Behaviour and ecology of mating in the jumping spider

Servaea incana (Araneae: Salticidae)

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March 2015

This thesis is presented for the degree of Doctor of Philosophy

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Abstract

Female sexual receptivity has been studied in great detail in insects, but very little is known about female sexual receptivity in spiders. I studied female sexual receptivity and associated life history traits in *Servaea incana* (Salticidae), a common jumping spider of temperate Australia. *S. incana* inhabit the trunks of eucalypt trees, where they build retreats and nests under loose pieces of bark. The display repertoire of *S. incana* is typical of salticids, although relatively simple compared to the displays of some of *Servaea*'s closest relatives. Context-dependent courtship versatility was evident in *S. incana* although not as pronounced as in many other jumping spiders. Adult males cohabit with subadult females at retreats, copulating shortly after females mature. Cohabiting pairs tended to assort by size and this may reflect size-dependent outcome of contests between males for access to larger females. In a population where virgin females were most abundant at the beginning of the mating season, males of *S. incana* were found to mature earlier than females (protandry).

I report for the first time in a jumping spider the number of copulations a female had over her entire life, and the occurrence and latency of sexual inhibition in this species. Mating status was found to have a potent effect on receptivity. Virgin females were highly receptive, but after only a single mating became aggressive towards males. Context was also important; virgin females in the open encountered more males before mating than was the case if they were in a retreat. However, after an initial mating, females in the open required a fewer number of exposures to males prior to remating, compared to females in retreats. Mating-induced sexual inhibition of females appears to

have consequences for life history; protandry and cohabitation likely increases a male's access to receptive virgin females.

The findings of empirical studies of *S. incana* are placed in the broader literature through a detailed review of mating patterns of female spiders in an ecological context, and likely mechanisms underpinning mating-induced sexual inhibition. Although little explicit research exists on receptivity and the mechanism that induces sexual inhibition in spiders, a range of studies was found that included species that, like *S. incana*, tend to copulate only once in their life (e.g., many Lycosidae) and species that mate readily even after many copulations (e.g., many Nephiliidae). Variation is also very common within species as in *S. incana*, with some females mating only once and others mating repeatedly.

Here we present the first study on mating-induced sexual inhibition during the entire life of a female spider. We used the observations on female remating to understand better the phenology, natural history and mating decisions of males and females in a population.

Statement of Candidate

I certify that this thesis entitled "Behaviour and ecology of mating in the jumping spider *Servaea incana* (Araneae: Salticidae)" has not previously been submitted for a degree nor has it been submitted as part of requirement for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

This thesis was not subject to approval by the Macquarie University Ethics review Committee since the organism studied is an invertebrate, the jumping spider *Servaea incana.*

Vivian Mendez (Student ID 40488020)

March 2015

Acknowledgements

Over the past three and a half years I have been supported by many people and I feel in debt to all of them. First, I want to thank my supervisor, Phil Taylor. Phil has not only been an excellent supervisor, but also a good friend that was always quick to help me and my family and for which we are very grateful.

All the people (past and present) participating in Phil Taylor's lab have also contributed in their own special way to the completion of my PhD, and I thank them. Special recognition goes to Rowan McGinley, I thank you for all your help, cheerful attitude during a long year of field work and for providing the front page photo of this thesis. I also thank Rowan's parents, Roger and Marcia, for the invitations to their house; they made us feel right at home.

I consider myself fortunate for the special friends I have made in Sydney. Thank you Cata, Randol, Maria, Alex, Dona Olga, Bibiana, Miguel and Michaela for making us feel a part of your families, and feeding us with your amazing cuisine. You are a wonderful family and always made us feel loved and in good company. Many thanks to my friends: Rocio, Alejandra, Martha, Vivian, Natalia, Caty, Ruth, Lis, Karla, Alvaro, Laura, Anita, Luis and Jeani.

To my family in Costa Rica, thank you for being so supportive. Special thanks to my mum, Ana Maria, and my mother-in-law, Flor Alicia, for making the long trip to

Australia to help take care of my son, Santi, precisely when I needed them most. Also, I wish to thank my sister, my father, my brother in law, my nephew and nieces for taking care of Santi , which allowed me time to focus on my research.

And finally, I am very grateful for the loving support of my partner, Carlos Porras, and my son, Santiago, who have been integral members of this Ph.D. adventure. Carlos, I am grateful for your love, kindness and understanding throughout this process. You and Santi have given me a very beautiful life.

Chapter One: General Introduction

For many years after Darwin (1871) first coined the term, the study of 'sexual selection' has focused almost entirely on precopulatory events, specifically (1) the elaborate ornamentation and courtship routines of male animals that may be used by females to assess their quality as mates and (2) weapons and displays used by males to resolve contests over access to females as a mating resource (Andersson 1994). Mating is of course a crucial step toward reproduction in most sexually reproducing species, but it is important to also bear in mind that other steps remain before the act of mating can yield a functional offspring. In recent decades we have gained greatly increased appreciation that males and females both commonly possess post copulatory mechanisms that are important in determining which male's sperm fertilizes a female's eggs (Parker 1984 and Eberhard 1996). Both female choice and male-male competition persist beyond copulation. Females may mate with more than one male and then choose which male's sperm to use for fertilizations ('cryptic female choice', *sensu* Eberhard 1996). Also, males often have adaptations that function in biasing fertilizations in their favour in females that accept more than one male as a mate ('sperm competition', see Parker 1998, Simmons 2001).

There are several benefits for females that copulate with more than one male. Usually the benefits are divided in two categories, direct benefits and indirect benefits (Andersson 1994). Direct benefits are the ones obtained by the female for herself such as acquisition of food during courtship or copulation, enough sperm for fertilization of eggs and better partners. Indirect benefits are the ones obtained by the female for her

offspring such as offspring genetic diversity, attractiveness and viability (Birkhead and Pizzari 2002). However, mating with multiple partners can also have costs associated (Jennions and Petrie 2000, Lehtonen et al. 2012). Mating increases the risk of physical damage (den Hollander and Gywnne 2009) and of being infected with sexually transmitted diseases (Hurst et al. 1995). Substances transfered by males with the sperm during copulation can also have detrimental effects on females (Chapman et al. 1995). Females are expected to mate with multiple partners only if the benefits of polyandry outweight the costs. The scope for expression of post-copulatory sexual selection is highly dependent on the number of males that a female mates with, and so studies of female remating tendency are crucial to our understanding of the extent to which post-copulatory sexual selection can operate in a species.

In spiders, sexual selection has been studied in detail, in fact, jumping spiders (Araneae: Salticidae) were one of the groups of animals used first to study sexual selection (Huber 2005). There is evidence, for example, that the behaviour and morphology of some species of jumping spiders have evolved as a result of sexual selection (Jackson 1980, Masta and Madison 2002). However, although precopulatory behaviours have been studied in detail (e.g., Elias et al. 2005; Su and Li, 2006; Cerveira and Jackson, 2013), much remains to be studied regarding post copulatory events in jumping spiders. Information on precopulatory activities studied in Salticidae now provides the foundation necessary to study and understand postcopulatory behaviours affecting male's fertilization success, such as female remating patterns.

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Female mating-induced sexual inhibition, a reduction in female sexual receptivity after a copulatory event, and its mechanisms have been reported for numerous insects (for reviews, see Thornhill and Alcock 1983, Chen, 1984, Eberhard 1996, Ringo 1996, Chapman et al. 1998), and while the expression and mechanisms are far less known, female mating-induced sexual inhibition has also been reported in arachnids. Mating-induced sexual inhibition has been found in ticks (Kiszewski and Spielman 2002), and spiders in the families Agelenidae (Masumoto 1991, 1993, Singer and Reichert 1995), Linyphiidae (van Helsdingen 1965, Austad 1984, Watson 1986), Salticidae (Jackson 1980, Taylor and Jackson 1999), Lycosidae (Norton and Uetz 2005, Aisenberg and Costa 2005, Jiao et al. 2011), Theridiidae (Perampaladas et al. 2005, Molina and Christenson 2008), Tetragnathidae (Aisenberg 2009), Pholcidae (Schäffer and Uhl 2002, 2005), Eresidae (Maklakov et al. 2003) and Thomisidae (Dodson and Beck 1993). There are other characteristics of female spiders that have been associated with their willingness to mate. Age (Uetz and Norton 2007, Wu et al. 2013), size (Prenter et al. 1994, Schäfer and Uhl 2005), feeding history (Herberstein et al. 2002, Bilde et al 2007), including sexual cannibalism (Andrade 1996, Snow and Andrade 2004) can affect female receptivity in spiders.

Because remating tendency can have a powerful influence on post-copulatory events such as cryptic female choice and sperm competition, in this thesis I focus on female mating-induced sexual inhibition of *Servaea incana*, a common Australian jumping spider found in temperate Australia. Previous studies on *S. incana* have focused on vision (Zurek and Nelson 2012; Zurek et al. 2010) and little is known about the mating behaviours of this species. Moreover, studies of mating behaviour of Australian jumping spiders have largely focused on tropical species.

Jumping spiders are known for having acute vision (Land, 1969; Williams and McIntyre, 1980) and elaborate visual courtship displays (Crane 1949; Richman 1982; Clark and Morjan 2001). However, male jumping spiders also have courtship displays that are not vision-dependent and are used when females are found inside a nest or a retreat where vision cannot be used as effectively (Jackson 1977). Similar to other spiders, virgin females tend to be highly receptive while mated females tend to be aggressive and unreceptive (Jackson 1980; 1981; 1982; Taylor and Jackson 1999). It has been suggested that females become chosier after their first mating because once-mated females have secured their reproduction, choosiness in virgin females might be costly because it might result in failure to mate (Jackson 1981). However, as for studies of other spider taxa, all reports of such mating-induced sexual inhibition in jumping spiders only include observations made during the first weeks following a female's first mating.

Female receptivity is predicted to be linked with female availability for males at the population level, especially in species with a well-defined seasonality. If highly receptive virgin females are common at the beginning of the reproductive season, then males are predicted to also be abundant at this time when there are higher chances of finding receptive females (Wiklund and Fagerström 1977, Bulmer 1983, Andersson 1994, Morbey and Ydenberg 2001). Mating with virgin females will be particularly critical for males in species with females that store sperm and have first male sperm precedence (Parker 1970) or low levels of remating. To increase mating opportunities with virgin females, males of some spiders tend to mature before females ('protandry') and even to guard subadult females that are about to moult to maturity ('cohabitation'). A guarding male will mate with the female when the female moults to adulthood (Jackson 1986). Populations of the salticids *Phidippus johnsoni* and *Phidippus clarus* have been

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reported to have protandry and cohabitation (Jackson 1981, 1986, Hoefler 2007), which suggests that virgin females are a highly valuable reproductive resource for the males. A better understanding of the evolution of mating systems and mating startegies can be achieved by studying the effect that ecological factors have on the development rates and times of maturation of the study species (Elias et al. 2012). However, in jumping spiders little is known about the links between temporal variation in population structure, protandry, mating-induced sexual inhibition and cohabitation. These are major issues that I address in this thesis.

Information on the natural history of *Servaea inca*na is provided in Chapter 2. We described the display repertoire and sequences of behaviour performed by *S. incana* in intraspecific interactions between spiders of different age and sex. In this chapter we also give information on prey and predator for this species. Details of the natural history of *S. incana* were unknown and with this study we were able to better understand and interpret the mating behaviours of this spider. Chapter 3 is also a descriptive study and provides information on the change in numbers of spiders of different sex and ages through the year. We wanted to know if female availability changed seasonally, if males were maturing ealier than females and if males were cohabiting with penultimate females, as it ocurs in other species of spiders. This chapter provides critical information on the ecology and mating strategies of males and females. Once we collected enough information on the ecology of the species in Chapter 2 and 3, we used this information to design experiments in Chapter 4. In Chapter 4 we wanted to know if female mating-induced sexual inhibition was occurring in *Servaea incana* and if so, we wanted to study how context afftected remating inhibition and the persistence of remating inhibition through the life of the female spider. We also wanted to know if the onset of sexual

inhibition occurred immediately after the first mating of the female. We related the data on remating inhibition we collected in Chapter 4 to the patterns of cohabitation and protandry found in Chapter 3. Finally in Chapter 6 we compile information on female sexual receptivity in spiders. We wanted to know if there were patterns within families that would be explained by the ecological context of each species as seems to be the case for *S. incana*. In this chapter we also discussed the mechanisms that have been suggested to be responsible for the lack of receptivity after first mating in female spiders.

1.1 Research objectives

The aim of this thesis was to study the mating behaviour of the jumping spider *Servaea incana* with a particular emphasis on mating-induced sexual inhibition in females, and to place the species' mating behaviour in an ecological context. *Servaea incana* is a common jumping spider found across temperate regions of Australia (Richardson and Gunter 2012). Here, I reported for the first time the natural history, display behaviour, demography of sexually available females, and tendency and timing of remating in *S. incana*, and place this in the broader context of receptivity patterns of spiders that have been reported in the literature.

1.2 Thesis organization

The chapters of this thesis have been written by me and improved with comments on all sections by Phil Taylor. Phil Taylor will be author in all four manuscripts. The following is a description of each chapter:

Chapter Two is a description of the intraspecific interactions and the natural history of *S. incana* in the field and the laboratory. Information on predators and prey is also discussed in terms of the selection pressures on *S. incana*. This chapter is co-authored by Rowan McGinley and Phil Taylor, who both contributed to the concept and design of this chapter. Rowan McGinley and I collected the data. Rowan McGinley analysed the data and wrote the first draft of the manuscript. I revised and worked substantially on subsequent versions of the manuscript. This chapter is formatted for the *Australian Journal of Zoology*.

Chapter Three is a field study, a census that ran for 13 months in a population of *S. incana.* During the census, we recorded the number of individuals in every developmental stage to determine the phenology of this species and examine the seasonality in the availability of mating opportunities for males. This chapter is co-authored by Rowan McGinley and Phil Taylor. Phil Taylor, Rowan and I established which variables were to be measured in the field. Rowan McGinley and I collected data together. I analysed the data and wrote the manuscript. Phil Taylor

provided suggestions for both the statistical analysis and the writing of the manuscript. This chapter is formatted for the *Journal of Natural History*.

Chapter Four consists of the results of two laboratory experiments on remating inhibition in *S. incana*. The aim of the first experiment was to establish the occurrence and persistence of mating-induced sexual inhibition in *S. incana*. The aim of the second experiment was to determine the timing for the onset of sexual inhibition after the female's first mating. This chapter is co-authored by Phil Taylor and Rowan McGinley. Phil Taylor and I designed the experiments. I collected the data with the help of Rowan McGinley. I carried out the statistical analysis and wrote the paper. Phil Taylor provided suggestions on experimental design, statistical analysis and the writing of the manuscript. This chapter is formatted for *Animal Behaviour*.

Chapter Five is a review on receptivity in female spiders and the factors that can affect female willingness to mate. The aim of the review was to link the patterns of receptivity reported for female spiders with aspects of the biology of the species. Phil Taylor is co-author in this manuscript. Phil Taylor and I developed the concept, I collected the information and wrote the review. Phil Taylor improved the manuscript with comments. This chapter was formatted for *Biological Reviews*.

Chapter Six   is the general discussion of the biology, phenology and behaviour of *S. incana.* I discuss the effect of female availability in the field and mating-induced remating inhibition in females on the mating opportunities of males and the reproductive strategies of both sexes. I also examine the effect of ecological pressures such as predators and hunting prey on the behaviours of *S.incana*. Finally, I discuss the taxonomic patterns of sexual receptivity in spiders and the potential mechanisms involved in mating-induced sexual inhibition.

References

Aisenberg, A. 2009. Male performance and body size affect female re-mating occurrence in the orb-web spider *Leucauge mariana* (Araneae, Tetragnathidae). *Ethology* **115**: 1127-1136.

Aisenberg, A. and Costa, FG. 2005. Females mated without sperm transfer maintain high sexual receptivity in the wolf spider *Schizocosa malitiosa. Ethology* **111**: 545-558.

Andersson, MB. 1994. *Sexual selection*. Princeton University Press. 624 pp.

Andrade, MCB. 1996. Sexual selection for male sacrifice in the Australian redback spider. *Science* **271**: 70-72.

Austad, SN. 1984. Evolution of sperm priority patterns in spiders. In: *Sperm competition and the evolution of animal mating systems* (Ed. RL Smith), pp. 233-249. Academic Press, London.

Bilde, T., Tuni, C. Elsayed, R., Pekar, S. and Toft, S. 2007. Nuptial gifts of male spiders: Sensory exploitation of the female's maternal care instinct or foraging motivation? *Animal Behaviour* **73**: 267-273.

Birkhead, TR. and Pizzari, T. 2002. Postcopulatory sexual selection. *Nature Reviews Genetics* **3**: 262-273.

Bulmer, MG. 1983. Models for the evolution of protandry in insects. *Theoretical Population Biology* **23**: 314–322.

Cerveira, AM. and Jackson, RR. 2013. Love is in the air: olfaction-based mate-odour identification by jumping spiders from the genus *Cyrba*. *Journal of Ethology* **31**: 29-34.

Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F., & Partridge, L. (1995). Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature* **373**: 241-244.

Chapman, T., Miyatake, T., Smith, H. and Partridge. L. 1998. Interactions of mating, egg production and death rates in females of the Mediterranean fruit fly, *Ceratitis capitata* females. *Proceedings of the Royal Society B* **265**: 1879-1894.

Chen, PS. 1984. The functional morphology and biochemistry of insect male accessory glands and their secretions. *Annual Review of Entomology* **29**: 233-255.

Clark, DL. and Morjan, CL. 2001. Attracting female attention: the evolution of dimorphic courtship displays in the jumping spider *Maevia inclemens* (Araneae: Salticidae). *Proceedings of the Royal Society B* **268**: 2461-2465.

Crane, J. 1949. Comparative biology of salticid spiders at Rancho Grande, Venezuela. Part IV. An analysis of display. *Zoologica* **34**: 159-214.

Darwin, C. 1871. *The Descent of Man, and Selection in Relation to Sex.* John Murray,
London.

den Hollander, M. and Gwynne, DT. 2009. Female fitness consequences of male
harassment and copulation in seed beetles, *Callosobruchus maculatus*. *Animal
Behaviour* **78**: 1061-1070.

Dodson, GN. and Beck, MW. 1993. Pre-copulatory guarding of penultimate females by
male crab spiders, *Misumenoides formosipes*. *Animal Behaviour* **46**: 951-959.

Eberhard, WG. 1996. *Female control: sexual selection by cryptic female choice*.
Princeton, New Jersey: Princeton University Press.

Elias, DO., Hebets, EA, Hoy, RR. and Mason, AC. 2005. Seismic signals are crucial for
male mating success in a visual specialist jumping spider (Araneae: Salticidae).
Animal Behaviour **69**: 931-938.

Elias, DO., Andrade, MC., and Kasumovic, MM. 2011. Dynamic population structure
and the evolution of spider mating systems. *Advances in Insect Physiology*, **41**: 65.

Herberstein, M., Schneider, JM. and Elgar, MA. 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.

Hoefler, CD. 2007. Male mate choice and size-assortative pairing in a jumping spider,
Phidippus clarus. *Animal Behaviour* **73**: 943–954.

Huber, BA. 2005. Sexual selection research on spiders: progress and biases. *Biological
Reviews* **80**: 363-385.

Hurst, GD., Sharpe, RG., Broomfield, AH., Walker, LE., Majerus, TM., Zakharov, IA. and Majerus, ME. 1995. Sexually transmitted disease in a promiscuous insect, *Adalia bipunctata*. *Ecological Entomology* **20**: 230-236.

Jackson, RR. 1977. Courtship versatility in the jumping spider, *Phidippus johnsoni* (Araneae: Salticidae). *Animal Behaviour* **25**: 953-957.

Jackson, RR. 1980. The mating strategy of *Phidippus johnsoni* (Araneae, Salticidae): II. Sperm competition and the function of copulation. *Journal of Arachnology* **8**: 217-240.

Jackson, RR. 1981. Relationship between reproductive security and intersexual selection in a jumping spider *Phidippus johnsoni* (Araneae: Salticidae). *Evolution* **35**: 601-604.

Jackson, RR. 1982. The biology of ant-like jumping spiders: intraspecific interactions of *Myrmarachne lupata* (Araneae, Salticidae). *Zoological Journal of the Linnean Society* **76:** 293-319.

Jackson, RR. 1986. Cohabitation of males and juvenile females: a prevalent mating tactic of spiders. *Journal of Natural History* **20**: 1193-1210.

Jennions, MD., and Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biological Reviews of the Cambridge Philosophical Society* **75**: 21-64.

Jiao, X., Guo, L., Che, Z., Wu, J., Che, J., Liu, F. and Li, D. 2011. Experimental evidence for female-driven monandry in the wolf spider, *Pardosa astrigera. Behavioral Ecology and Sociobiology* **65**: 2117-2123.

Kiszewski, AE. and Spielman, A. 2002. Preprandial inhibition of re-mating in *Ixodes* ticks (Acari: Ixodidae). *Journal of Medical Entomology* **39**: 847-53.

Land, MF. 1969. Structure of the retinae of the principal eyes of jumping spiders (Salticidae: Dendryphantinae) in relation to visual optics. *Journal of Experimental Biology* **51:** 443-470.

Lehtonen, J., Jennions, MD. and Kokko, H. 2012. The many costs of sex. *Trends in ecology & evolution*, **27**: 172-178.

Maklakov, AA. Bilde, T. and Lubin, Y. 2003. Vibratory courtship in a web-building spider: Signalling quality or stimulating the female? *Animal Behaviour* **66**: 623-630.

Masta, SE. and Maddison, WP. 2002. Sexual selection driving diversification in jumping spiders. *Proceedings of the National Academy of Sciences* **99**: 4442–4447.

Masumoto, T. 1991. Males' visits to females' webs and female mating receptivity in the spider *Agelena limbata* (Araneae Agelenidae). *Journal of Ethology* **9**: 1-7.

Masumoto, T. 1993. The effect of the copulatory plug in the funnel-web spider, *Agelena limbata* (Araneae: Agelenidae). *Journal of Arachnology* **21**: 55-59.

Molina, Y. and Christenson, T. 2008. Effects of mating experience on subsequent reproductive behaviour and sperm release in the spider *Nesticodes rufipes*. *Animal Behaviour* **76**: 1327–1334.

Morbey, YE. and Ydenberg, RC. 2001. Protandrous arrival timing to breeding areas: a review. *Ecology Letters* **4**: 663-673.

Norton, S. and Uetz, GW. 2005. Mating frequency in *Schizocosa ocreata* (Hentz) wolf

    spiders: Evidence for a mating system with female monandry and male polygyny.

    Journal of Arachnology **33**: 16-24.

Parker, GA. 1970. Sperm competition and its evolutionary consequences in the insects.

    Biological Reviews **45**: 525-567.

Parker, GA. 1984. Sperm competition and the evolution of animal mating strategies. In:

    Sperm Competition and the Evolution of Animal Mating Systems (Ed. RL. Smith),

    pp. 1-60. Academic Press, London.

Parker, GA. 1998. Sperm competition and the evolution of ejaculates: towards a theory

    base. In: *Sperm Competition and Sexual Selection*. (Eds.TR. Birkhead and AP.

    Møller), pp. 3-54. Academic Press, London.

Perampaladas, K., Stoltz JA. and Andrade, MCB. 2008. Mated redback spider females

    re-advertise receptivity months after mating. *Ethology* **114**: 589-598.

Prenter, J., Elwood, RW. and Montgomery, WI. 1994. Assessment and decisions in

    Metellina segmentata (Araneae:Metidae): evidence of a pheromone involved in

    mate-guarding. *Behavioural Ecology and Sociobiology* **35**: 39-43.

Richardson, BJ. and Gunter, NL. 2012. Revision of Australian jumping spider genus

    Servaea Simon 1887 (Aranaea: Salticidae) including use of DNA sequence data

    and predicted distributions. *Zootaxa* **3350:** 1-33.

Richman, DB. 1982. Epigamic display in jumping spiders (Araneae, Salticidae) and its

    use in systematics. *Journal of Arachnology* **10**: 47-67.

Ringo, J. 1996. Sexual receptivity in insects. *Annual Review of Entomology* **41**: 473-494.

Schäfer, MA. and Uhl, G. 2002. Determinants of paternity success in the spider *Pholcus phalangioides* (Pholcidae: Araneae): The role of male and female mating behaviour. *Behavioral Ecology and Sociobiology* **51**: 368-377.

Schäfer, MA. and Uhl, G. 2005. Sequential mate encounters: Female but not male body size influences female remating behaviour. *Behavioral Ecology* **16**: 461-466.

Simmons, LW. 2001. *Sperm competition and its evolutionary consequences in the insects.* Princeton University Press, Princeton.

Singer, F. and Riechert, SE. 1995. Mating system and mating success of the desert spider *Agelenopsis aperta*. *Behavioral Ecology and Sociobiology* **36**: 313-322.

Snow, LS. and Andrade, MCB. 2004. Pattern of sperm transfer in redback spiders: implications for sperm competition and male sacrifice. *Behavioral Ecology* **15**: 785-792.

Su, KF. and Li, D. 2006. Female-biased predation risk and its differential effect on the male and female courtship behaviour of jumping spiders. *Animal Behaviour* **71:** 531-537.

Taylor, PW. and Jackson, RR. 1999. Habitat-adapted communication in *Trite planiceps*, a New Zealand jumping spider (Araneae, Salticidae). *New Zealand Journal of Zoology* **26**: 127-154.

Thornhill, R. and Alcock, J. 1983. *The evolution of insect mating systems.* Harvard University Press.

Uetz, GW. and Norton, S. 2007. Preference for male traits in female wolf spiders varies with the choice of available males, female age and reproductive state. *Behavioral Ecology and Sociobiology* **61**: 631-641.

van Helsdingen, PJ. 1965. Sexual behaviour of *Lepthyphantes leprosus* (Ohlert) (Araneida, Linyphiidae), with notes on the function of the genital organs. *Zoologische Mededelingen* **41**: 15-42.

Watson, PJ. 1986. Transmission of a female sex pheromone thwarted by males in the spider *Lyniphia litigiosa* (Linyphiidae). *Science* **233**: 219-221.

Wiklund, C. and Fagerström, T. 1977. Why do males emerge before females? A hypothesis to explain the incidence of protandry in butterflies. *Oecologia* **31**: 153-158.

Williams, DS. and McIntyre, P. 1980. The principal eyes of a jumping spider have a telephoto component. *Nature* **288**: 578-580.

Wu, L., Zhang, H., He, T., Liu, Z. and 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.

Zurek, DB. and Nelson, XJ. 2012. Hyperacute motion detection by the lateral eyes of jumping spiders. *Vision Research* **66**: 26-30.

Zurek, DB., Taylor, AJ., Evans, CS. and Nelson, XJ. 2010. The role of the anterior lateral eyes in the vision-based behaviour of jumping spiders. *Journal of Experimental Biology* **213**: 2372-2378.

Chapter Two: Natural history and display behaviour of *Servaea incana*, a common and widespread Australian jumping spider (Araneae, Salticidae)

ABSTRACT

The natural history and intraspecific interactions of *Servaea incana*, a common jumping spider of temperate Australia, are described. Particular attention is paid to observations of displays and behaviours in nature and in the laboratory, including interactions between male and female adults, subadults and juveniles both at and away from retreats and nests. *Servaea incana* is most often found on the trunks of eucalypt trees, where they build silken retreats and nests under loose bark. A 'retreat' is a thin silken structure that might be constructed as a shelter by any stage and sex of *S. incana* and a 'nest' is denser silken structure only constructed by adult females. In most respects, the behaviour of *S. incana* is typical for jumping spiders. Like other jumping spiders, *S. incana* males use elaborate visual displays (Type I courtship) when they encounter females in the open. Male jumping spiders usually rely on silk borne vibrations to communicate with females residing within retreats and nests (Type II courtship). *Servaea incana* instead relies largely on visual displays in this context, likely because the retreats tend to be in well-lit locations and have thin silken walls through which the resident can be easily seen. Adult males that encounter subadult females at retreats often build their own retreat nearby and cohabit until the subadult females moult to maturity, copulating shortly afterwards. Ants make up a large portion of the diet, but *Servaea*

incana prey upon a variety of small arthropods and exhibit versatility in predatory behaviour. Identified enemies of *S. incana* include spiders, a pompilid wasp and a mantispid.

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INTRODUCTION

Jumping spiders (Araneae, Salticidae) are a widespread and speciose family of spiders that is distinguished from other spiders by their acute vision and vision-mediated diurnal behaviour. Salticids characteristically rely on vision-mediated behaviour in contexts where spiders from other families rely on other senses (Land 1985). Vision is used extensively in navigation, allowing salticids to plan long and circuitous routes that may include sophisticated detouring behaviour (Jackson, Pollard *et al.* 2002; Tarsitano and Jackson 1994; Tarsitano and Jackson 1997). Rather than building webs, salticids are cursorial hunters that rely on vision to detect, assess, and target their prey (Harland and Jackson 2002; Jackson, Nelson *et al.* 2005; Li, Jackson *et al.* 2003; Nelson and Jackson 2011). Some tend toward sit and wait predation, lunging at or pursuing prey that comes close (Jackson 1985b; Jackson 1990). Others are more active hunters, foraging over long distances and actively stalking their prey (Forster 1977; Jackson 1985a; Jackson 1988). Some salticids exhibit versatile hunting behaviour, using different strategies for different types of prey (Jackson and Pollard 1996; Nelson *et al.* 2005). Salticids are perhaps best known, however, for their elaborate use of complex visual displays to mediate intraspecific interactions (Clark 1994; Cross, Jackson *et al.* 2008; Nelson and Jackson 2007). Intraspecific interactions, especially courtship, typically entail complex communication at a distance through sequences of postures involving legs, pedipalps, and body positions, and 'dances' whereby the spiders make a series of stepping movements while posturing or waving their appendages. In addition to their more readily discernable visual displays, some salticids also incorporate seismic elements as multi-modal signalling into their otherwise visual display routines (Edwards 1981; Elias, Maddison *et al.* 2012; Girard, Kasumovic *et al.* 2011; Gwynne and Dadour

1985; Maddison and Stratton 1988; Sivalinghem, Kasumovic *et al.* 2010; Taylor and Jackson 1999).

Vision-mediated display behaviour is effective when salticids are in the light away from their nests, but is not usually a suitable means of communication for males that encounter females in nests, where visual signals are usually precluded by the dense silken nest or dark location. Courtship versatility that accommodates the diverse sensory environment of interactions appears to be typical of salticid spiders (Jackson 1977, Jackson 1992, Taylor and Jackson 1999); males primarily use visual displays when females are away from nests but instead use seismic and tactile signals when females are inside their nests. When males encounter sub-adult females in retreats they commonly use seismic and tactile signals, but soon cease display and instead cohabit until the subadult female matures (Jackson 1992).

Comparative studies of diverse species are key to understanding both the evolution of salticid behaviours and the evolution of this group of spiders as a whole (Jackson and Blest 1982). The present study is the first to describe natural history and display behaviour of a spider from the genus *Servaea*. *Servaea incana* is a common Australian salticid inhabiting eastern and southern eucalypt forests of mainland Australia, from Queensland to Western Australia, as well as Tasmania, and is the largest and most widespread species of its genus (Richardson and Gunter 2012). The genus *Servaea*, members of which are primarily from temperate regions and inhabit the trunks of eucalypt trees, was revised recently (Richardson and Gunter 2012). Previously known as *Plexippus validus* and *Servaea vestita*, *S. incana* has been the subject of some

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important studies of arthropod vision (Blest 1983; Blest, Hardie *et al.* 1981; Blest and Maples 1979; Blest, McIntyre *et al.* 1988; Zurek and Nelson 2012; Zurek, Taylor *et al.* 2010). Despite being important as a model in these studies, detailed descriptions of this species' natural history and behaviour have not been published previously. Studies of the natural history and behaviour of Australian jumping spiders have largely focused on tropical species.

According to the most recent phylogeny (Zhang and Maddison 2013), the genera most closely related to *Servaea*, for which studies of behaviour are available include; *Lycidas* (Gwynne and Dadour 1985), *Hypoblemum* (Jackson and Willey 1995), *Saitis* (Hill 2009), *Maratus* (Girard, Kasumovic *et al.* 2011; Hill and Otto 2011) and *Thiania* (Chan, Lau *et al.* 2008; Jackson 1986b; Li, Yik *et al.* 2002). These genera all form a sister clade to *Servaea*. Within this clade, the iridescent *Thiania* are part of a separate branch to the other genera mentioned above. The genus *Lycidas* currently appears to be paraphyletic and *Lycidas michaelseni,* the only '*Lycidas*' species for which some elements of behaviour have been described (Gwynne and Dadour 1985), was not included in the phylogeny. The appearance and behaviour of *L. michaelseni* does not match the other species in the clade for which behaviour has been described. Males of the remaining genera (*Maratus*, *Saitis* and *Hypoblemum*) have ornamented third legs, which are used extensively in their visual display repertoires. *Maratus*, the peacock spiders, are known for particularly striking displays, making use of unusual morphological features as well as vibratory signals (Girard, Kasumovic *et al.* 2011; Hill and Otto 2011). *Thiania* are iridescent and build unusual rivet-like nests (Li, Yik *et al.* 2002). Compared with many related genera, *Servaea* has a comparatively plain

appearance. Based on appearance and position in the phylogeny, *Servaea* may represent a more primitive state from which these other more colourful groups are derived.

Here we describe the display repertoire used by *S. incana* in intraspecific interactions, including interactions between males and female adults, subadults and juveniles. Sequences of behaviour are described for interactions between each sex and age class of spiders. We also present field observations of prey, predators and predatory behaviour.

METHODS

Collection & Maintenance

Servaea incana were observed on, and collected from, the trunks of eucalypt trees in parks in Sydney, NSW, Australia, between September 2010 and September 2013. Field observations were made during collecting trips and also during regular surveys carried out as part of another study (Chapter 3). Prey items were collected from spiders found in the field. Spiders always dropped the prey item during or after being collected. Prey items were taken to the laboratory and identified under a miscroscope. Spiders were maintained in a laboratory under controlled temperature ($25 \pm 0.5°C$) and humidity ($65 \pm 5\%$) on an 11:1:11:1 h light:dusk:dark:dawn cycle. During the light phase the laboratory was illuminated with a 50:50 mix of metal halide and halogen lights. At the commencement of the dusk phase, the metal halide lights turned off and

the halogen lights lowered in intensity until switching off after 1h. At the commencement of the dawn phase, the halogen lights switched on at 5% output and then increased in intensity until at 100% output after 1h, at which time the metal halide lights turned on.

Spiders were housed individually in 1.125 L ventilated plastic cages that contained half a sheet of crumpled white A4 paper as environmental enrichment. Environmental enrichment has been found to improve the performance of jumping spiders in behavioural research (Carducci and Jakob 2000). Spiders were maintained on a two-week feeding schedule, receiving two Queensland fruit flies (*Bactrocera tryoni*) and two houseflies (*Musca domestica*) on alternate weeks. Small juveniles were fed a mixed diet of laboratory-reared *Drosophila melanogaster* and various small wild insects caught with a sweep net. Water was provided from a soaked cotton wick that was replaced regularly, or by spraying water through the cage at least three times each week.

Procedures for observing interactions

We use the established conventions that the terms 'usually' or 'generally', 'sometimes' or occasionally', and 'infrequently' or 'rarely' refer to frequencies of more than 80%, 20-80% and less than 20%, respectively (Jackson and Hallas 1986). 'Juvenile' includes all active stages prior to the penultimate instar. Spiders in their penultimate instar are referred to as 'subadults', and at this stage can be readily identified as male or female by enlarged terminal segments of pedipalps and developing

epigyne, respectively. Other than when qualified as 'subadult', the terms 'male' and 'female' refer only to adults.

Interactions were staged between randomly selected pairs of spiders in the laboratory, with the restriction that individual spiders were never used more than three times in any particular interaction type (e.g., male-female), never more than once with the same individual and never more than once in the same day. Interactions were never staged within two hours of the end or beginning of the laboratory light phase. Spiders were separated after one of the spiders decamped. Interactions were recorded with Panasonic HDC-HS700 and HS-900 High Definition digital video cameras (resolution: 1920x1080, 25 fps, codec: H264-MPEG-4-AVC) with supplementary close-up lenses. High-speed photographs were taken with the Panasonic cameras (50 s^{-1}) and high-speed photos and video (240 fps) were recorded with a Casio EX-FH25 camera. Opportunistic observations were made in the field and these were photographed and video-recorded using a Panasonic DMC-FT1 camera.

