is the common link between the cannibalised and headless male treatments, we suggest that removal of the head (either during the initial stages of cannibalism or through artificial decapitation) may act as a trigger additional sperm transfer.

While it is easy to understand how increasing sperm transfer may be an adaptive male behaviour in cannibalistic species, we must also consider the possibility of non-adaptive explanations. Roeder (1998), previously observed that surgical decapitation or cutting the nerve cord transversely causes increased sexual activity in some insects. For example, in praying mantids (e.g. *Mantis religiosa*), decapitation or removal of the oesophageal ganglion resulted in continuous sexual activity (S-bending of male abdomen and walking movements to position themselves along the female body) in males and increased egg laying in females (Roeder, 1935, Roeder, 1998). This decapitation effect has been interpreted as an endogenous behaviour resulting from an intrinsic instability of the central nervous system (Roeder, 1998). Therefore, it is possible that increased sperm transfer by cannibalised/headless males may be the result of a loss of coordination in the central nervous system. This proposal is supported by observations of non-cannibalistic

species such as cockroaches, where the removal of the terminal ganglion results in intense sexual behaviour similar to that seen in male mantids (Roeder, 1998). Thus, the most likely explanation for the increased sperm transfer by cannibalised male mantids is that it was initially a non-adaptive response due to a lack of neuron control, but that the nature of this mating system (i.e. occurrence of sexual cannibalism) makes it secondarily adaptive by improving the reproductive success for cannibalised males.

Whether decapitation specifically, or damage more generally, is the cue responsible for the observed increased sperm transfer is not entirely clear. Therefore, we are planning to extend this study by implementing various injury treatments and a focal cooling of brain (using a thermoelectric device without damaging the head) and counting the subsequent number of sperm transferred.

## **Acknowledgements**

We thank Darshana Rathnayake and Barbara Dean for their support in collecting juvenile mantids, to Kelton Jarvis, Zachary Sheehan and Andrew Ly for their help in the rearing of mantids and to Nicole Vella from the Microscopy unit, Macquarie University for assistance with fluorescence microscopy. Hermon Slade Foundation funded this research.

## References

- ALLEN, L. E., BARRY, K. L., HOLWELL, G. I. & HERBERSTEIN, M. E. 2011. Perceived risk of sperm competition affects juvenile development and ejaculate expenditure in male praying mantids. *Animal Behaviour*, 82, 1201-1206.
- ANDRADE, M. C. B. 1996. Sexual selection for male sacrifice in the Australian redback spider. *Science*, 271, 70-72.
- BARRY, K. L. 2010. Influence of female nutritional status on mating dynamics in a sexually cannibalistic praying mantid. *Animal Behaviour*, 80, 405-411.
- BARRY, K. L. 2013. You are what you eat: food limitation affects reproductive fitness in a sexually cannibalistic praying mantid. *PLoS One*, 8, e78164.
- BARRY, K. L. 2015. Sexual deception in a cannibalistic mating system? Testing the Femme Fatale hypothesis. *Proceedings of the Royal Society of London B: Biological Sciences*, 282, 20141428.
- BARRY, K. L., HOLWELL, G. I. & HERBERSTEIN, M. E. 2008. Female praying mantids use sexual cannibalism as a foraging strategy to increase fecundity. *Behavioral Ecology*, 19, 710-715.
- BARRY, K. L., HOLWELL, G. I. & HERBERSTEIN, M. E. 2009. Male mating behaviour reduces the risk of sexual cannibalism in an Australian praying mantid. *Journal of Ethology*, 27, 377-383.
- BARRY, K. L., HOLWELL, G. I. & HERBERSTEIN, M. E. 2010. Multimodal mate assessment by male praying mantids in a sexually cannibalistic mating system. *Animal Behaviour*, 79, 1165-1172.
- BARRY, K. L., HOLWELL, G. I. & HERBERSTEIN, M. E. 2011. A paternity advantage for speedy males? Sperm precedence patterns and female re-mating frequencies in a sexually cannibalistic praying mantid. *Evolutionary Ecology*, 25, 107-119.

- BARRY, K. L. & WILDER, S. M. 2013. Macronutrient intake affects reproduction of a predatory insect. *Oikos*, 122, 1058-1064.
- BONDURIANSKY, R. 2001. The evolution of male mate choice in insects: A synthesis of ideas and evidence. *Biological Reviews*, 76, 305-339.
- BROWN, W. D., MUNTZ, G. A. & LADOWSKI, A. J. 2012. Low mate encounter rate increases male risk taking in a sexually cannibalistic praying mantis. *Plos One*, 7, e35377.
- DEWSBURY, D. A. 1982. Ejaculate cost and male choice. *American Naturalist*, 119, 601-610.
- DHOLE, S. & PFENNIG, K. S. 2014. Age-dependent male mating investment in *Drosophila pseudoobscura*. *Plos One*, 9, e88700.
- ELGAR, M. A. & NASH, D. R. 1988. Sexual cannibalism in the Garden spider *Araneus diadematus*. *Animal Behaviour*, 36, 1511-1517.
- ENGQVIST, L. & SAUER, K. P. 2001. Strategic male mating effort and cryptic male choice in a scorpionfly. *Proceedings of the Royal Society B-Biological Sciences*, 268, 729-735.
- ENGQVIST, L. & SAUER, K. P. 2003. Determinants of sperm transfer in the scorpionfly *Panorpa cognata*: male variation, female condition and copulation duration. *Journal of Evolutionary Biology*, 16, 1196-1204.
- FROMHAGE, L. & SCHNEIDER, J. M. 2005. Safer sex with feeding females: sexual conflict in a cannibalistic spider. *Behavioral Ecology*, 16, 377-382.
- FROMHAGE, L., UHL, G. & SCHNEIDER, J. M. 2003. Fitness consequences of sexual cannibalism in female *Argiope bruennichi*. *Behavioral Ecology and Sociobiology*, 55, 60-64.
- FULLER, R. C. 1998. Sperm competition affects male behaviour and sperm output in the rainbow darter. *Proceedings of the Royal Society B: Biological Sciences*, 265, 2365-2371.
- GAGE, A. R. & BARNARD, C. J. 1996. Male crickets increase sperm number in relation to competition and female size. *Behavioral Ecology and Sociobiology*, 38, 349-353.



- GAGE, M. J. G. 1991. Risk of sperm competition directly affects ejaculate size in the Mediterranean fruit fly. *Animal Behaviour*, 42, 1036-1037.
- GAGE, M. J. G. & BAKER, R. R. 1991. Ejaculate size varies with socio-sexual situation in an insect. *Ecological Entomology*, 16, 331-337.
- GALVANI, A. & JOHNSTONE, R. 1998. Sperm allocation in an uncertain world. *Behavioral Ecology and Sociobiology*, 44, 161-168.
- GRESS, B. E. & KELLY, C. D. 2011. Is sperm viability independent of ejaculate size in the house cricket (*Acheta domesticus*)? *Canadian Journal of Zoology*, 89, 1231-1236.
- HERBERSTEIN, M., SCHNEIDER, J., UHL, G. & MICHALIK, P. 2011. Sperm dynamics in spiders. *Behavioral Ecology*, 22, 692-695.
- HERBERSTEIN, M., WIGNALL, A., NESSLER, S., HARMER, A. & SCHNEIDER, J. 2012. How effective and persistent are fragments of male genitalia as mating plugs? *Behavioral Ecology*, 23, 1140-1145.
- HERBERSTEIN, M. E., SCHNEIDER, J. M. & ELGAR, M. A. 2002. Costs of courtship and mating in a sexually cannibalistic orb-web spider: female mating strategies and their consequences for males. *Behavioral Ecology and Sociobiology*, 51, 440-446.
- JAYAWEERA, A. & BARRY, K. L. 2015. The Effect of female quality on male ejaculatory expenditure and reproductive success in a praying mantid. *Plos One*, 10, e0124209.
- JAYAWEERA, A., RATHNAYAKE, D. N., DAVIS, K. S. & BARRY, K. L. 2015. The risk of sexual cannibalism and its effect on male approach and mating behaviour in a praying mantid. *Animal Behaviour*, 110, 113-119.
- KRALJ-FISER, S., SCHNEIDER, J. M., JUSTINEK, Z., KALIN, S., GREGORIC, M., PEKAR, S. & KUNTNER, M. 2012. Mate quality, not aggressive spillover, explains sexual cannibalism in a size-dimorphic spider. *Behavioral Ecology and Sociobiology*, 66, 145-151.

- LELITO, J. P. & BROWN, W. D. 2006. Natural history miscellany - Complicity or conflict over sexual cannibalism? Male risk taking in the praying mantis *Tenodera aridifolia sinensis*. *American Naturalist*, 168, 263-269.
- LELITO, J. P. & BROWN, W. D. 2008. Mate attraction by females in a sexually cannibalistic praying mantis. *Behavioral Ecology and Sociobiology*, 63, 313-320.
- LUPOLD, S., MANIER, M. K., ALA-HONKOLA, O., BELOTE, J. M. & PITNICK, S. 2011. Male *Drosophila melanogaster* adjust ejaculate size based on female mating status, fecundity, and age. *Behavioral Ecology*, 22, 184-191.
- MALLARD, S. T. & BARNARD, C. J. 2003. Competition, fluctuating asymmetry and sperm transfer in male gryllid crickets (*Gryllus bimaculatus* and *Gryllodes sigillatus*). *Behavioral Ecology and Sociobiology*, 53, 190-197.
- MARCONATO, A. & SHAPIRO, D. Y. 1996. Sperm allocation, sperm production and fertilization rates in the bucktooth parrotfish. *Animal Behaviour*, 52, 971-980.
- MAXWELL, M. R. 1999. The risk of cannibalism and male mating behavior in the Mediterranean praying Mantis, *Iris oratoria*. *Behaviour*, 136, 205-219.
- MAXWELL, M. R., GALLEGU, K. M. & BARRY, K. L. 2010. Effects of female feeding regime in a sexually cannibalistic mantid: fecundity, cannibalism, and male response in *Stagmomantis limbata* (Mantodea). *Ecological Entomology*, 35, 775-787.
- NESSLER, S. H., UHL, G. & SCHNEIDER, J. M. 2009. Sexual cannibalism facilitates genital damage in *Argiope lobata* (Araneae: Araneidae). *Behavioral Ecology and Sociobiology*, 63, 355-362.
- PARKER, G. A. 1990. Sperm competition games: Raffles and roles. *Proceedings of the Royal Society B: Biological Sciences*, 242, 120-126.
- PARKER, G. A. & PIZZARI, T. 2010. Sperm competition and ejaculate economics. *Biological Reviews*, 85, 897-934.

- PARKER, G. A., SIMMONS, L. W. & KIRK, H. 1990. Analysing sperm competition data: simple models for predicting mechanisms. *Behavioral Ecology and Sociobiology*, 27, 55-65.
- REINHOLD, K., KURTZ, J. & ENGQVIST, L. 2002. Cryptic male choice: sperm allocation strategies when female quality varies. *Journal of Evolutionary Biology*, 15, 201-209.
- ROEDER, K. D. 1935. An experimental analysis of the sexual behavior of the praying mantis (*Mantis religiosa* L.). *Biological Bulletin*, 69, 203-220.
- ROEDER, K. D. 1998. *Nerve Cells and Insect Behavior*, Harvard University Press.
- ROGGENBUCK, H., PEKAR, S. & SCHNEIDER, J. M. 2011. Sexual cannibalism in the European garden spider *Araneus diadematus*: the roles of female hunger and mate size dimorphism. *Animal Behaviour*, 81, 749-755.
- SCHNEIDER, J. M. & ELGAR, M. A. 2001. Sexual cannibalism and sperm competition in the golden orb-web spider *Nephila plumipes* (Araneoidea): female and male perspectives. *Behavioral Ecology*, 12, 547-552.
- SCHNEIDER, J. M. & ELGAR, M. A. 2002. Sexual cannibalism in *Nephila plumipes* as a consequence of female life history strategies. *Journal of Evolutionary Biology*, 15, 84-91.
- SHAPIRO, D. Y., MARCONATO, A. & YOSHIKAWA, T. 1994. Sperm competition in a coral-reef fish, *Thalassoma bifasciatum*. *Ecology*, 75, 1334-1344.
- SIMMONS, L. W. 2001. *Sperm Competition and its Evolutionary Consequences in the Insects*, Princeton, New Jersey: Princeton University Press.
- SIMMONS, L. W., DENHOLM, A., JACKSON, C., LEVY, E. & MADON, E. 2007. Male crickets adjust ejaculate quality with both risk and intensity of sperm competition. *Biology Letters*, 3, 520-522.
- SIMMONS, L. W. & KVARNEMO, C. 1997. Ejaculate expenditure by male bushcrickets decreases with sperm competition intensity. *Proceedings of the Royal Society B-Biological Sciences*, 264, 1203-1208.

