New Insights in the Evolutionary Maintenance of Male Mate Choice Behaviour using the Western Black Widow, *Latrodectus hesperus*

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Ecology and Evolutionary Biology
University of Toronto

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Abstract

Mate choice among males is relatively understudied, despite recent evidence supporting its ubiquity. Theory predicts male mate choice in response to variation in female quality, and male mating strategies that limit polygyny. However empirical research investigating these connections, particularly under natural conditions, is generally lacking. Using the Western black widow spider, *Latrodectus hesperus*, I investigated male mate choice, male investment, and the advantage of mating with females that differ in their potential to deliver fitness benefits to males via higher fecundity and/or reduced risk of sperm competition. Males were found to be preferentially attracted to larger, high-diet virgin females over females that were smaller due to a lower diet, or having been previously mated, or both. Through a three-year field study on females I found that males likely benefit from selectivity, as smaller, low-diet females often failed to deposit any egg sacs throughout the breeding season, likely due to their shortened web-site tenure and/or lack of bodily resources. I investigated the costs and benefits to male genital breakage in *L. hesperus*, a strategy typically assumed to result in male sterility while providing paternity protection. I found that genital mutilation in *L. hesperus* did not cause sterility and that males were capable of inseminating multiple females, likely because of the comparatively low
amount of genital damage and the likelihood of the efficient placement of genital fragments. Evidence from double mating trials supported the efficacy of broken genital fragments as plugs, but first male sperm precedence was often maintained in cases where male genital breakage failed or fragments were positioned incorrectly, which occurred frequently. Together, these laboratory and field experiments contribute to a more complete view of mate choice.
Acknowledgments

I am very grateful for the support of my mentor Maydianne Andrade. Her guidance and direction as well as her faith in my ideas and abilities led me to be able trust myself enough to complete a project that I am truly proud of. Maydianne’s passion for her work, her intelligence, her personal integrity, and her feminist perspective on the scientific world has been inspirational to me. I am also thankful to my committee members, Peter Abrams and Deborah McLennan, for their insights and perspectives over the past five years; their comments and criticism have greatly improved this manuscript. Gard Otis introduced me to the subject of behavioural ecology, and I will be forever grateful for his guidance, friendship, and consistent support and encouragement. My academic achievements are rooted in his faith in me.

I could not have completed my work without the assistance of Maria Modanu. Her piercing insight, her help, her support and her friendship over the past five years has been invaluable. I am also extremely grateful to my three field assistants: Madeline Girard, Katie Keough, and Sheena Fry. I was so fortunate to be able to spend my field seasons in the company of such intelligent, hard working, and interesting friends. I am particularly indebted to Maddie Girard and Damian Elias for both their practical assistance and friendship during my California days. I consider myself very fortunate to have had the opportunity to conduct my field research at Hastings Natural History Reservation in California, and am particularly grateful for all the support I received over four years there.

Finally I would like to acknowledge my extreme privilege that having supportive friends and family has afforded me during my degree. My sister, my extended family, and especially my parents, Rod and Cathy MacLeod, have provided me with endless love and encouragement, despite their apprehension towards my study species. The patience, calmness, love and practical assistance of Simon Ford has been instrumental in my achievements, as have the friendships of Stephanie Moynagh, Viktoryah Lapp and Jennifer Code. I am also grateful for the conversation and commiseration of Tiffany Schreiver, Devin Bloom and Caroline Tucker, and the advice and support of Luciana Baruffaldi.

I would like to dedicate my thesis to my grandparents, all either officially or unofficially scientists. They continue to inspire me despite their absence.
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**Introduction**

Darwin (1859) proposed sexual selection to explain the evolution of traits that were costly to survival and thus challenged the main focus of his theory of evolution by natural selection. He envisioned two processes that lead to differential mating success among individuals of one sex. One of these, competition over mates, was overt, relatively easy to quantify, and rapidly accepted as an important determinant of mating patterns. The second mechanism was mate choice, and this was much more controversial when proposed. Debate about the existence and importance of this mechanism was partly because it appeared to require an ‘aesthetic sense’ among choosy individuals. Substantial study has since eliminated this objection, and it is clear that differential mating success arises from choice in a range of taxa. Mate choice has been shown to be an important evolutionary mechanism that can increase the fitness of both the chooser and the object of choice and thus change animal phenotypes over evolutionary time. Choosy behaviour is expected to evolve and persist when the costs of missed mating opportunities from being selective are outweighed by the benefit of pairing with a high quality mate (Andersson 1994). Mate choice has been a strong focus of research in sexual selection, as many aspects of an individual’s environment or life-history may theoretically promote or restrict its expression (e.g., Trivers 1972; Emlen and Oring 1977; Real 1990; Kokko, Brooks et al. 2003). Empirical work testing theoretical predictions about the occurrence of mate choice continues to inform our perception of its complex role in determining lifetime fitness. While the vast majority of this work has concerned mate choice by females, recent studies suggest choice by males may be prevalent and an important source of variation in mating patterns across taxa. The main goal of this thesis is to examine predictions and assumptions underlying mate choice theory when applied to male choice in a species that is tractable for study in the field and laboratory.

Central to the prediction of mate choice behaviour is individual investment into mating and reproduction. High amounts of time and energy directed towards one mating or reproductive event can limit an individual’s potential reproductive rate, and therefore increase the proportional contribution of each offspring towards total parental fitness (Trivers 1972; Clutton-Brock and Parker 1992; Bonduriansky 2001). Individuals with low expected mating rates that are able to discriminate among potential mates that differ in their contributions to reproductive fitness are then favoured by selection. Potential mates may be preferred because they either ensure increases
to the fitness of the choosy individual, e.g. through parental care (Trivers 1972; Saetre, Fossnes et al. 1995; Forsgren, Karlsson et al. 1996), or towards the fitness of their shared offspring, through offspring inheritance of advantageous phenotypes (Moller and Jennions 2001; Kokko, Brooks et al. 2003). Early theory led to predictions that mate choice would occur in only one sex in a given population, as inequalities in investment lead to differences in the availability of each of the sexes for mating, creating a bias in the sex ratio towards the lower-investing sex (Emlen and Oring 1977). Members of the lower-investing sex are expected to compete for access to mates, as their fitness is then limited by access to the less-available, higher-investing sex (Trivers 1972; Emlen and Oring 1977; Clutton-Brock and Vincent 1991). The higher-investing sex thus has the opportunity to choose among many potential mates. In sexual species, females typically have a more limited reproductive potential than males due to higher investment in gametes, gestation and/or parental care (Trivers 1972; Jennions and Petrie 1997). Moreover, females generally stand to benefit from mating selectively as males vary in traits that affect their fitness, or that of their offspring (e.g., Hasselquist and Bensch 1991; Petrie 1994; Godin and Dugatkin 1996; David, Bjorksten et al. 2000). In contrast, males are generally the lower-investing sex and male choice has not been expected in most common mating systems. The exception arises in cases of sex-role reversal, where extreme male investment into reproduction limits male reproductive rate (Gwynne 1981; Vincent, Ahnesjo et al. 1994). In species with conventional sex roles however, males gain more fitness per mating event than females, and males are not expected to be selective about their mates as such a strategy would decrease a male’s reproductive options and potentially lower fitness (Bateman 1948; Arnold and Duvall 1994). This basic theory of sex roles and mate choice explains many aspects of the sexual behaviour of many species. However, more recent evidence and theory suggests male mate choice exists, and may be common, in species with traditional sex-roles. Thus choice and competition within one sex may coexist. Such work is expanding the way in which relative investment into mating and reproduction is conceptualized and quantified, thereby increasing the predictive power of mate choice theory (Bonduriansky 2001).

The sexes typically differ in the form of their behavioural or physiological investment in mating and reproduction. These differences may affect the likelihood of observers recognizing links between fitness and investment, which shapes assessments of the higher-investing sex, and predictions about the occurrence of mate choice. For example, parental care and gamete
provisioning are common forms of female investment (Trivers 1972) which have clear and direct implications for fitness. This direct link may therefore lead to the perception that females are the higher-investing sex. Male investment can take different forms, some which are less directly related to fitness. The ‘parental effort’ of males that directly affects offspring has been distinguished from male ‘mating effort’ (sensu: Edward and Chapman 2011) which is investment required to produce offspring, but which does not contribute directly to offspring fitness (Gwynne 1991). While parental effort was predicted to cause sex role reversal, mating effort was not (Gwynne 1991). However, the time and energy required for a male to secure access to a female and ensure paternity (mating effort) may also constitute a sufficient level of investment to affect male potential reproductive rate (Bonduriansky 2001; South, Steiner et al. 2009). Recent work provides evidence to support this more complete perspective on male investment. Male ‘mating effort’ may include costly courtship or competitive behaviours, mate guarding, and time and energy requirements for spermatogenesis, all of which may reduce a male’s total number of mates and increase the benefits to be gained from mating selectively (Bonduriansky 2001; Edward and Chapman 2011). Most levels of male mating effort will not result in sex-role reversal, as reversal is only predicted where male investment (parental effort) limits female reproduction (Gwynne 1991). High levels of male mating effort may predict the co-occurrence of choice and competitive behaviour however, as males compete selectively for preferred females (Fawcett and Johnstone 2003; Hardling and Kokko 2005; Hoefler 2007; Bel-Venner, Dray et al. 2008; Venner, Bernstein et al. 2010). This ‘partial sex role reversal’ is likely common (Bonduriansky 2001), and the resulting patterns of mating may be influenced as much by male responses to variation in female quality as by female responses to male investment (Parker 1983; Johnstone, Reynolds et al. 1996; Bonduriansky 2001). Male preferences for females with indicators of high fecundity and low sperm competition risk would be advantageous under partial role reversal, as in complete reversal, and promoted through natural and sexual selection (Bonduriansky 2001). Thus a more realistic theoretical framework for male mate choice requires considering influences of male mating effort on polygyny as well as understanding patterns of variation in female quality. Such a framework will more accurately predict the occurrence and importance of this common behaviour, and determine how male choice shapes patterns of mating in nature.
Despite the advances in theory that have facilitated new perspectives on male mate choice (Parker 1983; Bonduriansky 2001), important aspects of this behaviour remain under-investigated. The goals of my research were to examine 1) the potential for male mate choice in the wild, 2) natural patterns of variation in female quality and the opportunity for male choice to exert sexual selection on females, and 3) assumptions surrounding the level of investment required to engage in a strategy of male paternity protection through mate plugging and its ensuing benefits. The first two goals address aspects of mate choice that have been well-studied from the perspective of female mate choice, but lack comparable research in male mate choice systems. The last goal seeks to investigate male investment in a partially role reversed species and the potential for variability in a male strategy that has been consistently associated with high male investment. I combined laboratory studies with field data to allow analysis of factors underlying male mating preferences, how these preferences are manifest in the field, and the potential fitness payoffs for choosy males in nature. Informed by new perspectives on male mate choice, my research offers novel insights into factors influencing male choice--male investment and variation in female quality--through the examination of a partially sex-role reversed focal organism.

**Focal taxon and organism**

My research utilized laboratory and field populations of the Western black widow spider, *Latrodectus hesperus* (Theridiidae). Studies of spiders have provided important evidence for male mate choice in the absence of parental effort (e.g. Schulte, Uhl et al.; Riechert and Singer 1995; Gaskett, Herberstein et al. 2004; Roberts and Uetz 2005; Moskalik and Uetz 2011; Rittschof 2011; Tuni and Berger-Tal 2012). In some species, male spiders have high mating investment in few matings (Herberstein, Gaskett et al. 2005; Berger-Tal and Lubin 2011; Schneider and Michalik 2011; Welke, Zimmer et al. 2012; Zimmer, Welke et al. 2012), or are monogynous (mate with only one female, (Elgar and Nash 1988; Forster 1992; Andrade 1996; Foellmer and Fairbairn 2003; Fromhage, Jacobs et al. 2007; Segoli, Arieli et al. 2008; Kralj-Fiser, Gregoric et al. 2011). Some male spiders are considered to invest highly in mating due to the common occurrence of genital mutilation during copulation. In these species, portions of the male’s genitalia are left behind where they block rival males’ access to the female’s sperm storage organs (reviewed in: Uhl, Nessler et al. 2010). Some genital mutilating species make
extreme investments into mating, either by dying spontaneously during copulation (Foellmer and Fairbairn 2003), or by being consumed by females through self-sacrifice (Forster 1992; Andrade 1996; Segoli, Arieli et al. 2008) or predation (Elgar and Nash 1988; Elgar 1992; Sasaki and Iwahashi 1995; Elgar and Fahey 1996; Spence, Zimmermann et al. 1996; Elgar, Schneider et al. 2000; Schneider, Gilberg et al. 2006).