Interactions in the open

Interactions in the open were staged in an open roofed acrylic arena with 300 x 150 mm floor and 80 mm high, 5 mm thick walls (following Taylor *et al.* 2001). The arena consisted of two sections; a transparent base and opaque upper walls. The base consisted of the floor and 15 mm high transparent walls, enabling filming of interactions at ground level. The upper walls were made of white acrylic, 65 mm high and sat on top of the base. The upper walls were lightly coated with petroleum jelly to prevent spiders

climbing out. A sheet of white paper covered the arena floor, and this was replaced for every interaction. Between interactions, the arena floor was wiped down with water to remove any silk and chemical cues that might have been left by previous pairs (Jackson 1987). For each interaction one spider was placed in each side of the arena, separated by an opaque divider. Spiders were given two minutes to settle down before the divider was removed. Interactions commenced when the spiders had oriented toward each other and at least one displayed. All spiders oriented toward each other. Spiders were allowed to interact until one spider walked or ran away from the other. Individuals of the same age class and sex were distinguished by size and natural markings. Some additional male-female interactions away from nests were staged in a cylindrical transparent acrylic arena (150 mm diameter, 120 mm height), the upper portion of which was lightly coated with petroleum jelly and surrounded with paper, leaving a 15mm window to film through at the bottom. The cylindrical transparent acrylic arena allowed more detailed observations of the spiders' movements during copulation.

Interactions at retreats and nests

The terms 'retreat' and 'nest' have been used inconsistently in the literature, and we here define 'retreat' as a thin silken structure that might be constructed as a shelter by any stage and sex of *S. incana* and define 'nest' as a denser silken structure only constructed by adult females (see Results for further details of retreat and nest structure).

Interactions at retreats and nests were staged in 150 mm diameter Petri dishes. Subadult and adult females were housed in these Petri dishes with a 50 x 40 mm piece of

brown paper folded into a tent like shape, with an entrance at each end, and fixed to the base of the Petri dish with Blu-tack adhesive putty (Bostik, Australia). Interactions were staged in open Petri dishes after resident spiders had built retreats or nests under the pieces of paper. To start a trial, conspecifics were introduced to the Petri dish while the resident was in the retreat or nest. Interactions began when the spiders oriented towards each other or when the intruder interacted with the retreat or nest. Trials ended when one spider left the Petri dish. Trials were terminated after 1 hour if both spiders remained in the Petri dish but were not interacting, except in interactions between males and subadult females. To observe cohabitation between males and subadult females these trials were terminated when one spider left the Petri dish or if males were not under the paper with the female by the end of the light phase on the first day.

Seismic communication during visual displays

We used laser vibrometry to determine whether *S. incana* include seismic elements in their 'visual' display routines. Vibrations were recorded using a digital laser vibrometer (Polytec PDV100, Germany) and encoded using a Digital Rapids DC 1500 board using Stream 1.5.23 (Digital Rapids, Canada) on a Windows computer (Dual 3.0 GHz Xeon, 4 GB RAM). Video was recorded with a 540TVL GoVideo camera (Digital Products International Inc., USA). The AES output of the laser vibrometer was converted to EBU (Midiman CO3, M-Audio, USA) and synchronised to the audio track of the video. Vibrations were recorded at 44.1 KHz/16 bits. Nylon fabric, a material that has been used before to record seismic signal production in spiders (Elias et al. 2006), was stretched over the base and window section of the rectangular acrylic arena that was

used to observe interactions in the open. Three rows of 16 equally spaced spots of reflective paint were applied onto the fabric as target points for the vibrometer. Pairs of males or males and females were placed on the nylon in the arena and allowed to interact. An interaction started when the spiders had oriented toward each other and at least one displayed and finished when one of the spiders walked or ran away from the other.

Morphological measures

Spiders were restrained against the lid of a Petri dish with cling film and then photographed with a ProgResC10 digital camera (Jenoptik LOS GmbH, Germany) focussed through an Olympus SZX12 stereo-microscope (Olympus Corporation, Tokyo, Japan). Measurements of cephalothorax width and length, to the nearest 0.1 mm, were taken from digital images using ImageJ 1.36b (National Institute of Health, Bethesda, MD, USA).

OBSERVATIONS

Morphology and appearance

Servaea incana is a medium sized salticid, adult female body length ranges between approximately 6.9 and 10 mm while adult male body length ranges between 5.4

and 10.3 mm. Compared with more gracile salticids, *S. incana* has a 'box shaped'
cephalothorax and a quite round abdomen. The abdomen is of similar size in males and
females, but can become much larger when females are gravid. Sexual size dimorphism
is modest in *S. incana*; in terms of size, the sexes have similar mean cephalothorax
width, but males are more variable (male: $N = 286$, range: $1.97 – 3.84$ mm, Mean $\pm$ SD
$= 2.80 \pm 0.37$; female: $N = 286$, range: $2.33 – 3.25$ mm Mean $\pm$ SD $= 2.81 \pm 0.16$; fig.
1). Males tend to be darker, usually grey in colouration. Chelicerae and Legs I and II are
larger and thicker in males than in females, and almost black in colouration (figs. 2, 3).
Juveniles are similar in appearance to adult females, being pale to dark brown in colour
(Richardson and Gunter 2012). Spiders tended to be redder for the first few days after
moulting. Variation in colouration, patterns and markings on the dorsal surface of the
body were substantial enough that similar sized pairs of spiders could be easily be
distinguished by eye.
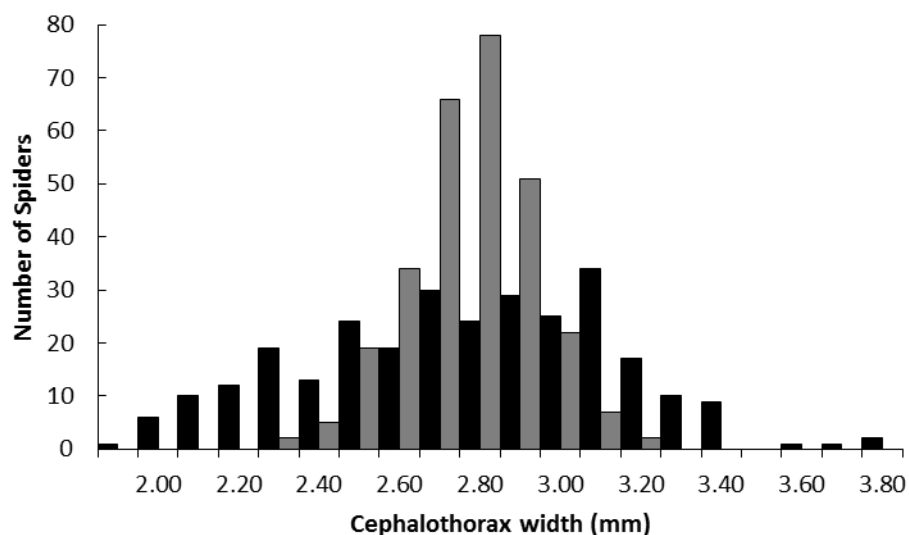
Figure 1: Size distribution of 286 adult male (black) and 286 adult female (grey) *S. incana*.

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Figure 2: Adult female *S. incana* standing with cephalothorax tilted upwards, the right palp in the neutral position and the left palp slightly lowered.

Figure 3: Adult male *S. incana* standing with retracted palps.

Locomotion

Routine locomotion typically was by intermittent bouts of walking, usually of distances less than 200 mm. Pauses usually lasted between 1 and 30 seconds, during which spiders would often pivot about and tilt their cephalothorax, apparently surveying their surroundings. Palps were usually held still in the neutral position while walking and during pauses. Spiders rarely jumped, usually only doing so to move from one surface to another. When startled in the field, *S. incana* typically ran across the trunk of the tree and then hid under loose bark. Startled spiders occasionally would leap off the tree and either land on the trunk further down or fall all the way to the ground. Once on the ground, *S. incana* would remain still for several minutes before moving towards the tree and climbing up the trunk again.

Location and structure of retreats and nests

Servaea incana were observed on the trunks of eucalypt trees including *Eucalyptus amplifolia, E. haemastoma, E. racemosa, E. saligna* and *Angophora costata*. They were most commonly found standing or walking on areas of smooth bark that were adjacent to areas of loose bark where they built their retreats and nests. In the field, retreats and nests were usually built between two pieces of loose bark or between loose bark and the trunk (fig. 4). Occasionally, retreats and nests were only attached to a single surface of bark. Retreats comprised a loosely spun tube of silk with an entrance at each end and were approximately 10 – 15 mm in length and width, for adults, just large enough for one spider to sit inside, although they were flexible and so could

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accommodate copulating pairs. Spiders were visible through the silk walls of the retreat (fig. 5). Subadult females moult to maturity inside their retreat. Adult males were observed building retreats next to the retreat of females at dusk or when the female was about to moult to maturity. When females were preparing to oviposit, they spun denser, larger opaque nests, so that the eggs and spider could not usually be seen from outside (fig. 6). Eggs were deposited under a layer of silk at the bottom of the nest and some females used the same nest to deposit up to three clutches of eggs, laying between 7 and 36 eggs per clutch. Nests had larger chambers than retreats, allowing the adult female to walk around inside. Nests were often roughly circular in shape, but could be more oval, usually with a length between 20 and 35 mm and width of approximately 20 mm. Males go inside the retreats and nests of females and if a female is willing to mate, copulation occurs inside the retreat or nest.

Figure 4: An adult female in a retreat in nature. The female's body is raised, her palps are downwards and her legs are semi-erect in position 3. A courting male is behind the curvature of the bark. Photograph taken from above, tree trunk is at the top of the photograph, the base of the female's retreat is on the bark.

Figure 5: Adult male above the retreat of a subadult female in nature. The female is visible inside the retreat.

Figure 6: Nest of an adult female *S. incana* in nature, between the bark and trunk of a eucalyptus tree.

Elements of behaviour in intraspecific interactions

We identified 41 body postures and movements that were associated with intraspecific interactions.

1. Raised and lowered cephalothorax – Spiders normally held their cephalothorax $1-2$ mm above, and parallel to, the substrate (figs. 3, 5, 7). A raised cephalothorax was held $2-4$ mm above the substrate, sometimes with legs fully extended (figs. 4, 7, 8, 9, 10, 11). When lowered, the sternum was positioned less than 1 mm above the substrate (fig. 7).

2. Lean, tilt & pivot – To lean, a spider flexed its legs, without moving its tarsi, so that the cephalothorax was moved anteriorly ('lean forward'), posteriorly ('lean backward') or to one side ('lean sideways'). To tilt, a spider raised the anterior end of its cephalothorax so that it angled up as much as 45° from the posterior end ('tilt upward'; figs. 2, 9) or rotated the cephalothorax to one side by as much as 30°. Spiders pivoted by lifting and repositioning their legs on the substrate to turn their body on the spot.

3. Neutral, lowered and bent abdomen – Spiders usually held their abdomen in the neutral position, in line with their cephalothorax, although gravid females sometimes let their distended abdomen rest on the substrate. When lowered, abdomens were flexed down $45-90°$ with the cephalothorax in the raised position, sometimes so that the spinnerets made contact with the substrate (figs. 7, 8). Lowered abdomens were

sometimes bent by positioning the distal end as much as 45°to the left or right of the sagittal plane.

Figure 7: Mounting sequence of *S. incana*. Receptive female with lowered body is on the left. Starting with body in the neutral position and Legs I in semi-erect position 3, the male approaches the female, extending Legs I forward into position 1 while raising the body.

4. Opened chelicerae – The chelicerae were held open with the basal segments spread apart and often with fangs extended between 0 - 90° (fig. 9).

5. Palp postures –

Normal palps – At rest, palps were held with the femur directed upward c. 60° to the front and the rest of the palp angled down in front of the chelicerae, slightly converging towards each other (figs. 2,8).

Retracted palps – Retracted palps were held with the femur directed upward and the rest of the palp flexed straight down so that the tarsi were positioned to the side of the chelicerae (fig. 3).

Arched palps – Arched palps were held with the femur c. 45° forward and c. 60° below horizontal. Segments distal to the patella were extended downwards with the tarsi slightly bent towards each other (fig. 11).

Lateral erect palps – All joints were fully extended, so that the palps were held approximately perpendicular to the sagittal plane and parallel to the substrate (fig. 12).

Forward erect palps – Palps were held with all joints extended forward, parallel to each other and the substrate, with the tarsi angled down slightly (fig. 9).

Downward erect palps – Palps were held at full extension angled down towards the substrate (figs. 4, 13, 14).

Downward lateral palps – With palps downwards, the tarsi were extended away from the sagittal plane of the body (figs. 10, 15).

6. Waving palps – Starting in the normal, arched or downward lateral palp positions, spiders made sweeping movements with their palps, moving them down then sweeping horizontally away from the centre of the body and then up and around to the starting position in a triangular pattern. These movements were made in an alternating phase pattern usually in bouts of 2 – 4 cycles in c. 0.5 s. Pauses between bouts of waving were highly variable.

Figure 8: Subadult female *S. incana* with raised body, hunched legs and abdomen lowered.

7. Palpate – To palpate, spiders usually move the palps similar to the waving palp pattern, but tarsi touched and moved along the substrate. If silk was present, spiders sometimes plucked at it by slowly raising tarsi away from the substrate, lifting the silk with it. Palps often moved independently of each other when plucking at silk. From the

normal palp position, palps were extended and lowered so that the tips of the tarsi touched the substrate.

8. Quiver palps – While embracing with locked chelicerae (see below), lateral erect palps rapidly moved (4 – 8 s^{-1}), primarily vertically, but also forwards and back, so that their tarsi touched (fig.12).

9. Erect legs – All joints distal to the femur were fully extended. Erect legs were held in 3 different positions.

Position 1 – Legs I held forwards parallel to each other and to the substrate (fig. 7)

Position 2 – Legs I held c. 45° to the side with the tarsi pointing down and either touching or slightly above the substrate.

Position 3 – Legs I held 20 - 60° to the side and 20-90° upward.

10. Semi-erect legs – Legs I were held with c. 135° flexion at the femur-patella joint and the distal joints fully extended, or slightly bent. Erect Legs positions 2 (fig. 15) and 3 (figs. 4, 7, 10, 13, 14, 16) were also observed with semi-erect legs.

11. Hunched legs – Hunched legs I were held 60 - 90° away from sagittal plane of the body with the femur angled upwards c. 45 - 90°, the femur-patella joint flexed at c. 90°, and the distal joints flexed slightly (figs. 8, 11, 16). Hunching spiders always held their cephalothoraxes in the raised position and usually with the abdomen lowered and bent.

Legs II and III were usually positioned perpendicular to the sagittal plane with similar flexion of joints as Legs I, although tarsi remained on the substrate. Legs IV angled rearward about 45°.

12. Posturing – Spiders postured by standing or stepping with erect, semi-erect or hunched legs.

Figure 9: The adult female *Servaea incana* on the right is fending off an adult male that is attempting to mount. The male's body is raised and palps and Legs I are extended forwards. The female's cephalothorax is tilted upwards with chelicerae open and fangs extended. The female has raised her first two pairs of legs to prevent the male from mounting.

13. Gesture – While displaying with either erect or semi-erect Legs I, spiders gestured by slightly raising and then lowering their legs by flexion and extension at the trochanter or femur-patella joint. This was often done while standing, but also while stepping. Legs usually moved up and down by c. 10°, and moved up faster than they came back down, the whole cycle taking c. 0.1 s. Pauses between gestures were highly variable.

Figure 10: Adult male *S. incana* posturing with raised body, downwards lateral palps and legs in semi-erect position 3.

14. Wag – Legs moved in matching phase and were extended from a hunched to semi-erect position. Wagging was usually performed while stepping forward or to the side. Legs moved smoothly throughout the motion and returned to the original hunched position once stepping stopped.

15. Quiver legs – Erect or semi-erect Legs I, were moved up and down rapidly (c. 50 s^{-1}) by movements of the trochanter in bouts of c. 0.5 s with c. 1 s between bouts. Palps often waved at the end of bouts, but were held relatively still in the downward lateral position during bouts.

Figure 11: An adult male posturing with hunched legs and arched palps.

16. Quiver body – With Legs I erect or semi-erect in position 3, spiders raised and lowered their body in approximately 0.05 s, followed by a pause of c. 0.05 s. Spiders performed up to c. 20 cycles per bout. Spiders usually pivoted away from the other spider at the end of a bout, keeping Legs IV in place. Spiders sometimes stepped as they quivered their body, giving them a jerky gait as they walked. Legs II were sometimes lifted and quivered briefly with Legs I.

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17. Stalk – Stalking spiders lowered their body and slowly approached conspecifics with legs held close to the body, similar to how they would stalk prey. Spiders usually only stalked while the other spider was turned away from them and would remain still if the other spider oriented.

18. Veer and sidestep – Rather than approach another spider directly, a spider veered by stepping to one side as it approached, while still facing the other spider. To sidestep, spiders stepped to one side without approaching.

19. Zigzag dance – While facing the female, males stepped to one side in an arc, quivering Legs 1 as they walked. Males would usually pause briefly before stepping in the other direction, but sometimes they would only slow down before performing another arc. Arcs became narrower as males approached females.

20. Long leap – Usually starting from a crouched position, a spider made a long leap by jumping 30 – 50 mm, usually landing very close to the other spider and sometimes making contact with them.

21. Truncated leap – The spider made a truncated leap by suddenly jumping 10 – 20 mm towards the other spider but without making contact. Spiders sometimes performed up to three truncated leaps in succession, or a long leap followed by truncated leaps.

22. *Charge* – Charging spiders ran rapidly 10-100 mm towards the other spider, sometimes with hunched legs or semi-erect legs in position 3, before stopping abruptly in front of the other spider without making contact. Truncated leaps were sometimes interspersed with charging.

23. *Ram* – Ramming was like charging, except that the spider continued so that they ran into the other spider, rather than stopping. If the other spider did not decamp, the spiders would end up with their faces in contact and often the ramming spider would push the other spider backwards.

24. *Lunge* – Spiders lunged at each other when within 20 mm. Lunging spiders moved rapidly forward c. 5 mm, without the tarsi of legs IV leaving the substrate. Legs I started either hunched or in a semi-erect position 3 and were extended and raised rapidly at all joints distal to the femur, as spiders moved forward, and then lowered to their original position as spiders moved back. Chelicerae were often open and extended forwards during lunges and spiders usually made contact with each other's legs or face.

25. *Propulsive displays* – These included long leaping, truncated leaping, charging, ramming and lunging.

26. *Embrace* – Two spiders approached each other, with Legs I hunched or in semi-erect position 3, and locked chelicerae usually so that each spider was holding one chelicera of the other in its own (fig. 12). Occasionally, they locked with the ends of the

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chelicerae touching, apparently holding on to each other with their fangs. Legs I were usually held out to the side, parallel to the substrate and 90° from sagittal with palps in the lateral erect position. Legs II, III and usually IV were held close to parallel to each other out to the side of the body. Embracing often occurred after bouts of lunging if neither spider decamped.

Figure 12: A pair of adult male *Servaea incana* embracing.

27. Duel – While embracing, spiders sometimes raised Legs I above parallel to the substrate, up to 90°, but in intervals of no more than 20° at a time. After each movement, spiders held their legs in the new position for 1 – 5 seconds before either raising or lowering them again. Spiders usually matched each other's movements while duelling. Duelling usually ended when spiders grappled (see below).

28. Push and pull – While embracing, spiders pushed by stepping, or by leaning forward and pulled by stepping or leaning backwards. Sometimes spiders pushed their opponent backwards.

Figure 13: An unreceptive female away from her retreat in nature. The female's body is in a neutral position with palps are downwards and Legs I semi-erect in position 3, although one is touching the substrate. Photograph taken from below. An adult male is out of focus in the foreground, oriented towards the female.

29. Grapple – While embracing, one or both spiders extended one or both Legs I, and sometimes Legs II, over the legs of the other spider. Spiders then pushed and pulled

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each other, repositioning their legs on the substrate. Grappling ended when spiders released their grip on each other's chelicerae and one decamped or when spiders clasped (see below).

30. Clasp – Clasping spiders were positioned venter-to-venter with their legs wrapped around each other and with open chelicerae. Both spiders moved their legs and body rapidly. They often rolled around on the substrate, apparently attempting to bite at each other. Clasping ended when one spider broke free and decamped. When they broke apart, spiders often had to right themselves and were no longer oriented towards each other. One spider would then decamp and the other would usually re-orient towards the other spider and pursue.

Figure 14: An adult female *Servaea incana* steps out of the entrance to her retreat in response to a courting male (out of focus, in front of and facing the female). The female's palps are held in the downwards erect position.

31. Decamp – Spiders usually decamped by turning and running away from the other spider. Sometimes spiders would step backwards while still facing the other spider before turning and running.

Figure 15: Adult male *Servaea incana* displaying towards a subadult female (out of frame). Legs I are in semi-erect position 2 and palps are in the downwards lateral position.

32. Watch and follow – Spiders watched conspecifics as they decamped by pivoting on the spot, maintaining orientation towards them. Sometimes spiders would follow conspecifics by running after them as they decamped.

33. Chew and tug – Spiders chewed by palpating silk of retreats or nests and pulling the silk towards their chelicerae with their palps and biting it. Spiders tugged by raising or tilting their cephalothorax away from the silk while holding the silk in their chelicerae. Chewing and tugging resulted in distinct tears in the silk.

34. Probe – While standing at retreats or nests, spiders probed by pushing and pulling silk with the tarsi of Legs I. Spiders also probed with their first two pairs of legs by making forward and lateral movements against the silk, usually in alternating phase.

35. Fend – Females prevented males from mounting by raising Legs I to erect or semi-erect position 3. Fending females often held their palps in the forward erect position and tilted their cephalothorax upward, sometimes with open chelicerae and fangs extended (fig. 9). Fending was generally combined with quivering body and legs.

36. Mount – Males mounted females by raising their body with Legs I erect in position 1 and palps in the forward erect position, and then walking forward until positioned over the female (fig. 7). Males usually started mounting a female from in front, while they were facing each other, but sometimes mounted from the side, reorienting after positioning themselves over the female's body. Once tarsi of the male's Legs I were in line with the distal end of the female's abdomen the male flexed Legs I curling them towards the female's body. Once the male's cephalothorax was over the female's pedicel the male then leaned to the left or right to engage the right or left palp respectively.

*37. **Post-mount tapping with legs*** – Males tapped at the female's abdomen with the tarsi of Legs I in alternating phase, but in no obvious pattern.

*38. **Post-mount tapping with palps*** – Starting with palps in the normal position, tarsi were lowered to palpate the female's body in alternating phase and an irregular pattern. Males usually leaned to one side of the female's body as they tapped.

Figure 16: Two adult male *Servaea incana* during an interaction. The male on the left is in a hunched posture, with lowered abdomen and hunched Legs I. The male on the right has Legs I semi-erect in position 3.

39. Lift leg IV and rotate abdomen – Males leaned to one side of the female and placed both Legs I between the female's Legs III and IV. Males then placed one Leg I against the female's Leg III and reached under the female's Leg IV and abdomen with their other Leg I, pulling the abdomen up. The female's Leg IV lifted as her abdomen rotated (fig. 17).

40. Apply palp – Males inserted their embolus into the female's genitalia by holding their palp against the ventral surface of the female's abdomen, posterior to her genitalia. The cymbium then bent towards the male's body as the hematodocha expanded allowing the embolus to engage with the female's epigynum. As males applied their palps, they were sometimes observed to open their chelicerae and extend the fangs slightly.

41. Copulation – As is typical of salticids, *Servaea incana* copulated with the male and female facing in opposite directions, with the male's ventral surface against the female's dorsal surface (fig. 13). After the first insertion, males would return to the female's midline, then switch to the other side to insert his palp into the other side. When the palp was initially inserted, hematodochae pulsed $4 - 6$ times over $3 - 4$ seconds and then usually once every 2-5 seconds after that. The bodies of both spiders rocked with each pulse and the spines of the male's legs also extended and then relaxed with each pulse.

Figure 17: A pair of *Servaea incana* copulating, the male is facing towards the camera, while the female is facing away. The male has just disengaged his left palp from the female's left genital opening and is holding the female's rotated abdomen with his left Leg I.

Organisation of behaviour

Interactions began when each spider oriented to the other, or when a spider walked onto the retreat or nest of another and began to display. Interactions ended when one spider decamped. A summary of the contexts in which each elements of behaviour was observed appears in table 1. Durations of interactions between adult *S. incana* are provided in table 2.

Table 1. Major elements of intraspecific behaviour of *Servaea incana* and the interactions in which they occurred, at (N) and away from (X) retreats and nests (M: adult male, F: adult female, SF: subadult female, SM: subadult male, J: juvenile. M-F is male behaviour.)

Behaviour	M-F	F-M	M-M	F-F	M-SF	SF-M	F-SF	SF-F	M-SM	SM-M	F-SM	SM-F	M-J	J-M	F-J	J-F	J-J
Charge	X	X	X	X	X	X	X	-	X	-	X	X	X	-	X	-	X
Chew	N	-	-	-	N	-	-	-	-	-	-	-	-	-	-	-	-
Clasp	-	-	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-
Embrace	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gesture	X,N	-	-	-	X,N	-	-	-	X	-	-	-	X	-	-	-	-
Grapple	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hunched legs	X,N	X,N	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Leg postures																	
Position 1	X,N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Position 2	X,N	-	-	-	X,N	-	-	-	X	-	-	-	X	-	-	-	-
Position 3	X,N	X	X	X	X,N	X	-	-	X	X	X	X	X	X	X	-	X
Long leap	-	X	X	X	-	-	-	-	-	-	X	X	-	-	-	-	-
Lunge	X	X,N	X	X	X	N	X	X	X	X	X	X	X	-	X	-	X
Palpate	X,N	-	X	-	X,N	-	-	-	X	-	-	-	X	-	X	-	-
Palp postures																	
Retracted	X	X,N	X	X	X	-	-	-	X	-	-	-	X	-	-	-	-
Arched	X	-	X	-	X	-	-	-	X	-	-	-	X	-	-	-	-
Lateral erect	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Forward erect	X,N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Downward erect	-	X,N	-	X	-	X,N	-	-	-	X	X	-	-	X	-	-	-
Downward lateral	X,N	-	-	-	X	-	-	-	X	-	-	-	X	-	-	-	-
Probe	N	-	-	-	X,N	-	-	-	-	-	-	-	-	-	-	-	-
Push	-	-	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-
Quiver body	-	X,N	-	-	-	X,N	-	-	-	X	-	-	-	X	-	-	-
Quiver legs	X,N	-	-	-	X,N	-	-	-	X	-	-	-	X	-	-	-	-
Ram	-	X	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-
Truncated leap	-	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tug	N	-	-	-	N	-	-	-	-	-	-	-	-	-	-	-	-
Wag	-	-	X	X	-	-	-	-	X	X	X	X	-	-	X	X	X
Zigzag dance	X,N	-	-	-	X	-	-	-	X	-	-	-	X	-	-	-	-

Table 2. Durations of intraspecific interactions between adult *Servaea incana* in the laboratory. Receptive females accepted male courtship and copulation while unreceptive females rejected the courting male persistently.

In the open	Male-unreceptive female	Male-receptive female	Male-male	Female-female
Number of interactions	31	10	36	48
Maximum duration	9 min 8 s	40 min 9 s	1 min 54 s	36 s
Minimum duration	2 s	3 min 42 s	1 s	1 s
Median duration	1 min 26 s	6 min 10 s	8 s	6.5 s
Mean duration	1 min 58 s	11 min 50 s	15.8 s	8.6 s
At retreats				
Number of interactions	13	13		
Maximum duration	56 min 59 s	80 min 32 s		
Minimum duration	1 min 27 s	9 min 5 s		
Median duration	6 min 20 s	14 min 47 s		
Mean duration	14 min 29 s	26 min 44 s		
At nests				
Number of interactions	20			
Maximum duration	20 min 20s			
Minimum duration	14 s			
Median duration	5 min 34 s			
Mean duration	7 min 15 s			

Male-male interactions

We staged a total of 36 male-male interactions. Upon orienting towards another male, males sometimes turned away prior to the other male orienting and continue to wander around the arena. Alternatively, they sometimes maintained orientation until the other male also oriented and an interaction would begin. After orienting towards another male, males usually postured with raised body, hunched legs, lowered abdomen and arched palps. Sometimes males adopted a neutral posture or lowered their body upon orientation and some walked toward the other spider before posturing. Once the two males were oriented towards each other, they usually postured with hunched legs, unless one spider immediately decamped. Sometimes only one spider hunched, while the other watched without displaying. During an interaction, males sometimes plucked at silk left by the other male on the substrate, and after doing so continued to pluck at silk and wave their palps throughout the interaction. However, if there was no silk, palps generally remained in the retracted or arched position.

After at least one spider began posturing, either one or both males usually walked towards the other, maintaining the posture that they were in. Males that lowered their body upon orienting occasionally made truncated leaps towards the other spider, rather than walking, and some males charged or rammed. Sometimes one male decamped when the other spider started to approach.

When within 20 – 30 mm of each other, males usually lifted their Legs I from a hunched position to erect or semi-erect position 3 and lunged, usually making contact

with their legs and sometimes also their faces. Between lunges males returned their legs to either a hunched position or semi-erect position 3. Often after $1-5$ lunges one spider decamped and the other usually pursued. Males often opened their chelicerae during lunges and would sometimes lock chelicerae and embrace. Embracing spiders extended legs I to position 4 and held and quivered their palps in the lateral erect position. Embracing spiders sometimes duelled, and pushed and pulled each other until one broke free, at which point they either clasped or one decamped. Clasping more often occurred directly after lunging, without the spiders embracing. Lethal attacks were never observed.

Female-female interactions

A total of 48 female-female interactions were observed. Interactions between adult females were generally simpler and shorter than those between males (table 2), with fewer displays, and they usually ended without any physical contact. When one female oriented towards the other she usually crouched and watched the other female, but sometimes adopted a hunched posture. Infrequently, the first female to orient stalked or made long or truncated leaps towards the other female. When the other spider oriented, often only one spider postured, but sometimes neither or both spiders postured. Rather than approach, some females hunched and sidestepped while wagging, usually while the other spider watched in a neutral posture. Females occasionally decamped immediately after orienting. Some females pursued decamping conspecifics but usually for no more than 50 mm. Females rarely came into contact with each other during

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interactions, and when they did it was usually through lunges. We never observed

females embracing and only observed clasping once.

Male-female interactions in the open

A total of 41 male-female interactions were staged in the open. Males usually

postured, gestured and waved palps within seconds of orienting towards a female. Males

raised their body and raised Legs I to semi-erect position 3 with downwards lateral

palps. If they were greater than 100 mm away, males often charged towards females,

with a raised body and waving palps, before they started to posture. After initially

posturing, males usually approached the female by walking and gesturing or by zig-zag

dancing. Males zig-zag danced with quivering legs and waving palps, and held legs and

palps still in between bouts of stepping. As males approached females, they tended to

bring Legs I forward from position 3 to position 1 and legs tended to become erect,

rather than semi-erect. Infrequently, males adopted a hunched posture.

If females oriented first, they usually watched the male briefly before turning

away. Infrequently, they hunched or started stalking the male. After males had

commenced displays, females often quivered their body as they walked towards the

male for a few steps and then turned away. Males usually kept their Legs I in position 3

while females performed these displays and sometimes approached or remained

stationary, but if the female was close they sometimes stepped backwards while

maintaining orientation. Palps were usually extended downwards as females quivered

their legs and body. Usually after a few bouts of quivering or fending, females walked

away from the male. Males usually pursued females, often positioning themselves in front of the female, but they also postured and zig-zag danced while the female was oriented away from them. Infrequently, interactions between males and unreceptive females were similar to intrasexual contests, with spiders exhibiting agonistic behaviours rather than courtship. Receptive females never performed propulsive displays.

As males got to within 20 mm of females, they usually extended their Legs I forward to position 1 erect and approached the female, while at the same time raising their body Unreceptive females usually fended off the male and turned away. Receptive females usually remained still and lowered their body to the substrate, allowing the male to mount. Some females allowed males to mount after initially fending them off. Males passed Legs I over the female's body as they mounted, often without touching the female. After males mounted, females occasionally started to step away. If this occurred, males usually moved to remain mounted on the female. If the female moved away from the male, the male usually recommenced courtship displays and immediately attempted to mount again.

Once mounted, males leaned to one side as they tapped the female's abdomen with their palps. Males then lifted Leg IV and rotated the female's abdomen to apply their palp, initiating copulation. Durations and numbers of palp applications are summarised in table 3. During copulation, both male and female bodies moved as the male's haematodocha inflated and deflated. The major hairs and spines on the male's legs extended as his haematodocha pulsed. After each palp application, the male and the

female's abdomen returned to the central position. The male then leaned to the other side to insert his palp into the female's other genital opening or dismounted. Females sometimes stepped after palp applications, causing males to dismount. After dismounting, females sometimes became aggressive, quivering and fending off the male, but occasionally decamped or remained still without display. Males sometimes recommenced courtship and approached to mount again, but usually desisted quickly and decamped.

Male-female interactions at retreats and nests

We staged 26 male-female interactions at retreats. As females inside retreats were visible from outside, both males and females sometimes started signalling visually prior to the male making contact with the retreat. Females usually raised and quivered their bodies while inside these retreats, with semi-erect legs in position 3 and downward palps (fig. 14). As females quivered, the silk of the retreat would also move and sometimes females stood in the entrance to their retreat while quivering. Occasionally, females lunged at males from inside the retreat when males touched the silk. Males postured and gestured in a manner similar to that observed during interactions in the open. Males palpated the silk at the periphery of retreats and sometimes probed at the retreat. Unreceptive females usually prevented males from entering the retreat by standing at the entrance and performing propulsive displays. Infrequently, unreceptive females abandoned the retreat. Receptive females sometimes performed propulsive displays but eventually permitted males to enter the retreat. Males extended their Legs I in position 1 through the retreat entrance and stepped forward to mount. After

copulating, females started stepping and pivoting and males decamped from the retreat. Some males left immediately whereas others stayed until females became aggressive, performing propulsive displays.

A total of 20 male-female interactions were staged at nests. Females at nests usually became aggressive when males stepped onto the nest. Lunging and quivering were the primary behaviours of females in nests. As males moved around the outside of nests, females moved around inside the nest to remain close to the male. The whole silk structure usually shook with the female's propulsive displays. Males palpated, probed, tugged and chewed at nests, but usually left soon afterwards. If females were visible, males sometimes used the same visual displays as were used during courtship interactions away from nests, including posturing, gesturing and zig-zag dancing. Females generally responded with quivering and lunging inside the nest. Females in nests were usually not receptive and males rarely entered nests. In one interaction a male entered a nest and appeared to mount the female but it was not clear whether they copulated. Both spiders remained in the nest for 4 hours, after which the female left and then re-entered the nest before the male decamped.

Table 3. Number of palp applications, duration of palp applications and total duration of copulations in the open, at retreats and with cohabiting pairs soon after females matured.

	In the open	At retreats	Cohabiting pairs
Number of copulations	10	13	4
Number of palp applications			
Maximum	3	6	2
Minimum	1	1	2
Median	2	2	2
Palp application duration			
Maximum	20 min 45 s	39 min 49 s	15 min 41 s
Minimum	4 min 48 s	4 min 23 s	6 min 15 s
Median	8 min 20 s	11 min 5 s	7 min 46 s
Copulation duration			
Maximum	38 min 23 s	54 min 59 s	28 min 50 s
Minimum	7 min 4 s	9 min 28 s	12 min 41 s
Median	19 min 4 s	22 min 10 s	15 min 36 s

Male-subadult female interactions in the open

A total of 28 male-subadult female interactions were staged in the open. After orienting toward a subadult female, males usually raised their body and approached while quivering with erect or semi-erect legs in position 3, often holding legs in position

2 between bouts. They either waved their palps or held their palps in the downwards lateral position. Zig-zag dances were rarely performed. Subadult females usually quivered their legs or body and decamped, or decamped without displaying. After these initial displays, some males pivoted to orient towards subadult females as they walked around but rarely followed, instead posturing while stationary with erect legs in position 2 and downwards lateral palps. Males rarely followed subadult females and continued to gesture. Subadult females walked about the arena, occasionally re-orienting to the male. Males eventually turned away from the subadult female. Infrequently, subadult females hunched and males usually responded with propulsive displays, after which the subadult females decamped.