- STEARNS, S. C. 1976. Life history tactics: A review of the ideas. *Quarterly Review of Biology*, 51, 3-47.
- UMBERS, K. D. L., HOLWELL, G. I., STOW, A. J. & HERBERSTEIN, M. E. 2011. Molecular evidence for variation in polyandry among praying mantids (Mantodea: Ciulfina). *Journal of Zoology*, 284, 40-45.
- WATANABE, E., ADACHI-HAGIMORI, T., MIURA, K., MAXWELL, M. R., ANDO, Y. & TAKEMATSU, Y. 2011. Multiple paternity within field-collected egg cases of the praying mantid *Tenodera aridifolia*. *Annals of the Entomological Society of America*, 104, 348-352.
- WU, L. B., ZHANG, H. P., HE, T., LIU, Z. L. & PENG, Y. 2013. Factors influencing sexual cannibalism and its benefit to fecundity and offspring survival in the wolf spider *Pardosa pseudoannulata* (Araneae: Lycosidae). *Behavioral Ecology and Sociobiology*, 67, 205-212.
- YUSA, Y. 1994. Factors regulating sperm transfer in an hermaphroditic sea hare, *Aplysia-Parvula* Morch, 1863 (Gastropoda, Opisthobranchia). *Journal of Experimental Marine Biology and Ecology*, 181, 213-221.
- ZIMMER, S. M., SCHNEIDER, J. M. & HERBERSTEIN, M. E. 2014. Can males detect the strength of sperm competition and presence of genital plugs during mate choice? *Behavioral Ecology*, 25, 716-722.

Table 1. Comparison of female age, male age, number of sperm transferred and copulation duration among treatments

	<b>Normal matings</b>	<b>Cannibalised &amp; mated</b>	<b>Headless matings</b>
<b>Female age/days</b>	22.55 ± 0.44	23.55 ± 0.54	26.53 ± 1.76
<b>Male age/days</b>	19.30 ± 1.24	22.50 ± 1.24	23.80 ± 1.56
<b>Total sperm transferred</b>	34155 ± 3903	64761 ± 9592	74488 ± 12406
<b>Copulation duration/minutes</b>	375.65 ± 12.45	384.75 ± 18.20	421.73 ± 30.00

Table 2. Relationship between the total number of sperm transferred and copulation duration, male morphology (size and condition) and female morphology (size and condition).

	<b>Treatment</b>	<b><i>F</i></b>	<b><i>P</i></b>	<b>Effect size</b>
<b>Copulation duration</b>	Normally mated without cannibalism	1.234	0.285	0.081
	Cannibalised and mated	0.210	0.654	0.015
	Headless and mated	0.860	0.378	0.087
<b>Male size</b>	Normally mated without cannibalism	0.160	0.695	0.011
	Cannibalised and mated	1.066	0.319	0.071
	Headless and mated	3.677	0.087	0.290
<b>Male condition</b>	Normally mated without cannibalism	2.287	0.153	0.140
	Cannibalised and mated	1.978	0.181	0.124
	Headless and mated	2.147	0.177	0.193
<b>Female size</b>	Normally mated without cannibalism	1.428	0.252	0.093
	Cannibalised and mated	1.696	0.214	0.108
	Headless and mated	2.115	0.180	0.190
<b>Female condition</b>	Normally mated without cannibalism	0.523	0.481	0.036
	Cannibalised and mated	0.442	0.517	0.031
	Headless and mated	2.310	0.163	0.204

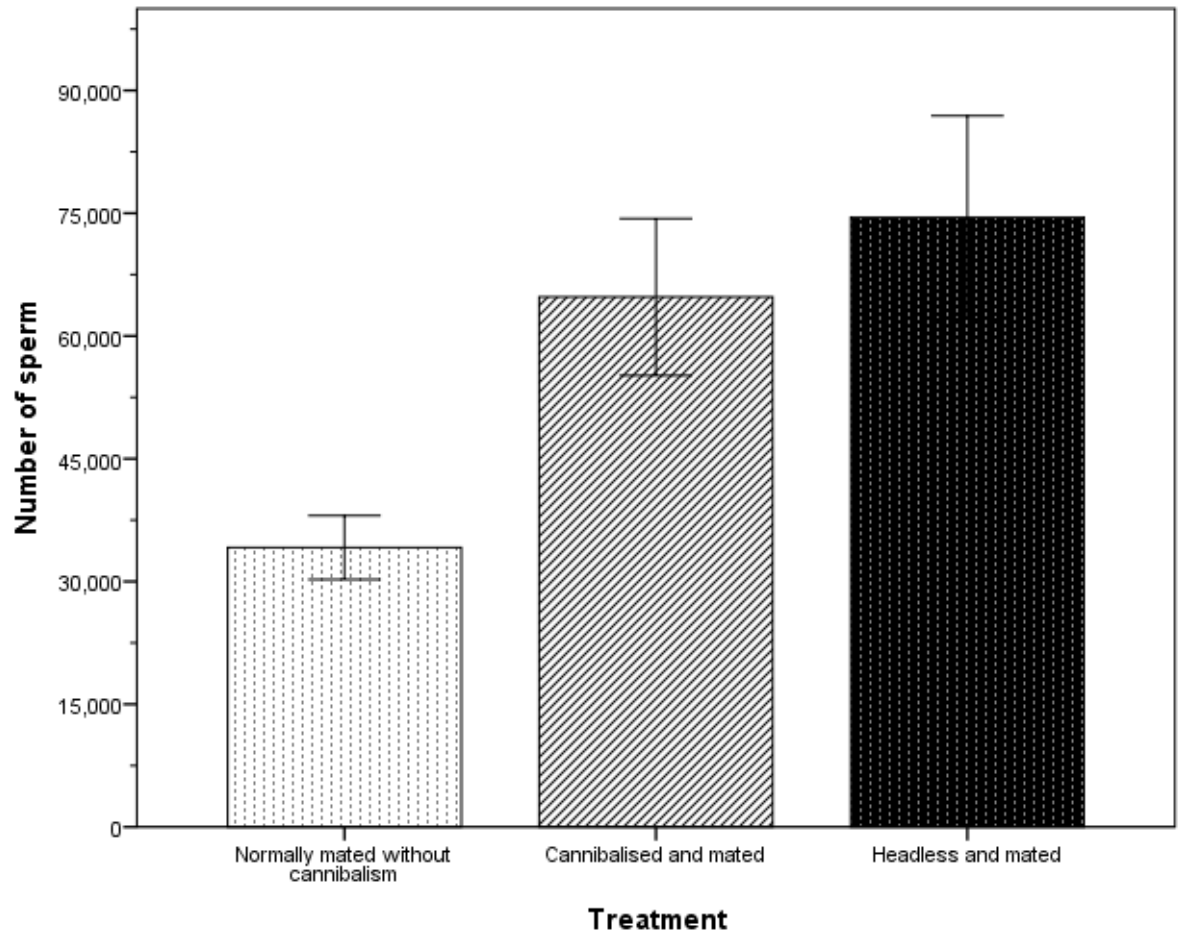


Figure 1. Total number of sperm transferred by normally mated, cannibalised and mated and headless mated male *P. albofimbriata*.

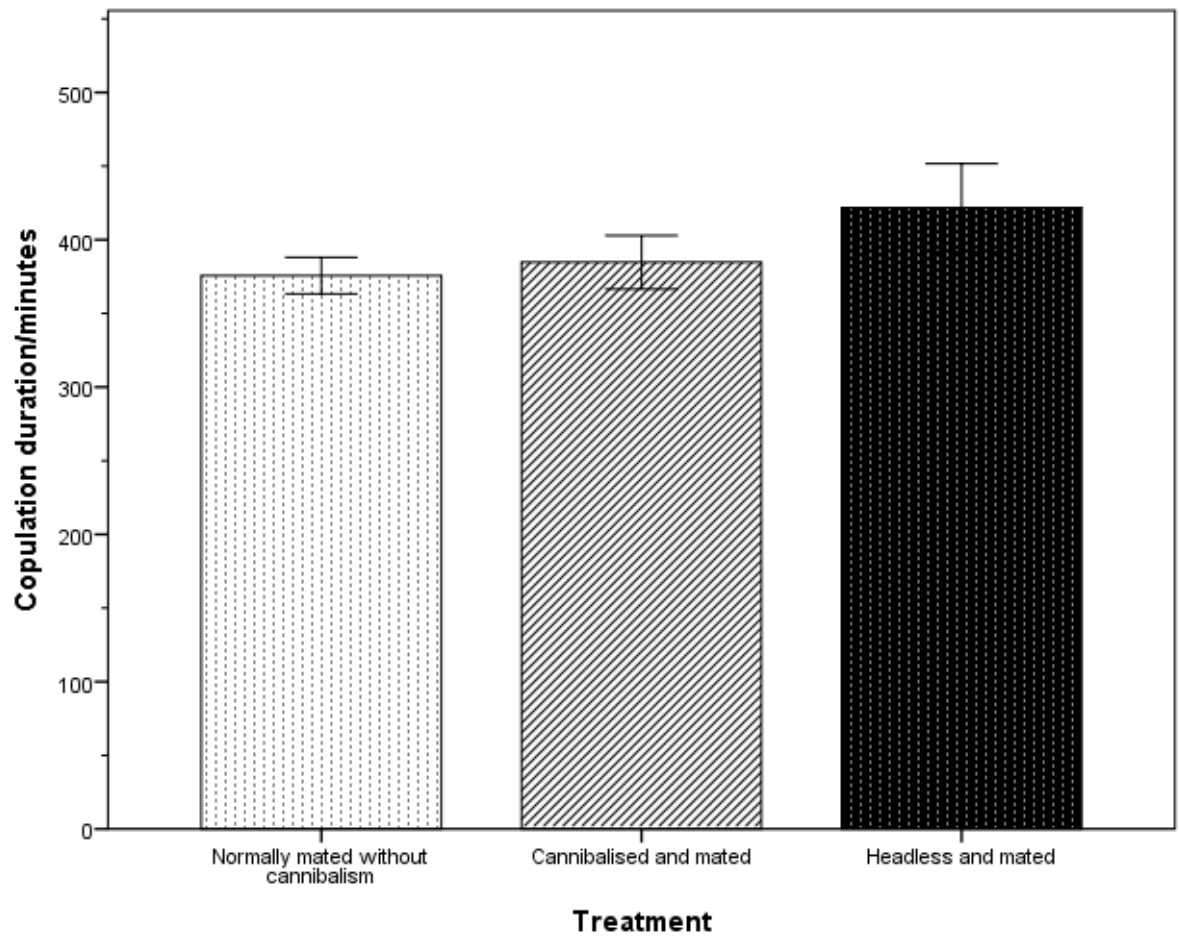


Figure 2. Copulation duration in normally mated, cannibalised mated and headless mated mating trials in *P. albofimbriata*



**The Effect of Female Quality on Male Ejaculatory Expenditure  
and Reproductive Success in a Praying Mantid**

Anuradhi Jayaweera & Katherine L. Barry

Published in *Plos One*

---

RESEARCH ARTICLE

# The Effect of Female Quality on Male Ejaculatory Expenditure and Reproductive Success in a Praying Mantis

Anuradhi Jayaweera\*, Katherine L. Barry

Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia

\* [anu.jayaweera@mq.edu.au](mailto:anu.jayaweera@mq.edu.au)



## Abstract

Strategic ejaculation is a behavioural strategy shown by many animals as a response to sperm competition and/or as a potential mechanism of cryptic male choice. Males invest more mating resources when the risk of sperm competition increases or they invest more in high quality females to maximize their reproductive output. We tested this hypothesis in the false garden mantid *Pseudomantis albobimbrata*, where females are capable of multiply mating and body condition is an indicator of potential reproductive fitness. We predicted male mantids would ejaculate strategically by allocating more sperm to high quality females. To determine if and how males alter their ejaculate in response to mate quality, we manipulated female food quantity so that females were either in good condition with many eggs (i.e. high quality) or poor condition with few eggs (i.e. low quality). Half of the females from each treatment were used in mating trials in which transferred sperm was counted before fertilisation occurred and the other half of females were used in mating trials where fertilisation occurred and ootheca mass and total eggs in the ootheca were recorded. Opposed to our predictions, the total number of sperm and the proportion of viable sperm transferred did not vary significantly between female treatments. Male reproductive success was entirely dependent on female quality/fecundity, rather than on the number of sperm transferred. These results suggest that female quality is not a major factor influencing postcopulatory male mating strategies in *P. albobimbrata*, and that sperm number has little effect on male reproductive success in a single mating scenario.

## OPEN ACCESS

**Citation:** Jayaweera A, Barry KL (2015) The Effect of Female Quality on Male Ejaculatory Expenditure and Reproductive Success in a Praying Mantis. PLoS ONE 10(5): e0124209. doi:10.1371/journal.pone.0124209

**Academic Editor:** Thérèse M. Jones, University of Melbourne, AUSTRALIA

**Received:** November 4, 2014

**Accepted:** March 10, 2015

**Published:** May 13, 2015

**Copyright:** © 2015 Jayaweera, Barry. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Funding:** The authors received no specific funding for this work.

**Competing Interests:** The authors have declared that no competing interests exist.

## Introduction

According to Darwin's theory of sexual selection, females are the choosy sex whereas males compete with each other for female mates [1]. However, it was subsequently suggested that males might also become choosy when the associated costs of reproduction are high; for example when parental investment is significantly higher for males [2] or if females vary greatly in quality [3,4]. Intrasexual competition and male mate choice may continue during and/or after copulation in the form of sperm competition [5] or cryptic male choice [6,7]. These

postcopulatory selective forces shape male mating strategies and are predicted to influence male reproductive success. Strategic ejaculation, where males tailor the ejaculate in response to variation in potential reproductive benefits [8], has been given much attention in the sperm competition and male mate choice literature during the last decade. Strategic ejaculation is likely to have evolved mainly as a defensive mechanism to overcome sperm competition [9–11], but also as a potential method of postcopulatory mate choice, where males cryptically invest more ejaculate into high quality females [12]. Although the sperm competition and cryptic male choice hypotheses are independent, it is important to note that they are not mutually exclusive. Indeed, except in rare instances where females are sperm limited, differential ejaculate expenditure as a result of cryptic male choice will coincide with sperm competition.