Selective mating among these high-investing male spiders may be favoured if low potential reproductive rates and high costs of mating facilitate the maximizing of male fitness through pairing with high quality females. Also critical is the variation in fitness benefits that results from mating with different females. Males may prefer to mate with virgin females (Schulte, Uhl et al.; Bukowski, Linn et al. 2001; Gaskett, Herberstein et al. 2004; Roberts and Uetz 2005; Baruffaldi and Costa 2010; Aisenberg and Gonzalez 2011; Tuni and Berger-Tal 2012), as the genital fragments or glandular secretions of previous mates in the reproductive organs of mated females may reduce or prevent paternity gains. Also, males may prefer larger females (Hoefler 2007; Bel-Venner, Dray et al. 2008; Moskalik and Uetz 2011; Welke, Zimmer et al. 2012), as female size predicts female fecundity in spiders (Marshall and Gittleman 1994; Head 1995; Uhl, Schmitt et al. 2004; Opell, Berger et al. 2007; Drapela, Frank et al. 2011). The evolution of male mate choice among spiders may be constrained if the rate of encountering females is low due to risks associated with mate searching (but see: Kasumovic, Bruce et al. 2007) or low population density (Elias, Andrade et al. 2011). However release of airborne pheromones by female spiders (reviewed in: Gaskett 2007) may facilitate male choice, as it may enable simultaneous detection of mates (Barry and Kokko 2010), reducing the time required for finding and assessing females. Taken together, past work establishes partial role reversal and male mate choice in spiders, and suggests studies using spiders as models may allow analysis of more complex aspects of this behavioural phenomenon. In work outlined here I investigate aspects of male mate choice theory, such as the associated variation in female quality and the connection between male preference and the fitness benefits provided by females, that have been the subject of few empirical studies.

While mating behaviour of *Latrodectus hesperus* has not previously been well researched, the studies on this species and congeners suggested the possibility of male mate choice. Male *L. hesperus* use airborne-web based volatile chemicals to locate females in the field (Kasumovic and Andrade 2004), and may potentially use chemical cues to detect females phenotypes. Differences in male behaviour on webs (Johnson, Trubl et al. 2011), and chemical extracts from
web silk (Barrufaldi and Andrade, in prep.) of fed and unfed females show that males prefer larger, fed females from silk cues alone. Male discrimination may be facilitated through differences in web size and structure exhibited by fed and unfed females (Blackledge and Zevenbergen 2007; Salomon 2007), which may result in differences in the quantity or quality of volatile chemicals released from the web. As feeding history is related to fecundity in spiders (Marshall and Gittleman 1994; Head 1995; Uhl, Schmitt et al. 2004; Drapela, Frank et al. 2011), this preference may be promoted through natural selection. However, male preference for larger, well-fed mates may also be important as hungry females may prey upon courting males and consume them (a behaviour which is different from the ritualistic self-sacrifice and sexual cannibalism of other Latrodectus males which occurs during copulation, Forster 1992; Andrade 1996; Segoli, Arieli et al. 2008). Similar behavioural observations were made on congeneric males, L. hasselti, showing male preference for chemical extracts from the webs of virgin females over those of previously mated females (Stoltz, McNeil et al. 2007; Perampaladas, Stoltz et al. 2008). Mating with non-virgin females may result in low paternity gains by males in L. hesperus due to the potential for mate plugging through genital mutilation by previously mating males, as seen in congeners L. hasselti and L. tredecimguttatus (Snow and Andrade 2005; Neumann and Schneider 2011, respectively). Discrimination against mated females may increase male fitness through avoiding females whose sperm storage organs are inaccessible or avoiding the potential for sperm competition.

Latrodectus hesperus was also a suitable species for the work described here due to their amenability to both laboratory and field studies. Previous work on L. hesperus showed that this species is able to be reared en masse under laboratory conditions and that females are able to build webs on which normal sexual behaviour with males can be observed (Kaston 1970; Ross and Smith 1979; Blackledge and Zevenbergen 2007; Salomon 2007). This enabled me to experimentally manipulate mate number and observe paternity distributions in a controlled environment. Field work on L. hesperus is facilitated through the sedentary nature of females, who are averse to displacement from the position where they establish their large webs (Salomon 2009). As egg sacs are conspicuous in the webs of females, the reproductive output of individuals can be documented and monitored throughout a breeding season.
*Latrodectus hesperus* inhabits a range on the west coast of North America from Texas to British Columbia (Kaston 1970). Experiments conducted over the course of this thesis used *L. hesperus* populations native to the Hastings Natural History Reservation in Carmel Valley California, USA (36:37: N, 121:55 W). At this field site spiders are typically found in abandoned mammal burrows or ruts in the ground of low-lying treeless grassy fields.

**Morphology**

The morphology of *L. hesperus* is similar to other members of the genus (Kaston 1970). Females typically possess a red hourglass shaped marking on the ventral aspect of their abdomen (Foelix 1996). Although female body colouration may vary, the black body of *L. hesperus* is shared by several species (Kaston 1970). Male colouration is variable between light brown and black with a light brown hourglass shape on the ventral abdomen. *L. hesperus* also exhibits the female-biased sexual size dimorphism common to *Latrodectus* species, with females typically ranging in length from 8-15.5mm and in weight from 120-400mg and males from 3-6.5mm in length and between 8-18mg in weight (Kaston 1970) (Figure 1). Like all spiders, *L. hesperus* has paired genitalia. *Latrodectus* females have two copulatory openings on the anterior aspect of their ventral abdomen, above the hourglass, which lead to paired insemination ducts, and terminate in paired sperm storage organs, the spermathecae (Figure 4.1). Separate fertilization ducts leave the spermathecae and connect to a single uterus externus, presumably where fertilization occurs (although this is not well studied). Eggs exit the body through one gonopore on the ventral surface of the abdomen, and are deposited inside a rounded sac constructed of silk. *Latrodectus* males possess two copulatory organs, called palps, which are modified forelegs held anterior to the cephalothorax (Figure 4.2). They are separate from the testes, which are located in the abdomen. Sperm from the testes is excreted onto a male’s web, and taken up into the palps where it is stored until ejaculation. Attached to the bulbul part of the palp is thin, whip-like, sclerotized coil, the embolus, which is inserted into the female during copulation. Attached to the apical tip of the embolus is a sclerite which breaks off inside the female during copulation (Figure 4.2).

**Mating and Reproduction**

Males search for females in the landscape using airborne cues derived from volatile chemicals deposited on the web (Kasumovic and Andrade 2004). Male courtship involves stroking the
female’s legs, plucking and strumming of the female’s web, web reduction (cutting and condensing portions of the web), and in later stages, drumming the female’s venter with the palps (Kaston 1970; Ross and Smith 1979). Males insert one embolus at a time into the female contra-laterally, and engage in courtship between insertions. Unlike some of the other species in the genus, *Latrodectus hesperus* females are not usually sexual cannibals (Ross and Smith 1979), and males regularly survive mating (Ross and Smith 1979). Female consumption of males has been observed, but it not a ritualized part of mating and is likely the result of female hunger (Johnson, Trublet et al. 2011) or a response to unwanted courtship.

Within one to two weeks after mating, females begin to deposit egg sacs which typically contain between 160-225 eggs (Kaston 1970), and offspring hatch within approximately three weeks after deposition (E.C.M. personal observation). The typical number of egg sacs deposited by females in the field is between 1 and 2 with 4 being the maximum number observed (Chapter 2), but females in the laboratory have been documented to deposit up to 21 egg sacs (Kaston 1970). Females are able to store sperm and require only one mating bout to achieve enough stores to fertilize their lifetime reproductive output (pers. obs., Andrade and Banta 2002 in a congener).

**Chapter 1. Male mate choice in the Western black widow**

Observations of male mate choice in the field remain relatively rare despite a recent renewed focus on this topic (Edward and Chapman 2011). However, field studies of male mate choice are necessary as preferences expressed by males in a controlled environment may not translate into common decisions under natural circumstances (e.g., Jennions and Petrie 1997). Theory posits that male mate choice is highly affected by environmental variables such as search time, mate encounter rate, search risk, and the operational sex ratio, which determines levels of intra-male competition. The documentation of choice behaviour in the field is significant as it would indicate that the benefits to selective mating and/or the high cost of mating are sufficient to favour choice despite environmentally determined costs of choice, and effects of individual variation in potential payoffs for choice (e.g., low quality males may be less likely to mate with preferred females). The examination of male mate choice behaviour among males with different levels of male investment into mating, or different expected benefits from choice may also be particularly informative as to the relative importance of these factors to the expression and evolutionary maintenance of male mate choice.
Male mate choice of some form has been documented in several spider species, with observed male preferences typically for female phenotypes that indicate either a reduction in sperm competition or a high level of female fecundity. Several laboratory studies have documented both male mate choice for virgin females (Elgar, Bruce et al. 2003; Gaskett, Herberstein et al. 2004; Andrade and Kasumovic 2005; Roberts and Uetz 2005; Baruffaldi and Costa 2010; Aisenberg and Gonzalez 2011; Tuni and Berger-Tal 2012), and larger, well-fed females in the lab (Hoefler 2007; Johnson, Trubl et al. 2011; Moskalik and Uetz 2011, but see: Schulte, Uhl et al. 2010), however results from the field are often more complex. Field studies are concordant with laboratory studies of male preference for mature virgin females (Riechert and Singer 1995; Gaskett, Herberstein et al. 2004; Schulte, Uhl et al. 2010; Tuni and Berger-Tal 2012; Welke, Zimmer et al. 2012). In contrast male preference for larger, more fecund females has not been found in cases where it was predicted in the field (Riechert and Singer 1995; Schulte, Uhl et al. 2010) apparently because of size-assortative mating due to intra-male competition (Hoefler 2007; Bel-Venner, Dray et al. 2008). This difference in the field-observed male choice behaviour may be due to insufficient power in field observations, or due to actual differences in the propensity for male discrimination to focus on female size versus risk of sperm competition.

The focus of this Chapter was an assessment of male mate choice in *L. hesperus* under controlled (semi-natural) and natural conditions. I tested the ability of males to discriminate between females that varied in both mating status and size—the two lines of common preference in spiders. In my design, I eliminated complications arising from interactions of males with females (e.g., assortative mating driven by female preferences) by measuring male responses to airborne volatile chemical cues from females and their webs.) This study established whether male mate choice occurred in nature, and the female traits that were the focus of male choice in field populations.

Chapter 2. Male mate choice and variable female quality in field populations

Surprisingly, few studies exist that document natural variation in female phenotypes and reproductive success (but see: Griggio, Tavecchia et al. 2003; Hanssen, Folstad et al. 2006; Hamel, Gaillard et al. 2009). Moreover, natural levels of variation in female quality, though
integral to the evolution and maintenance of male mate choice behaviour, have rarely been documented. To my knowledge, only three field studies have documented aspects of female quality in the wild in systems with male mate choice, however these studies either focused on documenting male preference for particular female qualities (Zhang, Li et al. 2010; Welke, Zimmer et al. 2012), or the connection between phenotype and reproductive success among mated females (Reading and Backwell 2007), rather than the natural variation among females. Evidence for choosy males deriving benefits due to variation in female quality have mostly arisen from experiments which manipulate female quality (e.g., Kvarnemo and Simmons 1999; Barbosa 2011; Schneider, Lucass et al. 2011; Nandy, Joshi et al. 2012). Without insight from long-term studies of natural populations we are unable to evaluate the effects of different aspects of variation among females, such as the consistency of variation in female quality, or the reliability of phenotypic indicators of fitness across years, on male mate choice.

In this Chapter, I report results of a three year study of female phenotypes and reproductive output in the field. The goal was to gain insight into the benefits of male mate choice under natural circumstances. I quantified variability among females in their phenotypes and reproductive success, both within and across years and I examined the relationship between the male-preferred phenotype (Chapter 1) and female reproductive output. Finally, I asked whether male choice might exert sexual selection on females. Tests for the potential of male choice to exert sexual selection pressure are rare. Though necessary to understand the nuances of the variations in female quality, work of the kind presented in this Chapter is novel and thus provides perspective on an important factor promoting male mate choice.