Male-subadult female interactions at retreats

A total of 22 male-subadult female interactions were staged at retreats. Subadult females moult to maturity inside their retreat. Interactions between males and subadult females at retreats were similar to interactions between males and adult females. Spiders usually oriented before the male made contact with the retreat. Subadult females often oriented first and started displaying. Sometimes males commenced courtship displays as they did during interactions with adult females. Subadult females usually quivered their legs and body while remaining inside the retreat. Infrequently, subadult females left the retreat. Males sometimes pursued subadult females that left their retreats while quivering their legs and performing zigzag dances, but sometimes they instead remained next to or entered the retreat. More often, after displays by both spiders, the male remained next to the retreat for the rest of the day, sometimes posturing again. Males sometimes tugged

and pulled silk at the periphery of the retreat, but did not enter the retreat while the female was inside. Males and females sometimes began spinning silk towards the end of the laboratory light phase. We observed the duration of cohabitation for five pairs of spiders in the laboratory. Each of the subadult females in these pairs had failed to cohabit with a male on the day prior to cohabiting, but on the following opportunity cohabited until they moulted inside the retreat. The duration of these five cohabitations were 3, 4, 4, 7 and 8 days.

On four occasions we observed subadult females moult to maturity in the presence of a male. Two of these observations were with pairs that had been cohabiting, the other two were during interactions at subadult female nests where pairs had not cohabited but males were nearby when females began moulting. Males remained outside retreats while females moulted. After separating from the moult, females initially remained relatively motionless with a lowered abdomen and all legs extended ventrally for approximately 10 minutes. Females then began moving about within the retreat, extending and flexing legs independently of each other in an irregular pattern for approximately one minute before curling all of their legs close to the body. Females remained in this curled position for approximately 30 minutes, with their legs slightly twitching and gradually extending from tightly curled to a loosely flexed position. Throughout this period, males remained outside the retreat, occasionally stepping around the periphery of the retreat but usually maintaining orientation towards the female. Females then pivoted or walked about within the retreat and males then started to probe, chew and tug on silk to get access to the female. Females did not display and only moved slightly as the male entered the retreat and mounted the female. Copulations were similar to the previously described copulations at retreats (table 3). After mating, one

male remained nearby and inducted sperm into his palps (see below), two males left the retreat but remained nearby for the rest of the day and one male walked away immediately, leaving the Petri dish.

Female-subadult female interactions in the open

A total of 20 female-subadult female interactions were staged in the open. Interactions between adult and subadult females in the open were similar to interactions between adult females, although subadult females generally decamped first. Both adult and subadult females sometimes hunched Legs I, but occasionally neither spider displayed during an interaction. Subadult females never charged and rarely approached adult females. Adult females sometimes performed propulsive displays and sometimes stalked subadult females.

Male-subadult male interactions in the open

We staged a total of 34 male-subadult male interactions in the open. Interactions between adult and subadult males were sometimes similar to interactions between males and subadult females, and were sometimes similar to female-female interactions. Some males courted subadult males, raising their semi-erect legs to position 2 or 3 and gesturing or zig-zag dancing. When males performed courtship behaviours, some subadult males quivered their legs and body and then decamped while others hunched their legs before decamping. Adult males usually continued to court as the subadult

males decamped. In some interactions either the male or the subadult male adopted a hunched posture. Usually this was immediately followed by the subadult male decamping, but sometimes one or both spiders approached the other first. Infrequently, spiders stalked each other. Adult males rarely decamped before the subadult males.

Female-subadult male interactions in the open

A total of 30 female-subadult male interactions were staged in the open**.** Interactions between adult females and subadult males were similar to interactions between adult females, as adult females and subadult males exhibited similar behaviours. When subadult males approached females, they usually performed propulsive displays. Females sometimes approached without displaying and then long leaped towards the subadult males. Subadult males tended to hunch more and also decamped more often than females. Spiders sometimes hunched their legs but in some interactions neither spider displayed. Spiders rarely came into contact with each other in these interactions.

Male-juvenile interactions in the open

A total of 23 male-juvenile interactions were staged in the open. Males sometimes hunched upon orienting towards juveniles but sometimes commenced courtship displays. If males hunched, juveniles generally decamped immediately but infrequently also hunched briefly, before decamping. Some juveniles waited until the

male stepped toward them before decamping. If males gestured or quivered Legs I juveniles either hunched or quivered Legs I before decamping. Infrequently, juveniles charged or long leaped towards males before decamping. Males sometimes pursued decamping juveniles.

Female-juvenile interactions in the open

We staged a total of 27 female-juvenile interactions in the open. Most interactions between adult females and juveniles proceeded in one of two ways. After orienting towards each other, some females showed little interest in the juvenile and turned away after several seconds. Juveniles usually continued to watch the female and as the female started to walk, juveniles sometimes hunched their legs and usually approached the female. Females usually reoriented and then the juvenile decamped. In other interactions, females were more aggressive and stalked, charged and long leaped towards juveniles. In these interactions the juveniles usually decamped as soon as the female approached and females usually pursued them.

Juvenile-juvenile interactions in the open

A total of 30 juvenile-juvenile interactions were staged in the open. Interactions between juveniles were similar to interactions between adult females. Interactions often started after a period of one spider watching the other. Occasionally, spiders simultaneously oriented and one or both immediately fled. The spider that oriented first

often maintained the same neutral body position and usually did not approach the other spider. Sometimes juveniles adopted a hunched posture. Juveniles rarely made contact with each other and rarely performed propulsive displays.

Recordings of vibrations

There was no evidence of seismic elements to visual displays other than those generated in the production of those displays. In particular, impact sounds were recorded from the substrate during footsteps. When males gestured towards females the movements of the legs generated weak seismic components, which had much lower amplitude than footsteps. All detected vibrations in male-male interactions appeared to be associated with footsteps.

Sperm Induction

On four occasions males were observed recharging their palps with sperm after copulation. In one instance the male was observed after copulating with a female in a retreat in a Petri dish, two other males were placed in Petri dishes after they mated with recently moulted females and one male inducted his palps while standing adjacent to a recently moulted female. Males first spun a small sheet of silk, shorter than their body length and slightly wider than their body width, between the wall and floor of the Petri dish. Males stood in the same spot while spinning the web, they lowered their abdomen and bent it from side to side, but their legs were not repositioned. Males spent

approximately two minutes spinning the web. Males then lowered and raised their abdomen so that it tapped against the posterior end of the web. As they moved their abdomen they also slightly flexed their legs, so that their cephalothorax moved both forward and downwards less than 1 mm then up and back. These movements were performed 10 – 20 times over approximately 30 seconds. Spiders then paused for 10 - 20 seconds before lowering their body again as they expelled a white drop containing sperm from their genital pore and deposited it onto the web. Males then stepped backwards so that their chelicerae were positioned over the drop of ejaculate and alternated touching the drop with the end of each palp for 1 – 10 seconds at a time. When touching the drop, males moved the palp up and down less than 1 mm at a rate of 10 s^{-1}, causing the drop to move as they did so. These movements were repeated about 20 times per palp, the drop becoming smaller as they did so.

Observations of interactions in nature

Although we often found many spiders inhabiting the same tree, interactions were rarely observed in the field. We only observed one male-male interaction in the field. A large male was walking on the trunk of a tree when he encountered a piece of bark under which there were two retreats of *S. incana*. As the male approached the retreats from below, another, smaller, male exited from the uppermost opening of the higher retreat and approached the intruder. Both males hunched their legs and approached each other. The two spiders lunged at each other and the smaller resident decamped, running around to the other side of the tree trunk. The larger intruder then entered the retreat that had previously been occupied by the smaller male. An adult

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female with a soft cuticle, indicating that she had recently moulted, was found inside the other retreat.

We observed one interaction between an adult male and a subadult female in the field. Both spiders were on the outside surface of a loose piece of bark, oriented towards each other, approximately 20 mm apart with the male above the subadult female. The subadult female turned away and after about 35 seconds the male stepped forward and to his right while quivering Legs I. The subadult female oriented to the male and displayed with quivering legs, the male stepped back and both spiders paused before the subadult female turned away again. After about 10 seconds the subadult female walked away, moved underneath the piece of bark and entered a retreat. Approximately 90 seconds later the male walked off the bark in the opposite direction to the female and onto the tree trunk. Later the male walked back around the bark to where the female had entered a retreat and stopped in front of the retreat while the female displayed from inside. There was at least one empty retreat visible under the bark close to the retreat containing the female.

All other observed interactions in nature were between adult males and adult females. We observed one male visit two females at their retreats and interact with a female in the open twice, all within 18 minutes. This male was first observed peering around the edge of a piece of bark to where a female's retreat was located, with a female inside. The male then left and had an aggressive interaction with an unreceptive adult female in the open. The female quivered her body and legs as the male approached. The male approached rapidly with semi-erect legs I in position 3 but then hunched before

lunging at the female, making contact and immediately returning to the original position. Both spiders then decamped. The female walked under and moved to under an adjacent piece of bark and the male walked away across the tree trunk. The male next encountered an adult female in a retreat under a piece of bark. The male entered the nest and the female backed away while facing the male and quivering her body and legs. The female then left the nest. The male walked through one entrance of the nest and came out the other entrance in front of the female. The female decamped around to the opposite side of the loose piece of bark under which the retreat was built and then returned to the inside but decamped again as the male approached. The female returned once more to the lower surface of the bark and quivered her body and legs in front of the male then decamped, leaving the bark and walking into the open on the tree trunk. The male then left the piece of bark in the opposite direction and encountered the same female that he had previously interacted with in the open. This time the male raised Legs I to position 3 and quivered while sidestepping to position himself above the female on the trunk. The female sidestepped c. 10mm from side to side while quivering her body and legs, and then retreated to under the piece of loose bark that she was standing on. The female came back out and quivered again before moving to the trunk underneath the bark. The male approached and looked under the bark but the female continued to quiver and the male did not move to beneath the bark. The male passed the female on the outer side of the bark and then left to walk across the tree trunk.

We observed another two interactions between males and females in the open and 3 at retreats, all of which proceeded in a similar manner to that described above. Males palpated the tree trunk as they approached retreats. Once oriented towards a female, males displayed with quivering legs, while the females quivered their body and

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legs and decamped if they were outside their nest. In one interaction that occurred close to dusk at the retreat of an adult female, a male began to spin silk, apparently building his own retreat.

We found males cohabiting with both subadult and adult females in the field. On one occasion we observed three retreats together under a single piece of bark. In one retreat was a subadult male, below a subadult female and in the lowest retreat there was an adult male.

Prey and hunting behaviour

In the field, we found 72 individual prey items from 6 different arthropod orders (Table 4). Hymenoptera were the most common prey items; 33 of the 72 prey items were ants (fig. 18), while four were wasps. Three of the ant specimens were alate. Other prey included Hemiptera (13), other spiders (10), including other salticids, Diptera (7), Coleoptera (5) and Neuroptera (1). Spiders were usually larger than their prey, in terms of body length. Although *Iridomyrmex* ants were common in many areas where *S. incana* were collected, we never found *S. incana* feeding upon them.

Foraging behaviour seems to primarily involve waiting on the trunk of a tree until a potential prey item comes into the spider's field of view. When outside their nests or retreats, females and juveniles were usually observed on the trunks of trees within 50 mm of a piece of bark where a retreat was located. Spiders usually oriented towards the

ground and adopted either a neutral or lowered body posture. Cannibalism was never observed in the field. We observed five prey capture attempts by *S. incana* in the field. Three of these occurred while *S. incana* was standing on a tree trunk close to a piece of bark and flying insects landed close to the spider. The spiders oriented and leaped without stalking, however, only one of these attempts was successful. On two occasions we observed *S. incana* hunting ants as they ran up the trunks of trees. On both occasions, the spider tracked the ant as it passed, and then ran around in front of the ant and lunged down over the head of the ant. In the laboratory, when prey was stationary *S. incana* engaged in the typical salticid hunting sequence of orienting, pursuing until within striking distance, crouching and then jumping (Forster 1977). If prey was running or struggling with silk in the cage spiders usually ran and leaped onto it.

Figure 18: Female *Servaea incana* feeding upon an ant on the trunk of a *Eucalypt* tree.

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Table 4. Observed prey items of *S. incana*

ORDER	TOTAL
Hymenoptera	**37**
Ant	33
Wasp	4
Hemiptera	**13**
Cicadellidae	11
Pentatomidae	2
Araneae	**10**
Salticidae	3
Thomisidae	2
Sparassidae	1
Theridiidae	1
Unknown	3
Diptera	**7**
Coleoptera	**4**
Elateridae	3
Weevil	1
Unknown	1
Neuroptera	**1**

Figure 19: Adult male *Sandalodes superbus* feeding upon subadult *S. incana*.

Enemies

We observed two instances of predators feeding upon *S. incana* in the field. One was a juvenile huntsman spider (Sparassidae) feeding upon an adult male *S. incana*, and the other was an adult male salticid (*Sandalodes superbus*) feeding upon a juvenile *S. incana* (fig. 19). We also observed an adult female *Sandalodes superbus* chase an adult female *Servaea incana* across the trunk of a tree. Adult *S. superbus* are generally larger than adult *S. incana*, however, the size of the adult males do overlap. We have observed *S. incana* feeding upon both juvenile *S. superbus* and juvenile sparassids.

Figure 20: Larva of the mantispid *Spaminta minjerribae* on the ventral surface of an adult male *Servaea incana*. The anterior end of the larva is positioned near the spider's pedicel, and the larva's body extending over the spider's left book lung.

The most commonly observed enemy of *S. incana* was the mantispid *Spaminta minjerribae*. In the field we found *S. incana* nests containing mantispid pupae, and some adults and subadult *S. incana* of both sexes collected from the field were later found to be carrying mantispid larvae (Fig. 20). Adult mantispids also emerged from nests built in the laboratory by female spiders collected from the field. Adult mantispids did not appear to interact with *S. incana* in the laboratory, but they did feed on the flies provided as food for the spiders. Under a microscope we observed mantispid larvae feeding on the haemolymph of *S. incana* at the pedicel. We did not observe any obvious behaviours in *S. incana* that may help to remove mantispids from their body or nest. We also observed

mantispid larvae on *Sandalodes superbus*, a salticid that was both a predator and prey of *S. incana*.

We staged an interaction in the open between an adult male *Servaea incana* that was harbouring a mantispid larva and an adult female that had recently moulted. The pair copulated, the male inserting each palp once, and the mantispid could be seen crawling on the side of the female's abdomen during the second palp application. The male was mounted on the female for a total of 1254s, the palp applications lasting 394s and 699s. Both spiders were checked underneath a microscope after the copulation and the mantispid larva was positioned on the dorsal side of the female's pedicel. An interaction was also staged between a female *S. incana* that was harbouring a mantispid larva and a female *Sandalodes superbus.* The larger *S. superbus* attacked and consumed the *S. incana*. The mantispid larva was observed leaving the body of the *S. incana* and crawling across the chelicerae and then cephalothorax of the *S. superbus.*

We also found adult and subadult *S. incana* of both sexes in the field that had been parasitised by the pompilid wasp *Epipompilus sp.* indet. Larvae of these wasps were attached to the side of the spider's abdomen, near the pedicel. Spiders remained active with the pompilid larva attached (Fig. 21). In the laboratory, larvae always consumed and killed the spiders while in the retreat, leaving behind only the dorsal carapace. Larvae then pupated within the silk left behind by the spider. Parasitised female *S. incana* that were brought into the laboratory were consumed before they laid any eggs, therefore, we did not observe any predation of *S. incana* eggs by *Epipompilus* larvae. We successfully removed larvae from three *S. incana*. Larvae were pierced at the

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anterior end of the body and then removed with forceps, while the spiders were restrained with cling film. All three *S. incana* survived and appeared to behave normally after removal of the larvae.

Figure 21: This subadult *S. incana* has been parasitised by a pompilid wasp, of the genus *Epipompilus,* but remains active. The larva is attached to the right side of the spider's abdomen.

DISCUSSION

Interactions between *Servaea incana* are more or less typical of jumping spiders and this allowed us to adopt similar terminology to that used for previous studies of

salticids. Generally, all individuals used the same sets of displays, except in interactions with adult males. Only males gestured, zig-zag danced and quivered their legs, and they used these displays with females and immature conspecifics. Adult females and immature spiders quivered their legs and body in response to these courtship displays from males, and this quivering was only used towards adult males. Immature spiders shared the same display repertoire as adult females. All aggressive intraspecific interactions were similar at the early stages, however, ritualised fighting was only pronounced at the later stages of male-male interactions. Therefore, it appears that males undergo a marked shift in their behaviour and display repertoire upon maturity, while females retain the same repertoire through at least the later juvenile instars.

Aggression towards adult males by both immature males and females was generally reciprocated, resulting in the subadult or juvenile spiders needing to flee. Immature spiders that were not aggressive and either did not display at all or only performed quivering displays were often able to continue wandering around the arena as they were, while the male watched and displayed at a distance. Subadult males quivered their body in the same way that adult females did, but this behaviour was never observed in adult males. It appears that by behaving like females, immature spiders may be able to avoid engaging in aggressive interactions with adult males, possibly similar to how males of some animals avoid aggression from other males by behaving like females (Norman, Finn *et al.* 1999; Whiting, Webb *et al.* 2009). While juveniles may not represent as significant a threat to males as another adult male would, they may still represent costs in terms of competition for food and retreat sites, so we would not expect males to tolerate them.

As is typical of jumping spiders, *S. incana* exhibit courtship versatility, although it may not be as pronounced as in some other species. When they encounter females (and subadults and juveniles) males engage in visual courtship displays (type 1 courtship; Jackson 1977). At retreats, most salticids studied so far primarily interact via silk vibrations (type 2 courtship), but these behaviours appear minimal in *S. incana*. Instead, *S. incana* seems to rely largely upon visual communication at retreats.

Males and females are capable of communicating via visual signals while the female is still within the retreat and prior to the male coming into contact with the silk. Presumably, ample light levels between the loose layers of bark facilitate this behaviour in *S. incana*, unlike in other salticids, such as *Trite planiceps* (Taylor and Jackson 1999) and *Phidippus johnsoni* (Jackson 1977) that build retreats and nests in darker places. Like many other spiders (Jackson 1986a), when male *S. incana* encounter subadult females at retreats, they cohabit with the female, copulating soon after the female moults to maturity.

Among its closest relatives, *Servaea* appears more similar to *Thiania* in both morphology and behaviour, although only male-male interactions have been described for *Thiania*. Males of both *Servaea* and *Thiania* possess dimorphic first pairs of legs which they make use of during their displays, unlike *Maratus, Saitis* and *Hypoblemum* which primarily use their dimorphic third pair of legs. *S. incana* were never observed raising their abdomen, while the rest of these genera are all known to raise their abdomen while displaying during interactions. *Maratus* are also known to produce

vibratory signals (Girard, Kasumovic *et al.* 2011). Vibratory signalling is an important feature of communication in the majority of spiders, including some salticids. However, we did not find any evidence that *S. incana* produces sounds or substrate borne signals away from retreats or nests and the use of tactile signals via silk appears to be minimal. Whether the presence of these behaviours in *Maratus* or the absence in *Servaea* represents the ancestral state remains to be seen, as the behaviour of many species still needs to be described. Aside from the Euophryinae, sound production or substrate borne signals have also been documented for the subfamilies Pelleninae and Dendryphantinae. The salticids that produce these signals all live on leaf litter or foliage, which may permit better transmission of sounds than a tree trunk (Elias, Mason *et al.* 2004). In addition, more complex habitats may necessitate multi-modal signalling for effective communication (Hebets and Papaj 2005).

Bright colouration and extravagant courtship, as seen in *Maratus*, are evidently not required for *S. incana* and could be costly in their relatively exposed microhabitat. Conspicuous visual signals may serve to attract unwanted attention from visually guided enemies such as other salticids (Roberts, Taylor *et al.* 2007). Parasitoid wasps are known to eavesdrop upon their host's intraspecific signals (Zuk and Kolluru 1998) and may use visual as well as chemical cues to find their hosts (Uma, Durkee *et al.* 2013). *S. incana* are attacked by parasitoid wasps of the genus *Epipompilus,* members of which are known to search under bark on the trunks of trees, attacking and consuming both the adults and eggs of clubionids, salticids and desids (Austin 1985; Harris 1987; Harris 1999; Pollard 1982). Like other salticids (Taylor 1998), *S. incana* appear to use cues from silk draglines to detect the presence of nearby conspecifics. Like other predators (Wignall and Taylor 2009), *Epipompilus* may also use these cues to locate hosts. We did

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not observe any predation of *S. incana* eggs by *Epipompilus* larvae. Adult and subadult males and females were attacked and all parasitised females that were brought in to the laboratory were consumed before they laid any eggs.

Mantispids were the most frequently observed enemy of *S. incana*. Bearing mantispid larvae is potentially costly for *S. incana* as larvae sustain themselves by feeding on the spider's haemolymph. When larvae of the mantispid, *Dicromantispa sayi*, parasitise subadults of the lycosid spider, *Rabidosa rabida,* the spiders take longer to mature and mature smaller, with females maturing with one less instar (Redborg 1982). To complete their life-cycle, mantispids must feed upon spider's eggs, so they are required to find the nest of a female spider and enter the egg sac, however, as we found with *S. incana*, mantispid larvae board adults and subadults of both sexes (Redborg 1998). Some species of mantispid are known to transfer between spiders during copulation (Redborg 1998; Scheffer 1992) and this was the case with *Spaminta minjerribae* that parasitised *S. incana*. If females mate with multiple males, they may increase the risk of acquiring a larval mantispid, representing a cost of polyandry. A single mantispid is capable of eating all the eggs in a spider's nest (Rienks 2000) and guarding of the eggs by females likely has little effect upon egg predation by mantispids (Vieira and Romero 2008; Willey and Adler 1989). As *S. incana* females only produce up to three batches of eggs, losing one batch represents a very high fitness cost. However, females that mate once and produce temporally spaced clutches of eggs may minimise the impact of mantispid predation on their eggs. For males, the benefits of polygyny are increased as they can get rid of mantispid larvae during copulation. Long copulation times will also benefit males and be more costly to females as mantispid larvae will have more time to transfer (O'Brien and Redborg 1997). This possibly results

in sexual conflict over both the number and duration of copulations. Female spiders would be advantaged by an ability to detect whether males are carrying mantispid larvae, however, both males and their mantispids would benefit by being able to conceal such cues from females. It is unknown whether mantispids influence display behaviour or mate choice of either sex in *S. incana* or any other spider.

We also observed mantispid larvae on a predator of *S. incana*, the salticid *Sandalodes superbus*. Some species of mantispid are known to transfer between prey and predator during araneophagy (O'Brien and Redborg 1997) and in this study we observed *Spaminta minjerribae* transfer from *S. incana* to another species of jumping spider. Therefore, *S. incana* and other cursorial spiders may also be at risk from acquiring mantispids when they prey upon each other. Despite predation of the eggs of all major families of cursorial spiders and the implications for sexual and natural selection highlighted here, there has been little research into the effects of mantispids on the behaviour and ecology of spiders.

Salticids are an abundant and diverse family of spiders and many interesting questions remain to be answered about the evolution and ecology of salticid behaviour. The behaviour of many more species still remains to be described and more studies are required in order to map the evolution of behaviour onto newly available phylogenies (Zhang and Maddison 2013). Further research is also required to determine what selection pressures could cause often-substantial variation in behaviour of closely related species.

ACKNOWLEDGEMENTS

REFERENCES

Austin, A.D. (1985) The function of spider egg sacs in relation to parasitoids and predators, with special reference to the Australian fauna. *Journal of Natural History* **19**, 359-376.

Blest, A. (1983) Ultrastructure of secondary retinae of primitive and advanced jumping spiders (Araneae, Salticidae). *Zoomorphology* **102**, 125-141.

Blest, A.D., Hardie, R.C., McIntyre, P., and Williams, D.S. (1981) The spectral sensitivities of identified receptors and the function of retinal tiering in the principal eyes of a jumping spider. *Journal of Comparative Physiology* A **145**, 227-239.

Blest, A.D., and Maples, J. (1979) Exocytotic shedding and glial uptake of photoreceptor membrane by a salticid spider. *Proceedings of the Royal Society B* **204**, 105-112.

Blest, A.D., McIntyre, P., and Carter, M. (1988) A re-examination of the principal retinae of *Phidippus johnsoni* and *Plexippus validus* (Araneae: Salticidae): implications for optical modelling. *Journal of Comparative Physiology A* **162**, 47-56.

Carducci, J.P., and Jakob, E.M. (2000) Rearing environment affects behaviour of jumping spiders. *Animal Behaviour* **59**, 39-46.

Chan, J.P.Y., Lau, P.R., Tham, A.J., and Li, D.Q. (2008) The effects of male-male contests and female eavesdropping on female mate choice and male mating success in the jumping spider, *Thiania bhamoensis* (Araneae: Salticidae). *Behavioral Ecology and Sociobiology* **62**, 639-646.

Clark, D.L. (1994) Sequence analysis of courtship behavior in the dimorphic jumping spider *Maevia inclemens* (Araneae: Salticidae). *Journal of Arachnology* **22**, 94-107.

Cross, F.R., Jackson, R.R., and Pollad, S.D. (2008) Complex display behaviour of *Evarcha culicivora*, an East African mosquito-eating jumping spider. *New Zealand Journal of Zoology* **35**, 151-187.

Edwards, G. (1981) Sound production by courting males of *Phidippus mystaceus* (Araneae: Salticidae). *Psyche* **88**, 199-214.

Elias, D.O., Maddison, W.P., Peckmezian, C., Girard, M.B., and Mason, A.C. (2012) Orchestrating the score: complex multimodal courtship in the *Habronattus coecatus* group of *Habronattus* jumping spiders (Araneae: Salticidae). *Biological Journal of the Linnean Society* **105**, 522-547.

Elias, D.O., Mason, A.C., and Hoy, R.R. (2004) The effect of substrate on the efficacy of seismic courtship signal transmission in the jumping spider *Habronattus dossenus* (Araneae: Salticidae). *Journal of Experimental Biology* **207**, 4105-4110.

Forster, L.M. (1977) A qualitative analysis of hunting behaviour in jumping spiders (Araneae: Salticidae). *New Zealand Journal of Zoology* **4**, 51 - 62.

Girard, M.B., Kasumovic, M.M., and Elias, D.O. (2011) Multi-modal courtship in the peacock spider, *Maratus volans* (OP-Cambridge, 1874). *PloS ONE* **6**, e25390.

Gwynne, D.T., and Dadour, I.R. (1985) A new mechanism of sound production by courting male jumping spiders (Araneae: Salticidae, *Saitis michaelseni* Simon). *Journal of Zoology* **207**, 35-42.

Harland, D.P., and Jackson, R.R. (2002) Influence of cues from the anterior medial eyes of virtual prey on *Portia fimbriata*, an araneophagic jumping spider. *Journal of Experimental Biology* **205**, 1861-1868.

Harris, A.C. (1987) Pompilidae (Insecta: Hymenoptera). *Fauna of New Zealand* **12**.

Harris, A.C. (1999) The life histories and nesting behaviour of the Pompilidae (Hymenoptera) in New Zealand: a comparative study. *Species diversity* **4**, 143-235.

Hebets, E.A., and Papaj, D.R. (2005) Complex signal function: developing a framework of testable hypotheses. *Behavioral Ecology and Sociobiology* **57**, 197-214.

Hill, D.E. (2009) Euophryine jumping spiders that extend their third legs during courtship (Araneae: Salticidae: Euophryinae: *Maratus, Saitis*). *Peckhamia* **74**, 1-27.

Hill, D.E., and Otto, J.C. (2011) Visual display by male *Maratus pavonis* (Dunn 1947) and *Maratus splendens* (Rainbow 1896). (Araneae: Salticidae: Euophryinae). *Peckhamia* **89**, 1-41.

Jackson, R.R. (1977) Courtship versatility in the jumping spider, *Phidippus johnsoni* (Araneae: Salticidae). *Animal Behaviour* **25**, 953-957.

Jackson, R.R. (1985a) The biology of *Euryattus sp*. indet., a web‑building jumping spider (Araneae, Salticidae) from Queensland: utilization of silk, predatory behaviour and intraspecific interactions. *Journal of Zoology* **1**, 145-173.

Jackson, R.R. (1985b) The biology of *Simaetha paetula* and *S. thoracica*, web‑building jumping spiders (Araneae, Salticidae) from Queensland: co-habitation with social spiders, utilization of silk, predatory behaviour and intraspecific interactions. *Journal of Zoology* **1**, 175-210.

Jackson, R.R. (1986a) Cohabitation of males and juvenile females: a prevalent mating tactic of spiders. *Journal of Natural History* **20**, 1193-1210.

Jackson, R.R. (1986b) Silk utilisation and defensive behaviour of *Thiania*, an iridescent jumping spider (Araneae: Salticidae) from Malaysia. *New Zealand Journal of Zoology* **13**, 553-561.

Jackson, R.R. (1987) Comparative study of releaser pheromones associated with the silk of jumping spiders (Araneae, Salticidae). *New Zealand Journal of Zoology* **14**, 1-10.

Jackson, R.R. (1988) The biology of *Jacksonoides queenslandica*, a jumping spider (Araneae: Salticidae) from Queensland: intraspecific interactions, web-invasion, predators, and prey. *New Zealand Journal of Zoology* **15**, 1-37.

Jackson, R.R. (1990) Ambush predatory behaviour of *Phaeacius malayensis* and *Phaeacius* sp. indet., spartaeine jumping spiders (Araneae: Salticidae) from tropical Asia. *New Zealand Journal of Zoology* **17**, 491-498.

Jackson, R.R. (1992) Conditional strategies and interpopulation variation in the behavior of jumping spiders. *New Zealand Journal of Zoology* **19**, 99-111.

Jackson, R.R., and Blest, A.D. (1982) The biology of *Portia fimbriata*, a web-building jumping spider (Araneae, Salticidae) from Queensland: utilization of webs and predatory versatility. *Journal of Zoology* **196**, 255-293.

Jackson, R.R., and Hallas, S.E.A. (1986) Comparative biology of *Portia africana*, *P. albimana*, *P. fimbriata*, *P. labiata*, and *P. shultzi*, araneophagic, web-building jumping spiders (Araneae: Salticidae): Utilisation of webs, predatory versatility, and intraspecific interactions. *New Zealand Journal of Zoology* **13**, 423-489.

Jackson, R.R., Nelson, X.J., and Sune, G.O. (2005) A spider that feeds indirectly on vertebrate blood by choosing female mosquitoes as prey. *Proceedings of the National Academy of Sciences* **102**, 15155-15160.

Jackson, R.R., and Pollard, S.D. (1996) Predatory behavior of jumping spiders. *Annual Review of Entomology* **41**, 287-308.

Jackson, R.R., Pollard, S.D., Li, D.Q., and Fijn, N. (2002) Interpopulation variation in the risk-related decisions of *Portia labiata*, an araneophagic jumping spider (Araneae, Salticidae), during predatory sequences with spitting spiders. *Animal Cognition* **5**, 215-223.

Jackson, R.R., and Willey, M.B. (1995) Display and mating behavior of *Euophrys parvula*, a New Zealand jumping spider (Araneae, Salticidae). *New Zealand Journal of Zoology* **22**, 1-16.

Land, M. (1985) The morphology and optics of spider eyes. In 'Neurobiology of arachnids.' (Ed. Barth, F.G.) pp. 53-78. (Springer-Verlag: Berlin).

Li, D.Q., Jackson, R.R., and Lim, M.L.M. (2003) Influence of background and prey orientation on an ambushing predator's decisions. *Behaviour* **140**, 739-764.

Li, D.Q., Yik, S.H., and Seah, W.K. (2002) Rivet-like nest-building and agonistic behaviour of *Thiania bhamoensis*, an iridescent jumping spider (Araneae: Salticidae) from Singapore. *Raffles Bulletin of Zoology* **50**, 143-151.

Maddison, W.P., and Stratton, G.E. (1988) A common method of sound production by courting jumping spiders (Araneae, Salticidae). *Journal of Arachnology* **16**, 267-269.

Nelson, X.J., and Jackson, R.R. (2007) Complex display behaviour during the intraspecific interactions of myrmecomorphic jumping spiders (Araneae: Salticidae). *Journal of Natural History* **41**, 1659-1678.

Nelson, X.J., and Jackson, R.R. (2011) Flexibility in the foraging strategies of spiders. In 'Spider Behaviour: Flexibility and Versatility'. (Ed M. E. Herberstein) pp. 31-56. (Cambridge University Press: New York)

Nelson, X.J., Jackson, R.R., and Sune, G. (2005) Use of *Anopheles*-specific prey-capture behavior by the small juveniles of *Evarcha culicivora*, a mosquito-eating jumping spider. *Journal of Arachnology* **33**, 541-548.

Norman, M.D., Finn, J., and Tregenza, T. (1999) Female impersonation as an alternative reproductive strategy in giant cuttlefish. *Proceedings of the Royal Society B* **266**, 1347-1349.

O'Brien, L.C., and Redborg, K.E. (1997) Copulation duration in the spider *Philodromus vulgaris* (Hentz) (Araneae: Philodromidae) and its influence on the evolution of host transfer behavior during cannibalism by *Mantispa uhleri* banks (Neuroptera: Mantispidae). *Journal of Insect Behavior* **10**, 469-477.

Pollard, S.D. (1982) *Epipompilis insularis* (Hymenoptera: Pompilidae), a parasitoid of hunting spiders. *New Zealand Journal of Zoology* **9**, 37-39.

Redborg, K.E. (1982). Interference by the mantispid *Mantispa uhleri* with the development of the spider *Lycosa rabida*. *Ecological Entomology* **7**, 187-196.

Redborg, K.E. (1998) Biology of the mantispidae. *Annual Review of Entomology* **43**, 175-194.

Richardson, B.J., and Gunter, N.L. (2012) Revision of Australian jumping spider genus *Servaea* Simon 1887 (Aranaea: Salticidae) including use of DNA sequence data and predicted distributions. *Zootaxa* **3350**, 1-33.

Rienks, J.H. (2000) Extended nest residence and cannibalism in a jumping spider (Araneae, Salticidae). *Journal of Arachnology* **28**, 123-127.

Roberts, J.A., Taylor, P.W., and Uetz, G.W. (2007) Consequences of complex signaling: predator detection of multimodal cues. *Behavioral Ecology* **18**, 236-240.

Scheffer, S.J. (1992) Transfer of a larval mantispid during copulation of its spider host. *Journal of Insect Behavior* **5**, 797-800.

Sivalinghem, S., Kasumovic, M.M., Mason, A.C., Andrade, M.C.B., and Elias, D.O. (2010) Vibratory communication in the jumping spider *Phidippus clarus*: polyandry, male courtship signals, and mating success. *Behavioral Ecology* **21**, 1308-1314.

Tarsitano, M.S., and Jackson, R.R. (1994) Jumping spiders make predatory detours requiring movement away from prey. *Behaviour* **131**, 65-73.

Tarsitano, M.S., and Jackson, R.R. (1997) Araneophagic jumping spiders discriminate between detour routes that do and do not lead to prey. *Animal Behaviour* **53**, 257-266.

Taylor, P.W. (1998) Dragline-mediated mate-searching in *Trite planiceps* (Araneae, Salticidae). *Journal of Arachnology* **26**, 330-334.

Taylor, P.W., Hasson, O., and Clark, D.L. (2001) Initiation and resolution of jumping spider contests: roles for size, proximity, and early detection of rivals. *Behavioral Ecology and Sociobiology* **50**, 403-413.

Taylor, P.W., and Jackson, R.R. (1999) Habitat-adapted communication in *Trite planiceps*, a New Zealand jumping spider (Araneae, Salticidae). *New Zealand Journal of Zoology* **26**, 127-154.

Uma, D., Durkee, C., Herzner, G., and Weiss, M. (2013) Double deception: ant-mimicking spiders elude both visually- and chemically-oriented predators. *PLoS ONE* **8**, e79660.

Vieira, C., and Romero, G.Q. (2008) Maternal care in a neotropical jumping spider (Salticidae). *Journal of Zoology* **276**, 237-241.

Whiting, M.J., Webb, J.K., and Keogh, J.S. (2009) Flat lizard female mimics use sexual deception in visual but not chemical signals. *Proceedings of the Royal Society B* **276**, 1585-1591.

Willey, M.B., and Adler, P.H. (1989) Biology of *Peucetia viridans* (Araneae, Oxyopidae) in South Carolina, with special reference to predation and maternal care. *Journal of Arachnology* **17**, 275-284.

Wignall, A.E., and Taylor, P.W. (2009) Responses of an araneophagic assassin bug, *Stenolemus bituberus*, to spider draglines. *Ecological entomology* **34**, 415-420.

Zhang, J.X., and Maddison, W.P. (2013) Molecular phylogeny, divergence times and biogeography of spiders of the subfamily Euophryinae (Araneae: Salticidae). *Molecular Phylogenetics and Evolution* **68**, 81-92.

Zuk, M., and Kolluru, G.R. (1998) Exploitation of sexual signals by predators and parasitoids. *The Quarterly Review of Biology* **73**, 415-438.