Sperm competition models predict that males should increase their mating investment when the risk of sperm competition (i.e. probability of competition) increases [11,13,14], but that they should decrease their mating investment when the intensity of sperm competition (i.e. absolute number of competitors) increases [9]. Male-male competition, female mating history and female quality have been identified as the most probable indicators of sperm competition and likely relate to the subsequent potential for strategic ejaculation [8,15]. Males of some species do indeed manipulate their ejaculate when competitors are present before or during copulation [16–20]. Risk and intensity of sperm competition can vary greatly with female mating history and the average mating rate of females in a particular mating system. Therefore, as suggested by Engqvist and Reinhold [10], males should invest comparatively more sperm in virgin females when the mating rate is high and when the mating rate is low they should invest more sperm in already mated females. And finally, female quality is associated with sperm competition because body condition may predict a female's likelihood of re-mating with other males: higher quality females are more likely to receive attention from additional males than lower quality females. Empirical evidence coming mainly from insects indicates that males tailor their ejaculate strategically in response to female quality, presumably to counteract the risk or intensity of sperm competition [18,20–25].

Cryptic male choice plays a significant role in strategic ejaculation in animals [4,12]. It is more likely to evolve in response to the indicators of female fecundity, genetic compatibility, relatedness or female mating status (reviewed in [7]). Female quality is an indicator of potential reproductive ability/output of a particular female and can be measured in several ways such as age, size, condition or fecundity [4]. Since high quality females will have a higher potential reproductive fitness [6,26,27], it is selectively beneficial for males to invest more mating resources into those females that maximise reproductive success. For example, male scorpionfly *Panorpa cognata* produce larger salivary masses when copulating with highly fecund females, and females feed on the salivary masses during copulation [28]. Therefore, by increasing the size of the nuptial gift, males may copulate for longer and subsequently transfer more sperm.

Praying mantids are an ideal group in which to investigate the response to sperm competition and/or cryptic male choice because females are capable of multiple copulations [29–31], females store sperm in a sac-shaped spermatheca [32], and numerous males are often simultaneously attracted to the pheromones emitted by a single female [27,33–35]. However, very few studies have attempted to determine if and how male mantids respond to the risk of sperm competition (but see [36,37]). Prokop and Vaclav [36] found that male *Mantis religiosa* increase their copulation duration when the perceived risk of sperm competition is high (i.e. when the sex ratio is male biased). Copulation duration is often linked to the number of sperm transferred (reviewed in [6]), so the authors suggest that male *M. religiosa* mantids do indeed respond to the level of sperm competition risk. However, the link between copulation duration and sperm number is mostly relevant for species in which males transfer free-flowing sperm, so these results should be considered with caution. To date, there has only been one study that

has directly tested the effect of sperm competition on strategic sperm allocation in praying mantids. Allen et al. [37] found that false garden mantids *Pseudomantis albofimbriata* transfer more sperm when the perceived risk of sperm competition is high (i.e. when the sex ratio is male biased), indicating that males are indeed capable of strategic ejaculation when they perceive an increased risk of intrasexual competition. Studies that investigate other factors (i.e. female mating status and female quality) are still required to fully understand the range of male responses in praying mantids.

In the current study, we used the sexually cannibalistic false garden mantid *Pseudomantis albofimbriata* to investigate the effect of female quality (i.e. body condition/fecundity) on male ejaculatory expenditure and reproductive success. Female quality is a major factor shaping the precopulatory mating strategies of this species [26,34,38–40]. Female body condition is positively related to female fecundity and negatively related to the propensity for cannibalism [26], and therefore males prefer good quality females in both close range (visual or chemical cues; [39]) and long distance (only chemical cues; [34,40]) mate choice trials. However, little is known about the influence of female quality on postcopulatory male mating strategies in this species. Therefore, our primary objective in this study was to determine whether male mantids allocate their sperm strategically in response to female quality. We predict that male *P. albofimbriata* will allocate more mating resources to high quality females via (1) the total number of sperm transferred, and/or (2) the proportion of viable sperm transferred. Our second objective was to link the amount of sperm transferred with actual reproductive success by comparing egg sac mass, total eggs in the egg sac and the percentage of eggs in the egg sac between high and low quality females. Finally, we determined whether male traits had an effect on sperm transfer or on actual reproductive success.

## Methods

### Study species and study sites

The false garden mantid *Pseudomantis albofimbriata* is a common mantid species distributed throughout Eastern Australia. Juvenile mantids ( $N = 130$ ) were collected in December 2013–January 2014 from Ku-ring-gai Bicentennial Park, West Pymble ( $33^{\circ} 45' 37.76''\text{S}$ ,  $151^{\circ} 08' 20.88''\text{E}$ ) and Yamba Reserve, Ryde ( $33^{\circ} 49' 0''\text{S}$   $151^{\circ} 6' 0''\text{E}$ ), Australia. All individuals were found on the leaves and flowers of *Lomandra longifolia* bushes.

### Ethics statement

No permits were obtained for the described field collections/studies because New South Wales state law does not require specific permissions for the collection of invertebrates from locations outside of a national park. The study did not involve endangered or protected species.

### Rearing, sexing and measuring mantids

Mantids were reared individually within inverted transparent plastic cups of which the bottom end was replaced by mesh to facilitate better air flow. They were reared at a controlled temperature ( $25\text{--}26^{\circ}\text{C}$ ), light (14h) and humidity (55%) in laboratory conditions. Juvenile mantids were fed with two small crickets *Acheta domesticus* (body mass =  $0.040 \pm 0.001$  g,  $N = 20$ ) three times per week until maturity and watered daily. Once they became adults the pronotum length (i.e. fixed size) and mass were recorded, and individuals were sexed using differences in wing morphology. After adult eclosion, males continued with the usual diet of two small crickets three times per week, whereas females were allocated to one of the two feeding regimes: high quantity or low quantity. Females on the high quantity treatment ( $N = 30$ ) were given three

small crickets three times per week, and individuals on the low quantity treatment ( $N = 32$ ) received one small cricket three times per week. Both males and females were weighed immediately preceding mating trials using digital scales. Body condition was calculated as body mass divided by fixed size, and was used as a measure of female quality.

## Mating trials

Mating trials were conducted in a large, high-ceilinged laboratory during the morning hours (0830–1130) in February–March 2014, and individuals were introduced onto a potted *Lomandra sp.* plant. First, the female was introduced onto a plant and was given five minutes to settle into the novel environment. Then a male was introduced 5 cm behind the female and precautions were taken to avoid cannibalism. Copulation was interrupted 3.5 hours after intromission (spermatophore transferred at  $\sim 3$  hours [37]) and both the female and male were reweighed. After successful mating, approximately half of the females from each feeding treatment were used in sperm transfer trials ( $N = 29$ ) and the other half were used in reproductive success trials ( $N = 33$ ). In order to measure sperm number, females had to be euthanized and dissected which precluded collecting data on fertilisation rates. There was no significant difference in female body condition between sperm transfer trials ( $0.057 \pm 0.004$  g/mm) and reproductive success trials ( $0.053 \pm 0.003$  g/mm; Mann-Whitney  $U$  test:  $Z = -0.120$ ,  $N = 62$ ,  $P = 0.905$ ).

## Aim 1. Do males allocate more sperm to high quality females?

The age of individuals was controlled so that on the day of the trial, females were  $25.31 \pm 0.65$  ( $N = 29$ ) days post-adult emergence and males were  $26.17 \pm 1.09$  ( $N = 29$ ) days post-adult emergence. After mating and sperm counting was complete, the female was dissected (see below for [methods](#)), and the number of large mature and small immature eggs in both ovaries was counted under a stereomicroscope. On the day of the mating trial, high food quantity treatment females were in significantly better condition ( $0.080 \pm 0.003$  g/mm,  $N = 14$ ) than low food quantity females ( $0.035 \pm 0.001$  g/mm,  $N = 15$ ,  $t$ -test:  $t_{27} = 12.95$ ,  $P < 0.001$ ). High quantity females had also produced significantly more mature eggs ( $91.29 \pm 6.8$ ) than low quantity females ( $22.33 \pm 4.5$ ; Mann-Whitney  $U$  test:  $Z = -4.596$ ,  $N = 29$ ,  $P < 0.001$ ). Further, female body condition significantly predicted the number of mature eggs found in a female's ovaries (linear regression:  $b = 1453.80$ ,  $r^2 = 0.773$ ,  $F_{1, 28} = 91.787$ ,  $P < 0.001$ ). Therefore, high quantity and low quantity female groups were renamed as 'high quality' and 'low quality' respectively.

After successfully mating, the female was immobilized with CO<sub>2</sub> gas and dissected along the mid-dorsal line from posterior to anterior without damaging the interior parts. Her abdomen was fully opened by pinning the body wall to a wax tray. Next, the spermatophore was isolated and placed into a 1.5 mL Eppendorf tube containing 30  $\mu$ L of Grace's Insect Medium (Grace's Insect Medium, Supplemented ( $\times 1$ ), liquid, Life Technologies, Carlsbad, CA 92008). The spermatheca was then located and separated using fine forceps and placed onto a wax board containing 30  $\mu$ L of Grace's Insect Medium. The spermatheca was macerated very gently with small pins under the dissecting microscope. After maceration, the contents of the spermatheca was pipetted out with the Grace's Insect Medium into a 1.5 mL Eppendorf tube. An additional 70  $\mu$ L of Grace's Insect Medium was added to the spermatheca to make the total dilution 100  $\mu$ L. The spermatheca was soaked for 10 minutes in Grace's Insect Medium, allowing the majority of sperm to come into solution [41]. Twenty  $\mu$ L of the spermatheca sample was pipetted into a new 1.5 mL Eppendorf tube, taking care not to transfer large clumps into the new tube. The solution was then stained with 5  $\mu$ L of diluted Propidium Iodide (PI) (11.25  $\mu$ L of PI (1mg/mL) + 3.75  $\mu$ L of Grace's Insect Medium) for 15 mins in order to discriminate between live and dead sperm. Five  $\mu$ L of Synergy Brands (SYBR) Green 1 nucleic acid gel stain (1  $\mu$ L

SYBR Green 1 (10,000× concentrate in DMSO) + 49 µl of Grace's Insect medium) was added to the same sample and left for 10 mins. After that, 10 µL of the sample was pipetted out into a haemocytometer (Neubauer improved double net ruling SVZ2NIOU) and live/dead sperm were counted in all grid squares under a fluorescent microscope. Live and dead sperm were identified easily as live sperm fluoresced in green and dead sperm fluoresced in red. The same procedure was repeated to count the number of sperm within the spermatophore, except it was macerated by three quick ruptures with fine forceps. The total number of sperm found in the spermatheca and the spermatophore were calculated separately by dividing the sum of live and dead sperm by grid volume (0.9µL) and then multiplying by the dilution factor (100µL). Finally, the total number of sperm transferred by each male was calculated by adding the number of sperm found in the spermatheca to the number of sperm in the spermatophore.

## Aim 2. Do males mating with high quality females gain higher reproductive success?

The age of individuals was controlled so that on the day of the experiment, females were  $24.03 \pm 0.55$  ( $N = 33$ ) days post-adult emergence and males were  $21.94 \pm 0.79$  ( $N = 33$ ) days post-adult emergence. Immediately preceding mating trials, high food quantity treatment females were in significantly better condition ( $0.070 \pm 0.003$  g/mm,  $N = 16$ ) than low food quantity females ( $0.037 \pm 0.001$  g/mm,  $N = 17$ , Mann-Whitney  $U$  test:  $Z = -4.827$ ,  $N = 33$ ,  $P < 0.001$ ). Since female body condition positively correlates with fecundity in praying mantids ([26,34,42] current study), high food quantity and low food quantity female groups were renamed as 'high quality' and 'low quality' respectively. After successful mating, the pair was labelled as mated individuals and placed back into their individual plastic cups. Females continued with their original feeding treatment and were allowed to lay an ootheca (egg sac). Once laid, the number of days taken to lay an ootheca and its mass were recorded. In addition to absolute ootheca mass, we used the percentage of eggs in the ootheca as an additional measure of male reproductive success. Therefore, once laid, females were dissected under a stereomicroscope and the number of large dark yellow coloured mature eggs (immature eggs can be easily distinguished as they are small and white or pale yellow in colour) remaining in her ovaries were counted. A few weeks later, oothecae were also dissected and the total number of eggs in each ootheca was counted under a stereomicroscope. Finally, the proportion of total eggs in the ootheca was calculated by dividing the total number of eggs in the ootheca by the total number of mature eggs produced by the female (i.e. total eggs in the ootheca plus the number of mature eggs remaining in her ovaries).

## Aim 3. Does male phenotype affect sperm transfer and/or male reproductive success?

We determined whether male phenotype (i.e. fixed size and condition) had an effect on sperm allocation (i.e. total number of sperm transferred and proportion of viable sperm) and to total eggs in the ootheca and the percentage of eggs in ootheca in *P. albofimbriata* by relating male traits to sperm allocation and to percentage of eggs in oothecae.