Chapter 3. Genital mutilation need not cause monogyny

Blocking the access of rivals to females’ reproductive organs with copulatory or sperm plugs is known from many taxa. Many male spiders most commonly use broken genital fragments, or entire intromittent organs, to prevent rival males from inseminating females’ sperm storage organs (reviewed in: Uhl, Nessler et al. 2010). These broken genitals can represent a high male investment in mating (Andrade and Kasumovic 2005), as males employing this strategy have been found to be more likely to mate monogynously (Miller 2007). Generally, male genital mutilation is assumed to cause post-mating sterility. Thus, male mate choice is predicted in groups with genital mutilation as it is assumed that this represents high mating effort (Andrade
and Kasumovic 2005). However, differences in the amount of damage to males’ genitalia, and the lack of a common evolutionary origin to this behaviour (Uhl, Nessler et al. 2010) suggests the level of investment inherent to plugging may vary across taxa, as would predictions about male choice. Since very few studies have actually assessed whether genital plugs result in sterility, it is challenging to assess this hypothesis.

My goals for Chapter 3 were to 1) examine the potential for male *L. hesperus* to mate with and inseminate multiple females despite genital mutilation during copulation. This allowed inferences about the possibility that genital mutilation could be a low investment strategy, and 2) gain insight into variation in mating effort related to this mating behaviour by comparing the mechanics of plugging among genital-mutilating spider species. In this study, I staged matings in the laboratory between males and multiple females, recorded the patterns of plug loss by males and plug deposition in females, and examined whether there were phenotypic predictors of sclerite loss and placement. I also looked across spider taxa at patterns related to male investment in plugging. I focused on the size of palp breakage during mating as a measure of investment, and typical location of placement as an indicator of the potential for broken pieces to act as plugs. Although there are insufficient data for a controlled comparative analysis, this approach could yield general insights that may motivate future studies.

Multiple mating by males in species with genital mutilation has been documented in two congeneric species, *Latrodectus mactans* (Breene and Sweet 1985) and *Latrodectus pallidus* (Segoli, Lubin et al. 2008). However both studies failed to document whether remating males had actually lost apical sclerites in their first copulations. This allows the possibility that males mating multiple times failed to undergo genital mutilation. In another experimental study on *Latrodectus hasselti*, experimental amputation of apical sclerites was not found to prevent successful mating and insemination by males (Snow, Abdel-Mesih et al. 2006). However, since genital damage was not natural, this may not accurately reflect male behaviour after normal first-matings. Although a review was completed by Uhl et. al. in 2010 on the recorded occurrence of genital damage or genital fragments in female reproductive organs in spiders, differences in the amount of damage and plug placement among males has never been investigated. Comparing genital damage and plugging patterns among spider taxa may enable the identification of characteristics generally associated with low male investment in genital mutilation/plugging.
This work challenged established ideas about the ubiquity of an evolutionary connection between genital mutilation, monogyny, and high male investment.

Chapter 4. Strong first male sperm precedence despite frequent plug failure in the Western black widow spider *Latrodectus hesperus*

One factor thought to drive male mate choice in spiders is a lack of fitness benefits from pairing with previously-mated females in species with genital plugs. However, despite the extensive documentation of male genital mutilation in spiders, the function of most of these genital fragments as plugs has only been tested in a few species (Schneider, Thomas et al. 2001; Snow and Andrade 2005; Nessler, Uhl et al. 2007; Neumann and Schneider 2011). These studies generally found high variability in the paternity of the second male, which were attributed to variable plug deposition and placement by the first male (but see: Schneider, Thomas et al. 2001). The potential for males to gain paternity from copulating with mated females may reduce sexual selection pressure on males to mate selectively in response to cues indicating the potential for sperm competition.

The studies in this Chapter include laboratory work to (1) quantify the potential for successful plugging of *L. hesperus* females using genital fragments, and (2) assess the frequency with which second-mating males gain paternity. It also includes an indirect measure of the occurrence of polyandry in the wild, as an indicator of the importance of plugging to male fitness. Previous studies of plugging in congeneric species manipulated males in order to force inseminations into previously used and unused female insemination ducts (Snow and Andrade 2005; Neumann and Schneider 2011). This approach tests for effective plugging but does not allow assessment of the more complex patterns of paternity and plugging success that arise from natural variation in plug deposition. I used un-manipulated double mating experiments to gain insight into the variability of plug deposition and placement success, and related variation in the paternity shares of each male. I also estimated the proportion of polyandrous females in nature using plug deposition data, as a measure of the risk of sperm competition. Although polyandry among females that mate with genital-mutilating males has been demonstrated in the lab (Elgar, Schneider et al. 2000; Snow and Andrade 2005; Schneider, Gilberg et al. 2006; Schneider, Herberstein et al. 2007).
2008; Neumann and Schneider 2011) few studies have assessed this risk to paternity in the wild. The determination of the patterns of paternity in relation to plugging success and failure, and the natural levels of polyandry inform our understanding of the likely benefits are of both plugging and male preference for virgin females.

**Summary**

My work on *L. hesperus* addressed questions surrounding male mate choice behaviour and male investment that have rarely been tested in the literature, but are well formed through theoretical work on choice. I chose to work on a species in which there were indicators of the existence of male choice in nature, but which otherwise shows conventional sex roles. Moreover, in this species laboratory and field work could generate complimentary data to derive a complete perspective of male mate choice. By examining factors that influence the expression of male choice, this work contributes to a more broadly conceptualized view of mate choice behaviour.
References


Figure 1. A female (bottom) and male (top) *Latrodectus hesperus*
Chapter 1

Male mate choice in the western black widow

Abstract

Although male mate choice has been documented in numerous systems, relatively few studies have examined the importance of this behaviour under natural conditions. Using the western black widow spider, *Latrodectus hesperus*, I recorded the attraction of both laboratory-reared males in an enclosure, and wild males in a natural setting to treatment females in a 2 X 2 design where females varied in mating status (virgin or mated) and diet (fed or unfed). These treatments represent two aspects of male choice in spiders: preference for females with low associated sperm competition risk and for females with high fecundity. Males in both experiments were significantly more likely to be found near the webs of virgin/fed females; no differences in phenotype were found between males choosing virgin/fed females or other females. I conclude that male *L. hesperus* are using airborne chemical cues to evaluate these two aspects of female quality, however sexual selection pressure on females for increased expression of the associated preferred phenotypes is unlikely.

Introduction

The role of mate choice in the evolution of elaborate phenotypic traits and behavioural tactics is well established (Andersson 1994) and has been a strong focus of research in sexual selection. Studies have been dominated by examinations of female choice (Bonduriansky 2001), but recent extensions of theory have increased understanding of male mating decisions and how these affect selection on females.

Mate choice among males will evolve and persist whenever benefits to choosing outweigh costs of choice. Male mate choice is expected in systems in which females vary in reproductive value, costs of assessing females are low and accuracy of assessment is high, polygyny is limited due to male investment in mating and/or mating effort (sensu Edward and Chapman 2011) (Parker 1983; Bonduriansky 2001; Edward and Chapman 2011), and mate encounter rate is not constrained (Tuni and Berger-Tal 2012). Documentation of male choice behaviour in species
with traditional sex roles (i.e. no parental care by males, male biased operational sex ratio) and polygynous males (Amundsen and Forsgren 2001; Bonduriansky 2001; Dunn, Crean et al. 2001; Wong and Jennions 2003; Preston, Stevenson et al. 2005; Bel-Venner, Dray et al. 2008; Pruitt and Riechert 2009; Jeswiet, Lee-Jenkins et al. 2011) has provided empirical support for theory that posits sex-roles are more labile than expected under the traditional Darwinian paradigm (Bonduriansky 2001; Edward and Chapman 2011). In these types of taxa, polygyny may be limited by male investment into costly aspects of mating, such as time intensive courtship, which are necessary to secure access to females (Edward and Chapman 2011). Males able to detect indicators of female quality may thus compete for those females that would provide greater fitness returns on their mating investment (Arntzen 1999; Craig, Herman et al. 2002; Hoefler 2007). Consistent with this, male preference for large highly fecund females, or unmated females has been inferred from laboratory choice tests in species from invertebrates to fishes (Verrell 1985; Sargent, Gross et al. 1986; Preston, Stevenson et al. 2005; Saeki, Kruse et al. 2005; Hoefler 2007; Hoysak and Godin 2007; Reading and Backwell 2007; Xu and Wang 2009) and in field studies of Soay sheep (Preston, Stevenson et al. 2005).

While laboratory studies can establish the existence of male preferences and the ability of males to discriminate among female phenotypes, they cannot demonstrate the occurrence or importance of male mate choice in nature (e.g., Jennions and Petrie 1997). Laboratory-demonstrated preferences may not correspond to male choice in the field for several reasons. First, males may not exercise preferences if there are sufficiently high costs of choice imposed by factors such as environmental conditions, such as abiotic conditions that interfere with travel, or mate signaling or reception (Wong and Jennions 2003; Simcox, Colegrave et al. 2005), demography (Kemp and Macedonia 2007; Elias, Andrade et al. 2011), or variation in the distribution of high-quality females. Spatio-temporal variation in these factors may lead to seasonal or stochastic variation in the occurrence of choice. Second, despite universal mate preferences, males may mate assortatively in the field if high quality males monopolize access to high quality females (Hoefler 2007; Yu and Lu 2010), or lower quality males avoid the most preferred females to forestall competition with higher quality rivals (Bel-Venner, Dray et al. 2008; Candolin and Salesto 2009), or to minimize the risk of rejection (i.e., ‘prudent mate choice’ (Venner, Bernstein et al. 2010). Third, preference functions may shift with individual condition or mating status (Gaskett, Herberstein et al. 2004) in ways that are challenging to elucidate in the laboratory. Despite these
and other problems with extrapolating from laboratory results, relatively few field studies have been conducted on male mate choice and fewer still involve populations with traditional sex-roles (but see: Foote 1988; Craig, Herman et al. 2002; Kasumovic and Andrade 2004; Hoefler 2007; Bel-Venner, Dray et al. 2008; Jeswiet, Lee-Jenkins et al. 2011).

Despite the difficulty of observing choice in the wild, field-based studies are required to fully understand not only the causes but also the consequences of male mate choice. Theory predicts that male mate choice has evolved along two major lines of preference: for females with high reproductive potential (as indicated by body size), and for females with low associated sperm competition risk (Bonduriansky 2001). In nature, males evaluating both aspects of female quality may resolve these preferences in complex ways, and the resulting patterns of choice may or may not exert sexual selection pressure on females. In some cases, one aspect may be prioritized, changing the predicted pattern and consequences of male mate choice. For example, if males prioritize low sperm competition risk, even strong male choice will be unlikely to result in directional selection for female fecundity indicators or ornaments. Only a handful of studies of male preferences has tested both aspects of female value together (Lelito and Brown 2008; Callander, Backwell et al. 2012; Jeswiet, Lee-Jenkins et al. 2012). Thus field studies of male choice for more than one female feature are crucial to our understanding of how mate choice leads to sexual selection among females.

The sedentary nature of females of many web-building spiders makes them particularly accessible for studying male mate choice in the field, and male choosiness may be common for several reasons. First, high levels of investment in mating bouts are common, despite conventional sex roles (but see: Aisenberg and Costa 2008). For example, several species are monogynous due to genital mutilation or sexual cannibalism (e.g., Andrade 1996; Knoflach and Van Harten 2000; Herberstein, Gaskett et al. 2005; Fromhage, Jacobs et al. 2007; Foellmer 2008) and many exhibit mate guarding (Miyashita 1994; Prenter, Elwood et al. 1994; Elgar and Bathgate 1996; Herberstein, Barry et al. 2005; Bel-Venner and Venner 2006), risky mate-searching (Andrade 2003; Kasumovic, Bruce et al. 2007; Berger-Tal and Lubin 2011) and time or energy-intensive courtship behaviours (Stoltz, Elias et al. 2009; Sivalinghem, Kasumovic et al. 2010; Cady, Delaney et al. 2011). Second, pheromones may allow males to assess variation in female diet or mating status (sperm competition risk) (Gaskett 2007; Thomas 2011), perhaps with very little cost of assessment. Third, females often vary considerably along both of the
major predicted axes of male choice. In many species, male spiders leave “plugs” blocking access to female sperm storage organs, and first male sperm priority is relatively common (Uhl, Nessler et al. 2010), so virgin females may be much more valuable than mated females in terms of the likely outcomes of sperm competition. In addition, correlations between female fecundity, diet and size (Marshall and Gittleman 1994; Head 1995; Uhl, Schmitt et al. 2004; Drapela, Frank et al. 2011) means that size is a good indicator of female value in spiders.