Zurek, D.B., and Nelson, X.J. (2012) Hyperacute motion detection by the lateral eyes of jumping spiders. *Vision Research* **66**, 26-30.

Zurek, D.B., Taylor, A.J., Evans, C.S., and Nelson, X.J. (2010) The role of the anterior lateral eyes in the vision-based behaviour of jumping spiders. *Journal of Experimental Biology* **213**, 2372-2378.

Chapter 3: Seasonal variation in sexual opportunities of the jumping spider *Servaea incana*

Abstract

Male reproduction is commonly constrained by the availability of females, and more specifically by opportunities to fertilize the ova that females carry. For species in which availability and reproductive value of females vary seasonally, it is anticipated that male phenology will adapt to maximize access to the females with the highest reproductive value. In *Servaea incana* jumping spiders, virgin females are at a premium because once mated females of this species only rarely accept subsequent suitors. We studied a population of *S. incana* for 13 months and found strong seasonal patterns in population structure, with a distinct peak of sexual maturation in the autumn months. Consistent with the high value of virgin females, males matured earlier than females (protandry) such that most males were already mature and ready to mate as females began maturing. Further, males were found to cohabit with subadult females that were about to mature, waiting for them to mature before mating. As the season progressed subadult and virgin females became less abundant and males were then sometimes found to cohabit with mated females. We found that size of adult females increased through the year, which was predicted since early maturation most likely means less development time. However, despite protandry, male size decreased through the year, suggesting that males maturing at the beginning of the year tend to be larger than males maturing later. Cohabitation tended to be size-assortative; size of male and female in cohabiting pairs tended to be correlated, and this was the case for pairs involving both

subadult and adult females. We discuss the implications of protandry, cohabitation and mating-induced sexual inhibition in the mating system and life history of this spider.

Keywords: *Cohabitation, Seasonality, Protandry, Jumping spider*

Introduction

Reproductive opportunities can vary substantially both in space and in time, and mating systems evolve in response to the selection pressures imposed by this variation (Emlen & Oring 1977; Clutton-Brock & Parker 1992). Because females carry the burden of providing material resources for developing ova and offspring, variation in female reproduction is expected to be influenced by the suitability of the environment between locations and through the year for the material requirements associated with production of eggs and development of offspring (Davies 1991). On the other hand, the timing and success of male reproduction may be linked more to the availability or quality of mating partners (Emlen & Oring 1977; Ims 1988; Pröhl & Berke 2001; Pröhl 2002; Shuster & Wade 2003). Accordingly, male distribution and seasonality might come to match periods of peak sexual opportunity rather than material resources.

In species that show distinct seasonality, reproductive opportunities available to males can be linked to timing of maturation. For example, males that mature early in a season might have more mating opportunities than males that mature later simply because their period of sexual activity coincides to the greatest extent with the availability of females (Bulmer 1983; Thornhill & Alcock 1983; Andersson 1994). The benefits of early male maturation are even greater for species in which virgin females are more abundant early in a season and are at a premium (Thornhill & Alcock 1983; Simmons 2001).

There are several reasons why virgin females might be at a premium. In some

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species, virgin females show higher receptivity, lower choosiness or lower aggression than mated females (Carrière & McNeil 1990; Miyatake et al. 1999). Accordingly, males that mature early in the season when virgins are more abundant will encounter females that are on average more likely to mate and less likely to be aggressive. Benefits of male maturation that coincides with maximum availability of virgin females may extend beyond probability of copulation and risk of injury. In species that store sperm and show first male sperm precedence or low remating tendency, males that mate with virgins rather than previously mated females will be advantaged in terms of fertilization success (e.g. Simmons et al. 1994). Mated females may carry sexually transmitted diseases and parasites, and so males that mate with virgins might also benefit from lower risk of exposure (Hurst et al. 1995; Thrall et al. 1997).

Protandry, whereby males tend to mature and prepare for mating before females, is common in butterflies (Wiklund & Fagerström 1977), crickets (Murray & Cade 1995), and spiders (Gunnarsson & Johnsson 1990; Masumoto 1994; Maklakov et al. 2004) and is thought to arise as an evolutionary response to the benefits of access to virgin females (Wiklund and Fagerström 1977; Thornhill & Alcock 1983; Morbey and Ydenberg 2001; Simmons 2001). Supporting this view, protandry tends to be negatively associated with propensity of females of a species to mate with multiple males ('polyandry') (Wiklund and Forsberg 1991). Protandry can also be negatively correlated with male size because size and development time are interdependent (Zonneveld 1996). For species with determinate growth, if males and females grow at the same rate as juveniles, then to mature earlier males must shorten their development time and complete their growth at a smaller size (Singer 1982). For example, Maklakov et al. (2004) found that early maturing males of *Stegodyphus lineatus* (Eresidae) spiders were smaller than those that

matured later. On the other hand, selection for large size can result when male mating success increases with male size (Morbey 2013). For example, if large males are more likely to win in contests, and winning a contest allows them access to virgin females, then there will be selection pressure for large size in males. In this context, selection pressure for large male size may oppose selection for protandry (Zonneveld 1996).

Spiders have served as useful subjects for the study of mating system evolution. Protandry has been found in many spiders (for a review, see Elias et al. 2011), which suggests a premium on virgin females as a reproductive resource. Associated with protandry, many spiders exhibit 'cohabitation' whereby mature males guard immature females that are close to maturing, and then mate soon after the females mature (Jackson 1986). For example, the linyphiid *Neriene litigiosa* (Watson 1986) and the salticid *Phidippus johnsoni* (Jackson 1981) exhibit both protandry and cohabitation. Consistent with the expected premium on virgin females, these species have distinct reproductive seasons and exhibit high levels of monandry (Jackson 1980; Watson 1986).

In the present study we are interested in the seasonal nature of mating opportunities in a jumping spider, *Servaea incana* Karsch, 1878 (= *Servaea vestita* (L. Koch 1879)) (Araneae, Salticidae), which lives on eucalyptus trees in temperate regions of Australia (Richardson and Gunter 2012). We have observed cohabitation in the field (Chapter 2) and have also found virgin females to be much more receptive to mating than are mated females (Chapter 4). Here we describe seasonal demographic changes in a population of *S. incana* and discuss the implications of protandry, cohabitation and remating inhibition in the mating system and natural history of this spider.

Materials and Methods

Study sites and sampling

A population of *Servaea incana* at Mirambeena Regional Park, in Sydney, Australia (33° 54' 0.7482" S, 150° 58' 27.9876" E), was surveyed from December 2010 until December 2011. The study site contained 67 eucalyptus trees surrounded by areas of maintained lawn and short grass.

From December 2010 until June 2011 (summer, autumn) a census was carried out every two weeks. Pilot data from previous seasons indicated that this is the peak maturation period in *S. incana* and so this sampling frequency was estimated as sufficient to detect protandry. From June until December (winter, spring) a census was carried out once each month by two people.

During each census, trees were searched from ground level to 2.5 m, lifting loose pieces of bark where *S. incana* usually builds nests and retreats. On each census date we counted the number of adult males, adult females, sub-adult males, sub-adult females and juveniles. Sub-adult males and females were those that needed only one moult to become adults, as is evident from enlarged palps in males and visible development of epigynum beneath the cuticle in females. Juveniles included all earlier stages, for which it was not possible to reliably ascertain sex from morphology. We recorded any

instances of cohabitation, whereby males were found sheltering together with an adult or subadult female.

Sex and developmental stage were determined in the field, but genitalia of each spider were also photographed for later confirmation. Dorsal and ventral views of each collected spider were photographed in the field using a digital camera (Panasonic DMC-FT1, 12 megapixels). Each spider found on a tree was placed inside a plastic vial and held in place against the bottom of the vial with a circular piece of sponge. A paper scale marked in millimetres was placed next to the spider for each photograph. After photographing the ventral or dorsal side of the spider, the sponge was removed and the spider was flipped over to photograph the other side. Using ImageJ software these photographs were used to measure body length, using the paper scale to calibrate size. Ventral body length was measured from the tip of the labium to the end of the spinnerettes. Each spider found on a tree was kept individually in a vial until the counting of spiders on that specific tree was finished, thus avoiding double counting. After identification of species, stage and sex, and photographing, each spider was replaced on the tree where it had been collected.

Results

Population composition and protandry

All developmental stages of both males and females were recorded through most of the year. However, there were distinct seasonal patterns in absolute and relative abundance of the developmental stages and sexes.

Juveniles were by far the most prevalent class at the beginning of the study (December 2010) when they comprised approximately 90% of the recorded population. Abundance of juveniles declined sharply over following sampling dates until the second half of March, after which they remained always present but were of comparatively low abundance. The decline in abundance of juveniles over this period was matched by an increase in abundance of the subsequent stages of subadults and adults. Sex differences in the replacement of juveniles with these subsequent stages indicate a distinct pattern of protandry (Figure 1, 2).

The decline in abundance of juveniles in late December and January was matched by increased abundance of subadult and then adult males. As subadult males matured into adult males, the continuing decline in juvenile numbers was apparently maintained by continuing development of juveniles to subadult males. From February only small numbers of subadult males were recorded as abundance of adults males plateaued and declined only slightly through until October, when numbers dropped

sharply to match the low numbers recorded in the previous December (Figure 1, 2). No

males were found in December 2010 or in the first half of January 2011, and only two

males were found in December 2011 (Figure 1, 2).
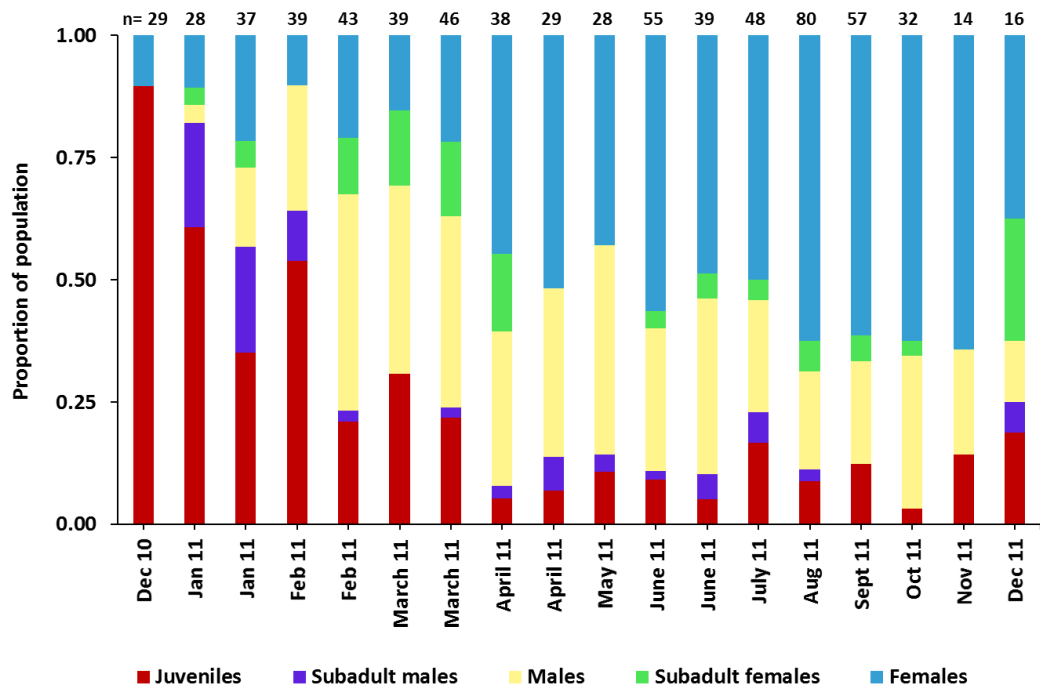
Figure 1. Relative abundance of each sex and age category of *Servaea incana* jumping

spiders over the year from December 2010 to December 2011

In February, just as abundance of sub-adult males declined and abundance of

adult males reached its peak, abundance of juveniles dropped with increased prevalence

of subadult females. In March the abundance of adult females increased sharply while

abundance of juveniles dropped to a similar extent. That abundance of subadult females

was maintained through this period indicates that the continuing decline in juvenile

numbers was at this time driven by ongoing development of juveniles to subadult

females. From late April only small numbers of subadult males and females were

recorded and the overall population profile remained comparatively stable through the autumn and winter months (Figure 1, 2). Mature females were most abundant in August, but after this peak the number of females declined sharply. All sex and age classes were found only in very low numbers in November and December.
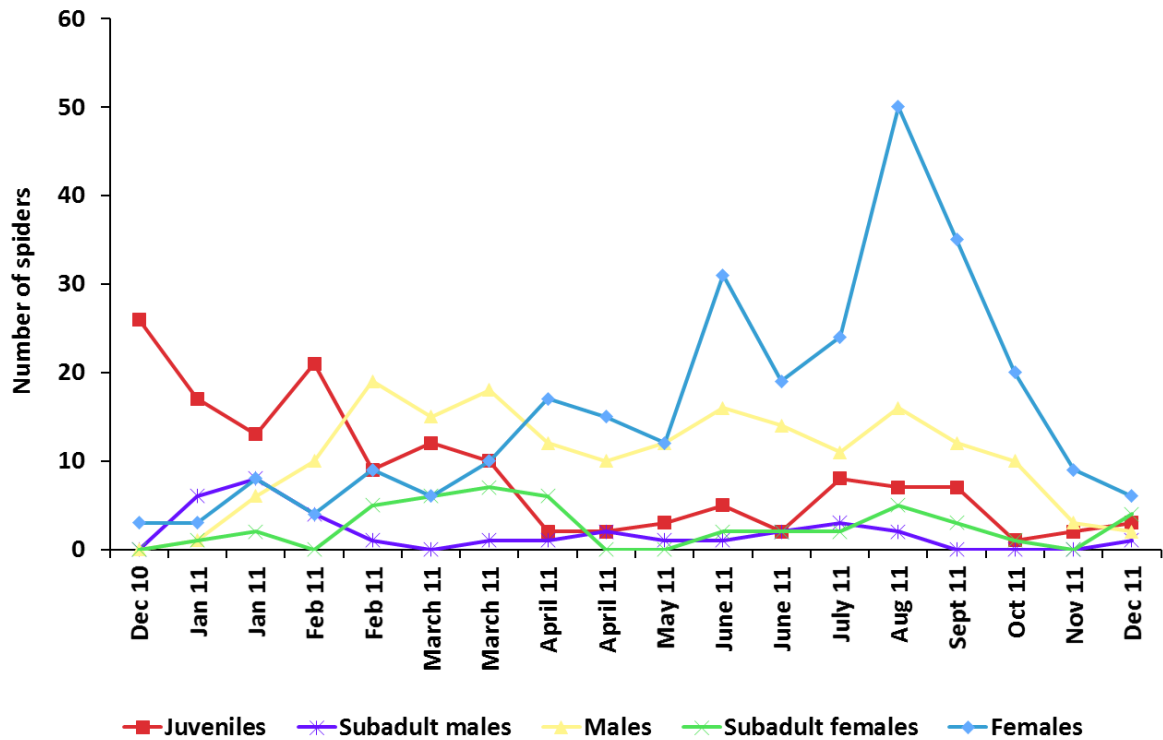

Figure 2. Absolute abundance of each sex and age category of *Servaea incana* jumping spiders over the year from December 2010 to December 2011.

Body size through the year

On average, females were larger (ventral length) than males (means + s.e.: females, 8.341 + 1.022 mm, N = 281; males, 7.237 + 1.005 mm, N = 187; t test = 11.527, d.f. = 466, P < 0.0001). Size of males and females varied through the year, but in opposite

directions. Ventral length increased through the year for both adult and subadult females (adult females: $F_{17,280} = 6.194$, $P < 0.0001$, N = 280, subadult females: $F_{13,46} = 2.345$, P = 0.024, N = 47) (Fig. 3a, c). In contrast, ventral length decreased through the year for adult males ($F_{16,186} = 1.741$, P= 0.043, N = 187) (Fig. 3b). That is, there was no evidence that early maturation by individual males entailed a trade-off of reduced body size. Ventral length for subadult males did not show significant variation through the season ($F_{12,32} = 1.3293$, P = 0.277, N = 33) (Fig. 3d).
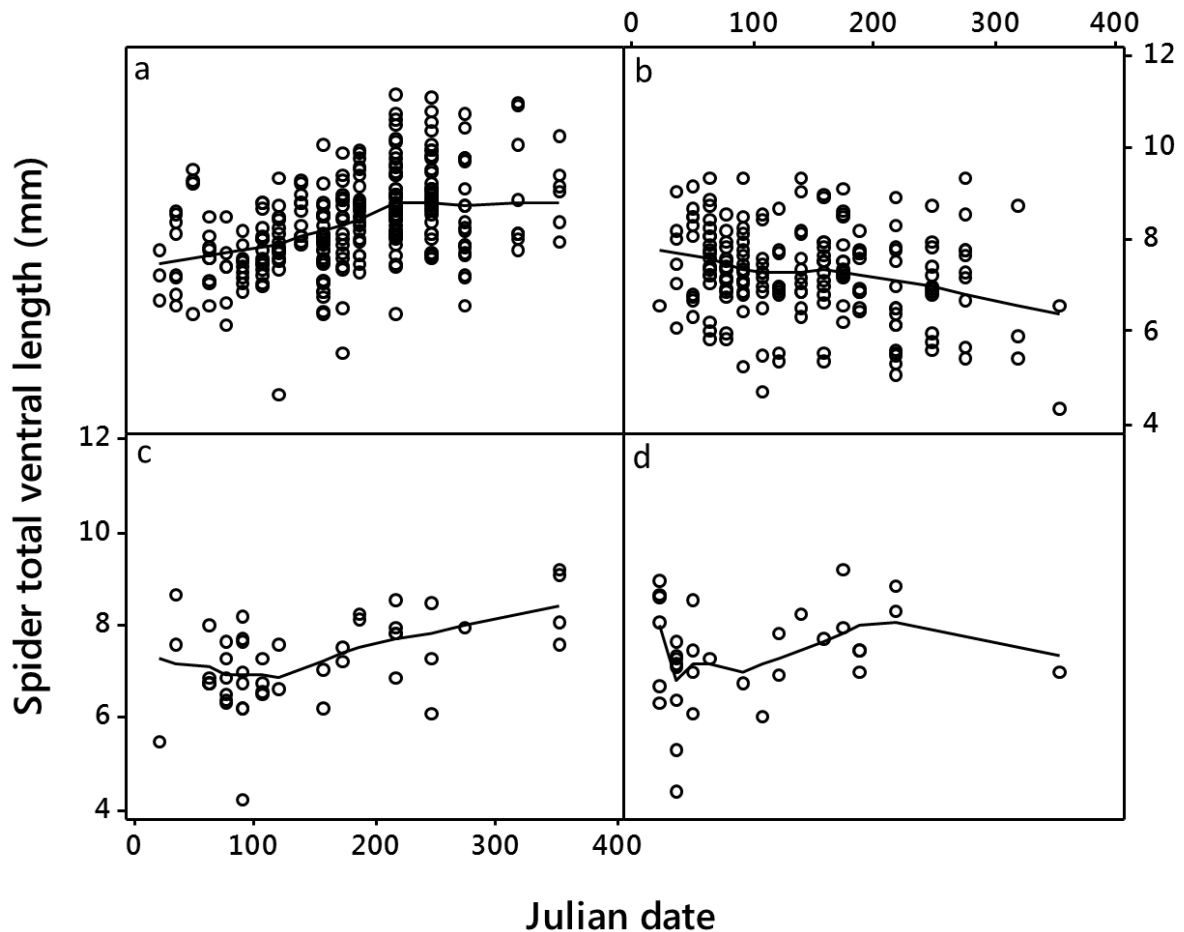
Figure. 3 Total ventral length of *Servaea incana* spiders and time of the year (Julian date) from December 2010 to December 2011. (a) Adult females, (b) Adult males, (c) Subadult females, (d) Subadult males

Cohabitation


Twelve instances of cohabitation were observed. Cohabiting pairs consisted of a subadult female and an adult male (N = 6), or an adult female and an adult male (N = 6). We only found cohabiting pairs during the first half of the year; the first pair was found in January and the last pair in June. In the first census of January 2011 we found a cohabiting pair of a subadult female and an adult male. In the second census of January 2011 we found a cohabiting pair of an adult female and an adult male. Between February and April 2011 we found four cohabiting pairs, all of which involved subadult females. In contrast, between April and June 2011 we found four cohabiting pairs, all of which involved adult females. We only found males cohabiting with adult females during times of the season when subadult females were rare (January, April, May, June) (Figure 1, 2).

Size-assortative cohabitation

There was evidence of size-assortative cohabitation in *S. incana*. In an ANCOVA predicting cohabiting male size as a function of female stage (subadult *vs.* adult) with female size as a covariate, we found that male size was positively associated with female size ($F_{1,11} = 6.340$, P = 0.036) and that this tendency was not significantly different for subadult and adult females (female size x stage $F_{1,11} = 1.193$, P = 0.307) (Figure 7). Males cohabiting with subadult females were not significantly different in size than males cohabiting with adult females ($F_{1,11} = 0.083$, P = 0.781) (overall model N = 12, Adjusted $R^2 = 0.40$, $F_{3,11} = 3.492$, P = 0.070).

To increase sample size to further explore this relationship, we added data of 10 cohabiting pairs of males and subadult females that had been opportunistically collected from other nearby populations in Sydney (additional pairings of males and adult females were not found). After increasing the sample size to 22, the relationships remained robust (female size $F_{1,18} = 8.558$, $P = 0.009$; female stage $F_{1,18} = 0.003$, P = 0.955; female size x stage $F_{1,18} = 2.033$, P = 0.171; overall model N = 22, Adjusted $R^2 = 0.26$, $F_{3,18} = 3.484$, P = 0.038) (Figure 4).

We were interested in comparing the size of males cohabiting with females with the size of males found alone. If male ability to defend females depends on his size, then cohabiting males would tend to be larger than single males. The size of males cohabiting with subadult females was 7.035 + 0.415 mm, N = 6, while the size for those cohabiting with adult females was 8.071 + 0.415 mm, N = 6. The size for males found alone was 7.215 + 0.995, N = 175. However, scarcity of cohabiting pairs precluded statistical analysis.
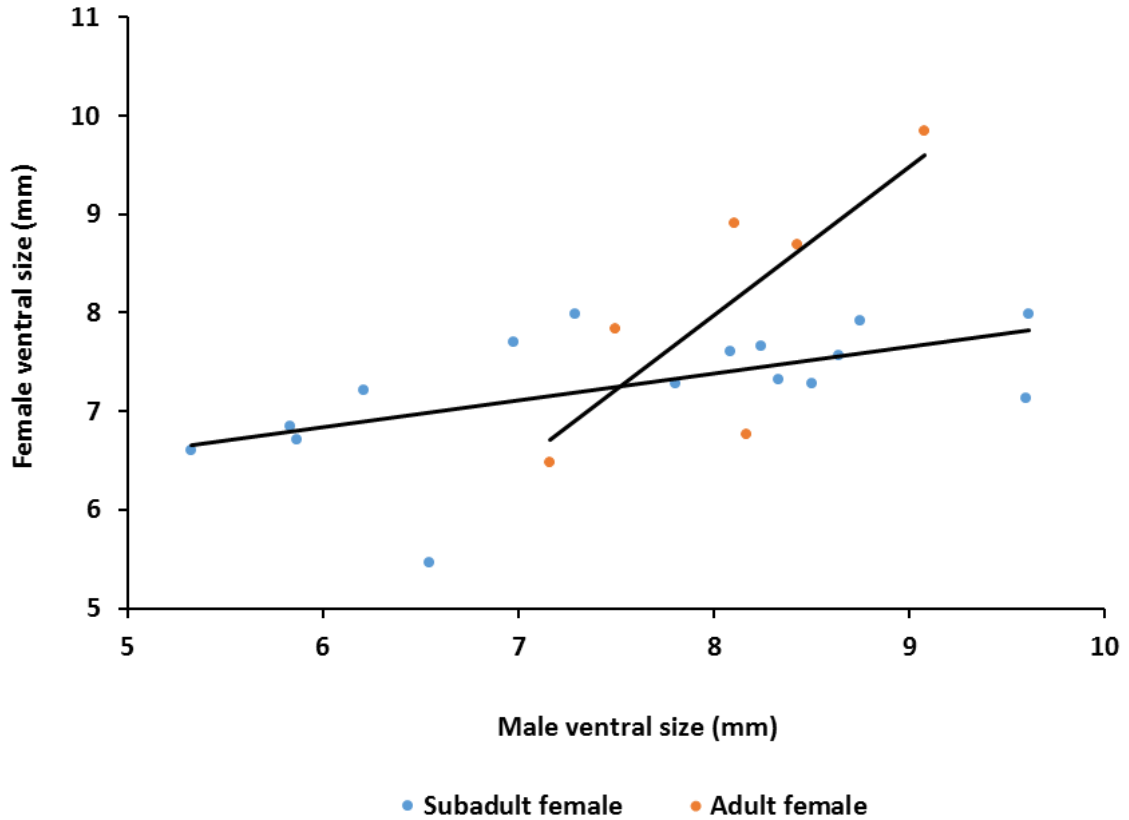
Figure 4. Relationship between total ventral length of *Servaea incana* females and males found cohabiting from December 2010 to December 2011 (Subadult females: R^2 = 0.323, $F_{1,15}$ = 6.689, P = 0.021, N = 16, Adult females: R^2 = 0.619, $F_{1,5}$ = 6.502 , P = 0.063, N = 6).

Discussion

Population composition and protandry

We found a distinct pattern of protandry in the studied population of *Servaea incana*; males tended to mature earlier in the season than females. *Servaea incana*

females are less receptive and more aggressive towards males after their first copulation and, in the laboratory, many females only mate once during their entire life (Chapter 4). Therefore, males maturing earlier in the season will be advantaged by greater access to subadult females that they can guard and later mate with. The evolution of protandry is most readily explained by these advantages to males.

Benefits to males are not the only potential drivers of protandry, however. Protandry might also be promoted by selection on females to delay maturation and prolong development (Thornhill and Alcock 1983), such as to achieve larger size that is also commonly associated with higher fecundity (Simpson 1995). Adult and subadult females were larger later in the season (Fig. 3a,c), raising the possibility of a link between phenology and fecundity. To mature later in the season, females may pass through more juvenile instars, spend longer in juvenile instars, or both (Levy 1970; Head 1995). In *Phiddippus johnsonii*, a North American jumping spider with protandry similar to that of *S. incana* (Jackson 1978), females undergo more instars than males before completing development. Lacking reliable information of the evolutionary starting point of protandry, we are currently unable to ascribe this aspect of life history to selection for early maturation in males (e.g., for increased access to subadult females) or late maturation of females (e.g., for greater fecundity through larger size).

The pattern of protandry found in *S. incana* naturally evokes questions of mechanism. The obvious possibilities include fixed sex differences in developmental requirements or schedule. Other possibilities include seasonal variation in sex ratio of eggs of the previous season. Sex bias in egg production is known to occur in spiders

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(Gunnarsson and Andersson 1996; Avilés *et al*. 1999, 2000; Gunnarsson et al. 2004) such that protandry could result from male bias in the early batches of eggs and female bias in later batches. Social factors may also play a role. Experience of subadult females with adult males can affect female mate choice (Hebets 2003) and propensity for pre-copulatory sexual cannibalism (Johnson 2005). Subadult females could base decisions of when to moult to maturity on the presence and abundance of adult males. By this mechanism, the final stages of female development would not proceed until the female encounters a large proportion of mature males.

Body size of spiders through the year

Size of subadult and adult females increased through the year. This trend is most likely driven mainly by females maturing at the beginning of the year being smaller than females maturing later in the year. The tendency for small female size at the beginning of the season may reflect the conditions experienced during development. If developmental time is comparatively fixed, then females maturing early might be those that began life earlier in the preceding season, and might have experienced developmental conditions that promoted smaller size at maturity. If developmental time is more flexible, as is likely the case, earlier maturation might mean less development time for growth of females maturing early in the year (Zonneveld 1996). Rather than time of maturation driving size, the causal pathway could be the other way around such that females destined for small size tend to have a propensity to mature earlier regardless of conditions. Slow and fast developing individuals have been reported by in another salticid. *Phidippus jonhsoni* (Jackson 1978). If females of *S. incana* also include distinct

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fast and slow developers then the variation in female size through the year might be due to fast developers maturing early at a smaller size and slow developers maturing later at a larger size. In addition to likely effects of size-linked developmental schedule, seasonal variation in the size of female *S. incana* might also arise in part from size-linked mortality patterns.

Whereas female size tended to increase towards the end of the year, male size decreased, suggesting that males maturing at the beginning of the year tend to be larger than males maturing later. While this trend was highly significant for females, variation in size for males through the year was less pronounced ($F_{16,186} = 1.741$, $P= 0.043$, $N = 187$) compared with adult females ($F_{17,280} = 6.194$, $P<0.0001$, $N = 280$) and subadult females ($F_{13,46} = 2.345$, $P = 0.024$, $N = 47$) (see Fig. 3a, b, c). Also, unlike subadult females, size of subadult males did not change significantly with time of year (Fig. 3d, $F_{12,32} = 1.3293$, $P = 0.277$, $N = 33$). Given the pattern of protandry in *S. incana* and that early maturation most likely means less development time, we expected a positive relationship between time of year and male size similar to what was observed for females. Seasonal variation in male size is not easily explained by developmental trade-offs associated with protandry in *S. incana*.

Male size may be partially explained by links between male size and performance in male-male contests early in the season, when subadult females are most abundant. Size is a very strong predictor of outcome in contests between male salticids (Wells 1988; Taylor et al. 2001; Taylor & Jackson 2003; Hoefler 2007, McGinley et al. 2014) and is also linked generally with physical performance capacity in *S. incana*

(McGinley et al. 2013). It is likely that male size would confer ability to defend

subadult females from rival males. For example, in *Zygiella x-notata* (Araneidae) large

males were more likely to succeed in their guarding attempts and are more likely to

succeed in evicting a guarding male (Bel-Venner and Venner 2006). We have observed

males of *S. incana* evicting guarding males both in the laboratory and in the field

(Chapter 2). If size advantage in male-male contests over subadult females was one of

the drivers for the variation in size we found in *S. incana* males then we would expect to

find cohabitation to be equally prevalent for both large and small males and a relation

between size of male and subadult females in cohabiting pairs; our findings of size-

assortative cohabitation are consistent with this hypothesis.

Males might be maturing as early as possible, to take advantage of the abundance

of subadult females; and as large as possible, to win in contests, as has been previously

suggested for males of *Stegodyphus lineatus* (Maklalov et al. 2004), therefore, males of

all sizes should be present throughout the year, as we observed in the study population

(Fig. 3b). Early maturing males are males that achieved a large size, plus those males

that could not obtain enough resources to be large but moult to maturity anyway to take

advantage of the time of the year when subadult females are more abundant. This is

supported by the observation of subadult males of all sizes early in the season (Fig. 3d).

Cohabitation

Male *S. incana* were sometimes found cohabiting with subadult females at the

beginning of the season, between January and April. In salticid spiders, such

cohabitation typically lasts one to two weeks (Jackson 1986) and so represents a very sizeable investment. The scale of this investment provides a measure of the high value male salticids place on copulations with virgin females. The time males spend guarding a female could be spent searching for and potentially mating with other females. However, if previously mated females are reluctant to mate compared to virgin females, or are aggressive, then guarding a subadult female would be a more rewarding tactic in terms of fitness (Jackson 1986). In general, pre-copulatory guarding is expected in species with first-male sperm precedence and post-copulatory guarding in species with last male sperm precedence (Miyashita 1993). The sperm precedence pattern for *S. incana* is not known. Based on the pattern of protandry, remating inhibition in females, cohabitation of males with females and studies of other salticids and spiders in general, first male sperm precedence or mixing seems likely. In *Phidippus johnsoni* (Salticidae) there is mixed sperm precedence, pre-copulatory cohabitation, protandry and remating inhibition (Jackson 1978, 1980), and this general pattern may hold for a large proportion of salticids, including *S. incana*.

In salticids, males mate with the subadult female they are guarding shortly after the subadult moults to maturity (Jackson 1980; Jackson and Hallas 1986; Taylor and Jackson 1999; Hoefler 2007), and this is the case for *S. incana* (Chapter 2). Remating inhibition is strong in *S. incana*, with females showing greatly reduced receptivity and increased aggressiveness immediately after their first mating (Chapter 4). Given high levels of mating-induced sexual inhibition in *S. incana* females, a male's ejaculate is quite well defended by female unwillingness to accept competing ejaculates from rival males. After mating with newly adult females, cohabiting males are hence expected to seek additional mating opportunities elsewhere rather than continue to guard a female

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they have already mated with. Indeed, during the times of year when subadult females were most abundant we found males cohabiting with subadult females but not with adult females.

Given the low reproductive value of previously mated females (low mating propensity, presence of competing ejaculate) and lack of precedents from other salticid species, we did not expect to find adult females being guarded by males. Yet six instances of adult males cohabiting with adult (presumably mated) females were recorded. It is important to note, however, that we only found males cohabiting with adult females when subadult females were rare (January, April, May, June) (Fig. 1, 2). Cohabitation of males with adult females was not observed during February and March, when subadult females were at their peak of abundance (Fig. 1, 2). At times of the year when subadult females are scarce, the lack of preferred mating partners may make males more inclined to invest in guarding of adult females. Although mated females are generally reluctant to remate and offer a smaller paternity return than virgins, some females do remate (especially if within their nest) (Chapter 4) and so this might be the best prospect of reproductive returns at times when subadult females and virgins are not available. From our field data we cannot ascertain whether males cohabiting with adult females: (1) had previously cohabited with those females when they were subadult and were hence defending sole paternity of her offspring; (2) were cohabiting in speculation of a potential mating opportunity as the female's second mate; or (3) had already copulated as the female's second mate and were then defending their share of a mixed paternity.

Even at times when subadults were very scarce, most adult females were not attended by an adult male and most adult males were found alone. It would be interesting to consider whether the males and adult females of cohabiting pairs are in some way distinct from solitary conspecifics. For example, it might be that a comparatively high remating propensity of some mated females can be detected by males such that males are able to decide whether time invested in cohabitation with a particular females is warranted on the basis of likely payoff.

Size assortative cohabitation

Size assortative pairing has been reported in other spiders (Rubenstein 1987, Masumoto 1999, Maklakov et al. 2004), including one salticid (Hoefler 2007). In the present study we found size assortative cohabiting in *S. incana*. Size assortative cohabitation might be the result of male choice, with males having a preference for females of large size (Hoefler 2007). In *Phidippus clarus* (Salticidae)*,* Hoefler (2007) found a male preference for larger females and a large male advantage in male-male contests, and suggested that the combination of these two conditions could have resulted in size assortative pairing.

Given that we found size assortative cohabitation, it seems that all sizes of males have the chance of cohabiting with females. If large males are more likely to win in contest over mates, then smaller males may have no choice but to cohabit with smaller females (Hoefler 2007). Perhaps only large males can gain access to large and more

fertile females, while small males have to guard and mate with small females because that is what remains.

This is the first long-term study on phenology of a temperate Australian jumping spider. *S. incana* shows protandry and size-assortative cohabitation in the study population and it would be interesting to establish if the same is observed across populations. The mechanisms responsible for the pattern of protandry found in this study are unknown and future research should focus on understanding how males mature before females and the advantages that this confer to both sexes. In order to fully understand cohabitation and the pattern of size-assortative cohabitation reported here, field observations should be done on the behaviour of males and females before they start to cohabit and once cohabitation is established.

Acknowledgements

References

Andersson, MB. 1994. Sexual selection. Princeton University Press.

Avilés L, Mc Cormack J, Cutter A, Bukowski T. 2000. Precise, highly female–biased sex ratios in a social spider. Proc R Soc Lond, B. 267: 1445-1449.

Avilés L, Varas C, Dyreson E. 1999. Does the African social spider *Stegodyphus dumicola* control the sex of individual offspring? Behav Ecol Sociobiol. 46: 237-243.

Bel-Venner MC, Venner S. 2006. Mate-guarding strategies and male competitive ability in an orb-weaving spider: results from a field study. Anim Behav. 71: 1315-1322.

Bulmer MG. 1983. Models for the evolution of protandry in insects. Theor Popul Biol. 23: 314-322.

Carrière Y, McNeil JN. 1990. Effect of age and mating status on the mating behaviour and pheromone titre in alfalfa blotch leaf-miner females *Agromyza frontella* (Diptera: Agromyzidae). J Insect Physiol. 36: 457-461.

Clutton-Brock TH, Parker GA. 1992. Potential reproductive rates and the operation of sexual selection. Q Rev Biol 67:437-456.