## Data analysis

Data were analysed using SPSS 21.0 for Windows and were checked for normal distribution (Kolmogorov-Smirnov test) before analysis. All non-normally distributed variables were log transformed and checked again for normality before proceeding. All stated values are mean  $\pm$  standard error and all statistical tests are two tailed. General linear models (GLM) were



used to determine the effect of female treatment and male phenotype on the total number of sperm transferred and the proportion of viable sperm (in sperm transfer trials). Effect sizes and confidence intervals (at the 0.05 level) for female quality and male phenotype were also generated. Regression analyses were performed to determine the effect of female body condition on the number of sperm transferred in sperm transfer trials. We performed a t-test to determine the difference in mean number of days taken by high quality and low quality females to lay their first ootheca in reproductive success trials. Mann-Whitney tests were carried out to compare the ootheca mass, total eggs in the ootheca and the percentage of eggs in the ootheca between female treatments in reproductive success trials. We performed non-parametric correlations to figure out the relationships among female body condition, weight of first oothecae and the total eggs in oothecae in reproductive success trials and also to relate male traits (i.e. fixed size and body condition) to total eggs in oothecae and percentage of eggs in oothecae.

## Results

### Aim 1. Do males allocate more sperm to high quality females?

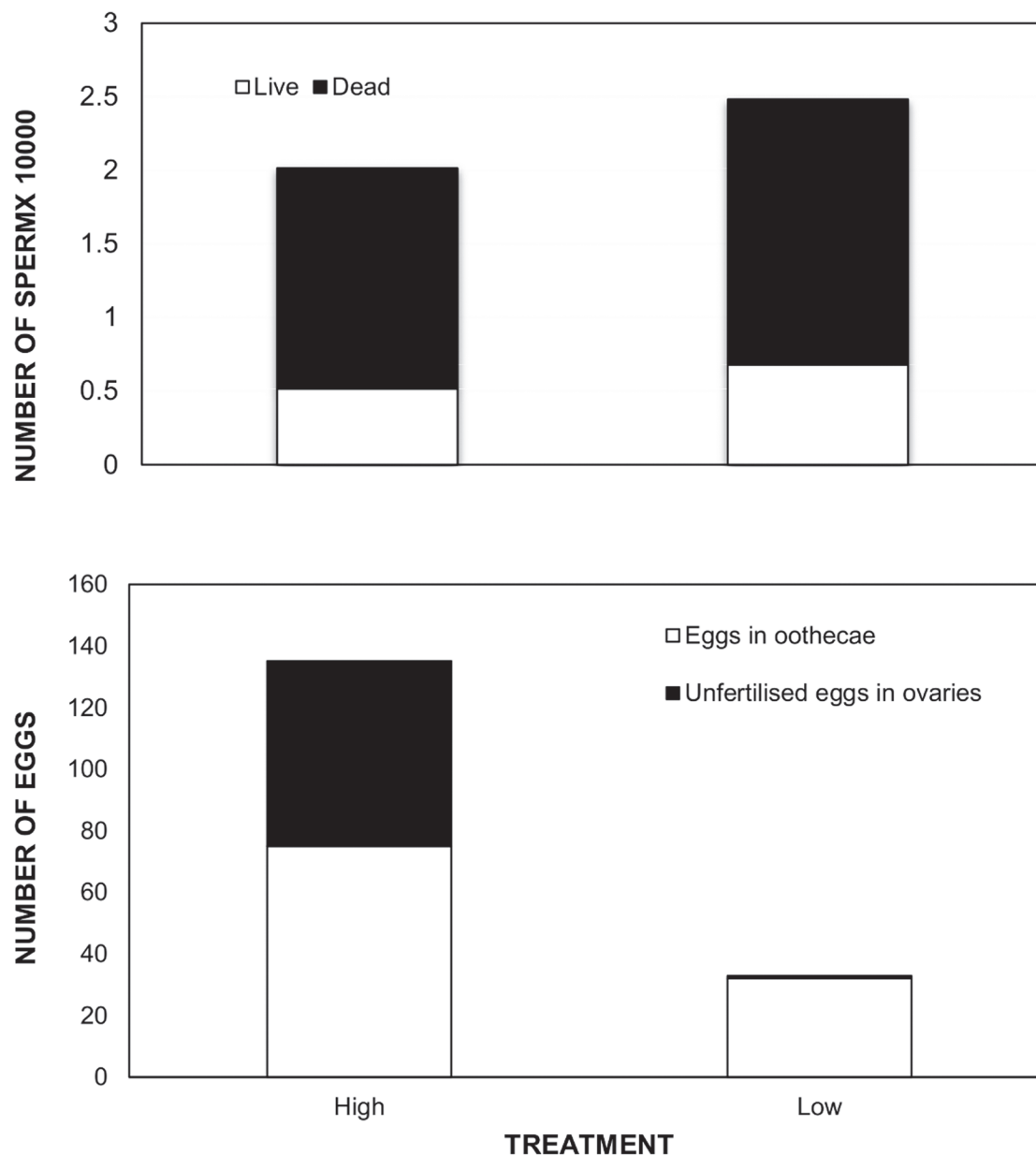
From the 29 trials, all males mounted females within the first hour of introduction and all males successfully transferred ejaculates to their mates within 3.5 hours. There was no significant difference in the log total number of sperm or the proportion of live sperm transferred ('percent sperm viability') between high quality and low quality females in *Pseudomantis albofimbriata* (Table 1; and see Fig 1). When comparing the variance (SE) as a percentage of the mean, variation of both the total number of sperm transferred (High quality -17.60%, Low quality—17.75%) and the percent sperm viability (High quality—14.69%, Low quality—14.33%) was very similar between treatments. We also found that effect sizes of female quality on log total number of sperm and on percent sperm viability were very close to zero and both confidence intervals overlapped zero, confirming that there was no effect of female quality on log total sperm transferred and on the percent sperm viability (Table 1). Female body condition did not predict the total number of sperm transferred across female treatments (linear regression:  $b = -2.035$ ,  $r^2 = 0.036$ ,  $F_{1, 28} = 0.996$ ,  $P = 0.327$ ) or within each female treatment (linear regression: [High quality]  $b = -0.575$ ,  $r^2 = 0.001$ ,  $F_{1, 13} = 0.007$ ,  $P = 0.937$ ; [Low quality]  $b = 14.429$ ,  $r^2 = 0.089$ ,  $F_{1, 14} = 1.273$ ,  $P = 0.280$ ).

**Table 1. Effect of female quality on sperm allocation (total sperm number and percent sperm viability) and on reproductive success (days taken to lay an ootheca, ootheca mass and percentage of eggs in ootheca) in *P.albofimbriata*.**

	High	Low	Statistics	Effect size	95% confidence intervals	
					Lower bound	Upper bound
Log total sperm number	4.21 ± 0.08	4.33 ± 0.06	$F_1 = 1.114$ , $P = 0.301$	0.043	-0.097	0.299
Percent sperm viability	26.24 ± 3.85	27.56 ± 3.95	$F_1 = 0.003$ , $P = 0.958$	0.001	-10.731	11.326
Days to lay an ootheca	4.50 ± 0.69	10.94 ± 1.31	$t_{31} = -4.366$ , <b><math>P &lt; 0.001</math></b>			
Ootheca mass	0.367 ± 0.03	0.135 ± 0.01	$Z = -4.901$ , <b><math>P &lt; 0.001</math></b>			
Total eggs in ootheca	75.00±5.31	32.06±1.98	$Z = -4.867$ , <b><math>P &lt; 0.001</math></b>			
Percentage eggs in ootheca	54.60 ± 1.85	96.41 ± 3.09	$Z = -4.616$ , <b><math>P &lt; 0.001</math></b>			

F-values were derived from general linear models using female treatment as a factor. Mann-Whitney tests were performed to compare the ootheca mass, total eggs in ootheca and percentage of total eggs in ootheca between female treatments. A t-test was used to compare the number of days taken to lay an ootheca between treatments. Significant P values are highlighted in bold.

doi:10.1371/journal.pone.0124209.t001



**Fig 1. Comparison of Sperm transfer and proportion of eggs laid between female treatments.** Female quality had no significant effect on the total number of sperm transferred by males ( $P = 0.301$ ) or on the percentage of viable sperm ( $P = 0.958$ ) in sperm trials. However, the percentage of eggs in oothecae was significantly different between female treatments ( $P < 0.001$ ) in reproductive success trials, where low quality females laid a higher proportion of eggs than high quality females.

doi:10.1371/journal.pone.0124209.g001

## Aim 2. Do males mating with high quality females gain higher reproductive success?

All pairs ( $N = 33$ ) were mated without sexual cannibalism and all females laid an ootheca successfully after mating. There was a significant difference in the number of days taken to lay the first ootheca between female treatments where high quality females laid ootheca significantly faster than low quality females (Table 1). Also high quality females produced a significantly heavier first ootheca compared to low quality females (Table 1). As expected, female body



**Table 2. Effect of male phenotype (fixed size and condition) on sperm allocation and reproductive success in *P. albofimbriata*.**

	Male phenotype	Statistics	Effect size	95% confidence intervals	
				Lower bound	Upper bound
Log total sperm number	Fixed size	$F_1 = 0.116, P = 0.736$	0.005	-0.144	0.202
	Condition	$F_1 = 2.749, P = 0.110$	0.099	-89.003	9.509
Percent sperm viability	Fixed size	$F_1 = 0.073, P = 0.790$	0.003	-10.841	8.397
	Condition	$F_1 = 1.931, P = 0.177$	0.072	-4612.640	868.722
Total eggs in oothecae	Fixed size	$P = 0.105$	0.287		
	Condition	$P = 0.884$	-0.026		
Percentage of eggs in oothecae	Fixed size	$P = 0.240$	-0.210		
	Condition	$P = 0.575$	0.101		

*F*-values were derived from general linear models using male fixed size and condition as covariates. *P*-values for the relationships between male traits with total eggs in ootheca and the percentage of eggs in ootheca were derived from non-parametric correlations.

doi:10.1371/journal.pone.0124209.t002

condition had a significant positive correlation with the mass of the first ootheca (Spearman's correlation:  $r^2 = 0.890, N = 33, P < 0.001$ ). We also found a significant difference in total eggs within the ootheca between female treatments, where high quality females had significantly more eggs in the ootheca than low quality females (Table 1). Further, there was a significant positive correlation between female body condition and the total eggs in the ootheca (Spearman's correlation:  $r^2 = 0.877, N = 33, P < 0.001$ ). The percentage of total eggs in the ootheca was significantly different between female treatments (Table 1). Furthermore, we found a significant positive correlation between ootheca mass and the total eggs in that ootheca (Spearman's correlation:  $r^2 = 0.958, N = 33, P < 0.001$ ).

### Aim 3. Does male phenotype affect sperm transfer and/or male reproductive success?

There was no significant effect of male phenotype (fixed size and condition) on the total number of sperm transferred, or on the percentage of viable sperm transferred (Table 2). Further effect sizes of male phenotype on the total number of sperm and percent sperm viability were very close to zero and both confidence intervals overlapped zero, confirming the fact that there was no effect of male phenotype on either of the two variables (Table 2). We also found no significant effect of male phenotype on the total eggs in the ootheca and on the percentage of total eggs in ootheca (Table 2).

## Discussion

Contrary to prediction, we found no effect of female quality on the ejaculatory expenditure of male praying mantids, *Pseudomantis albofimbriata*. That is, males did not significantly vary the total number of sperm or the proportion of viable sperm transferred when paired with a high quality or a low quality female. Effect sizes and confidence intervals confirm these results. Variation in the total number of sperm and percentage of viable sperm was also very similar between treatments. Further, we found no significant relationship between female body condition and the total number of sperm transferred by males. Our finding is inconsistent with the general pattern shown for many insect groups in which males allocate their ejaculate strategically in response to female quality [18,20,24,25].

Since polyandry in *P. albofimbriata* has not been directly observed in the wild (but see [29]) and mated females become chemically unattractive to males soon after mating [29], it may be

that polyandry, and hence sperm competition, is not a common occurrence and therefore males do not respond to its perceived risk. However, in *P. albobimbriata* [34] and in praying mantids more generally [33,35], multiple males may be simultaneously attracted to the initial pheromone plume of a single female, increasing the likelihood of multiple mating. Furthermore, a recent study found that males strategically ejaculate in response to the perceived risk of sperm competition associated with sex ratio biases [37], which suggests male *P. albobimbriata* do sense and respond to sperm competition via strategic ejaculation in certain contexts. Therefore, the absence of strategic ejaculation by male mantids in the current study is most likely due to female quality being a poor indicator of the risk of sperm competition in this system. Since praying mantids (including *P. albobimbriata*) exhibit a scramble competition polygynous mating system where males race to find virgin females and often arrive simultaneously [29,34,43,44], inter-male competition may act as a more appropriate indicator of the risk of sperm competition in this system. Alternatively, it may be that male mantids need longer than the 3.5 hours afforded to them in the current study to perceive sperm competition risk and modulate their ejaculate accordingly (see [18,20,45]). The treatments used in the aforementioned study showing strategic ejaculation in *P. albobimbriata* [44] were continued for an extended period during development (for nearly two weeks) and therefore, males had more time to modulate spermatophore production in response to the mating context.

Female condition is generally a good indicator of female fecundity in insects [4] and males may be able to achieve greater reproductive success by selectively allocating more sperm to good condition females as they produce more eggs that may require more sperm to fertilise them. Male mantids (including *P. albobimbriata*) have a preference for good-condition females rather than poor-condition females in a precopulatory scenario [27,34,39], but surprisingly males did not show a similar preference in the postcopulatory context during the current study. Differences in precopulatory and postcopulatory male mate choice have been observed in some other insect groups, for example male bush crickets *Kawanaphila nartee* prefer larger females in precopulatory choice [46], but transfer more sperm to smaller females [24]. In the current study (and see [34,47]), high quality females produced a significantly higher number of mature eggs compared to low quality females, and males transferring the least number of sperm (total sperm 3444) still transferred many more than was necessary to fertilize the most fecund female's egg load (total eggs 173). Therefore, it may not be selectively beneficial for males to allocate more sperm to high quality females, but still important to find a high quality female in the precopulatory arena as she will be more fecund and less likely to cannibalise [26,48].