Male spiders’ preference for virgin females (low sperm competition risk) has been established in several species (Riechert and Singer 1995; Gaskett, Herberstein et al. 2004; Andrade and Kasumovic 2005; Roberts and Uetz 2005; Baruffaldi and Costa 2010; Morse 2010; Schulte, Uhl et al. 2010; Aisenberg and Gonzalez 2011), and may be widespread. In contrast, there is no consistent evidence for the importance of female fecundity in male mate choice. Male *Pholcus phalangioides* and *Metellina segmentata* were both found to be differentially attracted to larger more fecund females in the field (Prenter, Elwood et al. 1994; Uhl 1998), and laboratory investigations of *Tetragnatha elongata* and *L. hesperus* demonstrated male preference for high body condition and females with prey cues on their webs respectively (Johnson, Trubl et al.; Danielson-Francois, Fetterer et al. 2002; Johnson, Trubl et al. 2011). However field studies by Riechert (1995) and Schulte et al (2010) found no significant effect of diet on male mate choice in two monogynous species, *Agelenopsis aperta* and *Argiope bruennichi*. Work by Bel-venner et al. (2008) on *Zygiella x-notata* and Hoefler (2007) on *Phidippus clarus* found that male preference for more fecund females was affected by context, with assortative mating occurring under higher levels of competition. Hence, only relatively sparse evidence exists that male spiders prioritize information relating to female reproductive potential in natural circumstances, which is interesting because (1) this is at odds with a major prediction of male mate choice theory (Bonduriansky 2001), and (2) this suggests that male choice in spiders may not exert selection pressure on female phenotypes.

In this study I examined mate choices made by both laboratory-reared and wild males in semi-natural and natural experiments in which females varied in both their reproductive value and their mating status (risk of sperm competition). I focused on a wild population of western black widow spiders (*Latrodectus hesperus*), a non-sexually cannibalistic species of spider whose range includes British Colombia and the West coast states of the USA (Kaston 1970; Ross and Smith 1979). Nomadic *L. hesperus* males locate females in the wild using airborne chemical cues.
from females’ webs (Kasumovic and Andrade 2004). Males invest in time and energy-intensive courtship bouts (Ross and Smith 1979), and potentially risky mate searching behaviour (as seen in a congener: Andrade 1996; Andrade 2003), which may dispose them to being choosy. However they are not sex-role reversed and they are capable of inseminating multiple females despite frequent genital mutilation during copulation (Chapter 3). I predicted that there would be mate choice for virgin females because access to the sperm storage organs of mated females is usually blocked by detached parts of the copulatory organs from their first partner (Kaston 1970), and because congeneric males discriminate between mated and virgin females from chemical cues found on females’ webs (Andrade and Kasumovic 2005; Stoltz, McNeil et al. 2007). Like most other insects and spiders, female *L. hesperus* vary in their fecundity depending on their diet (associated with larger size) (Marshall and Gittleman 1994; Head 1995; Uhl, Schmitt et al. 2004; Drapela, Frank et al. 2011). I predicted that *L. hesperus* males would be more likely than other male spiders to also exhibit choice for females with high reproductive potential for two reasons: 1. poorly-fed females are more likely to engage in pre-copulatory sexual cannibalism (Johnson, Trubl et al. 2011), and 2. laboratory trials show males discriminate between well-fed and unfed female webs containing prey cues (Johnson, Trubl et al. 2011). The use of chemical cues of body size has never been definitively demonstrated in spiders (Schulte, Uhl et al. 2010), although it has been documented in snakes (Lemaster and Mason 2002) and moths (Harari, Zahavi et al. 2011). If males could use airborne chemical cues to simultaneously evaluate body size (fecundity) of multiple potential mates, this could play a crucial role in the evolution of male mate choice in this species.

In two sets of choice experiments, I used a 2X2 design that crossed female mating status (mated/virgin) and diet (fed/unfed) to determine patterns of male discrimination among females based on these two aspects of quality in the field. In my first experiment, laboratory-reared males were presented with females from the four treatments in a field enclosure to examine choice in a low-risk environment. The same females were subsequently placed in a field with an existing population of spiders to examine choice by wild males under natural circumstances. I predicted males would prefer virgin/fed females over all other groups, and my treatments allowed us to determine how choice is affected when female characteristics diverge along these two axes (e.g., virgin/unfed).
Materials and Methods

Females used in these experiments were first or second generation descendents of individuals collected from Hastings Natural History Reserve (Carmel Valley, CA, USA) in June 2010. Mated females were transported to rearing facilities at the University of Toronto Scarborough (Toronto, Canada) where they were kept at 25°C and a 12:12 light cycle and allowed to produce egg sacs, which were moved to separate cages. After spiderlings emerged from egg sacs, they were reared communally with siblings and fed a diet of *Drosophila melanogaster* 2X per week until they reached their 3rd – 5th instar when they were moved to individual cages. Once female spiderlings were sufficiently large (~ 4th instar), they were fed one *Acheta domestica* cricket per week. Males were reared similarly as juveniles, but as adults they were given one drop of water 2X per week and were fed 1 small cricket (*A. domestica*) every 1.5 weeks (males are much smaller than females: Kaston 1970).

For the enclosure study (experiment 1), I used laboratory-reared males 6 – 14 days after their final moult. Once females reached adulthood they were divided into the two diet treatments; fed females were given one large cricket per week and unfed females were not given any food after their adult moult. Adult female *Latrodectus* spiders encounter food restriction in nature and can recover from up to 12 weeks of starvation (Forster and Kavale 1989; Stoltz, Hanna et al. 2010). Females were then separated into 19 experimental blocks of four spiders each (two unfed and two fed) based on the similarity of their adult moult dates. One fed and one unfed female from each block was assigned to the ‘mated’ treatment. All four females from the same block were placed on separate wire frames on the same day, given 24h to build a web, and then a single adult male was introduced to the web of the two ‘mated’ treatment females. After 24h males were removed and their genitalia inspected to ensure that copulation had occurred. Males of the genus *Latrodectus* typically lose a small portion of their genitalia inside the female during copulation (Kaston 1970), allowing visual confirmation of copulation. All females from the same block remained on the frames until ‘mated’ females had copulated, with new males introduced every day until copulation could be confirmed. After copulation of ‘mated’ females was confirmed, all females were returned to individual cages until they were used experimentally, a period of no shorter than two weeks and no longer than three weeks after their final moult.

Females used in the last sixteen experiments were mated in the laboratory at the field station; the
first three blocks of females were mated in the university laboratory. All spiders were reared until maturity at the university laboratory and were shipped to California for use in trials. Following trials females were immediately euthanized by hypothermia. All trials were conducted at the Hastings Natural History Reserve in May and June 2011.

Experiment 1: Enclosure study

I ran 19 replicate trials between May 8th and July 2nd 2012. Field enclosures were three square 3m x 3m x 0.5m arenas located in a flat open field with low growing vegetation (Figure 1.1). Arenas were framed using pvc tubing (1.5 inches thick) and plumbing joints which were set into troughs approximately 5cm deep. A skirt of fiberglass window screening was then threaded along the frame and tucked under the frame to prevent males from escaping. The centre of each arena was marked with a 0.5m high piece of 1.5 inch pvc tube, which served as a release point for males. At each inner corner, I placed one cage containing a treatment female. Cages were made of ¼ inch hardwire mesh, and were 20cm length x 20cm width x 39cm height in dimension, with a clear plastic tube (10cm diameter, ~15 cm long) attached to the bottom. Tubes were buried at an angle with the attached screen cage placed at ground level (Figure 1.1). This allowed females to construct naturalistic webs comparable in size to those I found in the field, and to include an underground refuge, as do many unconstrained females. Inter-web distance in the enclosures was within the range seen in natural populations in the field that year (mean nearest neighbor distance = 3.22m, Standard deviation = 1.80m; ECM, unpublished).

I placed females in cages six days prior to the start of trials to build webs. Each enclosure included four females from one block with their positions randomized. Cages were individually covered with fiberglass window screening to prevent intrusion by wild males.

The day prior to a trial, the enclosure was constructed around the females’ cages. A trial commenced with the release of four laboratory-reared males within an enclosure and ended 40 hours later. On the day of a trial four laboratory-reared males were weighed and painted with an identifying colour of non-toxic fluorescent paint (BioQuip). In each enclosure, males were released in two groups of two individuals from opposite sides of the centre pole, 30min. apart. In the first 4 trials (May 8- May 28), males were released at sunset (approx 8pm), however due to suspected high mortality from low overnight temperatures, males in the remaining 15 trials (May
20 - July 2) were released between 8-9am. Each treatment female was checked every hour from male release until midnight, then every hour starting the following day from 7am until midnight. The extra screens covering the cages were partially removed to allow males access to the top third of the cage and attached silk, but a protective layer of fiberglass window screening was attached inside the cage which blocked male from access to the female or her web directly in order to prevent them from destroying the web (web reduction is typically performed by males upon reaching a female's web: Ross and Smith 1979).

**Experiment 2: Wild males**

On the morning following an experiment 1 trial, cages containing females were removed from the enclosure site and transferred individually in (78cm X 51cmX 35cm) Sterilite™ plastic containers to a second trial site within the Hastings natural history reserve. Nine grassy fields which contained at least one active female *L. hesperus* web were used as second trial sites. Experiment 2 trials were never conducted in the same field at the same time, and trials using the same field locations were temporally separated by at least one week, no field sites were used more than twice. Cages were re-established in a similar arrangement (females placed in blocks, with cages separated by 3m) and tubes were re-dug into the ground. I placed strips of foamboard (15cm wide) coated in Catchmaster™ insect glue in a square surrounding the bottom of each cage between 8:30 and 9:30 in the morning. Cages and glue traps were subsequently checked for arriving males every 2 hours until 6pm. After the completion of experiment 2 trials, females were removed from cages and weighed, and all silk within the cage and the tube was removed and rated visually as a small, medium or large volume. I did not weigh the silk as it was impossible to get accurate measurements due to numerous small pieces of grass and seed adhering to the webs.

**Body size**

All females and all re-collected males used in experiment 1 were preserved in 70% ethanol and mean patella-tibia length and cephalothorax widths were subsequently measured as an indicator of size (Skow and Jakob 2003).

**Results**
Experiment 1: Enclosure study

In 16 of the 19 enclosure trials at least one male was found on a female’s web, and a total of 45 males (59% of released males) were recovered within the time designated for the experiments. In 2 of the 4 trials started at sunset no males were recovered. Males in the other 2 sunset trials were found on the virgin fed (3 males) and on the mated fed (2 males), and these outcomes are included in my analysis. Males were significantly more likely to be found on a virgin/fed web (individual males: \( \chi^2 = 73.13, p < 0.0001 \); \( \chi^2 = 27.50, p < 0.0001 \) by replicate) than any other treatment, and were least likely to be found with mated/unfed females, although there were no significant differences in attractiveness among the other treatments (Figure 1.2). Of the males recovered, 36 (80%) were found at a virgin/fed web, 4 (8.9%) at a virgin/unfed web, 4 (8.9%) at a mated/fed web and 1(2.2%) at a mated/unfed web. Moreover, in 12 of the 16 trials males were only found on webs of virgin/fed females. In four trials males were found on both virgin/fed webs and on other female webs, however it should be noted that in two of these cases virgin/fed females failed to build webs that extended beyond the very base of the tube.

Experiment 2

I ran 18 replicates of experiment 2 between May 19th and July 1st, and in 11 trials at least one wild male was found on the cage, or on the glue trap surrounding at least one experimental female. Of the 25 males recovered on experimental cages, all but three were found on the cages of virgin/fed females (individual males: \( \chi^2 = 50.33, p < 0.0001 \); by replicate: \( \chi^2 = 19.18, p=0.0003 \), Figure 1.2). Male choice was unanimous in all but one trial, in which males were found on multiple cages (2 on virgin/fed, 1 on virgin/unfed).

Body size

No differences were found between the weights of males that were recaptured and those that were not in the enclosure study, nor between the weights and sizes of males recaptured on virgin/fed webs compared to other treatments (Table 1.1).

Fed females were significantly heavier than unfed females (Mann Whitney U test = 49.00, \( p=0.00 \)), but there were no differences in female size among treatments (Mann Whitney U test
One of the mated females produced an egg sac prior to use in trials. However, trials using this female showed the same patterns of male choice as others, so data for this block are included in the analyses presented here.