Davies NB. 1991. Mating systems. In: Behavioral ecology: an evolutionary approach (Krebs JR, Davies NB, eds.), Oxford: Blackwell Scientific, pp 263-294.

Elias DO, Andrade MCB, Kasumovic MM. 2011. Dynamic population structure and the evolution of spider mating systems. In: Spider Physiology and Behaviour (Casas J. ed.). Adv Insect Physiol. 41: 65-114.

Emlen ST, Oring LW. 1997. Ecology, sexual selection, and the evolution of mating systems. Science. 197: 215-223.

Gunnarsson, B, Andersson A. 1996. Sex ratio variation in sheet-web spiders: options for female control? Proc R Soc Lond, B. 263: 1177-1182.

Gunnarsson B, Johnsson J. 1990. Protandry and moulting to maturity in the spider *Pityohyphantes phrygianus.* Oikos. 59: 205-212.

Gunnarsson B, Uhl G, Wallin K. 2004. Variable female mating positions and offspring sex ratio in the spider *Pityohyphantes phrygianus* (Araneae: Linyphiidae). J Insect Behav. 17: 129-144.

Head G. 1995. Selection on fecundity and variation in the degree of sexual size dimorphism among spider species (class Araneae). Evolution. 49: 776-781.

Hebets EA. 2003. Subadult experience influences adult mate choice in an arthropod: exposed female wolf spiders prefer males of a familiar phenotype. P Natl Acad Sci USA. 100: 13390-13395.

Hoefler CD. 2007. Male mate choice and size-assortative pairing in a jumping spider, *Phidippus clarus*. Anim Behav. 73: 943-954.

Hurst GD, Sharpe RG, Broomfield AH, Walker LE, Majerus TM, Zakharov IA, Majerus ME. 1995. Sexually transmitted disease in a promiscuous insect, *Adalia bipunctata*. Ecol Entomol. 20: 230-236.

Ims RA. 1988. Spatial clumping of sexually receptive females induces space sharing among male voles. Nature. 335:541–543.

Jackson RR. 1978. Life history of *Phidippus johnsoni* (Araneae, Salticidae). J Arachnol. 6: 1-29.

Jackson RR. 1980. The mating strategy of *Phidippus johnsoni* (Araneae, Salticidae): II. Sperm competition and the function of copulation. J Arachnol. 8: 217-240.

Jackson RR. 1981. Relationship between reproductive security and intersexual selection in a jumping spider *Phidippus johnsoni* (Araneae: Salticidae). Evolution. 35: 601-604.

Jackson RR. 1986. Cohabitation of males and juvenile females: a prevalent mating tactic of spiders. J Nat Hist. 20: 1193-1210.

Jackson RR, Hallas SE. 1986. Comparative biology of *Portia africana, P. albimana, P. fimbriata, P. labiata, and P. shultzi,* araneophagic, web-building jumping spiders (Araneae: Salticidae): utilisation of webs, predatory versatility, and intraspecific interactions. New Zeal J Zool. 13: 423-489.

Johnson JC. 2005. Cohabitation of juvenile females with mature males promotes sexual cannibalism in fishing spiders. Behav Ecol. 16: 269-273.

Levy G. 1970. The life cycle of *Thomisus onustus* (Thomisidae: Araneae) and outlines for the classification of the life histories of spiders. J Zool. 160: 523-536.

Maklakov AA, Bilde T, Lubin Y. 2004. Sexual selection for increased male body size and protandry in a spider. Anim Behav. 68: 1041-1048.

Masumoto T. 1994. Male emergence timing and mating success in the funnel-web spider, *Agelena limbata* (Araneae: Agelenidae). Ecol Res. 9: 159-165.

Masumoto T. 1999. Size assortative mating and reproductive success of the funnel-web spider, *Agelena limbata* (Araneae, Agelenidae). J Insect Behav. 12: 353-361.

McGinley RH, Prenter J, Taylor PW. 2013. Whole-organism performance in a jumping spider, *Servaea incana* (Araneae: Salticidae): links with morphology and between performance traits. Biol J Linn Soc. 110: 644-657.

McGinley RH, Prenter J, Taylor PW (Forthcoming 2014) Assessment strategies and decision-making in male-male contests of *Servaea incana* jumping spiders. Anim Behav.

Miyashita T. 1993. Male-male competition and mating success in the orb-web spider, *Nephila clavata*, with reference to temporal factors. Ecol Res. 8: 93-102.

Miyatake T, Chapman T, Partridge L. 1999. Mating-induced inhibition of remating in female Mediterranean fruit flies *Ceratitis capitata*. J Insect Physiol. 45: 1021-1028.

Morbey YE. 2013. Protandry, sexual size dimorphism, and adaptive growth. J Theor Biol. 339: 93-99.

Morbey YE, Ydenberg RC. 2001. Protandrous arrival timing to breeding areas: a review. Ecol Lett. 4: 663-673.

Murray AM, Cade WH. 1995. Differences in age structure among field cricket populations (Orthoptera; Gryllidae): Possible influence of a sex-biased parasitoid. Can J Zool. 73: 1207-1213.

Pröhl H. 2002. Population differences in female resource abundance, adult sex ratio, and male mating success in *Dendrobates pumilio*. Behav Ecol. 13: 175-181.

Pröhl H, Berke O. 2001. Spatial distributions of male and female strawberry poison frogs and their relation to female reproductive resources. Oecologia 129: 534-542.

Richardson BJ, Gunter NL. 2012. Revision of Australian jumping spider genus *Servaea* Simon 1887 (Aranaea: Salticidae) including use of DNA sequence data and predicted distributions. Zootaxa. 3350: 1-33.

Rubenstein DI. 1987. Alternative reproductive tactics in the spider *Meta segmentata*. Behav Ecol Sociobiol. 20: 229-237.

Shuster SM, Wade MJ. 2003. Mating systems and strategies. Princeton, New Jersey: Princeton University Press.

Simmons LW. 2001. Sperm competition and its evolutionary consequences in the insects. Princeton, New Jersey: Princeton University Press.

Simmons LW, Llorens T, Schinzig M, Hosken D, Craig M. 1994. Sperm competition selects for male mate choice and protandry in the bushcricket, *Requena verticalis* (Orthoptera: Tettigoniidae). Anim Behav. 47: 117-122.

Simpson MR. 1995. Covariation of spider egg and clutch size: the influence of foraging and parental care. Ecology. 76: 795-800.

Singer MC. 1982. Sexual selection for small size in male butterflies. Am Nat. 119: 440-443.

Taylor PW, Hasson O, Clark DL. 2001. Initiation and resolution of jumping spider contests: roles for size, proximity, and early detection of rivals. Behav Ecol Sociobiol. 50: 403-413.

Taylor PW, Jackson RR. 1999. Habitat-adapted communication in *Trite planiceps*, a New Zealand jumping spider (Araneae, Salticidae). New Zea J Zool. 26: 127-154.

Taylor PW, Jackson RR. 2003. Interacting effects of size and prior injury in jumping spider conflicts. Anim Behav. 65: 787-794.

Thornhill R, Alcock J. 1983. Evolution of Insect Mating Systems. Cambridge, Massachusetts: Harvard University Press.

Thrall PH, Antonovics J, Bever JD. 1997. Sexual transmission of disease and host mating systems: within-season reproductive success. Am Nat. 149: 485-506.

Watson PJ. 1986. Transmission of a female sex pheromone thwarted by males in the spider *Lyniphia litigiosa* (Linyphiidae). Science. 233: 219-221.

Wells MS. 1988. Effects of body size and resource value on fighting behaviour in a jumping spider. Anim Behav. 36: 321-326.

Wiklund C, Fagerström T. 1977. Why do males emerge before females? A hypothesis to explain the incidence of protandry in butterflies. Oecologia. 31: 153-158.

Wiklund C, Forsberg J. 1991. Sexual size dimorphism in relation to female polygamy and protandry in butterflies: a comparative study of Swedish Pieridae and Satyridae. Oikos. 60: 373-381.

Zonneveld C. 1996. Being big or emerging early? Polyandry and the trade-off between size and emergence in male butterflies. Am Nat. 147: 946-965.

Chapter Four: Mating-induced sexual inhibition in a jumping spider: a fast acting and long lasting effect

ABSTRACT

Female mating-induced sexual inhibition, a reduction in female sexual receptivity after a copulatory event, has been studied extensively in insects but this research area remains poorly developed in spiders. Once mated, females of many jumping spider species become unreceptive and aggressive toward males, but the persistence of this effect and speed of onset are not known. We studied the mating behaviour of *Servaea incana* females from maturation until death in two experiments: one that investigated mating tendency of virgins, latency to remating, and lifetime mating frequency, and one that investigated how quickly sexual inhibition is expressed after the first mating. Encounters between males and females took place in two different contexts that simulated locations where mating occurs in nature: within the shelter of silken retreats ('at a retreat') and away from retreats ('in the open'). Virgin females exhibited high receptivity levels in both contexts but sexual inhibition was induced immediately after their first copulation; mated females became aggressive towards their first mate and almost always rejected courtship from subsequent males. The most common tendency was for just one mating in a lifetime, and few females mated more than twice. Context also had an effect on female mating tendency. While the general pattern of reduced receptivity after mating was consistent between contexts, females in the open rejected more males before accepting their first mate than did females in retreats. Considering only those females that did remate, females in the open tended to

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reject fewer males before remating. Given low levels of female remating, virgin females are at a premium for male reproductive fitness in *S. incana* jumping spiders.

INTRODUCTION

Females of most species have more constrained reproductive potential than males such that the number of reproductive opportunities in a population is limited by the number of offspring that females can produce. Because it is difficult for females to increase fitness through number of offspring, they tend to invest in ensuring high sexual attractiveness and survival of offspring by choosing males that confer such qualities. These female mating decisions have consequences for male fitness; whereas preferred male types might fertilize many eggs of many females, this non-random mating advantage is at the expense of non-preferred males. Female decisions about how many mates to accept also have consequences for males. In some species, females may be advantaged by mating with more than one male through direct (material) resources provided by males (Richardson, Burke & Komdeur, 2002) or through indirect (genetic) benefits of 'upgrading' to a superior male or by gaining genetically diverse offspring (Newcomer, Zeh & Zeh, 1999; Fedorka & Mousseau, 2002; Klemme, Ylönen & Eccard, 2008).

Regardless of benefits for females, male fitness is threatened by female remating decisions. In species in which females are able to store sperm, a female's first mate's sperm may be displaced or diluted by subsequent mates. The presence of ejaculates from multiple males leads to sperm competition, whereby the sperm from two or more males compete for the fertilization of ova (Parker, 1970; Simmons, 2001). Given that male fitness can be severely reduced by competing ejaculates there is selection both for increased competitive ability of ejaculates and also for minimizing risks of sperm

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competition. For example, males might physically defend their mates against rival males to reduce the risk of sperm competition (Sherman, 1989; Frankino & Sakaluk, 1994; Brockerhoff & McLay, 2005), or may deposit a mating plug that impedes insemination by rivals (Dickinson & Rutowski, 1989; Masumoto, 1993; Shine, Olsson & Mason, 2000). Alternatively, a male's ejaculate can be protected from sperm competition in his absence if females are unwilling to accept subsequent suitors as mates.

Female mating-induced sexual inhibition, a reduction in female sexual receptivity after a copulatory event, has been reported for numerous insects (for reviews, see Chen, 1984; Eberhard, 1996; Ringo, 1996; Chapman, Miyatake, Smith & Partridge, 1998) and has been studied in fine detail in several insect systems, including drosophilid flies (Gromko, Newport & Kortier, 1984; Scott, 1986; Wolfner, 1997), tephritid flies (Miyatake, Chapman & Partridge, 1999; Harmer, Radhakrishnana & Taylor, 2006, Radhakrishnan & Taylor 2007, 2008) and bruchid beetles (Yamane, Miyatake & Kimura, 2008; Yamane & Miyatake, 2010). While the expression and mechanisms are far less known, mating-induced sexual inhibition has also been reported in arachnids, including ticks (Kiszewski & Spielman, 2002), and spiders of the families Agelenidae (Singer & Reichert, 1995; Masumoto, 1991; 1993), Linyphiidae (van Helsdingen, 1965; Austad, 1984; Watson, 1986), Salticidae (Jackson, 1980; Taylor & Jackson, 1999; Sivalinghem, Kasumovic, Mason, Andrade & Elias, 2010), Lycosidae (Norton & Uetz, 2005; Aisenberg & Costa, 2005; Gonzalez and Costa 2008; Jiao et al., 2011), Theridiidae (Perampaladas, Stoltz, Andrade, Schäffer & Uhl, 2005; Molina & Christenson, 2008), Tetragnathidae (Prenter, Elwood & Montgomery, 1994; Aisenberg, 2009), Pholcidae (Schäffer & Uhl, 2002, 2005), Eresidae (Maklakov, Bilde & Lubin, 2003) and Thomisidae (Dodson & Beck, 1993). Although reports of mating-induced

sexual inhibition in spiders span quite a broad taxonomic range, most studies have done little more than record the incidence of this phenomenon at the population level, and usually only over a few days following the female's first mating.

Jumping spiders (Araneae: Salticidae) are among the most studied spiders in terms of mating strategies and precopulatory courtship behaviours (Elias, Hebets, Hoy & Mason, 2005; Su & Li, 2006; Lim, Land & Li, 2007; Hoefler, 2007; Cerveira & Jackson, 2013). Jumping spiders have large 'primary' eyes that provide them acute vision (Land, 1969; Williams & McIntyre, 1980), as well as an array of 'secondary' eyes that serve as motion detectors (Land, 1971; Duelli 1978; Zurek & Nelson, 2012) and provide low acuity vision (Zurek, Taylor, Evans & Nelson, 2010). Rather than building webs, most jumping spiders are cursorial hunters that use their remarkable vision to navigate (Hoefler & Jakob, 2006) as well as to locate, identify, and pursue prey (Li, Jackson & Lim, 2003; Tarsitano & Jackson, 1992; Tarsitano, 2006; Bednarski, Taylor & Jakob, 2012). In accord with their visual abilities and active lifestyle, jumping spiders also rely on vision for communication and are best known for their elaborate courtship routines (Crane 1949; Richman 1982; Clark & Morjan, 2001; Cross, Jackson & Pollard, 2008). Jumping spider courtship routines typically entail complex postures and motions of legs, pedipalps and soma, as the males 'dance' and pursue a prospective mate and, if successful, mate in the open (Clark, 1994; Girard, Kasumovic & Elias, 2011). While jumping spiders are best known for their vision-mediated courtship routines, they are also known for their use of other sensory modalities to court females and then mate in sheltered locations or silken retreats where visual signals would be occluded by darkness or sheets of silk (Jackson & Macnab, 1989a). Given the marked differences in communication and assessment environment of these mating contexts (Jackson &

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Macnab, 1989b; Jackson & Whitehouse, 1989), there is a need to consider potential differences in efficacy of mating interactions both in terms of mating tendency and in terms of subsequent female mating decisions. *Phidippus johnsoni* (Jackson, 1981), *Trite planiceps* (Taylor & Jackson, 1999) and *Myrmarachne lupata* (Jackson, 1982) are more inclined to mate when in a sheltered location than when in the open and in *P. johnsoni* and *T. planiceps*, matings that take place in sheltered locations last longer than those that take place in the open (Jackson, 1980; Taylor & Jackson, 1999). The effects of such contextual differences in mating behaviour on subsequent female mating decisions also warrant further consideration.

Mating-induced sexual inhibition has been demonstrated in two jumping spiders; *Phidippus johnsoni* (Jackson, 1981) and *Trite planiceps* (Taylor & Jackson, 1999). As is the case for other spider taxa, all reports of mating-induced sexual inhibition in jumping spiders have only included observations made during the first days or at most weeks following a female's first mating. Given that female spiders might store and use sperm from their first mate throughout their remaining lifetime (Jackson, 1980), to understand the evolutionary significance of mating-induced sexual inhibition there is a need for studies that consider the persistence and efficacy of sexual inhibition over this time frame. Male jumping spiders typically decamp immediately after copulation ends but there are no studies of how quickly sexual inhibition is induced in females. In the present study we address both of these issues through studies of *Servaea incana* (Karsch, 1878) (= *Servaea vestita* (L. Koch 1879)), a common jumping spider in temperate regions of Australia (Richardson & Gunter, 2012).

Servaea incana is commonly associated with eucalyptus trees, where females build silken retreats in dry cavities that form under loose bark. Like many other jumping spiders, *S. incana* has a flexible courtship strategy (Chapter 2): if a male encounters an adult female in the open he courts her using elaborate visual displays; if a male encounters an adult female inside a retreat, he courts her using visual, seismic and tactile signals. In the present study, for courtship and mating that takes place in the open and at retreats we investigate (1) female mating tendency, (2) mating duration, (3) presence and persistence of mating-induced sexual inhibition in females following their first mating, and (4) how quickly mating-induced sexual inhibition is expressed.

MATERIALS AND METHODS

Spider origin and maintenance

Adult males and immature males and females of *S. incana* were collected from parks in Sydney, Australia, between December 2010 and September 2011. Collected spiders were housed individually in a controlled environment laboratory (25±0.5ºC, 65±5% RH). Lighting was a mix of metal halide and halogen. The halogen lights were set to slowly ramp up to full output over a 0.5 h dawn phase, remain on full for 12 h and then slowly ramp down again over a 30 min dusk phase (0.5 Dawn: 12 Light: 0.5 Dusk: 11 Dark). The metal halide lights were set to 12 Light: 12 Dark to coincide with the full illumination phase of the halogen lights. Spiders were housed in 1.125-L plastic cages of roughly cubic shape with a mesh covered opening on one side for ventilation. As a source of water, a 5-mL vial of water was placed in each cage. A cotton dental roll

inserted through a hole in the vial lid served as a wick that carried moisture into the cage. Jumping spiders can suffer reduced performance when maintained in simple laboratory cages, and this can be ameliorated by the inclusion of architecturally complex structure (Carducci & Jakob, 2000). As environmental enrichment, each cage was loosely filled with crumpled paper. Spiders were fed once each week with two fly pupae, alternating between Queensland fruit fly (*Bactrocera tryoni*) and housefly (*Musca domestica*). Every two weeks, spiders were transferred to clean cages, fresh paper was provided, and the vials of water and cotton wicks were replaced. Immature males (evident from enlarged terminal segments of the pedipalps) were reared through to adulthood and were then used in mating experiments along with the males that had been collected as adults.

Sex of immature *S. incana* females is readily apparent once in their penultimate instar ('subadult' stage) from the developing epigynum visible through the cuticle. At this stage, each immature female was assigned to one of two groups that would later be used in experiments that simulate encounters between adult males and females taking place in the light away from retreats ('in the open') and encounters taking place within the shelter of silken retreats that *S. incana* females construct under loose bark ('at the retreat'). Female *S. incana* to be tested in the open remained in 1.125-L maintenance cages. Female *S. incana* to be tested at the retreat were transferred to 150 mm diameter clear plastic Petri dishes. To simulate the typical site of shelters in nature, each Petri dish contained a shelter comprising a 5 x 2 cm sheet of brown paper folded to the shape of a tent. As a source of moisture, each Petri dish contained a 5-mL vial of water with a cotton wick. Spiders always moulted inside silken retreats that they constructed inside the shelter. In nature, nest sites may be occupied for weeks or months, leading to

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accumulation of silk and chemical cues. To maintain these cues, spiders maintained for tests at the retreat were not transferred to clean Petri dishes during the experimental period, but the remains of dead flies and excess silk were removed and the vials of water and cotton wicks were replaced every two weeks.

Experiment 1: Lifetime mating tendency

The aim of this experiment was to determine for each context (1) female mating tendency, (2) mating duration, (3) induction of sexual inhibition in females following their first mating, (4) whether loss of a brood increases female remating tendency, and (5) lifetime mating frequency. To ensure that all adult females used in these experiments were virgin and of known age for the first mating opportunity, subadult females maintained in 1.125-L cages (for trials 'in the open') and in Petri dishes (for trials 'at the retreat') were monitored every day until they moulted to maturity. Virgin females participated in their first mating trial on the day after they moulted to maturity. Each adult female was exposed to a different male of unknown mating status, every day for the first ten days of adult life and then every ten days for the rest of their lives.

Trials of matings in the open were run in a circular white arena. A clear acrylic cylinder (150 mm diameter, 120 mm high) was positioned over a 180 mm diameter disk of white plastic that was mounted on a tripod. So that spiders were not distracted by movement in the surroundings, the cylinder walls were covered with white paper, leaving a 10 mm gap at the bottom for video recording of interactions at floor level (Sony HDC HS700 High Definition camcorder with supplementary close-up lenses).

An opaque white plastic barrier was positioned across the middle of the arena, dividing the arena into halves. A male *S. incana* was guided gently out of its cage with a soft paintbrush and released on one side of the barrier. A female was released on the other side of the barrier. The spiders were left in the arena for three minutes to settle down before the trial was started by removing the barrier. If the pair did not mate within 10 min after removing the barrier, the trial ended. If the pair mated, the trial ended when the male dismounted and the pair separated. Fifty-four female *S. incana* were tested in this context.

Trials of matings at the retreat were run in the Petri dishes containing a female in her silken retreat, constructed in the provided shelter. Trials started when a male was gently coaxed out of its cage with a soft paintbrush and released into the Petri dish, and were video recorded in high definition for later analysis (Sony HDC HS700). If the pair did not mate within one hour the trial ended and the male was removed. If the pair mated then the entire copulation was video recorded through the opening of the shelter and the trial ended when the male dismounted and decamped from the female's retreat. Thirty-five female *S. incana* were tested in this context.

After they died, tested spiders were preserved in 70% ethanol. After preservation spiders were photographed using a ProgResC10 digital camera (Jenoptik LOS GmbH, Germany), focussed through an Olympus SZX12 dissecting microscope (Olympus Corporation, Tokyo, Japan). Cephalothorax width, a commonly used measure of spider size (Taylor & Jackson, 1999; Taylor, Hasson & Clark, 2001), was measured from digital images using ImageJ 1.36b (National Institute of Health, Bethesda, MD, USA).

Experiment 2: Timing of sexual inhibition onset

Having established the lifetime effects of mating-induced sexual inhibition in experiment 1, in experiment 2 we investigated how quickly following the first mating sexual inhibition is induced in *S. incana*. As in experiment 1, trials were carried out in the open and at retreats using virgin females that moulted to maturity the day before testing. All trials were video recorded, using a high definition video camera (Sony HDC HS700).

Thirty-seven females were used in this experiment, 22 in the open and 15 at the retreat. Each virgin female was sequentially provided the opportunity to mate with three males of unknown mating status. Only females that mated in the first trial were used in subsequent trials. The general approach for this experiment was the same as for Experiment 1 (see above for details). Immediately after the first mating finished, the male was removed. For trials in the open, the arena was wiped clean with water-moistened paper towel to remove silk and polar pheromones (Jackson, 1987) left by the previous male. For trials at the retreat, the Petri dish was wiped clean but cues inside the retreat remained.

Immediately after cleaning the arena a second male was released. There was a latency of approximately five minutes between the end of the first trial and the start of the second trial. If the pair did not mate within one hour the trial ended and the male was removed. If the pair mated, the trial ended when the male dismounted and the pair separated. Males were then removed from the arena. Three hours after the end of the

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second trial, the Petri dish around the retreat was wiped clean and each female was provided a third mating opportunity. The third trial was run to see if females remained unreceptive even after three hours of their first mating. In trials with spiders in the open and at the retreat, females remained in the arena continuously from the beginning of the first trial until the end of the second trail. At the end of the second trial, females used in trials in the open were returned to their cages until the start of the third trial, while females used in trials at the retreat stayed in the Petri dish that contained their retreat.

We used Wilcoxon signed-rank tests to compare females tested in the open with females tested in retreats with respect to the following dependent variables: a- the number of males rejected before accepting a first mating, b- the duration of mounting during the first mating, c- the number of males rejected before remating, d- the number of rematings over a lifetime. A Spearman's rank correlation was used to investigate if: a- the number of males rejected before first matings was related to female size in either context, b- the duration of mounting with virgin females was related to female size, male size or mating latency in either context, c- the number of times a female remated over a lifetime was related to size of the female or size of her first mate in either context, d- the number of males rejected before remating was related to female size or the size of her first mate in either context. Logistic regressions were ran to test if tendency to remate at least once over a lifetime varied with: a- context and b- the size of the female or her first mate in either context. We used Fisher's exact test to compare between context with respect to: a- the number of females that mated with the first male they encountered, b- the number of females that mated with one of the two first males they encountered and c- the number of females that mated after rejecting more than two males.

RESULTS

Experiment 1: Lifetime mating tendency

Of the 54 females tested in the open and 35 females tested at the retreat, all except three females mated at least once (Figure 1). All of the females that did not mate had been tested in the open, and had been exposed to 3, 11, and 14 males before they died.

Females tested in the open rejected a significantly greater number of males before accepting a mate than did females tested in retreats (N = 85, Z = -3.748, P = 0.0002; Figure 1). In trials carried out in the open 42% of females mated with the first male they encountered and 85% females tested at retreats mated with the first male they encountered (Fisher's exact test, P < 0.0001). In trials carried out in the open 66% of females mated with one of the first two males they encountered and 91% of females tested at retreats mated with one of the first two males they encountered (Fisher's exact test, P < 0.005). The remaining virgin females tested in the open (31%) encountered a greater number of males (up to 20) before accepting their first mate while all of the remainder females tested in the retreat (8%) mated with the third or the fourth male they encountered (Fisher's exact test, P < 0.0178).
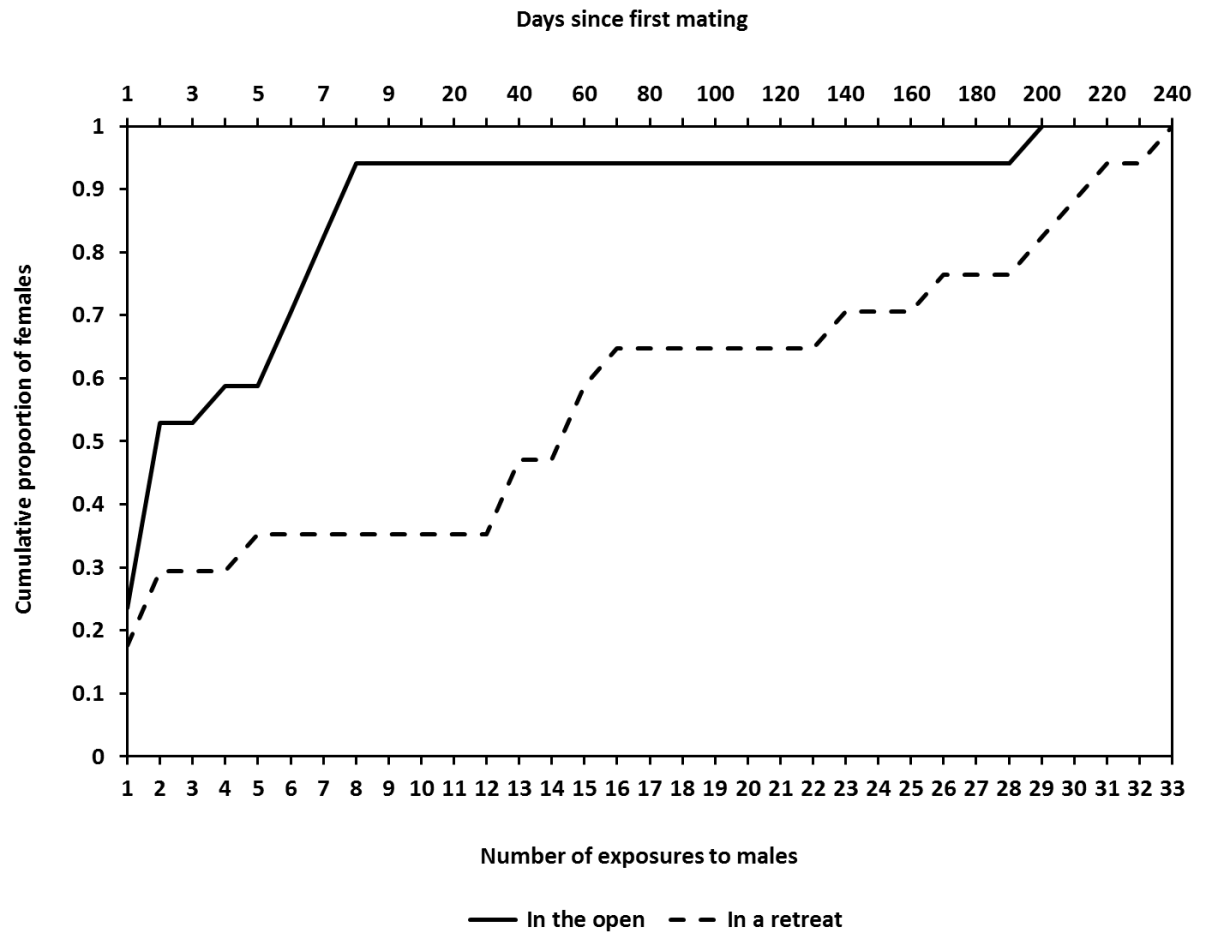
Days since first exposure
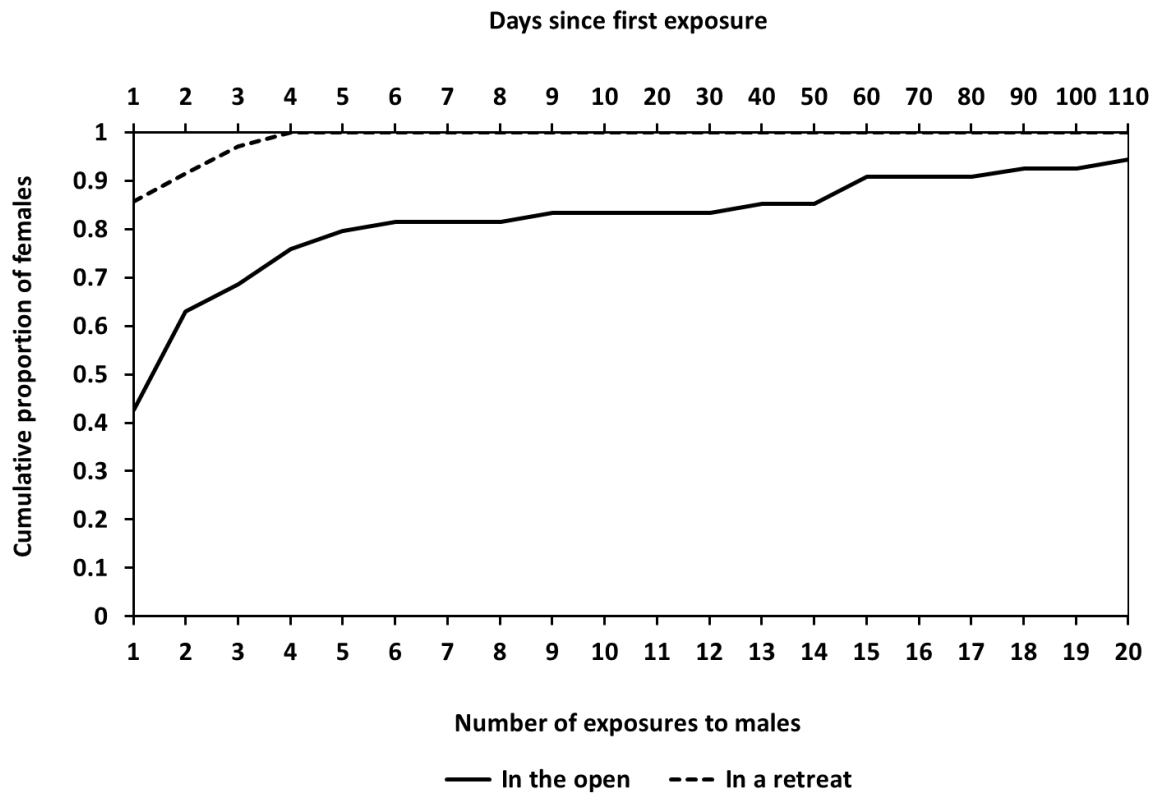
Figure 1. Cumulative proportion of female *Servaea incana* that mated following repeated exposure to males (a) in the open (N=54) or (b) in a retreat (N=35).

The number of times virgin females rejected males before mating was also related to female size, but in a context-specific manner. For trials carried out in the open there was a significant tendency for larger females to reject fewer males before mating than was the case for smaller females ($r_s = -0.426$, $P = 0.006$) but for trials carried out at the retreat there was no evidence of relationship between female size and number of males rejected before first mating ($r_s = 0.213$, $P = 0.277$).

Males remained mounted for a shorter duration when mating with virgin females in the open (N = 51, Median = 1209 s, Q_1 = 912, Q_3 = 1732, range 144 - 4891) than when in a retreat (N = 35, Median = 2433 s, Q_1 = 1885, Q_3 = 3569, range 1078 - 8295) (Z = 5.239, P < 0.001). The duration of mounting was not related to female size, male size or mating latency in either context (Spearman's rank correlation, for all P > 0.2).

Of the 51 females that had mated as virgins in the open, 17 (33%) remated at least once in their lifetime. Of the 35 females that had mated as virgins in a retreat, 17 (49%) remated at least once in their lifetime (Figure 2). Tendency to remate at least once over a lifetime did not differ significantly between the mating contexts (Logistic regression, G_1 = 2.010, N = 86, P = 0.156). Tendency to remate at least once over a lifetime also did not vary significantly with size of female or the size of her first mate in either context (Logistic regression, for all P > 0.5). Similarly, the number of times that females remated over a lifetime (Figure 3) did not differ significantly between the mating contexts (Z = 1.437, N = 86, P = 0.151), with size of the female or the size of her first mate in either context (Spearman's rank correlations, for all P > 0.2).

Figure 2. Cumulative proportion of once-mated female *Servaea incana* that remated following repeated exposure to males (a) in the open (N=17) or (b) in a retreat (N=17).
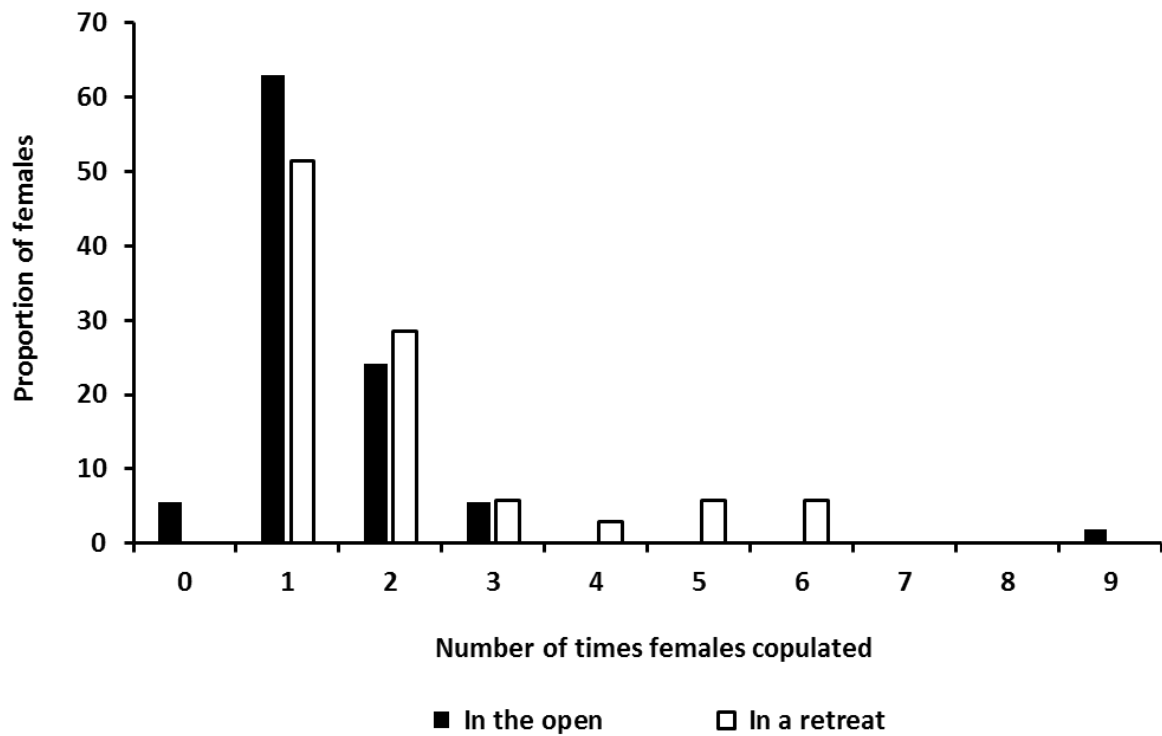
Figure 3. Number of times that female *Servaea incana* mated over a lifetime of repeated

exposure to males (a) in the open (N=54) or (b) in a retreat (N=35).