In our reproductive success trials, high quality females laid significantly heavier first oothecae within a shorter time period compared to low quality females. Poor condition females had fewer mature eggs in their ovaries compared to good condition females ([34] current study), hence, it is likely that poor quality females delay laying the ootheca to allow more time for further egg production. As expected we found a significant positive correlation between female body condition and first ootheca mass—this result is consistent with previous studies conducted on the same species [26] and on the praying mantid *Hierodula membranacea* [42]. Further, we found significant positive correlations between female body condition and total eggs in the ootheca and also between ootheca mass and total eggs in the ootheca. Also high quality females put significantly more eggs in their ootheca than low quality females. These results confirm that female condition has a significant effect on the reproductive output of praying mantids [26,42]. The percentage of eggs in the ootheca was significantly different between female treatments. That is, low quality females laid almost all available mature eggs and high quality females laid only half of their mature eggs (the other half of mature eggs remained unfertilised in their ovaries). Poor condition females may fertilise more mature eggs because

they are less attractive to males [27,39] and are therefore less likely to gain additional mating opportunities. High quality females may instead prefer to partially delay fertilisation so as to obtain additional mates, thereby increasing the genetic diversity of offspring [31]. Alternatively, high quality females might simply choose to lay multiple oothecae so as to literally not put ‘all of their eggs in one basket’, thereby reducing the risk of offspring predation. We found no significant link between the total number of sperm transferred and any of the indicators of actual reproductive success (i.e. first ootheca mass and number of eggs in ootheca), demonstrating a disconnect between sperm transfer and reproductive success for males in a single mating scenario. Reproductive success for male *P. albofimbriata* is mostly dependent on female condition/fecundity in a single-male scenario, however sperm number is likely to have an effect on reproductive success if females mate multiply. According to the fair raffle principle, males who transfer more sperm should gain a higher proportion of fertilisations in a multiple mating scenario ([49,50] reviewed in [11]).

Sperm allocation may vary with male phenotype [51–53]. For example in domestic crickets *Acheta domesticus*, sperm number correlates with male body size/pronotum length [52] or body weight [51]. However, we found no evidence for a relationship between male phenotype and sperm allocation in *P. albofimbriata* (see [54]). Effect sizes and 95% confidence intervals confirmed these results. We also found no significant effect of male phenotype on total eggs in the ootheca and on percentage of eggs in ootheca in reproductive success trials. Therefore it is unlikely that females bias fertilisation, favouring males with specific phenotypes. However, some studies have shown that females may exhibit cryptic choice based on male phenotype and favour the sperm of males with specific traits [55,56]. The lack of an effect of male phenotype on sperm transfer and also on fertilisation success in the current study may be due to minimal variation in male size and body condition, as we did not manipulate male body size/condition. Future studies that manipulate male size and condition will better determine if such a relationship exists in *P. albofimbriata*.

In summary, the current study suggests that male *P. albofimbriata* mantids do not strategically adjust their ejaculate in response to female quality, providing evidence of a disconnect between their precopulatory and postcopulatory strategies. Furthermore, the amount of sperm transferred does not play a significant role in the reproductive success of males in a single mating context. Male phenotype had no effect on sperm allocation or on reproductive success in *P. albofimbriata*. Future studies focusing on strategic ejaculation in a multiple mating scenario will be important in gaining a more complete understanding of postcopulatory male mating strategies in praying mantids.

## Supporting Information

**S1 Table. Underlying (raw) sperm transfer trials data.**  
(CSV)

**S2 Table. Underlying (raw) reproductive success trials data.**  
(CSV)

## Acknowledgments

We thank Kaytlyn Davis and Darshana Rathnayake for their help in collection and rearing mantids, to Debra Birch and Nicole Vella from the Microscopy Unit, Faculty of Science and Engineering, Macquarie University for help with microscopy and to Kasun Rathnayake for help with statistical analysis. Special thanks to Mariella Herberstein for her continuing support throughout experiments and manuscript preparation.

## Author Contributions

Conceived and designed the experiments: AJ KLB. Performed the experiments: AJ. Analyzed the data: AJ KLB. Contributed reagents/materials/analysis tools: AJ KLB. Wrote the paper: AJ KLB.

## References

1. Darwin C (1871) *The Descent of Man and Selection in Relation to Sex*. London: Murray.
2. Trivers R (1972) Parental investment and sexual selection.
3. Dewsbury DA (1982) Ejaculate cost and male choice. *American Naturalist* 119: 601–610.
4. Bonduriansky R (2001) The evolution of male mate choice in insects: A synthesis of ideas and evidence. *Biological Reviews of the Cambridge Philosophical Society* 76: 305–339. PMID: [11569787](#)
5. Parker GA (1970) SPERM COMPETITION AND ITS EVOLUTIONARY CONSEQUENCES IN THE INSECTS. *Biological Reviews of the Cambridge Philosophical Society* 45: 525–567.
6. Simmons LW (2001) *Sperm competition and its evolutionary consequences in the insects*. Princeton, New Jersey: Princeton University Press. i-xviii, 1–434 p.
7. Edward DA, Chapman T (2011) The evolution and significance of male mate choice. *Trends in Ecology and Evolution* 26: 647–654. doi: [10.1016/j.tree.2011.07.012](#) PMID: [21890230](#)
8. Kelly CD, Jennions MD (2011) Sexual selection and sperm quantity: meta-analyses of strategic ejaculation. *Biological Reviews* 86: 863–884. doi: [10.1111/j.1469-185X.2011.00175.x](#) PMID: [21414127](#)
9. Parker GA, Ball MA, Stockley P, Gage MJG (1996) Sperm competition games: Individual assessment of sperm competition intensity by group spawners. *Proceedings of the Royal Society B-Biological Sciences* 263: 1291–1297.
10. Engqvist L, Reinhold K (2006) Theoretical influence of female mating status and remating propensity on male sperm allocation patterns. *Journal of Evolutionary Biology* 19: 1448–1458. PMID: [16910976](#)
11. Parker GA, Pizzari T (2010) Sperm competition and ejaculate economics. *Biological Reviews* 85: 897–934. doi: [10.1111/j.1469-185X.2010.00140.x](#) PMID: [20560928](#)
12. Reinhold K, Kurtz J, Engqvist L (2002) Cryptic male choice: sperm allocation strategies when female quality varies. *Journal of Evolutionary Biology* 15: 201–209.
13. Ball MA, Parker GA (1998) Sperm competition games: a general approach to risk assessment. *Journal of Theoretical Biology* 194: 251–262. PMID: [9778437](#)
14. Ball MA, Parker GA (2007) Sperm competition games: the risk model can generate higher sperm allocation to virgin females. *Journal of Evolutionary Biology* 20: 767–779. PMID: [17305842](#)
15. Wedell N, Gage MJG, Parker GA (2002) Sperm competition, male prudence and sperm-limited females. *Trends in Ecology & Evolution* 17: 313–320.
16. Gage MJG (1991) Risk of sperm competition directly affects ejaculate size in the Mediterranean fruit fly. *Animal Behaviour* 42: 1036–1037.
17. Gage MJG, Baker RR (1991) Ejaculate size varies with socio-sexual situation in an insect. *Ecological Entomology* 16: 331–337.
18. Gage AR, Barnard CJ (1996) Male crickets increase sperm number in relation to competition and female size. *Behavioral Ecology and Sociobiology* 38: 349–353.
19. Fuller RC (1998) Sperm competition affects male behaviour and sperm output in the rainbow darter. *Proceedings of the Royal Society B: Biological Sciences* 265: 2365–2371.
20. Mallard ST, Barnard CJ (2003) Competition, fluctuating asymmetry and sperm transfer in male gryllid crickets (*Gryllus bimaculatus* and *Gryllodes sigillatus*). *Behavioral Ecology and Sociobiology* 53: 190–197.
21. Shapiro DY, Marconato A, Yoshikawa T (1994) SPERM ECONOMY IN A CORAL-REEF FISH, *THALASSEMA BIFASCIATUM*. *Ecology* 75: 1334–1344.
22. Yusa Y (1994) FACTORS REGULATING SPERM TRANSFER IN AN HERMAPHRODITIC SEA HARE, *APLYSIA-PARVULA MORCH*, 1863 (GASTROPODA, OPISTHOBRANCHIA). *Journal of Experimental Marine Biology and Ecology* 181: 213–221.
23. Marconato A, Shapiro DY (1996) Sperm allocation, sperm production and fertilization rates in the buck-tooth parrotfish. *Animal Behaviour* 52: 971–980.
24. Simmons LW, Kvarnemo C (1997) Ejaculate expenditure by male bushcrickets decreases with sperm competition intensity. *Proceedings of the Royal Society B-Biological Sciences* 264: 1203–1208.

25. Lupold S, Manier MK, Ala-Honkola O, Belote JM, Pitnick S (2011) Male *Drosophila melanogaster* adjust ejaculate size based on female mating status, fecundity, and age. *Behavioral Ecology* 22: 184–191.
26. Barry KL, Holwell GI, Herberstein ME (2008) Female praying mantids use sexual cannibalism as a foraging strategy to increase fecundity. *Behavioral Ecology* 19: 710–715.
27. Maxwell MR, Gallego KM, Barry KL (2010) Effects of female feeding regime in a sexually cannibalistic mantid: Fecundity, cannibalism, and male response in *Stagmomantis limbata* (Mantodea). *Ecological Entomology* 35: 775–787.
28. Engqvist L, Sauer KP (2001) Strategic male mating effort and cryptic male choice in a scorpionfly. *Proceedings of the Royal Society B-Biological Sciences* 268: 729–735. PMID: [11321062](#)
29. Barry KL, Holwell GI, Herberstein ME (2011) A paternity advantage for speedy males? Sperm precedence patterns and female re-mating frequencies in a sexually cannibalistic praying mantid. *Evolutionary Ecology* 25: 107–119.
30. Umbers KDL, Holwell GI, Stow AJ, Herberstein ME (2011) Molecular evidence for variation in polyandry among praying mantids (Mantodea: Ciulfina). *Journal of Zoology* 284: 40–45.
31. Watanabe E, Adachi-Hagimori T, Miura K, Maxwell MR, Ando Y, Takematsu Y (2011) Multiple Paternity Within Field-Collected Egg Cases of the Praying Mantid *Tenodera aridifolia*. *Annals of the Entomological Society of America* 104: 348–352.
32. Winnick CG, Holwell GI, Herberstein ME (2009) Internal reproductive anatomy of the praying mantid *Ciulfina klassi* (Mantodea: Liturgusidae). *Arthropod Structure & Development* 38: 60–69.
33. Lelito JP, Brown WD (2008) Mate attraction by females in a sexually cannibalistic praying mantis. *Behavioral Ecology and Sociobiology* 63: 313–320.
34. Barry KL (2010) Influence of female nutritional status on mating dynamics in a sexually cannibalistic praying mantid. *Animal Behaviour* 80: 405–411.
35. Maxwell MR, Barry KL, Johns PN (2010) Examinations of Female Pheromone Use in Two Praying Mantids, *Stagmomantis limbata* and *Tenodera aridifolia sinensis* (Mantodea: Mantidae). *Annals of the Entomological Society of America* 103: 120–127.
36. 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.
37. Allen LE, Barry KL, Holwell GI, Herberstein ME (2011) Perceived risk of sperm competition affects juvenile development and ejaculate expenditure in male praying mantids. *Animal Behaviour* 82: 1201–1206.
38. Barry KL, Holwell GI, Herberstein ME (2009) Male mating behaviour reduces the risk of sexual cannibalism in an Australian praying mantid. *Journal of Ethology* 27: 377–383.
39. Barry KL, Holwell GI, Herberstein ME (2010) Multimodal mate assessment by male praying mantids in a sexually cannibalistic mating system. *Animal Behaviour* 79: 1165–1172.
40. Barry KL, Wilder SM (2013) Macronutrient intake affects reproduction of a predatory insect. *Oikos* 122: 1058–1064.
41. Gress BE, Kelly CD (2011) Is sperm viability independent of ejaculate size in the house cricket (*Acheta domesticus*)? *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 89: 1231–1236.
42. Birkhead TR, Lee KE, Young P (1988) SEXUAL CANNIBALISM IN THE PRAYING MANTIS *HIERODULA-MEMBRANACEA*. *Behaviour* 106: 112–118.
43. Maxwell MR (1998) Lifetime mating opportunities and male mating behaviour in sexually cannibalistic praying mantids. *Animal Behaviour* 55: 1011–1028. PMID: [9632486](#)
44. Holwell GI, Barry KL, Herberstein ME (2007) Mate location, antennal morphology, and ecology in two praying mantids (Insecta: Mantodea). *Biological Journal of the Linnean Society* 91: 307–313.
45. Sakaluk SK (1985) SPERMATOPHORE SIZE AND ITS ROLE IN THE REPRODUCTIVE-BEHAVIOR OF THE CRICKET, *GRYLLODES-SUPPLICANS* (ORTHOPTERA, GRYLLIDAE). *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 63: 1652–1656.
46. Gwynne DT, Simmons LW (1990) Experimental reversal of courtship roles in an insect. *Nature* 346: 172–174.
47. Barry KL (2013) You Are What You Eat: Food Limitation Affects Reproductive Fitness in a Sexually Cannibalistic Praying Mantid. *PLoS ONE* 8.
48. Lelito JP, Brown WD (2006) Natural history miscellany—Complicity or conflict over sexual cannibalism? Male risk taking in the praying mantis *Tenodera aridifolia sinensis*. *American Naturalist* 168: 263–269. PMID: [16874635](#)
49. Parker GA (1990) Sperm competition games: Raffles and roles. *Proceedings of the Royal Society B: Biological Sciences* 242: 120–126.