**Discussion**

These results show *Latrodectus hesperus* males are choosy, with near universal preferences for virgin, well-fed females over mated and unfed females (Figure 1.2). Moreover, these preferences are expressed even in the presence of natural costs of choice, and without variation related to the phenotype of individual males (Table 1.1). Both the design of my test enclosures and spider biology indicate that male assessments were most likely based on female-specific airborne chemical cues. In terms of biology, spiders have very low visual acuity, making it improbable that males were differentiating female sizes from a distance (Foelix 1996). More importantly, there were no size differences among females in the four treatments; morphological differences were based on weight, which is a subtle character and something that males with poor visual acuity would be even less likely to perceive. Given that males are assessing females on the basis of chemical cues, could those cues have been contact chemicals located on the web itself? This is again very unlikely. Although males could contact the cages, the double layer of cage material prevented easy contact with the web. Males could only have touched the web if they had inserted one leg through the screening, a behaviour I never observed. Additionally, the low number of males caught near the cages of other females in experiment 2 (n=3) versus those caught near virgin/fed females (n=13) does not support a model of male assessment through contact web chemicals because the cages were surrounded by glue traps. If males were attempting to assess web cues, the number of glue-trapped males should have been evenly distributed across all four female treatments. Similarly, I would have expected more males to be caught on the cages of females other than the virgin/fed female in experiment 1 if males are required to contact female in order to make assessments of female quality. I thus conclude that males use airborne chemical cues to assess both feeding and mating status of females.

Not only are *L. hesperus* males able to use airborne chemical cues to assess females, they are selective for at least two different aspects of female quality: low sperm-competition risk and high fecundity/low cannibalism risk. Few studies have examined male choice with respect to both major aspects of male preference (but see: Bel-Venner and Venner 2006; Wong and McCarthy
2009; Callander, Backwell et al. 2012; Jeswiet, Lee-Jenkins et al. 2012), possibly because choice is generally studied in the laboratory, where it is difficult to realistically mimic the costs of such choice. In general the use of multiple criteria in mate evaluation may be limited in nature if the number of potential mates shrinks with each criterion added to the preference function. If a multi-criterion preference has an elevated cost relative to a single criterion, then each aspect of preference should evolve only if the additional fitness benefit of adding a new choice dimension outweighs the costs of further limiting the number of potential mating partners. *Latrodectus. hesperus* males, however, may be particularly likely to use two indicators of female quality because there may be costs to mating indiscriminately with regard to either aspect. The cost of mating with a non-virgin female is high because male spiders leave “plugs” blocking access to female sperm storage organs, reducing paternity gains from subsequently mating males (Chapter 4). The cost of mating with a low diet/low condition female is also high because these females are more likely to attack and consume males as prey items (Johnson, Trubl et al. 2011). Male choice for these same two criteria has also been demonstrated in natural populations of mantids (Lelito and Brown 2008), another group in which males benefit from mating with virgins and are at risk of pre-copulatory sexual cannibalism when pairing with low-diet females. It is possible that the risk of sexual cannibalism is required to elevate information about female fecundity to the same level of importance as mating status for choosy males. For example, in *Argiope bruennichi* spiders, sexual cannibalism does not prevent mating and in this species males are choosy with respect to female mating status but not diet (Schulte, Uhl et al. 2010). Further investigations comparing male preference for high diet females and the presence of multiple choice criteria within genera whose species vary in their propensity for pre-copulatory cannibalism would elucidate the influence of this behavior on the evolution of male mate choice in spiders.

Studies of male mate choice have frequently found assortative pairing, which can occur through high quality males outcompeting lower quality ones, or through prudent or reversed male mate choice (RMMC) (Venner, Bernstein et al. 2010) in which low quality males avoid competition by pairing with low quality females. Although assortative pairing seems to be a common outcome in male mate choice systems (Olsson 1993; Hunt, Cuthill et al. 1999; Shine, O'Connor et al. 2001; Preston, Stevenson et al. 2005; Hoefler 2007; Bel-Venner, Dray et al. 2008; Franceschi, Lemaitre et al. 2010; Yu and Lu 2010), prudent choice/RMMC has been
demonstrated in only a few species (Candolin and Salesto 2009; Wada, Arashiro et al. 2011), some of which are spiders (Hoefler 2007; Bel-Venner, Dray et al. 2008). Studies on *Latrodectus hasselti*, however, showed that although small males are outcompeted by larger rivals in direct competition (Stoltz, Elias et al. 2008), they may not be subject to the type of competitive disadvantage that leads to prudent male choice as small sneakers males can mate successfully after parasitizing the courtship effort of larger rivals (Stoltz and Andrade 2010). Thus direct intra-male competition for preferred females may not exert sufficient selection pressure on small/low quality males to alter their mate choice strategy and consequently all males maintain the same preferences. Working with a relative to *L. hasselti*, *L. hesperus*, I also did not find any evidence supporting prudent or reversed male mate choice. The two males captured near females other than the virgin-fed treatment in Experiment 2 represented opposite ends of the captured-male size distribution (the third male was too glue-covered to be accurately measured). Although enclosure (experiment 1) males may not have differed much in quality due to the similarity of their treatment and feeding schedule in the laboratory, wild caught males should be representative of natural variation. It is possible that assortative mating could arise from intra-male competition in nature but this would not have been detected given my experimental design because males were unable to access the female’s web, which is where intra-male contests take place in congers (Stoltz, Elias et al. 2009). Overall then, *Latrodectus* species in particular may be less likely to exhibit RMMC than other spiders and, despite the plausibility of the idea (Venner, Bernstein et al. 2010), it remains to be seen whether this is a common feature of male choice in general.

Although strong female choice is known to lead to the evolution of a wide range of male traits, there are contexts in which this is not the case. For example, when female choice is conditional, or for genetic compatibility, a lack of consensus on the preferred male phenotype will dilute selection on males. Here I show strong, highly consistent choice among males for well-fed (high condition) females that are unmated, but I do not expect this male behaviour to exert selection pressure on females. Firstly, with respect to mating status, selection cannot act on females to maintain their virginity as it is a state experienced by all females and because, obviously, virgin females do not gain fitness benefits over mated ones. One can ask whether this strong preference may lead to females attempting to conceal their mating status because of some benefit from continuing to attract males. However, no records of such behaviour exist, perhaps because males
induce changes in the chemical profile of mated females through substances transferred during copulation (Thomas 2011). Secondly, with respect to female nutritional condition, females are already under strong natural selection to be efficient predators; the addition of male choice is unlikely to add significant strength to that pressure. However, the preference for high condition females could select for female signaling of their feeding status. Female condition-dependent ornaments (Weiss 2006; Baldauf, Bakker et al. 2011), or those that exaggerate body size (Funk and Tallamy 2000) are known from other systems with male mate choice, and female condition-dependent pheromone signaling has been found in moths (Harari, Zahavi et al. 2011). Future work examining the persistence of volatile chemicals indicating body condition in mated females, who have no need to attract males, could indicate whether these volatile chemicals are advertised by mate seeking females as a sexually selected signal.

In summary, my work provides evidence that male mate preferences can lead to choice behaviour under natural conditions. Males of *L. hesperus* show near unanimous choice for females with high fecundity, low risk of cannibalism and low risk of sperm competition, despite differences in size and weight in lab-reared males and natural differences in developmental conditions and experience in wild males. Further examinations into male mate choice using field experimentation are required in order to confirm laboratory based tests of preference and to gain a more complete picture of the process of mate choice from a male perspective.
References


Figure 1.1. (A) Field enclosure, approx 3m x 3m with cages in corners (pictured without screen roof), and (B) individual cage made of wire mesh, covered with screening.
Figure 1.2. Males recovered in experimental release enclosure studies (Expt. 1, dark grey, n = 45) and in field trials with wild males (Expt. 2, light grey bar, n = 25) that were discovered on webs of females in each of 4 treatment groups. Data are shown as percent of total males recovered over the entire experiment (A) and as average (± S.E.) percent of males found on each treatment type within each replicate (B).
Table 1.1. Difference between the weights and sizes of males attracted to different female treatments or unrecovered at the end of experiment 1.

<table>
<thead>
<tr>
<th>Category</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Difference?</th>
<th>test</th>
</tr>
</thead>
</table>
| Weight   | Recaptured males         | Lost males               | No          | Mann-Whitney U        | \( U = 465.5 \) \\
|          |                          |                          |             | \( p = 0.894 \)      |
| Size     | Males found on VF females| Males found on other females | No          | Mann-Whitney U        | \( U = 40.5 \) \\
|          |                          |                          |             | \( p = 0.161 \)      |
| Weight   | Males found on VF females| Males found on other females | No          | T test                | \( T = -1.129 \) \\
|          |                          |                          |             | \( p = 0.266 \)      |
Chapter 2

Male mate choice and variable female quality in field populations

Abstract

Theory predicts that male mate choice is driven by variation in female quality. However, to my knowledge, no study to date has examined the connections or co-variations between female reproductive output and phenotypes preferred by males in the field. Whether male mate choice might exert sexual selection on females has also rarely been assessed. I address both of these issues in field populations of western black widow spiders (*Latrodectus hesperus*). Male western widows are choosy and prefer to settle with and court heavier, well-fed females under laboratory and semi-natural conditions. I assessed variation in these traits and related aspects of female phenotype and whether this variation predicted female reproductive success in nature. I monitored female size, web size, and reproductive output during spring breeding-season surveys over 3 consecutive years. I also quantified female reproductive failure, the potential that this was caused by a failure to mate, and whether reproductive and non-reproductive females differed in their expression of male-preferred phenotypes. I found that female size, web size, and female persistence on webs were all positively correlated with reproductive output (number of egg sacs). However, a more important source of variation in female success was the occurrence of reproductive failure, which was least likely for larger females that matured early in the season. I found evidence consistent with differential female reproductive success due to male rejection of less preferred phenotypes in only one year. My work suggests that choosy males are benefitting from selecting larger females as these females are more likely to reproduce, and in good years, may produce many more egg sacs than the average for smaller females. I argue that the inconsistent sexual selection pressure from male choice on female size is likely inconsequential for the evolution of female phenotypes due to the consistent fecundity and viability selection of females for the same trait. My work offers a field-based perspective on fitness effects of male mate choice for both sexes, and tests theoretical assumptions about choice under natural conditions.
Introduction

Mate choice is expected to evolve when fitness effects of variation in the quality or reproductive value of potential mates are sufficient to offset the costs of choice (Parker 1983; Owens and Thompson 1994; Bonduriansky 2001; Edward and Chapman 2011). When the number of matings is limited by high mating effort or similar costs, the likelihood of choice increases since the contribution of each interaction to total fitness also increases (Gwynne 1991; Bonduriansky 2001; Kokko and Monaghan 2001; Edward and Chapman 2011). Constraints on choosiness, including difficulty identifying high-quality mates (Parker 1983), decrease the likelihood of choice by attenuating the expected benefit of choosy behaviour (Bonduriansky 2001). This theory has been explored in models, laboratory and field studies of female choice (Parker 1983; Real 1990; Andersson 1994; Kvarnemo and Simmons 1999; Kokko and Johnstone 2002; Rudolfsen, Figenschou et al. 2005; Nandy, Joshi et al. 2012). Empirical support is now growing for the idea that male mate choice may also be widespread, may co-occur with inter-male competition and may be understood using the theory established for female choice (Bonduriansky 2001; Edward and Chapman 2011). Mate choice among males has been documented in a broad range of taxa including fishes (Jeswiet, Lee-Jenkins et al. 2011), snakes (Lemaster and Mason 2002), lizards (Olsson 1993), primates (Zhang, Li et al. 2010), other large and small mammals (Schwagmeyer and Parker 1990; Craig, Herman et al. 2002; Preston, Stevenson et al. 2005), and within polygynandrous (Costello, Pultorak et al. 2009), polygynous (South and Arnaqvist 2011), and monogynous (Schulte, Uhl et al. 2010) systems.