Although lifetime remating tendency did not differ between contexts, amongst

those females that did remate at least once the duration of sexual inhibition did differ.

Once-mated females tended to reject fewer males before remating when tested in the

open (N = 17, Median = 2, Q_1 = 1.5, Q_3 = 7, range 1 - 29) than when tested at a retreat

(N = 17, Median = 13, Q_1 = 2, Q_3 = 24.5, range 1 - 33) but the difference between the

two groups was marginally significant (Z = -1.929, N = 34, P = 0.05). Number of males

rejected by females before remating did not vary significantly with size of female or the

size of her first mate in either context (Spearman's Rank correlations, for all P > 0.6).

Experiment 2: Timing of sexual inhibition onset

Sexual inhibition of females was evident immediately following the female's first mating in both contexts. Of the 22 virgin females that mated in the open, none remated immediately after their first mating and only one remated three hours later giving an overall count of 1 female (4.5%) remating when provided two further opportunities on the day of their first mating. Of the 15 virgin females that mated in retreats, three remated immediately after their first mating and none remated three hours later giving an overall count of 3 females (20%) remating when provided two further opportunities on the day of their first mating.

DISCUSSION

Lifetime mating tendency

Receptivity over the entire lifespan of female spiders has not been studied previously. In the present study we report the number of times that female *S. incana* jumping spiders mated over a lifetime, and the occurrence and latency of sexual inhibition. *Servaea incana* females were mostly unreceptive immediately after their first copulation and for many this mating-induced sexual inhibition persisted for the entire remaining life of the spider (Fig. 2, 3). Most females mated only once or twice in a lifetime.

Mating-induced sexual inhibition in females may reflect the interests of either sex, but is most often considered from the male perspective, perhaps because the benefits for males are easier to identify (Arnqvist & Nilsson, 2000). For males at risk of sperm competition, an ability to induce sexual inhibition in mates would increase the effectiveness with which his ejaculates convert to fertilizations (Parker, 1970). Moreover, because the onset of sexual inhibition occurs immediately after the first mating in *S. incana*, there is no need for males to invest in mate guarding to prevent matings by rival males. This means that males can leave immediately after mating to search for more mating opportunities. In the field, *S. incana* males can be found wandering around on the trunks of trees (Chapter 2) and therefore it is likely that females often encounter other males soon after their first mating. In *Gasteracantha minax* orb-weaving spiders, mating-induced sexual inhibition is only expressed in females after 1 to 24 hours and males guard females during this post-mating period of receptivity (Elgar & Bathgate, 1996). Through rapid induction of sexual inhibition in females, *S. incana* males avoid this expense.

For *S. incana* males, virgin females are a far more valuable resource than are mated females; virgin females are more likely to mate, do not contain sperm from rival males, and are less aggressive. Under these conditions, male strategies designed to maximise access to virgin females are anticipated. In nature, males jumping spiders are commonly found cohabiting with juvenile females that just need one moult to become adult ('subadult females') (Jackson, 1982; Jackson, 1986; Taylor & Jackson, 1999; Hoefler, 2007). After as much as several weeks of cohabitation, these males mate when the subadult female matures (Jackson, 1986). The substantial amount of time that males dedicate to cohabiting provides an indication of the premium placed on virgin females.

In addition to benefits for males, mating-induced sexual inhibition can also be driven by benefits to females. Differences in mating tendency of virgin and mated females may represent changes in mating preferences of females that relate to reproductive security. While it might be important for females to have sperm from the best possible mate to fertilize her eggs, it is also important that females balance this benefit against the risk of failing to mate at all if they are overly discriminating or insufficiently receptive. A first mate is necessary for reproductive security, and so high receptivity levels of *S. incana* virgins might be interpreted as relaxation of mate preference criteria to ensure that females accept at least one mate. Such relaxation of criteria may be through a general lowering of thresholds for acceptance or may be through a less strict application of the same criteria used once mated. This possibility has been raised previously by Jackson (1981) in reference to another jumping spider, *P. johnsoni* that also exhibits high levels of mating-induced sexual inhibition. Once mated, however, females are released from the risk of outright reproductive failure and can turn their attention to selecting high quality mates. If this is the case then we would anticipate that those females that never remated tended to be those that had mated with a preferred male type when virgin, whereas those that did remate, and especially those that remated early, were those that would benefit from an 'upgrade'.

Male courtship persistence might also explain the reluctance of mated females to remate. Mated females might be evaluating and choosing males based on courtship persistence, those males that invest more time in courting might be the ones accepted by mated females. Male performance during courtship and copulation has been found to affect the remating decisions of females in the orb weaving spider *Leucauge mariana* (Aisenberg 2009). Similarly, courtship duration affects the receptivity to future matings

of female redback spiders *Latrodectus hasselti* (Stoltz et al 2009). Redback females are less likely to remate when the first male court for longer periods of time.

It is unclear at this time whether mating-induced sexual inhibition of jumping spiders represents a general sexual inhibition that applies regardless of male quality or instead represents a sharp increase in mate discrimination such that whereas most males can mate with a virgin only high quality males can mate with previously mated females. At this time, male traits associated with female preferences are poorly understood in jumping spiders. It may also be difficult to disentangle male and female influences. If ability to induce sexual inhibition is a key predictor of male mating success then it would be adaptive for females to fertilise their eggs with sperm from males that are more able to induce persistent sexual inhibition, if that trait is inherited by the male's offspring.

Context- and size-dependent mating behaviour

Matings inside retreats entailed longer periods of mounting than was observed for matings in the open. Inside the retreat the copulating pair is more protected and this might have influenced willingness of both the male and the female to remain mounted for longer. Silken retreats constructed by the female would contain odours (Jackson, 1987) that influence behaviour. Longer matings in the dark have also been reported for virgin females in the salticids *Phidippus johnsoni* (Jackson 1980) and *Trite planiceps* (Taylor & Jackson, 1999), as well as in tetragnathid (Eberhard & Huber, 1998; Danielson-François & Budkowski, 2005) and pholcid (Huber & Eberhard, 1997)

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spiders. At this time, it is unclear to what extent mating duration is an expression of male and female influences.

We did not find any evidence of a link between duration of mounting, context of copulation, male size and female size in female first copulation or in female remating tendency. However, we did find that females in the open rejected more males before their first mating than was the case for females in retreats. In nature, subadult females would commonly moult to maturity and copulate for the first time inside their retreat with a co-habiting male. In accord with this natural system, virgin females inside the retreat may show high receptivity because the male we provided serves the role of a male that would have cohabited with the female had she been in nature. Females in the open might be choosier before their first mating because they have not been cohabiting with the males they encounter and, in this context, they have the opportunity of assessing and choosing between the males. Copulating in the open is associated with a higher risk of attack by visually orienting predators (Jackson, 1976; Jackson, 1980; Taylor & Jackson, 1999) and this likely explains the lower mating tendency of females in this context.

After mating once, females tested in the open rejected fewer males before they remated than was the case for females at retreats. Females tested in the open might be remating sooner than females tested at retreats as a result of copulations in the open being less effective in inducing a long lasting sexual inhibition. We know that duration of mounting was not related to the probability of a female remating, therefore other characteristics of the copula, such as male performance, might be affecting the

effectiveness of the mating-induced sexual inhibition. Male performance during courtship and copulation in the open might be deficient compare to male performance at retreats and subsequently females that copulate in the open remate sooner. Male performance during courtship and copulation have been found to affect the remating decisions of females in the orbweaving spider *Leucauge mariana* (Aisenberg 2009) and *Schizocosa malitiosa* (Gonzalez and Costa 2008).

In the open, larger females tended to reject fewer males before mating, a tendency that was not found in trials at the retreat. In salticids, encounters that occurred in the open are more dependent on visual communication than are encounters that occurred inside retreats (Jackson, 1977). Males of *S. incana* might obtain more information about the size of the females in the open. A large size in female spiders is commonly associated with higher fecundity and males of some other spider species appear to show a preference for mating with larger and more fecund females (Metidae, Prenter, Elwood & Montgomery, 1994; Salticidae, Hoefler, 2007; Cross, Jackson & Pollard, 2008; Araneidae, Bel-Venner, Dray, Allainé, Menu, & Venner, 2008). If males of *S. incana* prefer larger females, then they might court large females more vigorously and persistently, and consequently large females will be more willing to mate in the open even at a higher risk of predation. In *Nephila clavipes* males preferentially cohabit with large females (Vincent & Lailvaux, 2006) and courtship displays are more vigorous when males court larger females (Christenson, Brown, Wenzl, Hill & Goist, 1985).

The mechanisms responsible for mating-induced sexual inhibition in females of *S. incana* are unknown, but as with insects and ticks, physical stimulus during

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copulation as well as sperm and substances in the seminal fluid transferred with the sperm during copulation might be involved (for reviews, see Chen, 1984; Eberhard, 1996; Simmons, 2001; Gillot, 2003). In *Schizocosa malitiosa* wolf spiders, females that have copulated with spermless males did not show a reluctance to mate afterwards and accepted all males presented to them (Aisenberg & Costa 2005). Given that all other stimuli during copulation were preserved in this study, the authors suggested that, as in many insects, substances in the seminal fluids are responsible for mating-induced sexual inhibition. Proteinaceous secretions have been found in the genital tract of the male spider *Pholcus phalangoides* and it has been suggested that these secretions are transferred as part of the ejaculate and then act on female physiology and receptivity after mating (Michalik & Uhl, 2005). Michalik et al (2013) also found secretory vesicles in the seminal fluid of the wolf spider *S. malitiosa*. The authors hypothesised that these vesicles could be responsible for secretions that inhibit female receptivity. However, the function of the secretions remains to be investigated.

The present study provides the foundations for further research on the mechanisms involved in mating-induced sexual inhibition in females of *S. incana,* and the implications of this inhibition for the reproductive strategies of females and males.

ACKNOWLEDGEMENTS

REFERENCES

Aisenberg, A. (2009). Male performance and body size affect female re-mating occurrence in the orb-web spider *Leucauge mariana* (Araneae, Tetragnathidae). *Ethology*, *115*, 1127-1136.

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.

Austad, S. N. (1984). Evolution of sperm priority patterns in spiders. In: R.L. Smith (Ed.), *Sperm competition and the evolution of animal mating systems.* (pp. 233-249). London: Academic Press.

Bednarski, J. V., Taylor, P. W. & Jakob, E. M. (2012). Optical cues used in predation by jumping spiders, *Phidippus audax* (Araneae). *Animal Behaviour*, *84*, 1221-1227.

Bel-Venner, M. C., Dray, S., Allainé, D., Menu, F. & Venner, S. (2008). Unexpected male choosiness for mates in a spider. *Proceedings of the Royal Society B*, *275*, 77-82.

Brockerhoff, A. M. & McLay, C. L. (2005). Mating behaviour, female receptivity and male-male competition in the intertidal crab *Hemigrapsus sexdentatus* (Brachyura: Grapsidae). *Marine Ecology Progress Series*, *290*, 179-191.

Carducci, J. P. & Jakob, E. M. (2000). Rearing environment affects behaviour of jumping spiders. *Animal Behaviour*, *59*, 39-46.

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Cerveira, A. M. & Jackson, R. R. (2013). Love is in the air and on the ground: olfactory and tactile cues elicit visual courtship behavior by *Cyrba* males (Araneae: Salticidae). *Journal of Arachnology*, *41*, 374-380.

Chapman, T., Miyatake, T., Smith, H. & Partridge, L. (1998). Interactions of mating, egg production and death rates in Mediterranean fruit fly, *Ceratitis capitata* females. *Proceedings of the Royal Society B*, *265*, 1879-1894.

Chen, P. S. (1984). The functional morphology and biochemistry of insect male accessory glands and their secretions. *Annual Review of Entomology*, *29*, 233-255.

Christenson, T. E., Brown, S.G., Wenzl, P. A., Hill, E. M. & Goist, K. C. (1985). Mating behavior of the golden-orb-weaving spider, *Nephila clavipes* 1: Female receptivity and male courtship. *Journal of Comparative Psychology*, *99*, 160-166.

Clark, D. L. (1994). Sequence analysis of courtship behavior in the dimorphic jumping spider *Maevia inclemens* (Araneae, Salticidae). *Journal of Arachnology*, *22*, 94-107.

Clark, D. L. & Morjan, C. L. (2001). Attracting female attention: the evolution of dimorphic courtship displays in the jumping spider *Maevia inclemens* (Araneae: Salticidae). *Proceedings of the Royal Society B*, *268*, 2461-2465.

Crane, J. (1949). Comparative biology of salticid spiders at Rancho Grande, Venezuela. Part IV. An analysis of display. *Zoologica*, *34*, 159-214.

Cross, F. R., Jackson, R. R. & Pollard, S. D. (2008). Complex display behaviour of *Evarcha culicivora*, an East African mosquito-eating jumping spider. *New Zealand Journal of Zoology*, *35*, 151-187.

Danielson-François, A. M. & Bukowski T. C. (2005). Female mating history influences copulation behavior but not sperm release in the orb-weaving spider *Tetragnatha versicolor* (Araneae, Tetragnathidae). *Journal of Insect Behavior*, *18*, 131–148.

Dickinson, J. L. & Rutowski, R. L. (1989). The function of the mating plug in the Chalcedon checkerspot butterfly. *Animal Behaviour*, *38*, 154-162.

Dodson, G. N. & Beck, M. W. (1993). Pre-copulatory guarding of penultimate females by male crab spiders, *Misumenoides formosipes*. *Animal Behaviour*, *46*, 951-959.

Duelli, P. (1978). Movement detection in the posterolateral eyes of jumping spiders (*Evarcha arcuata*, Salticidae). *Journal of Comparative Physiology*, *124*, 15-26.

Eberhard, W. G. (1996). *Female control: sexual selection by cryptic female choice.* Princeton, New Jersey: Princeton University Press. 472 pp.

Eberhard, W. G. & Huber, B. A. (1998). Courtship, copulation, and sperm transfer in *Leucauge mariana* (Araneae, Tetragnathidae) with implications for higher classification. *Journal of Arachnology*, *26*, 342-368.

Elias, D. O., Hebets, E. A., Hoy, R. R. & Mason, A. C. (2005). Seismic signals are crucial for male mating success in a visual specialist jumping spider (Araneae: Salticidae). *Animal Behaviour*, *69*, 931-938.

Elgar, M. A. & Bathgate, R. (1996). Female receptivity and male mate-guarding in the jewel spider *Gasteracantha minax* thorell (Araneidae). *Journal of Insect Behavior*, *9*, 729-738.

Fedorka, K. M. & Mousseau, T. A. (2002). Material and genetic benefits of female multiple mating and polyandry. *Animal Behaviour*, *64*, 361-367.

Frankino, W. A. & Sakaluk, S. K. (1994). Post-copulatory mate guarding delays promiscuous mating by female decorated crickets. *Animal Behaviour*, *48*, 1479-1481.

Gillot, C. (2003). Male accessory gland secretions: Modulators of female reproductive physiology and behavior. *Annual Review of Entomology*, *48*, 163–184.

Girard, M. B., Kasumovic, M. M. & Elias, D. O. (2011). Multi-modal courtship in the peacock spider, *Maratus volans* (OP-Cambridge, 1874). *PloS ONE*, *6*, e25390.

Gonzalez, M. & Costa, F. G. (2008). Persistence of sexual reluctance in mated females and the importance of regular copulation in a wolf spider. *Ethology Ecology & Evolution*, *20*, 115-124.

Gromko, M. H., Newport, M. E. & Kortier, M. G. (1984). Sperm dependence of female receptivity to remating in *D. melanogaster. Evolution*, *38*, 1273–1282.

Harmer, A. M. T, Radhakrishnana, P. & Taylor, P. W. (2006). Remating inhibition in female Queensland fruit flies: Effects and correlates of sperm storage. *Journal of Insect Physiology*, *52*, 179-186.

Hoefler, C. (2007). Male mate choice and size-assortative pairing in a jumping spider, *Phidippus clarus*. *Animal Behaviour*, *73*, 943-954.

Hoefler, C. D. & Jakob, E. M. (2006). Jumping spiders in space: movement patterns, nest site fidelity and the use of beacons. *Animal Behaviour*, *71*, 109-116.

Huber, B. A. & Eberhard, W. G. (1997). Courtship, copulation, and genital mechanics in *Physocyclus globosus* (Araneae, Pholcidae). *Canadian Journal of Zoology*, *75*, 905-918.

Jackson, R. R. (1976). Predation as a selection factor in the mating strategy of the jumping spider *Phidippus johnsoni* (Salticidae, Araneae). *Psyche*, *83*, 243-255.

Jackson, R. R. (1977). Courtship versatility in the jumping spider, *Phidippus johnsoni* (Araneae: Salticidae). *Animal Behaviour*, *25*, 953-957.

Jackson, R. R. (1980). The mating strategy of *Phidippus johnsoni* (Araneae: Salticidae): II. Sperm competition and the function of copulation. *Journal of Arachnology*, *8*, 217-240.

Jackson, R. R. (1981). Relationship between reproductive security and intersexual selection in a jumping spider *Phidippus johnsoni* (Araneae: salticidae). *Evolution*, *35*, 601-604.

Jackson, R. R. (1982). The biology of ant-like jumping spiders: intraspecific interactions of *Myrmarachne lupata* (Araneae, Salticidae). *Zoological Journal of the Linnean Society*, *76*, 293-319.

Jackson, R. R. (1986). Cohabitation of males and juvenile females: a prevalent mating tactic of spiders. *Journal of Natural History*, *20*, 1193-1210.

Jackson, R. R. (1987). Comparative study of releaser pheromones associated with the silk of jumping spiders (Araneae, Salticidae). *New Zealand Journal of Zoology*, *14*, 1-10.

Jackson, R. R. & Macnab, A. M. (1989a). Display, mating, and predatory behaviour of the jumping spider *Plexippus paykulli* (Araneae: Salticidae). *New Zealand Journal of Zoology*, *16*, 151-168.

Jackson, R. R. & Macnab, A. M. (1989b). Display behaviour of *Corythalia canosa*, an ant-eating jumping spider (Araneae: Salticidae) from Florida. *New Zealand Journal of Zoology*, *16*, 169-183.

Jackson, R. R. & Whitehouse, M. E. A. (1989). Display and mating behaviour of *Thorellia ensifera*, a jumping spider (Araneae: Salticidae) from Singapore. *New Zealand Journal of Zoology*, *16*, 1-16.

Jiao, X., Guo, L., Che, Z., Wu, J., Che, J., Liu, F. & Li, D. (2011). Experimental evidence   for female-driven monandry in the wolf spider, *Pardosa astrigera. Behavioral Ecology and Sociobiology*, *65*, 2117-2123.

Kiszewski, A. E. & Spielman, A. (2002). Preprandial inhibition of re-mating in *Ixodes* ticks (Acari: Ixodidae). *Journal of Medical Entomology*, *39*, 847-53.

Klemme, I., Ylönen, H. & Eccard, J. A. (2008). Long-term fitness benefits of polyandry in a small mammal, the bank vole *Clethrionomys glareolus*. *Proceedings of the Royal Society B*, *275*, 1095-1100.

Land, M. F. (1969). Structure of the retinae of the principal eyes of jumping spiders (Salticidae: Dendryphantinae) in relation to visual optics. *Journal of Experimental Biology*, *51*, 443-470.

Land, M. F. (1971). Orientation by jumping spiders in the absence of visual feedback. *Journal of Experimental Biology*, *54*, 119-139.

Li, D., Jackson, R. R. & Lim, M. L. (2003). Influence of background and prey orientation on an ambushing predator's decisions. *Behaviour*, *140*, 739-764.

Lim, M. L., Land, M. F. & Li, D. (2007). Sex-specific UV and fluorescence signals in jumping spiders. *Science*, *315*, 481-481.

Maklakov, A. A., Bilde, T. & Lubin, Y. (2003). Vibratory courtship in a web-building spider: Signalling quality or stimulating the female? *Animal Behaviour*, *66*, 623–630.

Masumoto, T. (1991). Males' visits to females' webs and female mating receptivity in the spider *Agelena limbata* (Araneae Agelenidae). *Journal of Ethology*, *9*, 1-7.

Masumoto, T. (1993). The effect of the copulatory plug in the funnel-web spider, *Agelena limbata* (Araneae:Agelenidae). *Journal of Arachnology*, *21*, 55-59.

Michalik, P. & Uhl, G. (2005). The male genital system of the cellar spider *Pholcus phalangioides* (Fuesslin, 1775) (Pholcidae, Araneae): development of spermatozoa and seminal secretion. *Frontiers in Zoology*, *2*, 1-12.

Michalik, P., Aisenberg, A., Postiglioni, R. & Lipke, E. (2013). Spermatozoa and spermiogenesis of the wolf spider *Schizocosa malitiosa* (Lycosidae, Araneae) and its functional and phylogenetic implications. *Zoomorphology*, *132*, 11-21.

Miyatake, T., Chapman, T. & Partridge, L. (1999). Mating-induced inhibition of remating in female Mediterranean fruit flies *Ceratitis capitata. Journal of Insect Physiology*, *45*, 1021-1028.

Molina, Y. & Christenson, T. (2008). Effects of mating experience on subsequent reproductive behaviour and sperm release in the spider *Nesticodes rufipes*. *Animal Behaviour*, *76*, 1327-1334.

Newcomer, S. D., Zeh, J. A. & Zeh, D. W. (1999). Genetic benefits enhance the reproductive success of polyandrous females. *Proceedings of the National Academy of Sciences*, *96*, 10236-10241.

Norton, S. & Uetz, G. W. (2005). Mating frequency in *Schizocosa ocreata* (Hentz) wolf spiders: evidence for a mating system with female monandry and male polygyny. *Journal of Arachnology*, *33*, 16-24.

Parker, G. A. (1970). Sperm competition and its evolutionary consequences in the insects. *Biological Reviews*, *45*, 525-567.

Perampaladas, K., Stoltz, J. A., Andrade, M. C. B., Schäffer, M. A. & Uhl, G. (2005). Sequential mate encounters: female but not male body size influences female remating behaviour. *Behavioral Ecology*, *26*, 127-154.

Prenter, J., Elwood, R. W. & Montgomery, W. I. (1994). Assessment and decisions in *Metellina segmentata* (Araneae: Metidae): evidence of a pheromone involved in mate-   guarding. *Behavioural Ecology and Sociobiology*, *35*, 39-43.

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.

Radhakrishnan, P. & Taylor, P. W. (2008). Ability of male Queensland fruit flies to inhibit receptivity in multiple mates, and the associated recovery of accessory glands. *Journal of Insect Physiology*, *54*, 421-428.

Richardson, B. J. & Gunter, N. L. (2012). Revision of Australian jumping spider genus *Servaea* Simon 1887 (Aranaea: Salticidae) including use of DNA sequence data and predicted distributions. *Zootaxa*, *3350*, 1-33.

Richardson, D. S., Burke, T. & Komdeur, J. (2002). Direct benefits and the evolution of female-biased cooperative breeding in Seychelles warblers. *Evolution*, *56*, 2313-2321.

Richman, D. B. (1982). Epigamic display in jumping spiders (Araneae, Salticidae) and its use in systematics. *Journal of Arachnology*, *10*, 47-67.

Ringo, J. (1996). Sexual receptivity in insects. *Annual Review of Entomology*, *41*, 473-494.

Schäfer, M. A & Uhl, G. (2002). Determinants of paternity success in the spider *Pholcus phalangioides* (Pholcidae: Araneae): The role of male and female mating behaviour. *Behavioral Ecology and Sociobiology*, *51*, 368–377.

Schäfer, D. & Uhl, G. (2005). Sequential mate encounters: female but not male body size influences female remating behavior. *Behavioral Ecology*, *16*, 461-466.

Scott, D. (1986). Inhibition of female *Drosophila melanogaster* remating by a seminal fluid protein (esterase 6). *Evolution*, *40*, 1084-1091.

Sherman, P. W. (1989). Mate guarding as paternity insurance in Idaho ground squirrels. *Nature*, *338*, 418-420.

Shine, R., Olsson, M. M. & Mason, R. T. (2000). Chastity belts in gartersnakes: the functional significance of mating plugs. *Biological Journal of the Linnean Society*, *70*, 377-390.

Simmons, L. W. 2001. Sperm competition and its evolutionary consequences in the insects. Princeton University. Press, Princeton, NJ. 434 pp.

Singer, F. & Riechert, S. E. (1995). Mating system and mating success of the desert spider *Agelenopsis aperta*. *Behavioral Ecology and Sociobiology, 36*, 313-322.

Sivalinghem, S., Kasumovic, M. M., Mason, A. C., Andrade, M. C. B. & Elias, D. O. (2010). Vibratory communication in the jumping spider *Phidippus clarus*: polyandry, male courtship signals, and mating success. *Behavioral Ecology*, *21*, 1308-1314.

Stoltz, J.A., Elias, D.O. & Andrade, M.C.B. 2009. Male courtship effort determines female response to competing rivals in redback spiders. *Animal Behaviour, 77*, 79-85.

Su, K. F. & Li, D. (2006). Female-biased predation risk and its differential effect on the male and female courtship behaviour of jumping spiders. *Animal Behaviour*, *71*, 531-537.

Tarsitano, M. (2006). Route selection by a jumping spider *Portia labiata* during the locomotory phase of a detour. *Animal Behaviour*, *72*, 1437-1442.

Tarsitano, M. S. & Jackson, R. R. (1992). Influence of prey movement on the performance of simple detours by jumping spiders. *Behaviour*, *123*, 106-120.

Taylor, P. W. & Jackson, R. R. (1999). Habitat-adapted communication in *Trite planiceps,* a New Zealand jumping spider (Araneae, Salticidae). *New Zealand Journal of Zoology*, *26*, 127-154.

Taylor, P. W., Hasson, O. & Clark, D. L. (2001). Initiation and resolution of jumping spider contests: roles for size, proximity, and early detection of rivals. *Behavioral Ecology and Sociobiology*, *50*, 403-413.

van Helsdingen, P. J. (1965). Sexual behaviour of *Lepthyphantes leprosus* (Ohlert) (Araneida, Linyphiidae), with notes on the function of the genital organs. *Zoologische Mededelingen*, *41*, 15-42.

Vincent, S. E. & Lailvaux, S. P. (2006). Female morphology, web design, and the potential for multiple mating in *Nephila clavipes*: do fat-bottomed girls make the spider world go round? *Biological Journal of the Linnean Society*, *87*, 95-102.

Watson, P. J. (1986). Transmission of a female sex pheromone thwarted by males in the spider *Linyphia litigiosa* Keyserling (Linyphiidae). *Science*, *233*, 219-221.

Williams, D. S. & McIntyre, P. (1980). The principal eyes of a jumping spider have a telephoto component. *Nature*, *288*, 578-580.

Wolfner, M. F. (1997). Tokens of love: functions and regulation of *Drosophila* male accessory gland products. *Insect Biochemistry and Molecular Biology*, *27*, 179-192.

Yamane, T. & Miyatake, T. (2010). Inhibition of female mating receptivity by male-derived extracts in two *Callosobruchus* species: Consequences for interspecific mating. *Journal of Insect Physiology*, *56*, 1565-1571.

Yamane, T., Miyatake, T. & Kimura, Y. (2008). Female mating receptivity after injection of male-derived extracts in *Callosobruchus maculatus*. *Journal of Insect Physiology*, *54*, 1522-1527.

Zurek, D. B. & Nelson, X. J. (2012). Hyperacute motion detection by the lateral eyes of jumping spiders. *Vision Research*, *66*, 26-30

Zurek D. B., Taylor, A. J., Evans, C. S. & Nelson, X. J. (2010). The role of the anterior lateral eyes in the vision-based behaviour of jumping spiders. *Journal of Experimental Biology*, *213*, 2372-2378.

Chapter Five: Sexual receptivity in female spiders: a review

ABSTRACT

Sexual receptivity in female spiders can be affected by female mating status. Virgin females are often highly receptive and copulate with the first male they encounter. After a first copulation, however, females in many species of spiders become aggressive and unreceptive. Cohabitation of males with subadult females that are about to moult to maturity seems to be a strategy adopted by males to increase access to receptive virgin females. In some spiders, females are less aggressive while feeding and attacks to males are less likely to occur during feeding bouts. Males in some species of the genus *Nephila* attempt to copulate females that are feeding, a behaviour that has been interpreted as a tactic to overcome female aggressiveness. Sexual receptivity after female's first copulation varies among spider families; whereas members of some families tend to be highly monandrous, such as Lycosidae, others are extremely polyandrous as is the case in Nephilidae. Post-copulatory guarding is often performed by males in species with highly polyandrous females. Besides female mating state, numerous other factors can also affect female receptivity and might interact with effects of mating state. Age, size, condition, feeding history of females and mating context have all been reported to influence female receptivity in spiders. Here we summarise and discuss the taxonomic patterns of sexual receptivity found in spiders, particularly with respect to female mating state. We also discuss potential mechanisms involved in the induction of remating inhibition, a field that remains largely unstudied in spiders.

I. INTRODUCTION

Copulation is an essential step toward fitness in most sexually reproducing animals, but it is by no means the last. In particular, subsequent female remating decisions are central in determining the success of a first mate's copulation in translating into fertilizations. From the male perspective, if a female remates then the first mate's sperm is likely to be exposed to competition for fertilizations from the sperm of subsequent males and the first male will then suffer a concomitant reduction in fitness (Parker 1998). The fitness consequences of sperm competition have given rise to a vast diversity of male tactics that function in increasing a male's share of paternity in the offspring of polyandrous females (Simmons 2001), as well as in reducing the risk that their mate would accept a subsequent suitor. However, just as there is selection on first mates to reduce the incidence of female remating there may also be selection on others to overcome the tactics of first mates. From the female perspective, remating might allow females an opportunity to increase the genetic diversity or quality of their offspring, or to gain access to material resources. Here we collate and review data on mating frequency of spiders, with a view to laying the foundations for subsequent studies investigating the taxonomic patterns, mechanisms and functions of female mating-induced sexual inhibition, defined as a reduction in female sexual receptivity after a copulatory event.

Spiders have served as useful subjects for the study of reproductive strategies (Eberhard 2004), but surprisingly little attention has been given to the question of how often females mate, the consequences of female mating frequency for males and

females, and the mechanisms determining how often females remate. Male spiders do not provide parental care and one copulation typically provides enough sperm to fertilize all the eggs a female produces (Schneider and Lubin 1998). However, female spiders may accrue genetic or material benefit from mating with several partners (Watson 1993, Uhl et al. 2005, Maklakov and Lubin 2006). Males of some spiders reduce the risk that their mates will receive sperm from another male through physical means of guarding females from other males after copulation (Calbacho-Rosa et al. 2010), plugging the female's genitalia (Uhl et al. 2010), or interrupting the release of pheromones by destroying associated web (Watson 1986, Andrade 1996). In some spiders, however, females show greatly reduced sexual receptivity after their first mating, thereby reducing or eliminating the need for physical means to defend mates against rival males. Mating-induced sexual inhibition has been widely reported and studied in insects (for reviews, see Chen 1984, Eberhard 1996, Ringo 1996, Chapman et al. 1998) but little is known about mating-induced sexual inhibition in spiders. In particular the adaptive benefits and mechanisms responsible for mating-induced sexual inhibition are poorly understood in spiders. Understanding the taxonomic distribution of mating-induced sexual inhibition is an important step toward resolving this knowledge gap.

The aims of this review are to: (1) assess the incidence of mating-induced sexual inhibition in spiders and identify taxonomic patterns; (2) assess other factors that can influence sexual receptivity of female spiders; (3) highlight strategies used by first mates to decrease sperm competition when females do remain receptive after the first copulation; and (4) consider mechanisms that might be responsible for mating-induced sexual inhibition in spiders.

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II. DIVERSE MATING PATTERNS IN SPIDERS

(1) Agelenidae

Female receptivity has been study in two species of Agelenidae. Singer and Riechert (1995) presented virgin males of *Agelenopsis aperta* to females every 3 days for the female's entire reproductive lifetime. Of 18 females tested, 1 did not mate, 7 mated once, 4 mated twice, 5 mated three times and 1 female mated four times. In the field, this species was found to exhibit a mainly monogamous mating system, with most males and females mating only once. In *A. aperta*, monogamy might have evolved as a consequence of high travel costs for males searching for receptive females that are widely dispersed (Riechert et al. 1973), and a decline in female receptivity after the first mating. Copulations are long and are thought to be costly for both females and males (Singer and Riechert 1995). However, some males and females mated more than once. The mating season for *A. aperta* is very short with most adults dying at the end of the mating season due to flooding. The eggs survive this flooding because they are protected by the impermeable silk of the egg sac (Riechert 1974). Moreover, females are not receptive immediately after moulting to maturity. Females have a delay of two weeks between moulting to maturity and becoming sexually mature and receptive (Papke et al. 2001), which reduces even more the time available for reproduction.

Mating-induced sexual inhibition has also been reported in *Agelena limbata* (Masumoto 1991). *Agelena limbata* males mature before females and after copulating with a virgin female the males deposit a plug in the female genitalia that impedes

copulation by rival males (Masumoto 1993). In this species some females do remain receptive after their first copulation and the second male that copulates with a female fertilizes c. 60% of the offspring. A total of 39 females were observed in the field by Masumoto (1993); 27 (70%) copulated with just one male while 12 (30%) copulated with two males. Mating plugs might serve as a backup if the female encounters another male and remains sexually receptive (Masumoto 1993). However, rival males can sometimes remove plugs deposited by previous males and copulate effectively with an already mated female (Masumoto 1993).

Cohabitation has not been reported in the Agelenidae included in this review. Since females of some species show low receptivity for several weeks after they moult to the adult stage and moulting to maturity is synchronised, cohabitation with subadult females would need to last until the female is willing to mate. In terms of missed mating opportunities, it might be too costly for males to cohabit with subadult females until the females become receptive.

(2) Araneidae

Protandry and cohabitation with subadult females have been found in the sexually cannibalistic *Argiope aurantia* (Foellmer and Fairbairn 2005). In this species, males mature around a week earlier than females and compete over access to females that have recently moulted. Once the female has started moulting, males fight fiercely over access to the female (Foellmer and Fairbain 2003). Most mated females are unreceptive or are not even courted by males (Foellmer 2008). Males attempt to mate

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with virgin females while females are undergoing their final moult to reach maturity at which time they cannot reject or cannibalise the male (Foellmer and Fairbairn 2005), although males also sometimes attempt to copulate already mated females. Part of the male's genitalia breaks off during copulation acting as a mating plug, therefore most mated females in the population are probably not available as mates either because they are not receptive or because their genitalia have been plugged (Foellmer 2008).

Mated females of the spider *Argiope keyserling* attack courting males more frequently than virgin females and they also cannibalise the male more often during the 24 h after the first mating (Herberstein et al. 2002). After finishing copulation with a virgin female males leap off the female to avoid being cannibalised but later return to the orb web where they remain for several hours. Males remaining in the web engage in physical contests to protect their reproductive investment. Some rival males do succeed in mating with the guarded female, although copulations tend to be shorter if the guarding male is present (Herberstein et al. 2002). Copulations by subsequent males are associated with a reduction of 20 to 30% in paternity of the first mate (Elgar et al. 2000). The first male guards the female for approximately 24 h after which the female becomes highly unreceptive, aggressive and cannibalistic (Herberstein et al. 2005). In this species, males break off part of the palp during copulation which becomes lodged in the female genitalia. However, the genital fragment does not always prevent successful copulation by subsequent males (Herberstein et al. 2012). Post-copulatory guarding in this species seems to be the main strategy to prevent remating while females remain receptive. Nonetheless, mating plugs might also prevent copulations with subsequent males right after the first mating, and also in the long term for the rest of the life of a female spider.

Ghione and Costa (2011) found that males of the spider *Argiope argentata* break off their genitalia during copulation with dead females, suggesting that in this species females are not responsible for the genital damage. However, it is not known whether the male's genital fragment logged in the female genitalia prevent copulations from subsequent males. In *A. argentata*, males died spontaneously after one or two palpal insertions and remain attached to the female epyginum (Ghione and Costa 2011). Females remove dead males attached to their genitalia after mating, which might be disadvantageous for the male if the dead body acts as a copulatory plug by preventing copulations with subsequent males (Ghione and Costa 2011)

Delayed onset of mating-induced sexual inhibition is also evident in *Gasteracantha minax*. Females of *G. minax* readily remate if provided an opportunity shortly after their first mating, but between 1 and 24 hours after their first mating females become unreceptive (Elgar and Bathgate 1996). As with *A. keyserling*, males guard females that they have just mated with until the onset of mating-induced sexual inhibition and then depart (Elgar and Bathgate 1996).