50. Parker GA, Simmons LW, Kirk H (1990) Analysing sperm competition data: simple models for predicting mechanisms. *Behavioral Ecology and Sociobiology* 27: 55–65.
51. Klaus SP, Fitzsimmons LP, Pitcher TE, Bertram SM (2011) Song and Sperm in Crickets: A Trade-off between Pre- and Post-copulatory Traits or Phenotype-Linked Fertility? *Ethology* 117: 154–162.
52. Worthington AM, Gress BE, Neyer AA, Kelly CD (2013) Do male crickets strategically adjust the number and viability of their sperm under sperm competition? *Animal Behaviour* 86: 55–60.
53. Anthes N, Werminghausen J, Lange R (2014) Large donors transfer more sperm, but depletion is faster in a promiscuous hermaphrodite. *Behavioral Ecology and Sociobiology* 68: 477–483.
54. Schaus JM, Sakaluk SK (2001) Ejaculate expenditures of male crickets in response to varying risk and intensity of sperm competition: not all species play games. *Behavioral Ecology* 12: 740–745.
55. Simmons LW (1986) Female choice in the field cricket *Gryllus bimaculatus* (De Geer). *Animal Behaviour* 34: 1463–1470.
56. Bateman PW, Gilson LN, Ferguson JWH (2001) Male size and sequential mate preference in the cricket *Gryllus bimaculatus*. *Animal Behaviour* 61: 631–637. PMID: [11768323](#)

**Chemical signalling and context dependent polyandry in a praying mantid**

Anuradhi Jayaweera, Darshana N. Rathnayake, Barbara Dean & Katherine L. Barry

Submitted to *Austral Entomology*

---

## Abstract

Multiple mating by females is a common phenomenon in nature. Polyandrous females may benefit from genetically diverse progeny that may survive better in a changing environment. Males in polyandrous systems, however, may not achieve their maximum paternity. Therefore, males are predicted to evolve traits that prevent or reduce female polyandry. Praying mantids are predatory insects where females can mate multiple times, predicting male counter-strategies. However, the rate of polyandry and male strategies against polyandry are rarely studied in these insects. In the current study, we used false garden mantids, *Pseudomantis albofimbriata*, to quantify the rate of multiple matings when several males are present within close visual range of an initially unmated female. We further determined how long mated females stay unattractive after mating. We found that in a scramble scenario the subsequently arriving males stay with a copulating pair and attempt mating once the first male has completed copulation. These second copulation attempts are often successful. If only one male is attracted then polyandry is unlikely because the female will remain chemically unattractive on average for eight days after mating, which is longer than the usual latency to lay the first egg sac. From previous studies, we know that single male attraction is the most common scenario in this system in both natural and semi-natural contexts. Therefore, polyandry seems to be context-dependent and a relatively uncommon phenomenon in this system.

Keywords: female unattractiveness - multiple mating – pheromone - *Pseudomantis albofimbriata* - reproductive success - scramble competition



## Introduction

Multiple mating by females (i.e. polyandry) is common among animals (Ridley, 1990, Petrie et al., 1998, Simmons, 2001a, Griffith et al., 2002). Polyandry is likely to occur in contexts where females gain material (e.g. in the form of nuptial gifts) or genetic benefits by copulating with multiple males (see Simmons, 2001a). Limited supply of viable sperm is another factor that could select for polyandry (Bonduriansky, 2001, Jones, 2001, Preston et al., 2001). However, polyandry can impose costs as well as benefits for females. For some species, mating with multiple males increases the energy spent in reproduction for females, increases predation risk, and the risk of physical injury (Rowe, 1994, Crudginton and Siva-Jothy, 2000, Arnqvist and Nilsson, 2000). For example, in bean weevils *Callosobruchus maculatus*, polyandrous females had increased genital tract damage and thereby reduced longevity (Crudginton and Siva-Jothy, 2000).

Apart from pre-copulatory mechanisms, the reproductive success of males in polyandrous mating systems mainly depends on two post-copulatory mechanisms: sperm competition and cryptic female choice. Sperm competition is the competition among sperm from two or more males for the fertilization of ova within the female reproductive tract (Parker, 1970). Female mate choice can continue after copulation in the form of cryptic female choice where females bias the fertilisation of ova by favouring sperm of preferred males (Eberhard, 1996). Therefore, males in polyandrous systems are predicted to evolve traits that may prevent or limit polyandry, maximize paternity through sperm competition and/or maximize the chance of fertilisations. For examples, male St Andrew's cross spiders (*Argiope keyserlingi*) remain on the female's web for several hours after mating as a mate guarding strategy which reduces female remating (Herberstein et al., 2005). Similarly, male accessory gland products reduce female receptivity and result in shorter copulations for mated female Queensland fruit flies (*Bactrocera tryoni*) (Radhakrishnan and Taylor, 2007). Male aggression can also

moderate female remating behaviour. For example, male chimpanzees behave aggressively towards females, hence limiting female polyandry (Muller et al., 2007). Finally, some male strategies do not moderate female behaviour but simply remove the sperm from previous males. For example, male earwig *Euborellia plebeja* insert their elongated genitalia into the female reproductive tract and remove the stored sperm from the spermatheca (Kamimura, 2000). Even though cryptic female choice is difficult to distinguish from other internal mechanisms such as sperm competition, some studies have cleverly demonstrated that females select the sperm of preferred males when they mate with multiple males (see Elgar et al., 2000, Schneider et al., 2015). For example, the polyandrous spider *Argiope lobata* stores sperm selectively by favouring the sperm of unrelated males (Welke and Schneider, 2009), and non-courting male *A. bruennichi* had a reduced paternity share compared to courting counterparts in experimentally manipulated courtship trials (Schneider and Lesmono, 2009).

Praying mantids are predatory insects in which females are capable of mating multiple times (Barry et al., 2011, Umbers et al., 2011, Watanabe et al., 2011). However, how common polyandry is in praying mantids is undocumented for the vast majority of species. To fully understand the mating dynamics of this group of insects, studies that investigate the frequency of female multiple mating and male behaviour are crucial. On the one hand polyandry in nature may be unlikely in praying mantids because in some species females become chemically unattractive to males after mating (*P. albofimbriata*: Barry et al., 2011), but on the other hand it is quite possible because numerous males are often attracted to the initial pheromone plume of a calling unmated female (Lelito and Brown, 2008, Barry, 2010, Maxwell et al., 2010a, Maxwell et al., 2010b, Barry, 2013, Barry and Wilder, 2013, Barry, 2015). Here, we investigate the occurrence and/or frequency of single versus multiple mating in false garden mantids *Pseudomantis albofimbriata* (Stal, 1860). Specifically, our study aims to (1) observe what happens after the first male begins

mating and there are other males in close proximity, (2) determine the length of time a female is chemically unattractive after mating, and (3) investigate how often single and multiple male attraction occurs (using data collected from previous studies) in the false garden mantid mating system.

## Methods

### *Study species and study sites*

The false garden mantid *Pseudomantis albofimbriata* is a common mantid species distributed throughout Eastern Australia. Juvenile mantids ( $N = 100$ ) were collected from December 2014 to January 2015 from Ku-ring-gai Bicentennial Park, West Pymble ( $33^{\circ} 45' 37.76''\text{S}$ ,  $151^{\circ} 08' 20.88''\text{E}$ ) and Yamba Reserve, Ryde ( $33^{\circ} 49' 0'' \text{S}$   $151^{\circ} 6' 0''\text{E}$ ), Australia. All individuals were found on the leaves and flowers of *Lomandra longifolia* bushes.

### *Ethics statement*

The New South Wales state law does not require specific permissions for the collection of invertebrates from locations outside of a national park, such as in our study. The study did not involve endangered or protected species.

### *Rearing, measuring and sexing mantids*

Mantids were reared individually in inverted transparent plastic cups of which the bottom end was replaced by mesh to facilitate better airflow. They were reared at a controlled temperature ( $25 - 26^{\circ}\text{C}$ ), light (14h) and humidity (55%). Juvenile mantids were fed two

small crickets *Acheta domestica* (body mass =  $0.040 \pm 0.001$  g,  $N = 20$ ) three times per week until maturity, and watered daily. Once they matured to adulthood, the pronotum length (in mm -i.e. fixed size) and mass (in g - measured to three decimal places using PB303-S DeltaRange) were recorded, and individuals were sexed using differences in wing morphology. Female body condition was calculated as body mass divided by fixed size. Adult males and females continued with the usual diet of two small crickets three times per week.

*Objective 1: Female behaviour in the presence of multiple males in close range*

The aim of this experiment was to observe what happens after the first male begins mating and there are other males in close proximity. Trials were conducted in March 2015, using unmated females approximately three weeks after adult eclosion. A single female was introduced on to a potted *Lomandra* sp. plant placed in the centre of an outdoor enclosure ( $3 \times 3 \times 2$  m) on the Macquarie University campus. The enclosure had mesh walls so that males could move freely. In each trial, five males randomly selected from the laboratory population were released onto the floor of the enclosure equidistant from the plant and each other (0.5 m). Males were marked carefully before release by writing a number on the wings with a permanent marker. Each trial began before sunrise, between the hours of 0500 and 0600. Regular behavioural observations (every 2 hours from 0700 to 2200) were made for two days, noting which males approached the female (a male was noted as approaching the female if it landed on the *Lomandra* sp. plant and keen on the female by directing and oscillating its antenna towards her), which males mounted the female and in what order, which males mated and whether or not they were cannibalised. Two trials were conducted simultaneously in two separate enclosures and the experiment was repeated four times each (total number of females: 8).

## *Objective 2: Duration of long range unattractiveness for mated females*

Mating trials ( $N = 15$ ) were conducted approximately three weeks after female adult eclosion, during the morning hours (0830-1130) in February-March 2015. Females and males were weighed immediately before the experiment using digital scales (to three decimal places using PB303-S DeltaRange). Mating trials were conducted on wood logs (30 cm  $\times$  50 cm) affixed to a Perspex plane that prevented the mantids from walking off the log. First the female was introduced onto the log and given five minutes to acclimatise, then a male was introduced 5 cm below the female. Occurrence of sexual cannibalism and the copulation duration (time to detach copula after the male initiated intromission) were recorded for each trial. Soon after the copulation finished the mated female was placed in a small cylindrical cage (30  $\times$  20 cm diameter), covered with a thick black mesh to obstruct visual cues. Subsequently, female cages were placed around the interior perimeter of a large field enclosure (6  $\times$  4  $\times$  3 m) located on the Macquarie University campus. The enclosures had mesh walls and gravel floor. We placed potted *Lomandra longifolia* plants ( $N = 20$ ) in the middle of the enclosure. A maximum of eight cages were placed in the enclosures for each experiment. Males were randomly selected from the laboratory population, released onto foliage in the centre of the enclosure, and allowed to move freely about the enclosure. The sex ratio was always 1:1, mimicking the natural sex ratio. Female cages were checked for incoming males and egg sacs (oothecae) two times a day (0800 and 2200) for a period of 14 days. Females were fed with small crickets *Acheata domestica* two times per week. We considered a female as attractive if a male was found on the female's cage. We counted the number of males found on a female's cage and subsequently removed both the male and female and returned their individual cups to the laboratory. The removed animals were replaced with new ones from the laboratory to maintain the experimental population size and sex ratio in the enclosure. Each animal was only used once in the experiment.

### *Objective 3: Frequency of single and multiple male attractions*

The aim of this analysis was to investigate how often single and multiple male attraction occurs in the *P. albobimbriata* mating system. Data collected from previous studies (Barry, 2010, Barry, 2013) on male attraction (the number of males attracted by a female in natural and semi-natural attractiveness trials) in the *P. albobimbriata* system were summarised as a way of estimating the likelihood of polyandry in natural (field) and semi-natural (field enclosure) conditions.

### *Data analysis*

Data were analysed using SPSS 21.0 for Windows and were checked for normal distribution (Kolmogorov-Smirnov test) before analysis. Unless otherwise stated, all values are mean  $\pm$  standard error and all statistical tests are two tailed.

## **Results**

### *Objective 1: Female behaviour in the presence of multiple males in close range*

In all trials, the female attracted her first male within 2 hours ( $57.12 \pm 12.51$  mins). Seven out of eight females attracted multiple males (Chi-square test:  $\chi^2 = 54.76$ ,  $P < 0.001$ ). Females did not attract any further males once they had started the first mating. When females attracted multiple males, these males generally mated with the female or lingered near the female until the end of two-day observation period.

We observed female multiple mating in four out of eight trials during the two-day observation period. When multiple males arrived at a female, we observed them mounted simultaneously on a female in three out of eight trials. In these three trials, the second and/or third males mounted while the female was already in copula with the first male.