Choice is driven by the fitness benefits from being choosy, and work to date shows that male mate preferences can largely be categorized as those for indicators of female fecundity and those for indicators of low potential sperm competition. These decisions will be constrained by the number and quality of mates encountered over a lifetime and the cost of acting on preferences (Jennions and Petrie 1997; Wong and Jennions 2003; Simcox, Colegrave et al. 2005; Svensson, Lehtonen et al. 2010). Thus, despite the existence of strong preferences for particular phenotypes, the density and spatial distribution of potential mates and rivals can have significant effects on the fitness consequences of being choosy, and thus on the evolution and maintenance of choice (Kokko and Rankin 2006; Kemp and Macedonia 2007; Elias, Andrade et al. 2011). Currently, there are relatively few studies that have examined male mate choice in the field (but
see: Foote 1988; Riechert and Singer 1995; Kasumovic and Andrade 2004; Preston, Stevenson et al. 2005), and few studies of male mate choice have determined potential benefits of choice in terms of natural variation in the reproductive value or quality of potential mates (but see: Kvarnemo and Simmons 1999; Nandy, Joshi et al. 2012). Insights about male choice mostly arise from laboratory studies with (usually binary) forced-choice protocols and/or manipulations of female quality; often paired with laboratory data linking female phenotypes to fecundity (e.g., Verrell 1985; Batorczak, Jablonski et al. 1994; Reading and Backwell 2007; Martel, Damiens et al. 2008; Liao and Lu 2009; Ala-Honkola, Saila et al. 2010; Barry, Holwell et al. 2010; Schulte, Uhl et al. 2010; Johnson, Trubl et al. 2011; Chen, Salcedo et al. 2012). While these can be valuable for documenting male preferences, inferences about choice from laboratory studies are not always reliable For example, although choosy males in many taxa prefer females with phenotypes indicating high fecundity (Cornwallis and Birkhead 2007; Barry 2010; Baldauf, Bakker et al. 2011; Harari, Zahavi et al. 2011), the strength of links between phenotypic cues and female reproductive output in nature (and thus male fitness) is unclear (e.g., female colouration: Doutrelant, Gregoire et al. 2008). Benefits from mating with females of high reproductive potential may be tempered by environmental factors that affect actual fecundity (Hampton 1986; Dunn, Crean et al. 2001; Rittschof 2011; Thompson, Hassall et al. 2011). This is particularly problematic for males because there is often a time lag between the occurrence of mate choice and the realization of the benefits from being choosy (Rittschof 2011). This time lag between male mating decisions and oviposition/birth may expose females to predation, infection, food or water limitation and weather conditions that reduce fecundity (Hampton 1986; Spencer 2002; Hasu, Valtonen et al. 2006). Variance in female quality as it relates to realized fecundity may thus be different from expectations based on laboratory studies. Similarly, fecundity may be effectively uncorrelated with phenotypic cues in nature, despite laboratory studies that document links under controlled conditions. This is in contrast to female choice since females generally have high confidence of maternity, and often receive benefits (whether genetic or material) from their mates at the time of mating. If the link between cues used for male choice and female reproductive value are consistently obscured in nature, conditions under which male mate choice evolves may be considerably more restrictive than female choice. It would thus be valuable to track female reproductive value over time in nature, and examine how this varies as a function of the traits that choosy males find attractive.
Understanding the effects of natural variation on male mate choice is not only important for predicting male reproductive tactics, but also for assessing whether male choice will impose sexual selection on females (Edward and Chapman 2011). There are three aspects of this interaction that require attention. First, we must know the proportion of females that remains unmated due to strong male choice. The relative size of this group will affect the opportunity for sexual selection (Shuster and Wade 2003; Jones 2009). Second, it is necessary to determine whether patterns of mating match those predicted by male preferences, as assortative mating may arise in the field even when male preferences are strong under controlled conditions (e.g., Hoefler 2007; Bel-Venner, Dray et al. 2008; Candolin and Salesto 2009). For example, when competition and choice co-occur in nature, smaller males may be competitively excluded from mating with high-quality females, or they may actively choose lower quality females to avoid intense competition over, or rejection by, high-quality females (prudent or reversed mate choice: Fawcett and Johnstone 2003; Yu and Lu 2010; Wada, Arashiro et al. 2011). Assortative mating can ease selection on females as few females will fail to mate under those conditions. Third, it is important to identify the traits favoured by males as these will not always lead to selection on females. For example, in many species of arthropods males prefer virgin females who present a relatively low risk of sperm competition (Martel, Damiens et al. 2008; Aisenberg and Gonzalez 2011; Tuni and Berger-Tal 2012). This preference does not impose selection on females, other than perhaps to advertise virginity. In contrast, sexual selection for fecundity may lead to females that advertise their body size or other correlates of high reproductive potential (Funk and Tallamy 2000; Lemaster and Mason 2002; Harari, Zahavi et al. 2011). Moreover, since in this case sexual and natural selection would be coincident, it is possible that male choice could contribute to exaggerated increases in female body size (e.g., in Nephila spiders: Kuntner and Coddington 2009). Longer term observational studies provide the opportunity to investigate the potential for male choice to exert sexual selection pressure through the examination of the connection between patterns of mating success and failure, and male-preferred phenotypes.

Here I report results of a 3-year longitudinal study of field populations of female Western black widow spiders (Latrodectus hesperus), which was designed to examine how female phenotype relates to male choice and reproductive success in nature. My goal was to gain insight into the theoretical predictions that (1) male mate choice is driven by variation in female quality, through examining variation in attractive phenotypic traits in females, and (2) preferred females should
deliver fitness benefits to males, through examining how phenotype relates to reproductive output across the season. Furthermore, I aimed to assess the consequences of male mate choice and the potential for sexual selection on females through examining female mating/reproductive success versus failure in the field.

Spiders are good subjects for this type of study. Males that are both choosy and competitive may be common among spiders and their relatives (Hoefler 2007; Kasumovic, Bruce et al. 2007; Bel-Venner, Dray et al. 2008), for three reasons. First, male avoidance of sperm competition has led many male spiders to invest in behaviours that limit polygyny, but block a rival’s access to females. These behaviours include mate guarding (Miyashita 1994; Prenter, Elwood et al. 1994; Herberstein, Barry et al. 2005), and both genital mutilation and self-sacrifice (Snow, Abdel-Mesih et al. 2006; Foellmer 2008; Uhl, Nessler et al. 2010). Such behaviours imply that virgin females have relatively high reproductive value. Second, as with many invertebrates, female fecundity is generally linked to body size and/or condition, which can be very variable (Marshall and Gittleman 1994; Head 1995; Uhl, Schmitt et al. 2004; Drapela, Frank et al. 2011). Male preference for heavier females has been documented several times in spiders, both in the laboratory and in the field (Prenter, Elwood et al. 1994; Uhl 1998; Danielson-Francois, Fetterer et al. 2002), including in *L. hesperus* (Johnson, Trubl et al. 2011, Chapter 2). Third, female web-building spiders produce pheromones that allow assessment of reproductive status (Roberts and Uetz 2005; Gaskett 2007; Stoltz, McNeil et al. 2007; Baruffaldi and Costa 2010; Thomas 2011), as well as feeding condition (Baruffaldi & Andrade unpublished, Chapter 1), so males may be able to assess females at a distance with relatively little cost.

I observed Western black widow spiders (*Latrodectus hesperus*) at Hastings Biological Reserve, near Carmel Valley California, in their natural habitat during their breeding season (late April-early July) from 2009 to 2011. Female *L. hesperus* are sedentary, building webs out of gopher holes or ruts in the ground of old fields, and adult males abandon web building and search for mates using web-based airborne cues from females’ webs (Kasumovic and Andrade 2004). Male *L. hesperus* are capable of polygyny (Chapter 3), but may have limited mating opportunities due to their short life spans compared to females, potentially risky mate search (as seen in a congener: Andrade 2003), high courtship investment (as seen in a congener: Stoltz, Elias et al. 2009) and post-mating refractory period (Chapter 3). *Latrodectus hesperus* males are choosy, preferring well fed, virgin females over poorly fed, virgin females and both well fed and poorly
fed, mated females (Chapter 1). Fecundity is likely governed by access to food in *L. hesperus*, as in many other spiders and arthropods (Marshall and Gittleman 1994; Head 1995; Uhl, Schmitt et al. 2004; Drapela, Frank et al. 2011). Female *L. hesperus* vary widely in abdomen size in the field, and in the number of egg sacs deposited over the breeding season (pers obs). Female web size also varies in the field and has been shown in the lab to be determined by feeding regime (Blackledge and Zevenbergen 2007; Salomon 2007). Through my observational study over three field seasons I examined the variation in female size and web size, the relationship of these phenotypes to egg sac production and compared the phenotypes of mated and unmated females.

### Natural history

*Latrodectus hesperus*, like all *Latrodectus* species, are cobweb-building spiders. Their webs are densely threaded, irregular 3-dimensional shapes that are defined by the surrounding vegetation. The web generally radiates from a thickly woven hole leading to a refuge, an unexposed annex to the web where mating likely occurs and where egg sacs and food items are stored, located either underground or under surrounding vegetation. Females actively maintain their webs by adding silk to increase density or volume or to repair damaged portions.

Female *L. hesperus* establish above-ground webs during the early spring (April – May) and lay eggs in May and June, with spiderlings hatching within two to three weeks of deposition. During pre-hatching development females keep the egg sacs suspended in their webs and are typically found in a defensive posture with one leg around each sac. After hatching spiderling dispersal behaviour varies. I have observed some dispersal by ballooning and some offspring remaining on females’ webs throughout the observation period. Laboratory-based work found the timing of female maturation was accelerated by access to increased food resources (Baskaran & Andrade in prep), and this is expected to be reflected in the field with females on nutrient-rich diets (i.e., successful foragers) maturing earlier in the season. Adult and sub-adult females were always present in webs by April 30th in all years.

Daily female activity is apparently linked to the light and temperature cycles (pers obs). Females typically sit with their suspended egg sacs at the entrance to their refuges in the early mornings and retreat underground with the sac as the day progresses and ground temperature increases. Females are often active again in the early evenings after dusk, maintaining webs or occupying
the refuge entrance hole, often with their egg sacs. Foraging occurred throughout the day and night.

**Methods**

Female Western black widow spiders were monitored in six discrete old field locations on the property of Hastings Natural History Reserve, in Carmel Valley California (Figure 2.1), from the last week in April until the first week in July in 2009, 2010 and 2011 (8 weeks total). The fields were all within close proximity to the main road of the field station, within 4km of each other, and differed in size. Fields were chosen based on observations from an investigation conducted the previous year into likely habitat for *L. hesperus*. Several different habitats types were investigated (riparian, oak savannah, forest, embankments/hill sides), with *L. hesperus* being found most often in open fields. All fields had consistent biotic and abiotic components typical of California grassland (Stromberg and Griffin 1996).

**Locating webs & females**

Prior to the start of the monitoring period, each field was scanned by a researcher (ECM or one of three field assistants) three times, and scanning also occurred once weekly during the monitoring period. Scanning consisted of walking transects spaced 1 meter apart looking for webs or spiders. Starting points for scan samples were changed regularly to minimize observation fatigue and reduce systematic biases. Black widow webs are distinctive in their size and in the strength and reflective properties of their silk, which makes them relatively easy to locate and identify. Every suspected web was marked with a surveyor’s flag adjacent to the web’s refuge and given an identifying number. Data were included in my analysis only if webs were confirmed by identification of black widow spiders in subsequent monitoring. Female sexual maturity was assessed by either evidence of reproduction in the web, the presence of a moult skin outside the refuge entrance hole, or by visual examination of the genitalia. Adult and sub-adult females have distinct colouration and body shape (Kaston 1970) but may be similar in size. They can be visually distinguished because the exoskeleton of sub-adult females covers the maturing female gonopore, and this can be detected with a trained eye.

**Monitoring webs**
Monitoring consisted of four activities: scanning the field for new females (once per week), and for every identified web in each field: egg sac checks (twice per week between 9am and 11am), female presence and size estimation checks (twice per week in 2009 & 2010, once per week in 2011, between sunset and 11pm) and web size measuring (once per week).

Female size estimation.

In spiders, abdominal dimensions are variable, and increase with feeding condition (‘fatness’), as well as, for females, whether they are gravid. Female abdomen size has been found to vary with diet in several species of spiders (Anderson 1974; Forster and Kavale 1989; Legrand and Morse 2000), and has been found to be an important factor in predicting male attendance on female’s webs (Vincent and Lailvaux 2006). To minimize disturbance to females, size estimations were made by comparing the abdomens of females to drawings of ovals representing small, medium and large-sized females. Drawings were based on the typical range of variation in laboratory-reared and field-surveyed females. To allow assessment, females were lured from their hidden refuge by making gentle, prey-mimicking vibrations on the visible portion of their webs using forceps. Female size was scored on a 7-point scale and ranged from extra-small (=0.5) to extra large (=3.5). To assess the accuracy of my relative size measure, at the end of every field season 20-30 females, consisting of 10% of the population of each surveyed field plus females from non-surveyed fields, were size-estimated in-situ then collected and weighed using a Mettler BB240 balance. Any unhatched egg sacs associated with collected females were also retrieved and returned to the lab.