In addition to effects of mating state, feeding history also affects sexual receptivity of *A. keyserling* females. Food-deprived females that have mated once tend to attack and cannibalise males more often than sated females, and only sated non-virgin females remate. In contrast, feeding history does not affect aggression in *A. keyserling* virgin females (Herberstein et al. 2002).

(3) Eresidae

Females of *Stegodyphus lineatus* exhibit mating-induced sexual inhibition (Maklakov et al. 2003). Males perform vibrating behaviour on the female's web during courtship; this courtship is effective in stimulating responses from virgin females, but not mated females. Males also vibrate less when courting mated females (Maklakov et al. 2003). The authors suggested that mated females cease production of a silk-borne sex pheromone and that this results in reduced male courtship. Polyandry is costly for female *S. lineatus* due to direct costs associated with mating and male cohabitation (Schneider and Lubin 1996, Maklakov and Lubin 2004, Maklakov et al. 2005, Erez et al. 2005) and so sexual inhibition appears to be in the interests of both sexes.

Stegodyphus lineatus females only produce one egg batch in their lives and the spiderlings eat their mother before leaving the nest (Schneider 1995). Some males of *S. lineatus* remove the egg sac from the female's nest and thereby coerce the female to remate. Females defend their egg sacs and engage in fierce fights with males. Once a male has succeeded in removing the egg batch, however, females produce a second egg batch using the infanticidal male's sperm to fertilize some of the eggs (Schneider and Lubin 1996, 1997). Coerced copulation and infanticide appear to have evolved in this species to overcome female resistance to remating. Protandry has evolved in this species probably in part because virgin females are a highly valuable resource; after mating, females become unreceptive and very aggressive.

(4) Linyphiidae

Complete matings in the sheet-web spider *Lyniphia triangularis* induce sexual inhibition, and females in this species are predominantly monandrous. Males cohabit with females that are about to moult to maturity, and the pair copulates once females moult (Watson 1990). As part of a strategy of reducing female remating tendency, males dismantle the pheromone-impregnated web of the subadult female being guarded, thereby preventing other males from finding the female (Watson 1986). Males charge their pedipalps twice during copulation and they use a sperm droplet each time they induct sperm (Rovner 1967). A maximum of two droplets are transferred into the female during a complete mating (Weldingh et al. 2011). It has been suggested that the long copulatory courtship associated with the transfer of the second droplet is responsible for induction of sexual inhibition in recently mated females (Weldingh et al. 2011).

In *Linyphia litigiosa*, females start to show attractiveness to males before maturing. Protandrous males visit the webs of subadult females. Two to five days before the female's final moult the behaviour of the female changes from staying away from the male on the web (avoidant behaviour) to actively trying to be close to the male (associative behaviour). Associative behaviour stops after the female mates (Watson 1990). Males use this behaviour to make decisions on whether to guard a female and on the length of the guarding. If males find an immature female or a mated female that is avoidant then they do not guard these female for longer than a day. If the immature female is associative then males remain with the female for several days and wait until the female moults and then the pair copulates (Watson 1990). During the period during

which a female shows associative behaviour, several males usually visit the web and engage in fights with the guarding male. Therefore by the time the immature female reaches maturity there have been a series of male-male fights, and the last winner copulates with the female. Associative behaviour of the female has been suggested to enhance male-male competition and thereby favour the selection of high quality, competitive, males as mates.

Watson (1998) found that 80-90% of *L. litigiosa* females in a field population were polyandrous. Polyandrous females gain genetic benefits from remating, and also foraging success (Watson 1993, 1998). It was found that female receptivity could be predicted by her recent foraging success; females with low foraging success were more likely to remate and copulations lasted longer. However, when females were under conditions of low food availability for long periods of time, their receptivity dropped. The number of previous mates was a strong predictor of female receptivity; the higher the number of mates the less likely the female would remate (Watson 1993). This pattern might reflect a cost of remating, because even that females are gaining benefits by remating they usually do not mate more than 2 to 4 times (Watson 1993).

In *Frontinella pyramitela* females remain receptive after their first copulation and throughout their lives (Austad 1982). The first male to copulate with the female fertilises the eggs of the first and second egg sac. If a second male copulates with the females immediately after the first copulation then he typically fertilises a few eggs in the third egg sac when spiders are maintained in the laboratory. However, in the field females generally do not live long enough to produce a third egg sac (Austad 1982).

Also, if more than 24h have passed since the first copulation, then the second male usually does not fertilise any of the eggs. Males do not transfer sperm during copulations with mated females, but apparently they need to copulate with these females to obtain information on the female's mating status (Austad 1982). In this species it seems that very low levels of sperm competition, a result of powerful first male sperm precedence, precludes the need to protect the ejaculate.

(5) Lycosidae

High levels of mating-induced sexual inhibition have been reported in Lycosidae. Reluctance to remate is so strong in females of *Pardosa astrigera* that remating trials intended to test the cost of polyandry required that the females be anesthetised in order to obtain a second copulation (Jiao et al. 2011). There is a cost associated with mating, as females that were forced to mate more than once had shorter lifespan than females that only mated once. In *P. astrigera*, females that have mated once become aggressive towards males that attempt to mate. A similar pattern has been found in *Hygrolycosa rubrofasciata,* in which females are highly monandrous and, according to Ahtiainen et al. (2006), never mate more than once and cannot be coerced by males into copulations. It has been suggested that copulation in some wolf spiders are costly for females because copulations are long and this can be associated with loss of foraging opportunities and an increase in the risk of predation and parasitism (Jiao et al. 2011, Scheffer 1992).

Pardosa astrigera and *H. rubrofasciata* both have mating seasons that are restricted to just a few weeks each year (Jiao et al. 2011, Ahtiainen et al. 2006). Females in species with such short seasons are expected to direct their time and energy toward reproductive activities other than mating such as foraging for nutrients to produce eggs.

Schizocosa ocreata is another lycosid species with highly monandrous females and a short breeding season. Norton and Uetz (2005) investigated whether females of *S. ocreata* mate more than once and the variables affecting their reproductive behaviour. Once-mated females were assigned to one of three remating treatments: paired after 24hr; paired after 3 days; or paired after approximately 30 days. Most females mated only once and only 7% mated twice. Similar results have been reported by Taylor et al. (2008). Moreover, mated females become more aggressive, a change in behaviour that might be the result of an increase in foraging activity before laying eggs (Uetz and Norton 2007).

Age is another variable associated with sexual receptivity in Lycosidae. Virgin *S. ocreata* females show low receptivity during the first and second week after moulting, the highest receptivity during the third week, and are less receptive afterwards (Uetz and Norton 2007; Taylor et al. 2008). It has been suggested that abundance of males early in the season allows virgin females to be more aggressive and choosy than older virgin females later in the season. Virgin females of *Pardosa pseudoannulata* are less receptive one day after moulting compare to ten and twenty days after moulting. Mated females don't remate even after laying eggs and they attack courting males more frequently than virgin females (Wu et al. 2013). Recently moulted females might be unreceptive due to

the risk of injury when the exoskeleton is still soft or perhaps the internal anatomy and physiology of the female is still not ready for reproduction (Wu et al. 2013).

An effect of age and mating status was also found in the Mediterranean tarantula *Lycosa tarantula* (Fernandez-Montraveta and Ortega 1990). In this species, old virgin females in their second year of life and previously mated females are less receptive than young, virgin females. Courtship was shorter for virgin females, regardless of age, which also suggests that willingness to mate decreases after the first mating or that mated females are choosier. Young mated females sometimes accepted a subsequent suitor, but old mated females tend to show a complete inhibition of sexual receptivity. Copulations in *L. tarantula* are long and potentially costly and the mating season is restricted to a short period of time (Fernandez-Montraveta and Ortega 1990). Males of this species mature earlier in the season than females (Fernandez-Montraveta and Ortega 1990, but see Fernandez-Montraveta and Cuadrado 2003), which might be an indication of males competing to find young virgin and very receptive females.

Age also affects female receptivity in the wolf spider *Rabidosa rabida*, although this varies with diet. Age did not have an effect on receptivity of virgin females provided a high-quantity diet, but younger virgin females tend to be less willing to mate if they have been provided a low-quantity diet (Wilgers and Hebets 2012). Older virgin females under low-quality diet tend to mate even under poor conditions probably because the risk of remain unmated is too high late in the season when males become rare (Wilgers and Hebets 2012).

(6) Nephiliidae

Multiple mating is common in females of many *Nephila* and males have evolved strategies to impede further copulations by their mates. Postcopulatory guarding and mating plugs are common in *Nephila*, although these physical measures are not always effective. Females of the golden web spider, *Nephila fenestrata*, remain receptive after their first copulation and males guard the female against rival males after mating, which reduces the likelihood of female remating by around 50% (Fromhage et al. 2007). Males of *N. frenestrata* approach and mate with females when they are feeding (Fromhage and Schneider 2004), a tactic termed 'opportunistic mating'. It has been suggested that opportunistic mating has evolved as a male tactic to reduce the risk of injury and cannibalism by females (Fromhage and Schneider 2004). Females that are feeding during their first copulation tend not to cannibalise males, so after mating the male can then guard them and fend off rival males. Males also break off part of their palps during copulation and these fragments can block the female genital openings and prevent remating (Fromhage and Schneider 2006). In the absence of mating-induced sexual inhibition, *N. frenestrata* rely on mate guarding and mating plugs to reduce the risk of sperm competition.

Cannibalistic females in the giant wood spider *Nephila pilipes* tend to be very aggressive towards males. To reduce risk of attack, males copulate with females while females are moulting to adulthood, and also while females are feeding. Males mature earlier in the season than females, presumably in order to increase chances of finding females in the process of moulting (Danielson-François et al. 2012). Females remate

readily after their first copulation and it this seems to be adaptive for the female, since they need several copulations to produce viable eggs. Kuntner et al. (2012) found that none of the females that copulated once oviposited, whereas 30% of females that copulated three times and 50% of female that copulated 5 times produced viable eggs. Part of the male's palp breaks off during copulation but does not function as an effective plug in this species; females collected from the field have been found with several palp fragments - indicating copulation by different males - in their genital openings (Kuntner et al. 2009). In this species, females produce amorphous plugs that cover the entire genitalia, and males cannot copulate successfully with plugged females. It has been suggested that females produce the amorphous plugs to prevent unnecessary copulations (Kuntner et al. 2012).

Receptivity also does not seem to be influenced by mating in the golden orb-web spider *Nephila senegalensis*; Schneider and Michalik (2011) found that each female copulated with 3 to 16 males in succession. In this species, the male's palp remains intact during copulation and therefore females are not plugged with genital fragments as happens in some other *Nephila*. Interestingly, males avoid females that have cues from other males on their webs, probably to avoid the high risk of sperm competition as a result of the very polyandrous females (Schneider and Michalik 2011).

At the other extreme, males of the nephilid *Nephilengys livida* castrate themselves by completely autotomizing the bulb of their pedipalps in order to plug the female's genitalia (Kuntner et al. 2009). Given that in this species females remain highly receptive after their first mating, females that are plugged on one genital opening might

copulate using the other opening with a different male (Kralj-Fišer et al. 2012). The highly cannibalistic females were less likely to kill aggressive and bold males in the first copulation compare to less aggressive males. Aggressive and bold males are favoured and allowed to copulate in both genital openings, plugging both openings and monopolizing the female (Kralj-Fišer et al. 2012). In addition to plugging the female's genitalia, eunuch males remain on the female's web after mating and guard the female from rival males (Kuntner 2007).

(7) Pholcidae

Like some araneids, mating-induced sexual inhibition is delayed in the pholcid *Holocnemus pluchei*. In this species females tend to copulate with more than one male and there is last male sperm precedence with the second male siring on average 74% of the offspring (Kaster and Jacob 1997). Females remain receptive for ca.14 h after their first mating and males defend females against rival males for this period (Calbacho-Rosa et al. 2010). Fertilization success is higher for the second male if the second copulation occurs within 6 h after the first mating. However, if the second copulation occurs after 24 h paternity is approximately even between first and second mates (Calbacho-Rosa et al. 2010). Guarding of the female by the first male through the period of greatest female receptivity, diminishes both the risk of female remating and also the proportion of fertilizations that the first male would lose should the female later remate.

Pholcus phalangioides females exhibit low levels of mating-induced sexual inhibition. Schäfer and Uhl (2005) found that 100% of virgin females copulated but after

the first mating the probability of mating dropped to 81.9%. A decrease in female receptivity after the first mating was also evident in a tendency of mated females to interrupt second copulations more often and sooner than was the case for virgin females. These results suggest that even when females accept a second mate, their behaviour during the second copulation might indicate some resistance to remating. Large *P. phalangioides* females show higher remating tendency than small females (Schäfer and Uhl 2005), and this may reflect more complete sexual inhibition in small females or elevated male mating effort during courtship with larger, more fecund, females. Males seem to be more attracted to large females and thus larger females might experience costs of higher harassment rates and sometimes remated to avoid the cost of resisting copulations of persistent males (Schäfer and Uhl 2005). In *P. phalangioides*, larger females have larger spermathecae (Uhl 1998) and they might need more copulations in order to fill these organs with sperm (Schäfer and Uhl 2005). However, females that received little sperm during a first copulation are not more likely to remate, therefore the amount of sperm stored in the spermathecae is not related to the remating tendency of a female (Uhl 1998).

(8) Pisauridae

Females of *Dolomedes triton* are very aggressive towards males. Virgin and mated females both attack and sometimes kill males, although mated females attack more often (Johnson 2005). Males attempt to copulate with females immediately after they moult to maturity while the cuticle is still soft and females are unable to kill the male (Zimmermann and Spence 1989). Females have been reported to copulate only

once (Johnson 2005), therefore there is substantial pressure on males to find females before or while moulting in order to copulate. Protandry and cohabitation of adult males with subadult females have probably evolved as a result of the pressure of finding females that have recently moulted (Zimmermann and Spence 1989). Receptivity of *D. triton* females can be affected by experience when immature; subadult females that have cohabited with a male are more likely to be unreceptive and cannibalize males when virgin adults (Johnson 2005).

The aggressive-spillover hypothesis has been used to explain precopulatory cannibalism in *Dolomodes fimbriatus* (Arnqvist 1992, Arnqvist and Henrikkson 1997) and *Dolomedes triton* (Johnson 2001, Johnson and Sih 2005). The hypothesis states that precopulatory sexual cannibalism can be explained as a spillover of high levels of aggression towards food that persists throughout ontogeny, from the juvenile foraging context to the adult mating context (Arnqvist and Henrikkson 1997). The hypothesis predicts that juveniles that are more aggressive towards prey will obtain more food, attain larger fixed adult size, be more fecund and be the most likely to attack their mates. The hypothesis explains how a behaviour that does not seem to be adaptive in the mating context can be explained by the greatest benefits gained in terms of adult size and fecundity (Arnqvist and Henrikkson 1997)

In addition to effects of mating state, feeding history has also been found to influence female receptivity in the nursery web spider *Pisaura miriabilis.* In this species, the male offers a prey wrapped in silk as a nuptial gift to the female during courtship. Once the female accepts the gift and starts feeding on it, the pair copulates (Drengsgaard

and Toft 1999). Females deprived of food show high receptivity levels regardless of mating state. In contrast, sated virgin females were more likely to copulate than are sated non-virgin females (Bilde et al 2007). Males that offer larger nuptial gifts copulate for longer and fertilize more eggs (Stålhandske 2001). Egg sacs of females that mate with two males have a higher hatching success than egg sacs from once-mated females. Thus, polyandrous females not only obtain nutritional benefits from polyandry (Bilde et al. 2007) but they also increase their fertilization success (Drengsgaard and Toft 1999).

(9) Salticidae

There is a general tendency for salticid females to become less receptive after their first copulation. In *Phidippus johnsoni*, males mature earlier than females and, like many other salticids (Jackson 1986a), cohabit with subadult females that are about to moult to maturity (Jackson 1978, 1980, 1986b). Once the female moults, the pair copulates (Jackson 1980, 1981). Compared with virgin females, mated females of *P. johnsoni* are less likely to mate, especially if the first copulation was long. Mating-induced sexual inhibition in *P. johnsoni* occurs immediately after the first mating and post copulatory guarding is not observed (Jackson 1980). Mating plugs are deposited by males of *P. johnsoni* on the female genitalia. These plugs effectively prevent copulations with some males, while others are able to overcome the plugs and mate with the female (Jackson 1980). Mixed sperm precedence patterns have been found in *P. johnsoni*, and so remating by females does impose fitness costs on first mates. Context also matters in *P. johnsoni*; females show higher receptivity when inside their retreat than when in the open (Jackson 1980). Copulations away from the retreat may be

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associated with a higher risk of predation, and females might be avoiding this risk by being less willing to mate away from the retreat.

Mating patterns of *Trite planiceps* are broadly similar to those of *P. johnsoni* (Taylor and Jackson 1999). Not only female mating status but also the context of copulation have an effect on female receptivity. In the dark, simulating encounters inside the shelter of rolled up leaves, all virgin females mated, but only 66% of non-virgin females mated. In the light, simulating encounters that would naturally occur on leaves in the open, 57% of virgin females mated while none of the non-virgin females mated.

Mendez et al. (Chapter 4) studied the mating frequency of 88 *Servaea incana* females from maturation until death. Virgin females were highly receptive but sexual inhibition was induced immediately after their first copulation; females became aggressive towards their first mate and almost always rejected courtship from subsequent males. As with studies of *P. johnsoni* and *T. planiceps*, encounters between males and females took place at two different contexts; away from nests ('in the open') and within the shelter of silken retreats ('at the retreat'). Virgin females in the open were less inclined to mate males than virgin females in the retreat, rejecting more males before mating. However, after their first mating, females in the open that did remate accepted their second mate after fewer exposures than females in the retreat. As with the pholcid *P. phalangioides*, larger females of *S. incana* were more likely to remate. In spiders female size is often associated with higher fecundity (Metidae: Prenter et al.1994b, Salticidae: Hoefler 2007, Cross et al.2008, Araneidae: Bel-Venner et al. 2008)

and larger females may be more inclined to remate owing to more vigorous courtship by males that prefer larger females (Christenson et al. 1985).

(10) Theridiidae

The subsocial spider *Anelosimus studiosus* show no evidence of sexual inhibition after their first mating. Despite complete first male sperm precedence, males court and attempt to copulate mated females but are very rarely successful. It is not clear whether the male is prevented from a normal copulation with mated females or if there was a change in male behaviour after assessing female mated state (Jones and Parker 2008). In another subsocial species of the same genus, *A. c.f. studiosus,* subadult and adult females adopt a sexual receptive posture after the male starts courtship (Viera and Albo 2002). Albo et al 2007, found that subadult females of *A. c.f. studiosus* are receptive to pseudocopulations and age does not affect their receptivity. It is suggested that pseudocopulations might be accelerating the maturation of the subadult female which would benefit both sexes. Alternatively, the authors suggest that subadult females might be deceiving the male. Showing receptiveness would retain a male that otherwise might leave to search for other females. Adult females remate, however in this study only 3 out of 14 adult non-virgin females remated.

Females in the spider *Tidarren cuneolatum* signal their receptivity by performing courtship movements as soon as they are in the presence of a male (Knoflach and van Harten 2000). Female courtship consist of continuous twanging with legs II, a behaviour that it is not common in Theridiidae. Males amputate one of their palps a few hours after

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their penultimate moult and hence copulations involves only one insertion; only one genital pore is inseminated. However, females collected in the field have sperm in both spermathecae, suggesting that remating is common in females. Females always terminate copulations by consuming the male. If a second male is offered 10-70 mins after the first copulation, the female interrupts consumption of the first male and copulates with the second one (Knoflach and van Harten 2000).

In the Australian redback, *Latrodectus hasselti*, females that have cannibalised the male during the first copulation are less likely to accept a second male. Cannibalised males mate for longer and obtain a higher fertilization success (Andadre 1996, Snow and Andrade 2004). It has been suggested that longer copulations might allow the male to transfer seminal substances that inhibit female sexual receptivity (Snow and Andrade 2004). Besides receptivity, these substances might also manipulate the production of pheromones that are deposited on the female's web in order to attract males. Immediately after their first mating, redback females stop producing a pheromone that elicits courtship in males (Stoltz et al. 2007). Males dismantle the web of virgin females during courtship (Forster 1995), and afterwards the female makes a new web that does not contain the attractive pheromones. Dismantling the female's web renders the female less detectable by rival males, and thereby reduces the chances of other males finding and copulating with the recently mated female. Approximately three months after their first copulation, female *L. hasselti* become receptive again and resume the production of sex pheromones (Perampaladas et al. 2008). Given that only 26% percent of all females that readvertised receptivity actually copulated, it is possible that mated females are also choosier (Perampaladas et al. 2008).

(11) Tetragnathidae

In *Metellina segmentata* the duration of courtship for females carrying many mature eggs tends to be shorter than for females with few eggs. Larger females also tend to contain more eggs and be more likely to mate (Prenter et al. 1994a). Similar to tendencies found in *P. phalangioides*, males of *M. segmentata* are more likely to court larger and more fecund females. Hence, as in *P. phalangioides*, larger females might mate more often as a result of higher rates of harassment. Male mate preferences need to be controlled for in studies of female receptivity in order to obtain results that actually reflects the female willingness to mate.

(12) Trechaleidae

Although data on remating tendency of trechaleid spiders are not available, there are some interesting patterns in the receptivity of virgins. Like some pisaurids, males of the trechaleid *Paratrechalea ornata* offer a nuptial gift during courtship and copulation (Costa-Schmidt et al. 2008). Virgin females courted by males with nuptial gifts are more likely to mate than are females courted by males with no gifts (Albo and Costa 2010). Males that offer a nuptial gift copulate for longer and longer copulations have been linked to earlier production of eggs (Albo and Costa 2010). Sexual receptivity in females of *P. ornata* is also age dependent. Females older than 15 days since their final moult are more likely to accept gifts offered by males than is the case for younger females (Klein et al. 2012). Also, female attractiveness seems to be affected by age, with older females eliciting more gift construction in males (Albo et al. 2009).

Attractiveness in female spiders is advertised through the emission of cuticular and silk-based sex pheromones as have been found for many species of spiders (for a review see Gaskett 2007). Attractiveness can be related to characteristics of the female such as size, as is the case in the cellar spider *Pholcus phalangioides* (Uhl 1998), age as in the wolf spider *Schizocosa malitiosa* (Uetz and Norton 2007) and mating status as in the dessert spider *Agelenopsis aperta* (Singer and Reichert 1995) and the redback *spider Latrodectus hasselti* (Stoltz et al 2007). Attractiveness and receptivity are expected to be closely related in spiders, females should advertise their attractiveness as soon as they are receptive to avoid the risk of remaining unmated (Watson 1990). However, attractiveness and receptivity are not necessarily synchronised, as the physiological changes needed to switch off attractiveness might occur slower compare to the ones that switch off receptivity or viceversa. In female spiders it should not be assumed that attractiveness and receptivity are the same, they must be addressed separately in studies on mating behaviour.

(13) Theraphosidae

Remating inhibition has only been reported for one species of theraphosid, the road tarantula *Eupalaestrus weijenberghi* (Perez-Miles et al 2007). Females live 8 to 10 years while the males only live 2 months (Perez-Miles et al. 2005). The mating season for *E. weijenberghi* is brief only lasting from the end of February to the beginning of March (Perez-Miles et al. 2005). Females show their receptivity by performing calls in the entrance of their burrows. Female calls consist of taping the first and second pairs of legs against the substrate. Adult females are not receptive every year and while males

mate several times, females only mate once. Unreceptive females show their reluctance to mate through piston behaviour, consisting of forward and backward movements usually performed inside the burrow. Females may also attack the male if they are not willing to mate. The lack of receptivity in once mated females might explain such a short mating season and the epehemeral life of males (Perez-Miles et al. 2007).

III. THE MECHANISMS OF MATING-INDUCED SEXUAL INHIBITION

The mechanisms responsible for mating-induced sexual inhibition have been studied in most depth in a lycosid, *Schizocosa malitiosa.* Aisenberg and Costa (2005) used males that were depleted of sperm in the palps and prevented from inducting fresh sperm and fluid from the genital pore. Females either copulated twice with a normal male or once with a treated male and then with a normal male. Females that copulated first with an untreated male showed high levels of sexual inhibition when exposed to the second normal male. On the other hand, females that copulated first with a spermless male remained receptive to a second copulation with a normal male. Genital stimulation and copulatory courtship without transferring an ejaculate was not enough to induce remating inhibition in this species. Substances present in the ejaculate - either sperm or other materials - appear to be responsible for mating-induced sexual inhibition of *S. malitiosa.*

Copulations of *S. malitiosa* comprise two copulatory patterns. Pattern I (PI) is characterised by a sequence of multiple insertions in the female epigynum with one palp, then side shift, and multiple insertions with the other palp. Pattern II (PII) follows and is

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characterised by single insertions with each palp in the female epigynum and alternate side shifts. Sperm are transferred during both patterns (Costa 1979, Costa and Toscano-Gadea 2003). Estramil and Costa (2007) tested remating inhibition in two groups of females, the first group consist of females that only experienced PI in their first copulation and the second group were females that only experienced PII. Three days after the first copulation, females in each treatment were exposed to new males to test whether they were receptive to a second copulation. The authors found that sexual inhibition is induced during the first part of the copulation (PI), since remating inhibition was present in females that only experienced PI in the first copulation, but was absent in females that only experienced PII. Further experiments with *S. malitiosa* showed that sexual inhibition was induced immediately after the female's first mating ended and persisted 30 days later (Gonzalez and Costa 2008). Irregularities during matings such a brief copulations, short insertions and few side shifts were associated with a higher tendency of the female to remate, suggesting that male performance during copulation is affecting the remating decisions of females (Gonzalez and Costa 2008)

Aisenberg et al. 2009 exposed copulating pairs of *S. malitiosa* to a second male at the beginning and the end of the pair's copula. When the second male was introduced at the beginning of the copula, the male in copula reduced the number of insertions and dismounted less frequently if copulating with a heavy female. A reduced number of insertions is associated with insertions that last longer and transfer more sperm and accessory substances. Heavy females might be more fecund as has been found for other spiders (Prenter et al 1994b, Uhl 1998, Huber 2005). If heavier females are higly valuable, males that mate with heavy females might be investing more energy in

transferring more accessory substances to induce a long-lasting remating inhibition and thus reduce the risk of sperm competition (Aisenberg et al. 2009).

Female mating-induced sexual inhibition has also been reported in the orbweaving spider *Leucauge mariana* (Tetragnathidae)*,* mated females are less willing to mate compare to virgin females (Aisenberg 2009). In this species, male performance during courtship and copulation have been found to affect the remating of females*,* an indication of cryptic female choice on the characteristics of courtship and copulation. Besides female assessment of male performance during courtship and copulation, substances transfered in the sperm during copulation might also be involved in the induction of remating inhibition (Aisenberg 2009). Similarly, courtship duration affects the receptivity to future matings of female redback spiders *Latrodectus hasselti* (Stoltz et al. 2009). Redback females are less likely to remate when the first male court for longer periods of time.

While numerous studies have suggested a role for secretions in the seminal fluid as mediators of mating-induced sexual inhibition in spiders, there has been almost no investigation of this topic. Two studies have given some insight into the substances that might be responsible of inducing remating inhibition in spiders. Three different types of roteinaceous secretions were identified in the male genital tract of the spider *Pholcus phalangoides*. It has been suggested that these secretions are transferred as part of the ejaculate, acting on the physiology and receptivity of the female after mating (Michalik & Uhl 2005). Also, Michalik et al (2013) found secretory vesicles in the seminal fluid of the wolf spider *Schizocosa malitiosa*, the authors hypothesised that these vesicles could

be responsible for secretions that inhibit female receptivity. However, the function of the secretionsremains to be investigated.

V. ACKNOWLEDGMENTS

VIII. REFERENCES

Aisenberg, A. and Costa, F. G. 2005. Females mated without sperm transfer maintain high sexual receptivity in the wolf spider *Schizocosa malitiosa*. *Ethology* **111**: 545-558.

Aisenberg, A. 2009. Male performance and body size affect female re-mating occurrence in the orb-web spider *Leucauge mariana* (Araneae, Tetragnathidae). *Ethology* **115**: 1127-1136.

Aisenberg, A., Estramil, N., Toscano-Gadea, C. and González, M. 2009. Timing of female sexual unreceptivity and male adjustment of copulatory behaviour under competition risk in the wolf spider *Schizocosa malitiosa*. *Journal of Ethology* **27***: 43-50.

Albo, M. J. and Costa, F. G. 2010. Nuptial gift-giving behaviour and male mating effort in the Neotropical spider *Paratrechalea ornata* (Trechaleidae). *Animal Behaviour* **79**: 1031-1036.

Albo, M. J., Costa-Schmidt, L. E. and Costa, F. G. 2009. To feed or to wrap? Female silk cues elicit male nuptial gift construction in a semiaquatic trechaleid spider. *Journal of Zoology* **277**: 284-290.

Albo, M. J., Viera, C. and Costa, F. G. 2007. Pseudocopulation and male-male conflict elicited by subadult females of the subsocial spider *Anelosimus cf. studiosus* (Theridiidae). *Behaviour* **144**: 1217-1234.

Ahtiainen, J. J., Alatalo, R. V., Kortet, R. and Rantala, M. J. 2006. Immune function, dominance and mating success in drumming male wolf spiders *Hygrolycosa rubrofasciata*. *Behavioral Ecology and Sociobiology* **60**: 826-832.

Andrade, M. C. B. 1996. Sexual selection for male sacrifice in the Australian redback spider. *Science* **271**: 70-72.

Arnqvist, G. 1992. Courtship behavior and sexual cannibalism in the semi-aquatic fishing spider, *Dolomedes fimbriatus* (Clerck) (Araneae: Pisauridae). *Journal of Arachnology* **20**: 222-226.

Arnqvist, G. and Henriksson, S. 1997. Sexual cannibalism in the fishing spider and a model for the evolution of sexual cannibalism based on genetic constraints. *Evolutionary Ecology* **11**: 255-273.

Austad, S. N. 1982. First male sperm priority in the Bowl and Doily spider, *Frontinella pyramitela* (Walckenaer). *Evolution* **36**: 777-785.

Bel-Venner, M. C., Dray, S., Allainé, D., Menu, F. and Venner, S. 2008. Unexpected male choosiness for mates in a spider. *Proceedings of the Royal Society B* **275**: 77-82.

Bilde, T., Tuni, C. Elsayed, R., Pekar, S. and Toft, S. 2007. Nuptial gifts of male spiders: Sensory exploitation of the female's maternal care instinct or foraging motivation? *Animal Behaviour* **73**: 267-273.

Calbacho-Rosa, L., Cordoba-Aguilar, A. and Peretti, A. V. 2010. Occurrence and duration of post-copulatory mate guarding in a spider with last sperm precedence. *Behaviour* **147**: 1267-1283.

Chapman, T., Takahisa, M., Smith, H. K. and Partridge, L. 1998. Interactions of mating, egg production and death rates in females of the Mediterranean fruitfly, *Ceratitis capitata*. *Proceedings of the Royal Society B* **265**: 1879-1894.

Chen, P. S. 1984. The functional morphology and biochemistry of insect male accessory glands and their secretions. *Annual Review of Entomology* **29**: 233-255.

Christenson, T. E., Brown, S. G., Wenzl, P. A., Hill, E. M. and Goist, K. C. 1985. Mating behavior of the golden-orb-weaving spider, *Nephila clavipes*: I. Female receptivity and male courtship. *Journal of Comparative Psychology* **99**: 160-166.

Costa, F. G. 1979. Análisis de la copula y de la actividad postcopulatoria de *Lycosa malitiosa* Tullgren (Araneae: Lycosidae). *Revista Brasileira de Biologia* **39**: 361-376.

Costa, F. G. and Toscano-Gadea, C. A. 2003. Experimental interruption and re-initiation of mating in a wolf spider: an analysis of behavioural patterns and resultant progeny. *Ethology Ecology & Evolution* **15:** 173-181.

Costa-Schmidt, L. E., Carico, J. E. and de Araújo, A. M. 2008. Nuptial gifts and sexual behavior in two species of spider (Araneae, Trechaleidae, Paratrechalea). *Naturwissenschaften* **95**: 731-739.

Cross, F. R., Jackson, R. R. and Pollard, S. D. 2008. Male and female mate-choice decisions by *Evarcha culicivora*, an East African jumping spider. *Ethology* **113**: 901-908.

Danielson-François, A., Hou, C., Cole, N. and Tso, I. M. 2012. Scramble competition for moulting females as a driving force for extreme male dwarfism in spiders. *Animal Behaviour* **84**: 937-945.

Drengsgaard, I. L. and Toft, S. 1999. Sperm competition in a nuptial feeding spider, *Pisaura mirabilis. Behaviour* **136**: 877-897.

Eberhard, W. G. 1996. Female control: sexual selection by cryptic female choice. Princeton. University Press, Princeton. 472 pp.

Eberhard, W. G. 2004. Why study spider sex: special traits of spider facilitate study of sperm competition and cryptic female choice. *Journal of Arachnology* **32**: 545-556

Elgar, M. A. and Bathgate, R. 1996. Female receptivity and male mate-guarding in the jewel spider *Gasteracantha minax* Thorell (Araneidae). *Journal of Insect Behavior* **9**: 729-738.

Elgar, M. A., Schneider, J. M. and Herberstein, M. E. 2000. Female control of paternity in the sexually cannibalistic spider *Argiope keyserlingi. Proceedings of the Royal Society B* **267**: 2439-2443.

Erez, T., Schneider, J. M. and Lubin, Y. 2005. Is male cohabitation costly for females of the spider *Stegodyphus lineatus* (Eresidae)? *Ethology* **111**: 693-704.

Estramil, N. and Costa, F. G. 2007. Female sexual receptivity after partial copulations in the wolf spider (*Schizocosa malitiosa*). *Journal of Zoology* **271**: 148-153.

Fernández-Montraveta, C. and Cuadrado, M. 2003. Cohabitation between an adult male and a subadult female in a burrowing wolf spider (Araneae, Lycosidae). *Journal of Arachnology* **31**: 135-138.

Fernández-Montraveta, C. and Ortega, J. 1990. Some aspects of the reproductive behaviour of *Lycosa tarantula fasciiventris* (Lycosidae). *Journal of Arachnology* **18**: 257-262.

Foellmer, M. W. 2008. Broken genitals function as mating plugs and affect sex ratios in the orb-web spider *Argiope aurantia*. *Evolutionary Ecology Research* **10**: 449-462.

Foellmer, M. W. and Fairbairn, D. J. 2003. Spontaneous male death during copulation in an orb-weaving spider. *Proceedings of the Royal Society B* **270** (Suppl 2): S183-S185.

Foellmer, M.W. and Fairbairn, D. J. 2005. Competing dwarf males: sexual selection in an orb-weaving spider. *Journal of Evolutionary Biology* **18**: 629-641.

Forster, L. M. 1995. The behavioural ecology of *Latrodectus hasselti* (Thorell), the Australian redback spider (Araneae: Theridiidae): a review. *Records of the Western Australian Museum Supplement* **52**: 13-24.

Fromhage, L., Jacobs, K. and Schneider, J. M. 2007. Monogynous mating behaviour and its ecological basis in the golden orb spider *Nephila fenestrata*. *Ethology* **113**: 813-820.

Fromhage, L. and Schneider, J. M. 2004. Safer sex with feeding females: sexual conflict in a cannibalistic spider. *Behavioral Ecology* **16**: 377-382.

Fromhage, L. and Schneider, J. M. 2006. Emasculation to plug up females: the significance of pedipalp damage in *Nephila frenastra. Behavioral Ecology* **17**: 353-357.

Gaskett, A. C. 2007. Spider sex pheromones: emission, reception, structures, and functions. *Biological Reviews* **82**: 27- 48.

Ghione, S., and Costa, F. G. 2011. Female attack is not necessary for male copulatory organ breakage in the sexually cannibalistic spider *Argiope argentata* (Araneae: Araneidae). *Journal of Arachnology* **39**: 197-200.

Gonzalez, M. and Costa, F. 2008. Persistence of sexual reluctance in mated females and the importance of regular copulation in a wolf spider. *Ethology Ecology and Evolution* **20**: 115-124.

Herberstein, M. E., Gaskett, A. C., Schneider, J. M., Vella, N. G. F. and Elgar, M. A. 2005. Limits to male copulation frequency: sexual cannibalism and sterility in St Andrew's Cross spiders (Araneae, Araneidae). *Ethology* **111**: 1050-1061.

Herberstein, M., Schneider, J. M. and 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.

Herberstein, M. E., Wignall, A. E., Nessler, S. H., Harmer, A. M. T. and Schneider, J. M. 2012. How effective and persistent are fragments of male genitalia as mating plugs? *Behavioral Ecology* **23**: 1140-1145.