These second and third males ( $N = 4$  from three different trials) remained on the female until she finished her first mating and they were successful in mating with the female once the first male had moved away. In the 14 male-female interactions we observed, two males were cannibalised and failed to mate. Of the twelve copulatory interactions, only one encounter resulted in cannibalism while the remaining 11 males mated with the female in the absence of cannibalism (Chi-square test:  $\chi^2 = 67.8$ ,  $P < 0.001$ ).

*Objective 2: duration of chemical unattractiveness for mated females*

From the total of 15 mated females, eight females attracted a male and seven females failed to attract a male during the observation period. We did not find a significant difference in female body condition and size (i.e. pronotum length) between attractive ( $N = 8$ , body condition:  $0.036 \pm 0.002 \text{ gmm}^{-1}$ , pronotum length:  $15.48 \pm 0.278 \text{ mm}$ ) and unattractive females ( $N = 7$ ; body condition:  $0.032 \pm 0.002 \text{ gmm}^{-1}$ , Mann-Whitney  $U$  test:  $Z = -1.157$ ,  $P = 0.247$ ; pronotum length:  $14.90 \pm 0.31 \text{ mm}$ , t-test:  $t_{13} = 1.417$ ,  $P = 0.180$ ). Females that attracted a male within the observation period ( $N = 8$ ) took  $8 \text{ days} \pm 1.07$  on average to become attractive again after their initial mating. There was no difference in the period of unattractiveness between cannibalistic ( $N = 4$ ,  $7.75 \pm 0.95 \text{ days}$ ) and non-cannibalistic females ( $N = 4$ ,  $8.25 \pm 2.10 \text{ days}$  t-test:  $t_6 = -0.217$ ,  $P = 0.835$ ).

Fourteen out of 15 females used in trials laid an egg sac. Of those mated females that attracted a male within the experimental period ( $N = 8$ ), two females laid their first egg sac on the same day they attracted a male, and the other five females laid one after they attracted a male (one died without laying). Females who attracted a male during the observation period laid their first egg sac  $13.14 \pm 1.64$  ( $N = 7$ ) days after mating and females that did not attract a male during the observation period laid their first egg sac  $16.00 \pm 1.44$  ( $N = 7$ ) days after mating. We also found no significant difference in the number of days taken to lay the first egg sac between cannibalistic ( $N = 6$ ,  $13.50 \pm 1.89$

days) and non-cannibalistic females ( $N = 8$ ,  $15.38 \pm 3.37$  days, Mann-Whitney  $U$  test:  $Z = -0.260$ ,  $P = 0.795$ ).

### *Objective 3: Frequency of single and multiple male attractions*

According to the male attractiveness data collected from previous studies on *P. albofimbriata*, single male attraction was more common than multiple male attractions in both natural and semi-natural conditions (Table 1). The percentage of multiple male attractions varied from 16% in the field to 24% in field enclosure conditions, whereas the percentage of single male attractions varied from 76% in field enclosures to 84% in the field.

## **Discussion**

This study demonstrates that for *P. albofimbriata*, if multiple males are present within close visual range, they generally linger near the female until their turn for mating and females will generally mate with multiple males. However, if the female only attracts a single male and subsequently mates with him, she will become chemically unattractive to other males for a period of eight days on average. Therefore, these males may be achieve 100% paternity as females lay their first (and largest) egg sac 4-5 days after mating (Jayaweera and Barry, 2015). These findings suggest that the occurrence of polyandry depends on how many males an unmated *P. albofimbriata* attracts simultaneously. If a female attracts only a single male from the initial pheromone plume, then polyandry is unlikely. Since single male attraction is the most common scenario under both natural and semi-natural conditions, polyandry is likely to be a reasonably uncommon phenomenon in the false garden mantids.



Female praying mantids are capable of multiple male attractions from their initial pheromone plume (Barry, 2010, Maxwell et al., 2010b), and also able to mate multiply (Watanabe et al., 2011, Jayaweera et al., 2015). Even though it is known that female *P. albofimbriata* are capable of mating multiple times in the laboratory (Barry et al., 2011), this is the first empirical evidence in a semi-natural environment. Multiple paternity within a single egg sac was reported for the praying mantids *Tenodera aridifolia* (Watanabe et al., 2011) and *Ciulfina klassi* (Umbers et al., 2011). Collectively, this suggests that polyandry is a possibility for praying mantids, setting the stage for the selection of males through post-copulatory sexual selection mechanisms such as sperm competition and cryptic female choice. Female unattractiveness/unreceptivity after mating has been identified primarily as a male strategy that reduces the likelihood of sperm competition in polyandrous mating systems and has been observed in many different taxa of insects (Simmons, 2001b). Decreasing the chance of female remating increases the probability of sole paternity, which subsequently maximises male lifetime reproductive success. This is especially relevant in the *Pseudomantis* mating system since there is a significant paternity advantage to the second male to mate (Barry et al., 2011) suggesting that unattractiveness in mated females may be a male strategy.

Males use different strategies/mechanisms to reduce female attractiveness/receptivity after mating. Sperm itself, accessory gland proteins, mating plugs and physical stimulation of the female reproductive tract can cause unreceptivity in mated females (Eberhard, 1996, Simmons, 2001b, Wedell, 2005, Radhakrishnan and Taylor, 2007). For example, the presence of sperm in the female reproductive tract of female wolf spider *Schizocosa malitioza* (Aisenberg and Costa, 2005), seminal fluid proteins in Queensland fruit fly *Bactrocera tryoni* (Radhakrishnan and Taylor, 2007) and mating plug proteins in *Drosophila melanogaster* (Bretman et al., 2010) reduce female receptivity in mated females. Further, physical stimulation associated with mating,

together with the presence of sperm in the spermatheca of the Gypsy moth *Lymantria dispar* cause permanent unreceptivity of mated females (Raina et al., 1994). There is some evidence that cannibalised males generally transfer more sperm to their mates (Schneider and Elgar, 2001, Fromhage et al., 2003, Jayaweera & Barry Unpublished data – Chapter 3), suggesting that they are more effective in reducing female receptivity (through more sperm and possibly through large amounts of accessory gland proteins). However, the limited data in the current study do not indicate that this is actually the case in *P. albofimbriata*.

While our study has demonstrated that female unattractiveness following mating can effectively secure male paternity, future studies are required to identify the exact mechanism that induces female unattractiveness.

## **Acknowledgements**

We thank Kelton Jarvis, Zachary Sheehan and Andrew Ly for their help in the rearing of mantids and Marie Herberstein for comments on the manuscript. Hermon Slade Foundation funded this research.

## References

- AISENBERG, A. & COSTA, F. G. 2005. Females mated without sperm transfer maintain high sexual receptivity in the wolf spider *Schizocosa malitiosa*. *Ethology*, 111, 545-558.
- ARNQVIST, G. & NILSSON, T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behaviour*, 60, 145-164.
- BARRY, K. L. 2010. Influence of female nutritional status on mating dynamics in a sexually cannibalistic praying mantid. *Animal Behaviour*, 80, 405-411.
- BARRY, K. L. 2013. You are what you eat: food limitation affects reproductive fitness in a sexually cannibalistic praying mantid. *PLoS One*, 8, e78164.
- BARRY, K. L. 2015. Sexual deception in a cannibalistic mating system? Testing the Femme Fatale hypothesis. *Proceedings of the Royal Society of London B: Biological Sciences*, 282, 20141428.
- BARRY, K. L., HOLWELL, G. I. & HERBERSTEIN, M. E. 2011. A paternity advantage for speedy males? Sperm precedence patterns and female re-mating frequencies in a sexually cannibalistic praying mantid. *Evolutionary Ecology*, 25, 107-119.
- BARRY, K. L. & WILDER, S. M. 2013. Macronutrient intake affects reproduction of a predatory insect. *Oikos*, 122, 1058-1064.
- BONDURIANSKY, R. 2001. The evolution of male mate choice in insects: A synthesis of ideas and evidence. *Biological Reviews*, 76, 305-339.
- BRETMAN, A., LAWNICZAK, M. K., BOONE, J. & CHAPMAN, T. 2010. A mating plug protein reduces early female remating in *Drosophila melanogaster*. *Journal of Insect Physiology*, 56, 107-113.

- 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.
- EBERHARD, W. G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*, Princeton, New Jersey, Princeton University Press.
- ELGAR, M. A., SCHNEIDER, J. M. & HERBERSTEIN, M. E. 2000. Female control of paternity in the sexually cannibalistic spider *Argiope keyserlingi*. *Proceedings of the Royal Society B-Biological Sciences*, 267, 2439-2443.
- FROMHAGE, L., UHL, G. & SCHNEIDER, J. M. 2003. Fitness consequences of sexual cannibalism in female *Argiope bruennichi*. *Behavioral Ecology and Sociobiology*, 55, 60-64.
- GRIFFITH, S. C., OWENS, I. P. F. & THUMAN, K. A. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, 11, 2195-2212.
- HERBERSTEIN, M., BARRY, K., TUROCZY, M., WILLS, E., YOUSSEF, C. & ELGAR, M. 2005. Post-copulation mate guarding in the sexually cannibalistic St Andrew's Cross spider (Araneae Araneidae). *Ethology Ecology & Evolution*, 17, 17-26.
- JAYAWEERA, A. & BARRY, K. L. 2015. The Effect of female quality on male ejaculatory expenditure and reproductive success in a praying mantid. *Plos One*, 10, e0124209.
- JAYAWEERA, A., RATHNAYAKE, D. N., DAVIS, K. S. & BARRY, K. L. 2015. The risk of sexual cannibalism and its effect on male approach and mating behaviour in a praying mantid. *Animal Behaviour*, 110, 113-119.
- JONES, T. M. 2001. A Potential cost of monandry in the lekking sandfly *Lutzomyia Longipalpis*. *Journal of Insect Behavior*, 14, 385-399.
- KAMIMURA, Y. 2000. Possible removal of rival sperm by the elongated genitalia of the earwig, *Euborellia plebeja*. *Zoological Science*, 17, 667-672.

- LELITO, J. P. & BROWN, W. D. 2008. Mate attraction by females in a sexually cannibalistic praying mantis. *Behavioral Ecology and Sociobiology*, 63, 313-320.
- MAXWELL, M. R., BARRY, K. L. & JOHNS, P. N. 2010a. Examinations of Female Pheromone Use in Two Praying Mantids, *Stagmomantis limbata* and *Tenodera aridifolia* sinensis (Mantodea: Mantidae). *Annals of the Entomological Society of America*, 103, 120-127.
- MAXWELL, M. R., GALLEG0, K. M. & BARRY, K. L. 2010b. Effects of female feeding regime in a sexually cannibalistic mantid: fecundity, cannibalism, and male response in *Stagmomantis limbata* (Mantodea). *Ecological Entomology*, 35, 775-787.
- MULLER, M. N., KAHLENBERG, S. M., THOMPSON, M. E. & WRANGHAM, R. W. 2007. Male coercion and the costs of promiscuous mating for female chimpanzees. *Proceedings of the Royal Society B-Biological Sciences*, 274, 1009-1014.
- PARKER, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. *Biological Reviews*, 45, 525-567.
- PETRIE, M., DOUMS, C. & MOLLER, A. P. 1998. The degree of extra-pair paternity increases with genetic variability. *Proceedings of the National Academy of Sciences of the United States of America*, 95, 9390-9395.
- PRESTON, B. T., STEVENSON, I. R., PEMBERTON, J. M. & WILSON, K. 2001. Dominant rams lose out by sperm depletion - A waning success in siring counters a ram's high score in competition for ewes. *Nature*, 409, 681-682.
- RADHAKRISHNAN, P. & TAYLOR, P. W. 2007. Seminal fluids mediate sexual inhibition and short copula duration in mated female Queensland fruit flies. *Journal of Insect Physiology*, 53, 741-745.
- RAINA, A. K., KINGAN, T. G. & GIEBULTOWICZ, J. M. 1994. Mating-induced loss of sex pheromone and sexual receptivity in insects with emphasis on *Helicoverpa zea* and *Lymantria dispar*. *Archives of Insect Biochemistry and Physiology*, 25, 317-327.

- RIDLEY, M. 1990. The Control and Frequency of Mating in Insects. *Functional Ecology*, 4, 75-84.
- ROWE, L. 1994. The costs of mating and mate choice in Water Striders. *Animal Behaviour*, 48, 1049-1056.
- SCHNEIDER, J., UHL, G. & HERBERSTEIN, M. E. 2015. Cryptic female choice within the genus *Argiope*: a comparative approach. *Cryptic Female Choice in Arthropods*. Springer.
- SCHNEIDER, J. M. & ELGAR, M. A. 2001. Sexual cannibalism and sperm competition in the golden orb-web spider *Nephila plumipes* (Araneoidea): female and male perspectives. *Behavioral Ecology*, 12, 547-552.
- SCHNEIDER, J. M. & LESMONO, K. 2009. Courtship raises male fertilization success through post-mating sexual selection in a spider. *Proceedings of the Royal Society B-Biological Sciences*, 276, 3105-3111.
- SIMMONS, L. W. 2001a. The evolution of polyandry: an examination of the genetic incompatibility and good-sperm hypotheses. *Journal of Evolutionary Biology*, 14, 585-594.
- SIMMONS, L. W. 2001b. *Sperm Competition and its Evolutionary Consequences in the Insects*, Princeton, New Jersey: Princeton University Press.
- UMBERS, K. D. L., HOLWELL, G. I., STOW, A. J. & HERBERSTEIN, M. E. 2011. Molecular evidence for variation in polyandry among praying mantids (Mantodea: Ciulfina). *Journal of Zoology*, 284, 40-45.
- WATANABE, E., ADACHI-HAGIMORI, T., MIURA, K., MAXWELL, M. R., ANDO, Y. & TAKEMATSU, Y. 2011. Multiple Paternity Within Field-Collected Egg Cases of the Praying Mantid *Tenodera aridifolia*. *Annals of the Entomological Society of America*, 104, 348-352.
- WEDELL, N. 2005. Female receptivity in butterflies and moths. *Journal of Experimental Biology*, 208, 3433-3440.