Web persistence

Females frequently disappeared during the monitoring period. I scored disappearances when three criteria were met: (1) females were absent during diurnal and nocturnal checks; (2) females did not appear in response to web destruction. Females typically repair their webs nightly and are unlikely to abandon webs due to this kind of disturbance; and (3) females were not found during exploration of the refuge (following confirmation of criteria 1 and 2). Many refugia and webs were destroyed due to backfilling of holes by gophers. In these cases, if a new female was found in a refuge immediately adjacent to a filled hole and in contact with the snare of the old web, she was assumed to be the same individual. Females surviving these evictions and establishing a
new web at a later date or further from their original web would have been counted as new individuals. This could be problematic but I did not mark individual females because this would have required significant disturbance to females and their webs. I assume that in the majority of cases, my surveys track individual females inhabiting single websites. This is a reasonable assumption for the following reasons. (1) I am confident females were not switching webs, as over three field season I only saw two occurrences of a female taking over another female’s web and in both cases one female was killed, and the carcass found outside the entrance of the refuge. (2) Although females frequently disappeared during the season, this is more likely due to mortality than to moving to establish new webs. Western black widow females appear to rarely abandon established webs, but instead repair them each night when they are damaged (Salomon 2009), or change the density of the snare when prey are scarce (Zevenbergen, Schneider et al. 2008). This may be partly because females are slow, ungainly, and vulnerable when walking unless they are on a web. Moreover, within each field, ~50 - 60% of all webs were found during the searching period or the first week of monitoring and the number of new webs found each week declined sharply after this (see Figure 2.2). To be conservative, I discuss data on female disappearances in terms of the duration of female presence on webs during my monitoring period (= ‘web persistence’). However, it is likely that most ‘disappearances’ occur when females die, which means web persistence is an indicator of longevity.

Web size

In 2009 and 2010 I measured the ground area covered by females’ webs as an estimate of web size. *Latrodectus hesperus*’ cob webs are difficult to measure due to their highly variable, irregular size and complexity. However, females tend to add to their webs by enlarging the horizontal coverage of their snare, so the total ground area covered by the web is a reasonable estimate of web size. I measured the distance from the centre of the refuge to the farthest web attachment point in all the cardinal and sub-cardinal directions (i.e., 8 measurements with 45° angles between them) for all webs every week during the breeding season. I used compasses held over the web to determine direction and then marked the location of the edge of the web in that direction using colour-coded wood sticks. I then used a measuring tape to measure the distance along the ground from the refuge to the stick. Care was taken to do minimal damage to the web while taking these measurements. Web area was calculated as the sum of the areas of the eight
triangles created by the distance measurements. For the average web sizes in my analyses I used only the measurements taken when I could confirm a female was residing in the web. Webs were not measured in 2011 due to the high time requirements of this activity which interfered with other studies conducted during the field season.

**Statistical analyses**

I examined seasonal and yearly variation in all the measured variables, and correlations between reproductive success (number of egg sacs), possible correlates of fecundity (web size, female size, web persistence) and female size (the variable most closely linked to demonstrated male preferences, Chapter 1). Statistical calculations were made in SPSS 15.0 (2000), with tests as outlined below.

**Results**

**Overview**

I observed 214 females across the six fields (Figure 2.1) in 2009, 172 in 2010 and 304 in 2011 and per capita egg sac production hovered around 1 sac / female each year (Table 2.1). I first observed adult females during my scan samples beginning on April 26\(^{th}\) (2009, 2011) or April 27\(^{th}\) (2010). Average web persistence differed between years with the lowest persistence in 2010 both in terms of the % of females that disappeared through the monitoring period (which differed across years: 2009 = 68.0%, 2010 = 82.6%, 2011 = 69.6% , Kruskal Wallis test, \(\chi^2 = 20.875, p=0.001\)), and in terms of the average number of weeks females were present (Table 2.1). Web sizes were highly variable, and webs were larger in 2009 compared to 2010 (Table 2.1).

Because of the difficulty in marking individual females, I was unable to be certain that females that disappeared during the study were not the same individuals that were found in subsequent weeks. This error is likely to be relatively rare since the number of new webs found after the first few weeks of the monitoring period (Figure 2.2) was small compared to the number of females that disappeared, particularly in 2010. In order to quantify the potential for error in my data, I calculated the percent of my observations that could have mistakenly lowered my estimate of web persistence. To do this, I calculated the total number of females that disappeared that could have reappeared at a new location given the number of new females found every week. I did this
by assuming that all females that disappeared could have reappeared anytime after their
disappearance, anywhere within their home field. I found that the maximum potential error rates
were 33.61% in 2009, 35.82% in 2010, and 25.86% in 2011. I used the same conservative
method to estimate potential error in my estimate of egg sac production, except in this case I
included only the number of new females found every week that eventually produced egg sacs. I
found that the maximum proportion of females to which I could have attributed the wrong
number of sacs was 7.72% of females in 2009, 7.96% in 2010, and 10.4% in 2011. The
maximum proportion of females that could have been mistakenly classified as non-reproductive
was 12.65% in 2009, 14.03% in 2010 and 4.80% in 2011.

**Phenotypic variation among females**

The proportion of females that produced at least one egg sac (= reproductive females) varied
significantly across years (Figure 2.3) (Kruskal-Wallis test, $\chi^2 = 8.964, p = 0.011$). However, the
mean number of egg sacs produced per capita was not different across years (Kruskal-Wallis
test, $\chi^2 = 1.604, p = 0.448$) (Table 2.1). Most commonly, females either failed to reproduce or
produced a single egg sac during the monitoring period, although some produced up to four sacs
(Figure 2.3).

My categorical estimate of female size was a significant predictor of mass among the subset of
females collected at the end of the season in each year and across years (Table 2.2), and was
positively correlated with female mass in all years. The average and maximum estimated sizes of
females were significantly different across years (Table 2.1) and fields (appendix 1). For further
analyses, I took the estimates of size made throughout the monitoring period for each female,
and calculated a single variable to represent ‘size’. This was the first principal component of an
analysis that included the maximum estimated size and the average estimated size across the
monitoring period (PCA1 accounted for 89.89% of the variability). All analyses below use this
composite measure of female size.

Web size was significantly different between years ($\chi^2 = 11.33, p = 0.001$) (Table 2.1) and
between fields (appendix 1). In further analysis web size is a condensed variable consisting of
the first axis of a PCA of maximum web size and average web size (PCA1 accounted for 92.23%
of the variability).
Female size and web size were positively correlated in both 2009 (Pearson correlation=0.28, p<0.001) and 2010 (Pearson correlation= 0.375, p<0.001), webs were not measured in 2011.

**Connections between phenotype and reproductive output**

In all years, female reproductive output was predicted by female size (GLM, distribution=poisson, link=log, Figure 2.4, Table 2.3), but not web size (webs were not measured in 2011) (Table 2.3). In 2009 and 2010 this relationship was driven by the differences between females that deposited egg sacs and females that did not, as there was no size differences among females that produced different numbers of egg sacs (Kruskal-Wallis test: $\chi^2= 0.876$, p=0.645 in 2009, and $\chi^2= 1.177$, p=0.555 in 2010). However in 2011 females that deposited one egg sac were significantly smaller than females that deposited two and three egg sacs (Kruskal-Wallis test: $\chi^2= 12.975$, p=0.002).

I further investigated the relationship between female reproductive output and other variables that may influence either size or reproductive output. Continued presence at a web site was significantly correlated with reproductive output, female size and web size in all years (Table 2.4), with females persisting for less than five weeks having an average reproductive output of less than one egg sac (Figure 2.5). Reproductive output was different between fields in 2009 (Kruskal-Wallis test: $\chi^2= 34.406$, p<0.000) and in 2010 (Kruskal-Wallis test: $\chi^2= 24.440$, p<0.000), but not in 2011 (Kruskal-Wallis test: $\chi^2= 10.467$, p<0.063) (Figure 2.6). Female size was different between fields in all years (Table 2.5) (Figure 2.6).

**Differences between females that did and did not reproduce**

I intended to compare the phenotypes of females that did and did not reproduce throughout the study to determine whether or not female reproductive failure can be attributed to male mate rejection. However, I had to first exclude the possibility that female reproductive failure was due to a female having insufficient residency time at a web site. Females that failed to reproduce were typically present for three or fewer weeks within the study period (Figure 2.7), while females present for fewer than five weeks had an average reproductive rate lower than one egg sac (Figure 2.5). In order to remove cases of reproductive failure due to insufficient web residency time, I compared the subset of reproductive and non-reproductive females that were persistent on their webs for longer than five weeks (and so should have had sufficient time to
produce an egg sac if they were mated). In my comparison I also used two measures of female persistence, **absolute persistence**, which is the total number of weeks that a female was observed at a web site within the study (e.g., 4 weeks out of 8), and **relative persistence**, which is the number of weeks a female persisted at her web site, out of the total number of weeks she was able to persist given her date of first discovery (e.g., discovered in week 4, present for subsequent 4 weeks = complete persistence). In each year, approximately 10% of females present for more than five weeks did not produce egg sacs (2009 n=24, 2010 n= 15, 2011 n=28).

As indicated in Table 2.6, females that reproduced were found significantly earlier in the season than those that did not reproduce in 2009 and 2011, but not in 2010. Reproductive females also persisted for longer in terms of absolute persistence in 2009 and 2011, not in 2010, but in terms of relative persistence were present for longer in 2010 and 2011 but not in 2009. Reproductive females were significantly larger than females that did not reproduce in 2010, and showed a trend of being larger in 2011, but were not larger in 2009. No differences were found in web size of reproductive and non-reproductive females.

Females that failed to reproduce despite > 5 week persistence at their webs, may have been unmated due to male rejection, but it is also possible that they were under-nourished and thus had ceased egg production as reduced size is related to low fecundity (Stoltz, Hanna et al. 2010).

To examine whether these females were simply under-nourished, I asked whether non-reproductive females were large enough to be capable of reproducing by comparing their average and maximum observed sizes to the estimated minimum size of other females that successfully reproduced. I calculated this as the lowest mean size of reproducing females across the three years minus one standard deviation of that size (mean estimated size of reproducing females=1.4125, standard deviation = 0.0459) which was 1.0066. In most cases, non-reproductive females were equal to or larger than this estimated minimum size for successful reproduction (22 of 24 cases in 2009, and all cases in 2010 and 2011), suggesting they should have been able to produce egg sacs, but may not have been mated.

**Discussion**

The goal of this study was to use observations from natural populations of *L. hesperus* to inform our knowledge of male choice from the patterns observed in and among females. From this work I show novel evidence that supports basic theory concerning three different aspects of male mate
choice: (1) The Male mate choice among *L. hesperus* in Chapter 1 does exist within a population of females that are highly variable in their average size, web size, survival and reproductive success. However there is little consistency in the variation of these traits across years and proximate field locations. (2) *Latrodectus hesperus* males would gain fitness benefits by mating with preferred females, which in this case would be larger females, as they are more likely to survive long enough and have the resources to deposit an egg sac. (3) Female reproductive failure in *L. hesperus* in some cases may be the result of rejection by males due to the expression of a less preferred phenotype. To my knowledge no other study has tested some of the basic theoretical assumptions surrounding females in a conventional mating system with male mate choice, namely that females do vary in their reproductive output, and that this variation is predicted by the focal trait or traits determining male mate choice in nature.

The use of naturalistic observations posed certain challenges that must be considered in assessing this dataset. One of these was finding a non-intrusive way to measure female size. The high correlations and the predictive relationship between my estimated size and the weight of captured individuals validates this system as an adequate metric for assigning relative sizes to females in my monitored populations (Table 2.2). Another challenging aspect of data collection was the disappearance and potential reappearance of unmarked females throughout the study season. My maximum estimated error rate for all years was not negligible, however this estimate was calculated with the assumption that any female could appear at any location in her field at any time after her disappearance. As females are likely conspicuous and inept overland travelers, who rarely abandon their webs sites even when they are completely destroyed (Salomon 2009) my error rates are likely to greatly overestimate the actual proportion of females who were mistakenly identified as different individuals. Females are susceptible to attacks by small omnivorous mammals, particularly when they are away from their webs (Vetter 1980), and work on a congener found a high mortality cost to relocation (Lubin, Ellner et al. 1993). My analyses also showed that only a small proportion of females could have been assigned a low egg sac number (between 7-11%) or treated as non-reproductive females (between 4-14%) due to incorrect identification. The differences in these error rates compared to the risk of mistakenly assigning persistence reflects the fact that females found later in the season did not reproduce as successfully as those found earlier. If these females are reproducing later because they had to re-locate their webs, this difference could reflect the drain on energy resources needed to travel to a
new area and then build a new web. Alternatively differences in prey availability throughout the season may also bias resource accrual towards early arriving females. Although my estimates of female longevity (persistence) require confirmation through further observation of marked females, the relationship between egg sac production and residency at a web site is a reliable observation of a natural pattern.