Hoefler, C. 2007. Male mate choice and size-assortative pairing in a jumping spider, *Phidippus clarus. Animal Behaviour* **73**: 943-954.

Huber, B. A. 2005. Sexual selection research on spiders: progress and biases. *Biological Reviews* **80**: 363-385.

Jackson, R. R. 1978. Life history of *Phidippus* (Araneae, Salticidae). *Journal of Arachnology* **6**: 1-29.

Jackson, R. R. 1980. Mating strategy of *Phidippus johnsoni* (Araneae: Salticidae): II. Sperm competition and the function of copulation. *Journal of Arachnology* **8**: 217-240.

Jackson, R. R. 1981. Relationship between reproductive security and intersexual selection in a jumping spider *Phidippus johnsoni* (Araneae: Salticidae). *Evolution.* **35**: 601-604.

Jackson, R. R. 1986a. Cohabitation of males and juvenile females: a prevalent mating tactic of spiders. *Journal of Natural History* **20**: 1193-1210.

Jackson, R. R. 1986b. Use of pheromones by males of *Phidippus johnsoni* (Araneae, Salticidae) to detect subadult females that are about to molt. *Journal of Arachnology* **14**: 137-139.

Jiao, X., Guo, L., Che, Z.Wu, J. Che, J. Liu, F. and Li, D. 2011. Experimental evidence for female-driven monandry in the wolf spider, *Pardosa astrigera*. *Behavioral Ecology and Sociobiology* **65**: 2117-2123.

Johnson, J. C. 2001. Sexual cannibalism in fishing spiders (*Dolomedes triton*): an evaluation of two explanations for female aggression towards potential mates. *Animal Behaviour* **61**: 905-914.

Johnson, J. C. 2005. Cohabitation of juvenile females with mature males promotes sexual cannibalism in fishing spiders. *Behavioral Ecology* **16**: 269-273.

Johnson, J. C. and Sih, A. 2005. Precopulatory sexual cannibalism in fishing spiders (*Dolomedes triton*): a role for behavioral syndromes. *Behavioral Ecology and Sociobiology* **58**: 390-396.

Jones, T. C. and Parker, P. G. 2008. First male sperm precedence in multiply-mated females of the cooperative spider *Anelosimus studiosus* (Araneae, Theridiidae). *Journal of Arachnology* **36**: 527-532.

Kaster, J. L. and Jakob, E. M. 1997. Last-male sperm priority in a haplogyne spider (Araneae: Pholcidae): correlations between female morphology and patterns of sperm usage. *Annals of the Entomological Society of America* **90**: 254-259.

Knoflach, B., and van Harten, A. 2000. Palpal loss, single palp copulation and obligatory mate consumption in *Tidarren cuneolatum* (Tullgren, 1910)(Araneae, Theridiidae). *Journal of Natural History* **34**: 1639-1659.

Klein, A. L., Trillo, M. C. and Albo, M. J. 2012. Sexual receptivity varies according to female age in a Neotropical nuptial gift-giving spider. *Journal of Arachnology* **40**: 138-140.

Kralj-Fišer, S., Schneider, J. M., Justinek, Ž, Kalin, S., Gregorič, M., Pekár, S. and Kuntner, M. 2012. Mate quality, not aggressive spillover, explains sexual cannibalism in a size-dimorphic spider. *Behavioral Ecology and Sociobiology* **66**: 145-151.

Kuntner, M. 2007. A monograph of *Nephilengys*, the pantropical "hermit spiders" (Araneae, Nephilidae, Nephilinae). *Systematic Entomology* **32**: 95-125.

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Kuntner, M., Gregorič, M., Zhang, S., Kralj-Fišer, S. and Li, D. 2012. Mating plugs in polyandrous giants: which sex produces them, when, how and why? *PloS ONE* **7**: e40939

Kuntner, M., Kralj-Fišer, S., Schneider, J. M. and Li, D. 2009. Mate plugging via genital mutilation in nephilid spiders: An evolutionary hypothesis. *Journal of Zoology* **277**: 257-266.

Maklakov, A. A. and Lubin, Y. 2004. Sexual conflict over mating in a spider: increased fecundity does not compensate for the costs of polyandry. *Evolution* **58**: 1135-1140.

Maklakov, A.A. and Lubin, Y. 2006. Indirect genetic benefits of polyandry in a spider with direct cost of mating. *Behavioral Ecology and Sociobiology* **61**: 31-38.

Maklakov, A. A., Bilde, T. and Lubin, Y. 2003. Vibratory courtship in a web-building spider: Signalling quality or stimulating the female? *Animal Behaviour* **66**: 623-630.

Maklakov, A. A., Bilde, T. and Lubin, Y. 2005. Sexual conflict in the wild: elevated mating rate reduces female lifetime reproductive success. *The American Naturalist* **165**: S38-S45.

Masumoto, T. 1991. Males' visits to females' webs and female mating receptivity in the spider *Agelena limbata* (Araneae: Agelenidae). *Journal of Ethology* **9**: 1-7.

Masumoto, T. 1993. The effect of the copulatory plug in the funnel-web spider, *Agelena limbata* (Araneae: Agelenidae). *Journal of Arachnology* **21**: 55-59.

Michalik, P. and Uhl, G. 2005. The male genital system of the cellar spider *Pholcus phalangioides* (Fuesslin, 1775) (Pholcidae, Araneae): development of spermatozoa and seminal secretion. *Frontiers in Zoology* **2**: 12.

Michalik, P., Aisenberg, A., Postiglioni, R., and Lipke, E. 2013. Spermatozoa and spermiogenesis of the wolf spider *Schizocosa malitiosa* (Lycosidae, Araneae) and its functional and phylogenetic implications. *Zoomorphology* **132**: 11-21.

Papke, M. D., Riechert, S. E. and Schulz, S. 2001. An airborne female pheromone associated with male attraction and courtship in a desert spider. *Animal Behaviour* **61**: 877-886.

Parker, G.A. 1998. Sperm competition and the evolution of ejaculates: towards a theory base. In: Sperm Competition and Sexual Selection. (Eds.T.R. Birkhead, A.P. Møller), pp. 3-54 Academic Press, London.

Perampaladas, K., Stoltz, J. A. and Andrade, M. C. B. 2008. Mated redback spider females re-advertise receptivity months after mating. *Ethology* **114**: 589-598.

Pérez-Miles, F., Postiglioni, R., Montes-de-Oca, L., Baruffaldi, L. and Costa, F. G. 2007. Mating system in the tarantula spider *Eupalaestrus weijenberghi* (Thorell, 1894): Evidences of monandry and polygyny. *Zoology* **110**: 253-260.

Pérez-Miles, F., Costa, F. G., Toscano-Gadea, C. and Mignone, A. 2005. Ecology and behaviour of the 'road tarantulas' *Eupalaestrus weijenberghi* and *Acanthoscurria suina* (Araneae, Theraphosidae) from Uruguay. *Journal of Natural History* **39**: 483-498.

Prenter, J., Elwood, R. and Colgan, S. 1994a. The influence of prey size and female reproductive state on the courtship of the autumn spider, *Metellina segmentata*: a field experiment. *Animal Behaviour* **47**: 449-456.

Prenter, J., Elwood, R. W. and Montgomery, W. I. 1994b. Assessments and decisions in *Metellina segmentata* (Araneae: Metidae): Evidence of a pheromone involved in mate guarding. *Behavioural Ecology and Sociobiology* **35**: 39-43.

Norton, S. and Uetz, G. W. 2005. Mating frequency in *Schizocosa ocreata* (Hentz) wolf spiders: Evidence for a mating system with female monandry and male polygyny. *Journal of Arachnology* **33**: 16-24.

Riechert, S. E. 1974. The pattern of local web distribution in a desert spider: mechanisms and seasonal variation. *The Journal of Animal Ecology* **43**: 733-746.

Riechert, S. E., Reeder, W. G. and Allen, T. A. 1973. Patterns of spider distribution (*Agelenopsis aperta* (Gertsch)) in desert grassland and recent lava bed habitats, south-central New Mexico. *The Journal of Animal Ecology* **42**:19-25.

Ringo, J. 1996. Sexual receptivity in insects. *Annual Review of Entomology* 41: 473-494.

Rovner J. S. 1967. Copulation and sperm induction by normal and palpless male linyphiid spiders. *Science* **157**:835.

Schäfer, M. A. and Uhl, G. 2005. Sequential mate encounters: female but not male body size influences female remating behavior. *Behavioral Ecology* **16**: 461-466.

Scheffer, S. J. 1992. Transfer of a larval mantispid during copulation of its spider host. *Journal of Insect Behaviour* **5**: 797-800.

Schneider, J. M. 1995. Survival and growth in groups of a subsocial spider (*Stegodyphus lineatus*). *Insectes Sociaux* **42**: 237-248.

Schneider, J. M. and Lubin, Y. 1996. Infanticidal male eresid spiders. *Nature* **381**: 655-656.

Schneider, J. M. and Lubin, Y. 1997. Infanticide by males in a spider with suicidal

    maternal care, *Stegodyphus lineatus* (Eresidae). *Animal Behaviour* **54**: 305-312.

Schneider, J. M. and Lubin, Y. 1998. Intersexual conflict in spiders. *Oikos* **83**: 496–506.

Schneider, J. M. and Michalik, P. 2011. One-shot genitalia are not an evolutionary dead

    end-regained male polygamy in a sperm limited spider species. *BMC Evolutionary*

    Biology **11**: 197.

Simmons, L. W. 2001. Sperm competition and its evolutionary consequences in the

    insects. Princeton University Press.

Singer, F. and Riechert, S. E. 1995. Mating system and mating success of the desert

    spider *Agelenopsis aperta*. *Behavioral Ecology and Sociobiology* **36**: 313-322.

Snow, L. S. and Andrade, M. C. B. 2004. Pattern of sperm transfer in redback spiders:

    implications for sperm competition and male sacrifice. *Behavioral Ecology* **15**: 785-

    792.

Stålhandske, P. 2001. Nuptial gift in the spider *Pisaura mirabilis* maintained by sexual

    selection. *Behavioral Ecology* **12**: 691-697.

Stoltz, J. A., McNeil, J. N. and Andrade, M. C. B. 2007. Males assess chemical signals

    to discriminate just-mated females from virgins in redback spiders. *Animal*

    Behaviour **74**: 1669-1674.

Stoltz, J. A., Elias, D. O., and Andrade, M. C. B. 2009. Male courtship effort determines

    female response to competing rivals in redback spiders. *Animal Behaviour* **77:** 79-

    85.

Taylor, P.W. and Jackson, R. R. 1999. Habitat-adapted communication in *Trite planiceps*, a New Zealand jumping spider (Araneae, Salticidae). *New Zealand Journal of Zoology* **26**: 127-154.

Taylor, P. W., Roberts, J. A., Wignall, A. E. and Uetz, G. W. 2008. Autotomy of ornamented forelegs reduces mating success of male *Schizocosa ocreata* wolf spiders. *Journal of Insect Behavior* **21**: 193-202

Uetz, G. W. and Norton, S. 2007. Preference for male traits in female wolf spiders varies with the choice of available males, female age and reproductive state. *Behavioral Ecology and Sociobiology* **61**: 631-641.

Uhl, G. 1998. Mating behaviour in the cellar spider, *Pholcus phalangioides*, indicates sperm mixing. *Animal Behaviour* **56:** 1155-1159.

Uhl, G., Nessler, S. H. and 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.

Uhl, G., Schmitt, S. and Schäfer, M. A. 2005. Fitness benefits of multiple mating versus female mate choice in the cellar spider (*Pholcus phalangioides*). *Behavioral Ecology and Sociobiology* **59**: 69-76.

Viera, C., and Albo, M. J. 2002. Sexual behavior of *Anelosimus studiosus* (Araneae, Theridiidae). In: Abstracts 39 Annual Meeting of the Animal Behavior Society, Indiana University, Bloomington, Indiana, USA. Vol. 98.

Watson, P. J. 1986. Transmission of a female sex pheromone thwarted by males in the spider *Lyniphia litigiosa* (Lyniphiidae). *Science* **233**: 219-221.

Watson, P. J. 1990. Female-enhanced male competition determines the first mate and principal sire in the spider *Linyphia litigiosa* (Linyphiidae). *Behavioral Ecology and Sociobiology* **26**: 77-90.

Watson, P.J. 1993. Foraging advantage of polyandry for female sierra dome spiders (*Linyphia litigiosa*: Linyphiidae) and assessment of alternative direct benefit hypotheses. *The American Naturalist* **141**: 440-465.

Watson, P. J. 1998. Multi-male mating and female choice increase offspring growth in the spider *Neriene litigiosa* (Linyphiidae). *Animal Behaviour* **55**: 387-403.

Weldingh, D. L., Toft, S. and Larsen, O. N. 2011. Mating duration and sperm precedence in the spider *Linyphia triangularis*. *Journal of Ethology* **29**: 143-152.

Wilgers, D. J. and Hebets, E. A. 2012. Age-related female mating decisions are condition dependent in wolf spiders. *Behavioral Ecology and Sociobiology* **66**: 29-38.

Wu, L., Zhang, H., He, T., Liu, Z. and 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.

Zimmermann, M. and Spence, J. R. 1992. Adult population dynamics and reproductive effort of the fishing spider *Dolomedes triton* (Araneae, Pisauridae) in central Alberta. *Canadian Journal of Zoology* **70**: 2224-2233.

Chapter Six: General Discussion

Servaea incana is a jumping spider that inhabits eucalyptus trees in temperate forest of mainland Australia. Little is known about the biology and mating behaviour of this common spider, the most widespread species in the genus *Servaea* (Richardson and Gunter 2012). In this thesis, I describe the natural history of this species, interspecific and intraspecific interactions observed in nature and in the laboratory. I also studied a field population through an entire year to understand how seasonality affects the availability of mating opportunities due to changes in abundance of females and males. Availability of mating opportunities is also affected by female receptivity, an aspect of mating behaviour that I studied for the entire life of females in the laboratory. Mated females were less receptive than virgin females and many females mated only once. Mating-induced sexual inhibition of females only occurred after males intromitted and transferred an ejaculate during copulation.

Natural history

The displays observed in *S. incana* are simpler than those of some related jumping spiders. As in other salticids, and spiders in general, the most complex displays of *S. incana* are performed by males during contests and courtship. Immature spiders of both sexes and adult females presented similar, less ritualized, display repertoires. A lack of complexity in the tree trunks inhabited by this species might have made unnecessary the use of elaborated display in males and females. Contrary to what I

found in *S. incana*, other species of salticids that live in litter have developed sound production or substrate borne signals that are easily transmitted in the litter environment (Elias et al. 2004). There was also a low level of complexity in context-dependent courtship versatility of *S. incana*. Like *S. incana*, other salticids use visual courtship when in the open where there is high intensity of light (Jackson 1977) but also use seismic courtship transmitted via the silk when males court females that are inside retreats or nests (Jackson 1977). In *S. incana*, seismic components of courtship at retreats and nest were relied on less than is common in other salticids. *Servaea incana* retreats are built under pieces of bark that are partially detached from the trunk, and are hence are often subject to high light levels. Presence of high light levels, and positioning of the nest or retreat openings in alignment with points where bark is detached, might allow males and females to rely largely on visual communication in this context where other salticids tend to rely on seismic communication.

Servaea incana is a sit and wait predator, which is in agreement with the cryptic colouration of this species. Most of the prey captures observed in the field were insects coming nearby rather than being actively hunted by *S. incana*. There was a high availability of ants on the eucalyptus trees and most of the prey items of *S. incana* observed in the field were ants. Ants are very mobile foragers and use not only chemical but also visual cues to navigate (Collet et al. 1992). Therefore *S. incana* might benefit by crypsis that allows it to remain unnoticed while ants approach. As a diurnal predator that hunts on exposed tree trunks, crypsis likely also affords protection from visually orienting predators such as birds, wasps and other salticids.

Polyandry may be costly due to increased exposure to sexually transmitted diseases and parasites. The most commonly observed enemy of *S. incana* were mantispid larvae, which were found to parasitise both females and males. The larvae of some species of mantispid are transferred between spiders during copulation (Redborg 1998; Scheffer 1992) and we also observed this in our study with S. incana. Females that copulate with more than one male are at a higher risk of acquiring a mantispid larva from males. Mantispid larvae not only feed on the fluids of the host but may eat the entire egg batch of female hosts (Rienks 2000). Such egg predation represents a very high cost, as *S. incana* females rarely produce more than three batches of eggs during their entire life. More observations on the transmission of the mantispid larvae between *S. incana* spiders and the length of time they spend on the spider are needed to determine the costs of this parasite for females and males.

In this study, *S. incana* spiders were always found on eucalyptus trees, although movement between trees is likely. Future research on the movements of males between trees will help to understand the population dynamics of this species. For example, males living in trees where most females are mated will have an advantage if they succeed in travelling to trees where there is a higher availability of virgin females and fewer rival males. High mortality risk is likely to be associated with moving between trees and this is another variable that should be studied for a better understanding of the costs associated with mate search.

Mating-induced sexual inhibition

Females of *S. incana* exhibit high levels of sexual receptivity when virgin, but then exhibit a substantial, and near complete, inhibition of sexual receptivity after their first mating. As a consequence, there is a high tendency toward monandry in this species, with the large majority of females mating only once or twice in their lifetime. Assuming that such a marked behavioural change is adaptive, we are then led naturally to the question 'adaptive for who?'

At this point it is not possible to ascertain whether mating-induced sexual inhibition is under male or female control, although there are potential benefits for males and females that might favour either hypothesis. From the male perspective, by inducing sexual inhibition males would avoid sperm competition and the risk of losing paternity if a female remates. Additionally, mating-induced sexual inhibition would be an economic and safer solution than the alternative of guarding females to repel subsequent suitors. Because they do not need to guard an unreceptive female, males can leave immediately after mating to seek further mating opportunities. From the female perspective, perhaps the general term 'sexual inhibition' fails to capture a more subtle behavioural change. Rather than being a general reduction in sexual receptivity, the reduced mating tendency of mated females might instead stem from a sharp increase in choosiness. Females need to mate at least once in order to reproduce and so somewhat relaxed preferences might then make sense as a means of ensuring reproductive security. But once reproductive security is established, females might then seek 'upgrades', restricting their sexual interest to high quality males. Such a female reproductive strategy has been suggested

previously for *Phidippus johnsoni,* another jumping spider that exhibits mating-induced sexual inhibition (Jackson 1981), but this possibility remains to be tested. If mating-induced sexual inhibition is actually an increase in mating skew toward particular male types, then there are several predictions that would support this case. First, variance in male mating success should be greater for pairings that involve mated females than for pairings that involve virgin females. Second, if females agree on preferred male types, then owing to reduced variance from other sources there should be male traits that are much stronger predictors of mating success in pairings of mated females than in pairings of virgin females.

Remating decisions in females have been found to be based on the characteristics of the first male's courtship and copulation in spiders such a *Latrodectus haltessi* (Stoltz et al. 2009) and *Leucauge mariana* (Aisenberg 2009). Although we found that copula duration was not related to the remating decisions of females, we did not study male performance during courtship and copulation which could also be involved in inducing remating inhibition in *S. incana.* Female assessment of males through cryptic female choice based on male sexual performance might be acting in the study species.

In addition to intrinsic effects of mating state, context also affected the receptivity of *S. incana* females and hence the strength of sexual selection. Virgin females that were in a retreat generally showed greater receptivity than virgin females in the open. Similar context-dependent sexual receptivity has also been reported in the salticids *Phidippus johnsoni* (Jackson 1981) and *Trite planiceps* (Taylor and Jackson

1999). It has been suggested that the reduced receptivity of virgin females that are away from a retreat is an adaptive response to risk from visually orienting predators. This also agrees with my observations of shorter copulations in *S. incana* virgin females in the open. As was the case for effects of mating state, it is worth considering whether the reduced mating tendency of virgin females away from retreats is applied equally to all males or is instead an expression of greater female choosiness when away from retreats. It may be that a high quality mate justifies elevated predation risk but low quality males do not. If this is the case, then there should be certain male traits that are much stronger predictors of mating success when away from retreats than is the case at retreats. The present thesis does not focus on predictors of male sexual success, but does lay the foundations for such work by establishing some of the key sources of variation in the mating system of *S. incana* jumping spiders.

Sexual opportunities for males

In seasonal species, availability of mating opportunities varies through the year with availability of adults and this variation is particularly acute when mating-induced sexual inhibition restricts sexual activity mainly to virgin females. A field population of *S. incana* was studied over the course of a year to gain insight to when females and males are present in wild populations, and to consider the extent to which mating-induced sexual inhibition of females might further restrict mating opportunities.

Given that virgin females are highly receptive and mated females tend to be unreceptive and aggressive, virgin females of *S. incana* are a highly valuable

reproductive resource for males. The pattern of protandry found in the studied population - that is, males maturing earlier than females - likely stems from selection on males to monopolise virgin females at the beginning of the mating season. Subadult females close to their final moult to maturity were abundant at the beginning of the season, but were rare later. By maturing early, most males reached the adult stage when subadult females were abundant. Males were found to cohabit with subadult females, a tactic that has been interpreted as a means of securing copulations with newly moulted virgin females (Jackson 1980; Jackson 1986; Jackson and Hallas 1986; Taylor and Jackson 1999; Hoefler 2007).

Whereas cohabitation is commonly observed between male and subadult female jumping spiders, I made the surprising observation of males cohabiting with mature females. Such male tactics are generally not expected given that virgin females are more valuable and because non-virgin females have low mating tendency. However, cohabitation involving adult females was only observed at times of the season when subadult females were rare. Mating with a non-virgin female might be less rewarding in terms of paternity for a male, but given that absolute sperm precedence is uncommon in spiders, a second male might still gain some paternity by mating with a non-virgin female.

Male and female size changed through the year; the size of males decreased while the size of females increased. Protandry is usually expected to carry a trade-off between size and time to develop. The shorter the time to develop the smaller the size achieved (Zonneveld 1996) and consequently spiders that mature early in the season are

expected to be smaller than those that mature later. However, this trend was only found for subadult and adult females of *S. incana.* For males, change in size through the year might be explained by a strong selection for large male size at the beginning of the season when there is a higher incidence of cohabitation with subadult females. If males need to defend subadult females against other males, large size might be advantageous to win contests, as has been found in other jumping spiders (Wells 1988; Taylor et al. 2001; Taylor & Jackson 2003; Hoefler 2007). In the field males of *S. incana* fight for access to subadult females and sometime evict guarding males (Chapter 2). Alternatively, large males might be more vulnerable to predation while searching for mates, and therefore they would be eliminated first, decreasing the average size of males in the population as the season progresses. There are several questions that should be investigated in future projects. It would be useful to establish whether large males are more effective in defending subadult females. Also, it would be useful to determine the interaction between accumulation of resources during the subadult stage and time of maturation in males.

An association between the size of males and females found cohabiting might also result from male-male competition, with large males monopolizing access to large and more fecund females. With large females 'off the market', small males would then take the remaining opportunities of cohabitation with small females. Size assortative mating has been reported previously for spiders (Rubenstein 1987, Masumoto 1999, Maklakov et al. 2004), and has been associated with preference of males for large females. However, we do not know if *S. incana* males cohabit preferentially with large females or if large females are more fecund. Further observations on the cohabiting behaviour of this species are needed to shed light on how cohabiting pairs are formed

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and if male-male competition and male mate choice are involved. Female behaviour after males initiate cohabitation should also be studied in *S.incana*, because precopulatory female mate choice might be acting if females abandon their retreats after males initiate cohabitation.

Receptivity in female spiders

Receptivity patterns in female spiders are highly variable. Some are highly monandrous, such as some Lycosidae (*Schizocosa ocreata*: Norton and Uetz 2005; *Hygrolycosa rubrofasciata*: Ahtiainen et al. 2006; *Pardosa astrigera:* Jiao et al. 2011), whereas others are highly polyandrous as is the case of some Nephilidae (e.g. *Nephila senegalensis:* Schneider and Michalik 2011, *Nephila pilipes:* Kuntner et al. 2009) and Araneidae (e.g. *Argiope bruennichi*: Schneider et al. 2006, *Argiope lobata*: Nessler et al. 2009). Polyandry and monandry in the same species has also been reported in spiders, including the salticids *Phidippus johnsoni* (Jackson 1981) and *Trite planiceps* (Taylor and Jackson 1999), and this is the pattern I found in *S. incana* (Chapter 4). As in many other spiders, some females mate only once while others mate more often. The fact that the onset of female mating-induced sexual inhibition occurs immediately after the end of the female's first copulation suggest that in this species mate guarding by males is not necessary. Post –copulatory mate guarding has been found to occur in species in which females remain receptive after copulation as is the case for the pholcid *Holocnemus pluchei* (Calbacho-Rosa et al. 2010) and *Gasteracantha minax* (Elgar & Bathgate, 1996).

In my thesis I have explored the effect of female mating state on sexual receptivity, but mating state is not the only variable that might influence female mating tendency. In Chapter 4 I found that even extrinsic variables, such as mating context, can be important. There are also many intrinsic variables not addressed in this thesis that warrant attention in *S. incana*. For example, sexual receptivity of females varies with age in some spiders. In some lycosids, females reach maturity but are not receptive to copulations immediately after their final moult. For example, in *Schizocosa ocreata* (Uetz and Norton 2007) young virgin females are less receptive than old virgin females, whereas in the Mediterranean tarantula *Lycosa tarantula* (Fernandez-Montraveta and Ortega 1990) young virgin females are more receptive than old virgin and mated females. Diet can also be an important determinant of sexual receptivity in spiders (Watson 1998, Drengsgaard and Toft 1999, Bilde et al 2007, Wilgers and Hebets 2012).

Female size has been associated with receptivity in females of the pholcid spider *Pholcus phalangoides* (Schäfer and Uhl 2005) and the tetragnathid *Metelina segmentata* (Prenter et al. 1994). However, since male preferences for large females have also been found in these species, a higher remating tendency of large females might be the result of more vigorous courtship and higher persistence by the males. In Chapter 4, we also found that in the open larger females of *S. incana* tended to reject fewer males before mating, and we hypothesised that this might be the result of male preference for larger females. Larger females might be experiencing greater harassment and consequently might be mating more frequently to avoid the cost of resistance. Even that large females might be more capable of resisting a male than small females, the cost of resistance (i.e physical damage) might still be high.

The mechanisms responsible for mating-induced sexual inhibition in female spiders have been most directly studied in *Schizocosa malitiosa.* Aisenberg and Costa (2005) reported that females showed sexual inhibition after mating with a normal male but that females remained receptive after copulating with a male that did not have sperm or the substances that accompanied the sperm in the ejaculate. This suggested that a substance in the ejaculate is responsible for induction of sexual inhibition in this spider, and that mechanical stimulation during copulation is not sufficient. Two studies have given some insight into the substances that might be responsible of inducing remating inhibition in spiders. Proteinaceous secretions have been found in the male genital tract of the spider *Pholcus phalangoides* and it has been suggested that these secretions might be transferred as part of the ejaculate and then act on female physiology and receptivity after mating (Michalik & Uhl 2005). Also, Michalik et al (2013) found secretory vesicles in the seminal fluid of *Schizocosa malitiosa.* The function of these substances are still unknown, but it is very likely that they contain substances that are directly responsible for induction of sexual inhibition.

The present study used *Servaea incana* as a model to examine mating-induced sexual inhibition in female spiders in an ecological context. Further studies should investigate whether substances in the ejaculate are causing remating inhibition in this spiders and if male performance during courtship and copulation is involved. The effect of context in the mating strategies of *S. incana* spiders should also be studied by quantifying female fertility in the open and at retreats. Also, the relationship between female size and fecundity should be investigated. Behaviours observed in the laboratory

were interpreted under the light of information gathered in field studies, which was critical to understand the impact of sexual inhibition in the reproductive strategies of males and females at the population level. Future studies should not only focus in the mechanisms involved in the induction of remating inhibition, but also in the ecological factors influencing changes in sexual receptivity of female spiders. We yet do not know the mechanism by which protandry occurs in *S. incana* or the way cohabitating pairs are established, this critical information is needed to fully understand the behaviours reported in this thesis.

References

Ahtiainen, J. J., Alatalo, R. V., Kortet, R. and Rantala, M. J. 2006. Immune function, dominance and mating success in drumming male wolf spiders *Hygrolycosa rubrofasciata. Behavioral Ecology and Sociobiology* **60**: 826-832.

Aisenberg, A. and Costa, F. G. 2005. Females mated without sperm transfer maintain high sexual receptivity in the wolf spider *Schizocosa malitiosa. Ethology* **111**: 545-558.

Aisenberg, A. 2009. Male performance and body size affect female re-mating occurrence in the orb-web spider *Leucauge mariana* (Araneae, Tetragnathidae). *Ethology* **115**: 1127-1136.

Bilde, T., Tuni, C., Elsayed, R., Pekar, S. and Toft, S. 2007. Nuptial gifts of male spiders: Sensory exploitation of the female's maternal care instinct or foraging motivation? *Animal Behaviour* **73**: 267-273.

Calbacho-Rosa, L., Cordoba-Aguilar, A. and Peretti, A. V. 2010. Occurrence and duration of post-copulatory mate guarding in a spider with last sperm precedence. *Behaviour* **147**: 1267-1283.

Collett, T. S., Dillmann, E., Giger, A. and Wehner, R. 1992. Visual landmarks and route following in desert ants. *Journal of Comparative Physiology A* **170**: 435-442.

Drengsgaard, I. L. and Toft, S. 1999. Sperm competition in a nuptial feeding spider, *Pisaura mirabilis. Behaviour* **136**: 877-897.

Elgar, M. A. and Bathgate, R. 1996. Female receptivity and male mate-guarding in the jewel spider *Gasteracantha minax* Thorell (Araneidae). *Journal of Insect Behavior* **9**: 729-738.

Elias, D. O., Mason, A. C. and Hoy, R. R. 2004. The effect of substrate on the efficacy of seismic courtship signal transmission in the jumping spider *Habronattus dossenus* (Araneae: Salticidae). *Journal of Experimental Biology* **207**: 4105-4110.

Fernández-Montraveta, C. and Ortega, J. 1990. Some aspects of the reproductive behaviour of *Lycosa tarantula fasciiventris* (Lycosidae). *Journal of Arachnology* **18**: 257-262.

Hoefler, C. 2007. Male mate choice and size-assortative pairing in a jumping spider, *Phidippus clarus. Animal Behaviour* **73**: 943-954.

Jackson, R. R. 1977. Courtship versatility in the jumping spider, *Phidippus johnsoni* (Araneae: Salticidae). *Animal Behaviour* **25**: 953-957.

Jackson, R. R. 1980. The mating strategy of *Phidippus johnsoni* (Araneae, Salticidae): II. Sperm competition and the function of copulation. *Journal of Arachnology* **8**: 217-240.

Jackson, R. R. 1981. Relationship between reproductive security and intersexual selection in a jumping spider *Phidippus johnsoni* (Araneae: Salticidae). *Evolution* **35**: 601-604.

Jackson, R. R. 1986. Cohabitation of males and juvenile females: a prevalent mating tactic of spiders. *Journal of Natural History* **20**: 1193-1210.

Jackson, R. R. and Hallas, S. E. 1986. Comparative biology of *Portia africana, P. albimana, P. fimbriata, P. labiata, and P. shultzi,* araneophagic, web-building jumping spiders (Araneae: Salticidae): utilisation of webs, predatory versatility, and intraspecific interactions. *New Zealand Journal of Zoology* **13**: 423-489.

Jiao, X., Guo, L., Che, Z., Wu, J., Che, J., Liu, F. and Li, D. 2011. Experimental evidence for female-driven monandry in the wolf spider, *Pardosa astrigera. Behavioral Ecology and Sociobiology* **65**: 2117-2123.

Kuntner, M., Coddington, J. A. and Schneider, J. M. 2009. Intersexual arms race? Genital coevolution in Nephilid spiders (Araneae, Nephilidae). *International Journal of Organic Evolution* **63**: 1451-1463.

Maklakov, A. A., Bilde, T. and Lubin, Y. 2004. Sexual selection for increased male body size and protandry in a spider. *Animal Behaviour* **68**: 1041-1048.

Masumoto, T. 1999. Size assortative mating and reproductive success of the funnel-web spider, *Agelena limbata* (Araneae; Agelenidae). *Journal of Insect Behaviour* **12**: 353-361.

Michalik, P. and Uhl, G. 2005. The male genital system of the cellar spider *Pholcus phalangioides* (Fuesslin, 1775) (Pholcidae, Araneae): development of spermatozoa and seminal secretion. *Frontiers in Zoology* **2**: 12.

Michalik, P., Aisenberg, A., Postiglioni, R. and Lipke, E. 2013. Spermatozoa and spermiogenesis of the wolf spider *Schizocosa malitiosa* (Lycosidae, Araneae) and its functional and phylogenetic implications. *Zoomorphology* **132**: 11-21.

Nessler, S. H., Uhl, G. and Schneider, J. M. 2009. Sexual cannibalism facilitates genital damage in *Argiope lobata* (Araneae: Araneidae). *Behavioral Ecology and Sociobiology* **63**: 355-362.

Norton, S. and Uetz, G. W. 2005. Mating frequency in *Schizocosa ocreata* (Hentz) wolf spiders: evidence for a mating system with female monandry and male polygyny. *Journal of Arachnology* **33**: 16-24.

Prenter, J., Elwood, R. W. and Montgomery, W. I. 1994. Assessment and decisions in *Metellina segmentata* (Araneae: Metidae): evidence of a pheromone involved in mate- guarding. *Behavioural Ecology and Sociobiology* **35**: 39-43.

Redborg, K. E. 1998. Biology of the mantispidae. *Annual Review of Entomology* **43**: 175-194.

Richardson, B. J. and Gunter, N. L. 2012. Revision of Australian jumping spider genus *Servaea* Simon 1887 (Aranaea: Salticidae) including use of DNA sequence data and predicted distributions. *Zootaxa* **3350**: 1-33.

Rienks, J. H. 2000. Extended nest residence and cannibalism in a jumping spider (Araneae, Salticidae). *Journal of Arachnology* **28**: 123-127.

Rubenstein D. I. 1987. Alternative reproductive tactics in the spider *Meta segmentata*. *Behavioral Ecology Sociobiology* **20**: 229-237.

Schäfer, M. A. and Uhl, G. 2005. Sequential mate encounters: Female but not male body size influences female remating behavior. *Behavioral Ecology* **16**: 461-466.

Scheffer, S. J. 1992. Transfer of a larval mantispid during copulation of its spider host. *Journal of Insect Behavior* **5**: 797-800.

Schneider, J. M., Gilberg, S., Fromhage, L. and Uhl, G. 2006. Sexual conflict over copulation duration in a cannibalistic spider. *Animal Behaviour* **71**: 781-788.

Schneider, J. M. and Michalik, P. 2011. One-shot genitalia are not an evolutionary dead end - Regained male polygamy in a sperm limited spider species. *BMC Evolutionary Biology* **11**: 197.

Stoltz, J. A., Elias, D. O. and Andrade, M. C. B. 2009. Male courtship effort determines female response to competing rivals in redback spiders. *Animal Behaviour* **77:** 79-85.

Taylor, P. W., Hasson, O. and Clark, D. L. 2001. Initiation and resolution of jumping spider contests: roles for size, proximity, and early detection of rivals. *Behavioral Ecology Sociobiology* **50**: 403-413.

Taylor, P. W., and Jackson, R. R. 1999. Habitat-adapted communication in *Trite planiceps*, a New Zealand jumping spider (Araneae, Salticidae). *New Zealand Journal of Zooloogy* **26**: 127-154.

Taylor, P. W. and Jackson R. R. 2003. Interacting effects of size and prior injury in jumping spider conflicts. *Animal Behaviour* **65**: 787-794.

Uetz, G. W. and Norton, S. 2007. Preference for male traits in female wolf spiders varies with the choice of available males, female age and reproductive state. *Behavioral Ecology and Sociobiology* **61**: 631-641.

Watson, P. J. 1998. Multi-male mating and female choice increase offspring growth in the spider *Neriene litigiosa* (Linyphiidae). *Animal Behaviour* **55**: 387-403.

Wells, M. S. 1988. Effects of body size and resource value on fighting behaviour in a jumping spider. *Animal Behaviour* **36**: 321-326.

Wilgers, D. J. and Hebets, E. A. 2012. Age-related female mating decisions are condition dependent in wolf spiders. *Behavioral Ecology and Sociobiology* **66**: 29-38.

Zonneveld, C. 1996. Being big or emerging early? Polyandry and the trade-off between size and emergence in male butterflies. *American Naturalist* **147**: 946-965.