WELKE, K. & SCHNEIDER, J. M. 2009. Inbreeding avoidance through cryptic female choice in the cannibalistic orb-web spider *Argiope lobata*. *Behavioral Ecology*, 20, 1056-1062.



Table 1. Frequency of single male and multiple male attractions by female *P. albofimbriata*. Frequencies were derived based on data from previous studies on *P. albofimbriata*. It includes percentages of single and multiple male attractions under semi-natural (field enclosure) and natural (field) conditions recorded in a fixed observation time (Barry, 2010, Barry, 2013)

	<b>Single male attractions</b>	<b>Multiple males attractions</b>	<b>Total attractions</b>
<b>Field enclosure</b>	63 (75.9%)	20 (24.1%)	83 (100%)
<b>Field</b>	16 (84.21%)	3 (15.79%)	19 (100%)
<b>Total attractions</b>	79 (77.45%)	23 (22.55%)	102 (100%)



Figure 1. Multiple males simultaneously mounting a single female

## Summary and Conclusions

My Ph.D. thesis investigates the reproductive ecology and evolution in the false garden mantid *Pseudomantis albofimbriata* mating system. More specifically it aims to identify male mating strategies in response to sexual selection and sexual cannibalism, both prior to and during/after copulation. This mating system provides unique and interesting opportunities to study the evolution of male reproductive strategies in the existence of extreme sexual conflict in the form of pre-mating sexual cannibalism.

Scrambling systems are one of the most common type of mating systems found mainly among invertebrates, but, also in some vertebrates (Herberstein et al., 2017). Males in these systems are predicted to evolve traits that aid in effective and efficient mate location such as increased mobility and early maturation. Even though effective sensory systems are known to play a crucial role in scrambling mating systems, direct evidence of the influence of sensory systems on scramble outcomes is sparse. Results of my research suggest that male antenna morphology plays an important role in scramble competition in *P. albofimbriata*, where males with more trichoid sensilla located females quickly (Chapter 1). Effective mate location aids male false garden mantids not only in mating first with the female, but, also in securing future mating opportunities, as unmated females are less likely to cannibalise their partners compared to mated females (Chapter 2). In addition, mating with a female first can protect against sperm competition as mated females become chemically unattractive to males soon after mating for relatively a long period (Chapter 5) making re-mating by slower males prior to ovipositing less likely. Therefore, slower males may end up in risky mating interactions or even lose their opportunity for any reproductive success by failing to find a partner to successfully mate.

Once a male locates a female and visually fixes onto her, males do not seem to abandon a mating attempt, even , if the female is already mating with another male

(Chapter 5). Males approach females regardless of the risk of sexual cannibalism (Chapter 2). These findings suggest that cost of mate searching and rejecting a female for male *P. albofimbriata* may outweigh the cost of shared paternity or of engaging in high risk mating interactions, as predicted by a recently published model (Barry and Kokko, 2010). It is possible that the ecology of this species crucially affects the evolution and maintenance of its mating system. Females occur at low density in a relatively complex vegetation. Male *P. albofimbriata* may invest more energy in the mate searching phase to locate a female, travelling long distances following female emitted cues (Barry et al., 2010).

The most interesting and important result of this study is the differential mating resource investment by male *P. albofimbriata* relative to the mating context (Chapter 3 & 4). Previous studies on false garden mantids suggest that they are capable of strategic ejaculation in response to the risk of sperm competition associated with the juvenile sex ratio (Allen et al., 2011). However, results of my research suggest that strategic ejaculation is further context dependent relative to the risk of sexual cannibalism. As predicted by a terminal investment model (Stearns, 1976), male *P. albofimbriata* allocate sperm strategically depending on whether they are being cannibalised, but, not in response to the female quality. Even though there is some evidence that males adjust their pre-copulatory behaviour (e.g. mate choice or approach behaviour) to counter the risk of sexual cannibalism (Maxwell, 1998, Maxwell, 1999, Herberstein et al., 2002, Schneider and Elgar, 2002, Fromhage and Schneider, 2005, Lelito and Brown, 2006, Barry et al., 2009, Barry et al., 2010, Brown et al., 2012, Kralj-Fiser et al., 2012), post-copulatory strategies in such systems are scant (but see, Andrade, 1996, Schneider and Elgar, 2001, Fromhage et al., 2003, Nessler et al., 2009). For example, increased copulation duration (Andrade, 1996, Schneider and Elgar, 2001, Fromhage et al., 2003) and frequent genitalia break off within the female reproductive tract (Nessler et al., 2009) were observed for some cannibalised male spiders as a paternity assurance mechanisms. However, to our

knowledge this is the first empirical evidence of increased sperm transfer by cannibalised males in a praying mantid mating system (Chapter 3). Males are likely to maximize their paternity through the only or last mating attempt in their life time by transferring more sperm to their partners. More importantly, our results suggest that the loss of the head during sexual cannibalism may be the trigger to increased sperm transfer by cannibalised males (Chapter 3). Increased reproductive behaviour upon the decapitation or the removal of sub-oesophageal ganglion is reported for some other insects as an innate response for the lack of body control (Roeder, 1935, Roeder, 1998). Therefore, it may be that cannibalised/headless males transfer more sperm to their partners due to the loss of coordination in the central nervous system. Thus, it is likely that increased sperm transfer by cannibalised males may have evolved as a non-adaptive response, with a secondarily adaptive response in this cannibalistic mating system.

Mated female unreceptivity/unattractiveness is recorded for a wide range of insect taxa and it is mainly described as a male strategy (Simmons, 2001, Wedell, 2005, Bretman et al., 2010). Accordingly, it is likely that males induce the observed lengthy refractory period (eight days on average) in mated female *P. albofimbriata*. The selective advantage is likely to be sole paternity of the first and the largest egg sac produced by the female (Chapter 5). Male ejaculatory components (sperm and/or accessory gland proteins) are mainly responsible for the male induced female unreceptivity/unattractiveness for many insect taxa (Aisenberg and Costa, 2005, Radhakrishnan and Taylor, 2007, Bretman et al., 2010, Larson et al., 2012) and we suggest similar mechanism may result the mated female unattractiveness in false garden mantid mating system. However, the quantity of the ejaculate does not have an effect on the female unattractiveness as we did not find a difference in the period of unattractiveness between cannibalistic and non-cannibalistic females (Chapter 5), even though cannibalistic females received more sperm (and also may have received more accessory

gland proteins) compared to non-cannibalistic females (Chapter 3). Therefore, future studies isolating the actual mechanism that results in the unattractiveness of mated female *P. albofimbriata* are crucial.

Overall, my research describes a mating system where the cost of mate searching and rejecting the current mating opportunity may exceed the cost of engaging in high risk mating interactions for males, and males are likely to maximize their reproductive success through strategic sperm allocation and by reducing the probability for female remating. Therefore, findings of my thesis highlight the importance of conducting studies that investigate both pre- and post-copulatory mating strategies in a wide variety of mating contexts. Furthermore, my research illustrates the power of combining fine scale morphological studies with behavioural studies. For example, even though it is suggested that male antenna may play a role in scramble competition in insects, previous studies were mainly limited to the most prominent characteristics of the antennae such as overall length. However, my research shows that the density of antennal sensilla seems to be more important in scramble competition. In order to fully understand the mating dynamics, especially the actual mechanisms behind observed mating behaviour of *P. albofimbriata*, more detailed physiological, anatomical and molecular studies are necessary. For example, future studies could undertake to determine the effect of ejaculatory components on mated female unattractiveness by injecting females with isolated sperm and male accessory gland products and thereby observing the period of unattractiveness. Further, molecular analysis of multiple paternity in field collected egg sacs will confirm the observed frequency of polyandry in nature for *P. albofimbriata*.

## References

- AISENBERG, A. & COSTA, F. G. 2005. Females mated without sperm transfer maintain high sexual receptivity in the wolf spider *Schizocosa malitiosa*. *Ethology*, 111, 545-558.
- ALLEN, L. E., BARRY, K. L., HOLWELL, G. I. & HERBERSTEIN, M. E. 2011. Perceived risk of sperm competition affects juvenile development and ejaculate expenditure in male praying mantids. *Animal Behaviour*, 82, 1201-1206.
- ANDRADE, M. C. B. 1996. Sexual selection for male sacrifice in the Australian redback spider. *Science*, 271, 70-72.
- BARRY, K. L., HOLWELL, G. I. & HERBERSTEIN, M. E. 2009. Male mating behaviour reduces the risk of sexual cannibalism in an Australian praying mantid. *Journal of Ethology*, 27, 377-383.
- BARRY, K. L., HOLWELL, G. I. & HERBERSTEIN, M. E. 2010. Multimodal mate assessment by male praying mantids in a sexually cannibalistic mating system. *Animal Behaviour*, 79, 1165-1172.
- BARRY, K. L. & KOKKO, H. 2010. Male mate choice: why sequential choice can make its evolution difficult. *Animal Behaviour*, 80, 163-169.
- BRETMAN, A., LAWNICZAK, M. K., BOONE, J. & CHAPMAN, T. 2010. A mating plug protein reduces early female remating in *Drosophila melanogaster*. *Journal of Insect Physiology*, 56, 107-113.
- BROWN, W. D., MUNTZ, G. A. & LADOWSKI, A. J. 2012. Low mate encounter rate increases male risk taking in a sexually cannibalistic praying mantis. *Plos One*, 7, e35377.
- FROMHAGE, L. & SCHNEIDER, J. M. 2005. Safer sex with feeding females: sexual conflict in a cannibalistic spider. *Behavioral Ecology*, 16, 377-382.
- FROMHAGE, L., UHL, G. & SCHNEIDER, J. M. 2003. Fitness consequences of sexual cannibalism in female *Argiope bruennichi*. *Behavioral Ecology and Sociobiology*, 55, 60-64.

- HERBERSTEIN, M. E., PAINTING, C. J., HOLWELL, G. I. 2017. Scramble competition polygyny in terrestrial arthropods. *Advances in the Study of Behavior*. Cambridge, Massachusetts : Elsevier.
- HERBERSTEIN, M. E., SCHNEIDER, J. M. & ELGAR, M. A. 2002. Costs of courtship and mating in a sexually cannibalistic orb-web spider: female mating strategies and their consequences for males. *Behavioral Ecology and Sociobiology*, 51, 440-446.
- KRALJ-FISER, S., SCHNEIDER, J. M., JUSTINEK, Z., KALIN, S., GREGORIC, M., PEKAR, S. & KUNTNER, M. 2012. Mate quality, not aggressive spillover, explains sexual cannibalism in a size-dimorphic spider. *Behavioral Ecology and Sociobiology*, 66, 145-151.
- LARSON, E. L., ANDRES, J. A. & HARRISON, R. G. 2012. Influence of the male ejaculate on post-mating prezygotic barriers in field crickets. *Plos One*, 7, e46202.
- LELITO, J. P. & BROWN, W. D. 2006. Natural history miscellany - Complicity or conflict over sexual cannibalism? Male risk taking in the praying mantis *Tenodera aridifolia sinensis*. *American Naturalist*, 168, 263-269.
- MAXWELL, M. R. 1998. Lifetime mating opportunities and male mating behaviour in sexually cannibalistic praying mantids. *Animal Behaviour*, 55, 1011-1028.
- MAXWELL, M. R. 1999. The risk of cannibalism and male mating behavior in the Mediterranean praying Mantid, *Iris oratoria*. *Behaviour*, 136, 205-219.
- NESSLER, S. H., UHL, G. & SCHNEIDER, J. M. 2009. Sexual cannibalism facilitates genital damage in *Argiope lobata* (Araneae: Araneidae). *Behavioral Ecology and Sociobiology*, 63, 355-362.
- RADHAKRISHNAN, P. & TAYLOR, P. W. 2007. Seminal fluids mediate sexual inhibition and short copula duration in mated female Queensland fruit flies. *Journal of Insect Physiology*, 53, 741-745.
- ROEDER, K. D. 1935. An experimental analysis of the sexual behavior of the praying mantis (*Mantis religiosa* L.). *Biological Bulletin*, 69, 203-220.



- ROEDER, K. D. 1998. *Nerve Cells and Insect Behavior*, Harvard University Press.
- SCHNEIDER, J. M. & ELGAR, M. A. 2001. Sexual cannibalism and sperm competition in the golden orb-web spider *Nephila plumipes* (Araneoidea): female and male perspectives. *Behavioral Ecology*, 12, 547-552.
- SCHNEIDER, J. M. & ELGAR, M. A. 2002. Sexual cannibalism in *Nephila plumipes* as a consequence of female life history strategies. *Journal of Evolutionary Biology*, 15, 84-91.
- SIMMONS, L. W. 2001. *Sperm Competition and its Evolutionary Consequences in the Insects*, Princeton, New Jersey: Princeton University Press.
- STEARNS, S. C. 1976. Life history tactics: A review of the ideas. *Quarterly Review of Biology*, 51, 3-47.
- WEDELL, N. 2005. Female receptivity in butterflies and moths. *Journal of Experimental Biology*, 208, 3433-3440.