For mate choice to exist, potential mates must vary in their expected contribution to the chooser’s fitness. Although natural variability among males in their phenotype and contributions to female fitness have been documented in systems with female mate choice (e.g., Borgia 1981; Robertson 1986; Evans and Hatchwell 1992; Thornhill and Sauer 1992), to my knowledge variation among females in these same traits have rarely been documented in systems with male mate choice, with the exception of some sex-role reversed systems (Owens, Burke et al. 1994; Robson and Gwynne 2010). I uncovered consistent differences in reproductive output between L. hesperus females within each year (Figure 2.3), confirming that in this male choice system there is variation in fitness returns among potential mates. The pattern of female reproductive output also varied between years (Figure 2.3), even though per capita egg sac production was not significantly different between years. For example, in 2010 proportionally fewer females produced egg sacs, but among females that did reproduce the proportion that produced more than one egg sac was higher, than in other years. In 2009 and 2011 more females reproduced than in 2010, but a much lower proportion produced more than one egg sac with this trend being more pronounced in 2011. The most consistent aspect of female reproduction across years was the high proportion of females that did not deposit egg sacs; between 30% and 45% of females each year (Figure 2.3, between 16% and 31% with maximum error incorporated). These observations suggest that the fitness benefits of choosiness may not be derived by continuous increases in reproductive returns on male mating investment as female size increases, as those are not consistent from season to season. Instead male mate choice may be promoted by the costs to males of wasting mating investment on females that fail to produce any offspring.

Although selection on males may be a response to a positive relationship between phenotypic expression and fecundity, with the accrual of fitness benefits among males able to detect females of high fecundity, it may also be a more threshold-like response to phenotypic differences that indicate whether females have enough resources for reproduction or not. Male mate choice for fecundity-indicating female phenotypes has been established in many taxa (Verrell 1985; Olsson
1993; Uhl 1998; Bonduriansky 2001; Jones, Monaghan et al. 2001; Szykman, Engh et al. 2001; Chenoweth, Petfield et al. 2007), however the relationship between these phenotypes and fecundity have rarely been examined (but see: Szykman, Engh et al. 2001). I found that female size significantly predicted likelihood of reproducing in all years. This pattern arose from size differences between females that did and did not deposit egg sacs in 2009 and 2010; only in 2011 were there differences in total egg sac production among females that reproduced successfully (with significantly smaller females producing one egg sac and larger females producing multiple egg sacs) (Figure 2.4). These findings are suggestive of a threshold of mate attractiveness, with smaller females failing to secure mates due to their inability to achieve a level of attractiveness that elicits a male response. Alternatively, these results could indicate that smaller females that failed to deposit egg sacs did mate but did not achieve the bodily resources to produce eggs. However, selection should favour males able to avoid investing into mating with females that are less likely to contribute to their fitness, and the evidence of male preference for larger, high diet females over smaller, low-diet females (Chapter 1) supports a hypothesis of high mating failure among smaller females.

As survival ultimately determines reproductive output, phenotypes that indicate a female’s potential to survive until breeding and rearing are completed may also be favoured by male mate choice (Hampton 1986; Rittschof 2011). These may coincide with indicators of fecundity if both are dependent on high body condition. I found that the length of time a female was able to persist at a web site was also positively correlated with female size, web size and reproductive output in all years. This relationship is expected, as absent females cannot lay eggs, increase in size or build webs. However the correlations among these factors also suggest that by preferring larger females males may benefit from their increased survival. Male preference for female traits that are correlated with survival has been found in the mosquito fish, Gambusia affinis (Hampton 1986), but remains relatively poorly studied. This is surprising given that in many species there is a time lag between mating and oviposition/birth so the realized benefits of male mate choice depend entirely upon the health and survivability of the female during that lag period. In L. Hesperus, male choice for females with indicators of high persistence may be particularly important not only because mating is temporally separated from egg sac deposition, but because females continue to care for egg sacs until hatching (a period of approximately 2 weeks as observed in laboratory populations). This care is likely necessary for offspring development and
survival as abandoned egg sacs in the field did not hatch (ECM personal observation). As many females disappear over the course of the season, choosy males might be reducing the risk of losing their reproductive investment during the time when offspring survival is dependent on female survival. Male mate choice for a reduction in the lag between mating and spiderling hatching has been found in *Nephila clavipes*, with males preferring females whose condition indicated their temporal proximity to egg sac deposition (Rittschof 2011). Additional studies in *L. hesperus* may allow further confirmation of the connection between male choice and fitness losses due to female mortality. Whether female size and web size facilitate persistence or whether females that happen to persist accrue resources that lead to larger body and web sizes cannot be determined from my data, however the continued presence of a spider on her web is clearly related to reproductive output.

Despite the increase in studies of male mate choice, investigations into the potential for corresponding sexual selection through differential mating success among females are rare (for examples from sex-role reversed species see: Rosenqvist and Berglund 2011). My study enabled the examination and comparison of reproductive and non-reproductive females in a population with male choice where male preferences are known. I found that many females failed to produce an egg sac over the course of the observation period, however, for most of the non-reproductive females the exact cause of this failure was unknown. When, I examined females that were present for long enough to lay an egg sac (five weeks or more) I discovered that approximately 10% of those females failed to reproduce in each year. In 2009 there was no indication that male choice may have played a role in that failure as there was no difference between reproductive and non-reproductive females in terms of either body size or web size. Male mate choice might have influenced female reproductive success in the other two study years, because females present for five or more weeks that failed to reproduce were significantly (2010), or almost significantly (2011), smaller over that period. Importantly, they were not so small that they were physiologically incapable of reproduction, as their sizes were all within one standard deviation of the average minimum size of reproductive females. Could male preference for larger females have played a role in this pattern? Females found in 2010 had the highest average and maximum female size with the largest standard deviations (Table 2.1) and had the most extreme differences in reproductive success in all study years, with both the highest proportion of females that failed to deposit an
egg sac and the highest proportion of females in the multiple egg sac producing groups (Figure 2.3). Furthermore in 2010 the fewest number of females were found during the observation period (Table 2.1), which naturally resulted in lower densities and potentially longer distances between females. From a male’s perspective, these population parameters would have increased the costs of mate searching but the potential payoff would be the increased probability of discovering a very large female. Reproductive variability among females was more evenly distributed in 2011; females had the lowest average size of all years and females that produced one egg sac were significantly larger than those that failed to reproduce, but also significantly smaller than those that produced multiple egg sacs. 2011 also had the highest population density. These population parameters would lower the costs of locating a female, but would decrease the probability of locating a very large one. In other words, male searching costs and mate choice gains appeared to be very different in 2010 and 2011. This leads me to conclude that, while male mate choice may be operating in the field on some level, it seems unlikely that it is a strong selective force on females.

One important difference between sexual selection exerted by male mate choice on females and selection exerted by female choice on males is that traits preferred by males are often naturally selected as well as sexually selected. Female traits associated with increased fecundity or survival promote the fitness of females as well as the males that preferentially mate with them. Although my work shows evidence that male choice may exclude smaller, less preferred females from the breeding pool in *L. hesperus*, I did not discover a strong relationship between female size and production of egg sacs beyond the excluded small female threshold. Given this, it seems probable that female phenotype is most strongly and reliably influenced by natural selection for female fecundity. *L. hesperus* females are significantly larger than males (Kaston 1970), and such female-biased size dimorphism has been shown to be at least partly the result of selection on females for increased size (Head 1995). Although male choice reinforces natural selection in this system, I cannot infer how strong or important that reinforcement has been because, according to my data, male-based sexual selection may not be consistent across the years. It is possible that over a much longer period of time the reinforcing effects of sexual selection may play some role, albeit a weak one, in these spiders. It is also possible that the effects of such selection are only manifested under extreme conditions (for example the type of conditions seen in 2010). To my knowledge no study has attempted to quantify the relative and long term
contributions of choice-based sexual selection and natural selection when the selected phenotype is common to both, but such a study would be interesting, and feasible in *L. hesperus*.

Through the examination of a field population of female black widow spiders, I have been able to gain insight into the costs and benefits of male mate choice. My work has tested the connections between preferred phenotype, reproductive output and sexual selection under natural circumstances and has elucidated a more nuanced picture of how choosy males gain fitness benefits. In this species, male mate choice for larger females may increase fitness through the avoidance of females less likely to reproduce, and potentially through the selection of females that are more likely to survive until offspring hatch. The potential for male choice to exert sexual selection on females, however, appears to be much less important than natural selection for fecundity and survival, even though both forms of selection favour the same trait because the opportunities for mate choice are so unpredictable in the field. My work expands the study of male mate choice beyond the theoretical and laboratory controlled into the complex realm of naturally occurring phenomena.
References


Figure 2.1. Fields (1-6) monitored from 2009-2011 at Hastings Natural History Reservation, Carmel Valley, California. Field areas: 1 = 3008.92 m$^2$, 2 = 4574.60m$^2$, 3 = 2820.71m$^2$, 4 = 2959.42m$^2$, 5 = 727.93m$^2$, 6 = 687.11m$^2$. 
Figure 2.2. Percent of all females found within each observation period in each year broken down by period of discovery. Across all years, more than 60% of webs were found within the first two weeks of the monitoring period.
Figure 2.3. Percentage of females that produced 0-4 egg sacs throughout the breeding season within each year.
A

B
Figure 2.4. Sizes of females that produced 0-4 egg sacs in (A) 2009, (B) 2010, (C) 2011. Letters denote significant differences in female size between reproductive categories.
Figure 2.5. The mean number of egg sacs produced by females persisting between 1-8 weeks across years.
Figure 2.6. Relationships between field location and female size, and egg sac production in (A) 2009, (B) 2010 and (C) 2011
Figure 2.7. Distribution of the number of weeks that non-reproductive females persisted at a web site.
<table>
<thead>
<tr>
<th>Year</th>
<th>Observation start date</th>
<th>Observation end date</th>
<th>Number of females found within observation period</th>
<th>% of females that disappeared over observation period</th>
<th>Average number of weeks present (persistence)</th>
<th>Mean ± SD of egg sacs per capita</th>
<th>Mean ± SD of average female size (cm)</th>
<th>Mean ± SD of maximum female size (cm)</th>
<th>Mean ± SD of average web size (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>April 30</td>
<td>June 28</td>
<td>304</td>
<td>5.2%</td>
<td>0.93 ± 0.77</td>
<td>1.85 ± 0.45</td>
<td>1.36 ± 0.77</td>
<td>1.83 ± 0.72</td>
<td>69.7%</td>
</tr>
<tr>
<td>2010</td>
<td>April 30</td>
<td>June 27</td>
<td>172</td>
<td>4.3%</td>
<td>0.88 ± 0.95</td>
<td>2.33 ± 0.48</td>
<td>1.87 ± 0.95</td>
<td>4.08 ± 0.98</td>
<td>82.6%*</td>
</tr>
<tr>
<td>2009</td>
<td>May 1</td>
<td>June 29</td>
<td>214</td>
<td>5.52%</td>
<td>1.92 ± 0.92</td>
<td>0.94 ± 0.29</td>
<td>1.94 ± 0.29</td>
<td>2.19 ± 0.48</td>
<td>68.4%*</td>
</tr>
</tbody>
</table>

Table 2.1: Summary observations of female phenotypes (female size, web size), web persistence and reproductive output of females across the years of observation. Stars indicate significant difference of the starred value from the other values in the column.
<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>814.44 ± 499.82</td>
</tr>
<tr>
<td>2</td>
<td>1127.14 ± 792.00*</td>
</tr>
</tbody>
</table>

*Mean ± SD of maximum web size (cm²)
Table 2.2. Result of GLM using field female weight as the dependent variable and estimated female size as a covariate for females collected in each year separately and for all females collected combined. Correlation coefficients ($r^2$) are also reported for each year separately and all collected females combined.

<table>
<thead>
<tr>
<th>Year</th>
<th>$\chi^2$</th>
<th>df</th>
<th>p</th>
<th>$\beta$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>578.678</td>
<td>30.1</td>
<td>$&gt;0.001$</td>
<td>0.608</td>
<td>0.902</td>
</tr>
<tr>
<td>2010</td>
<td>2101.982</td>
<td>20.1</td>
<td>$&gt;0.001$</td>
<td>0.430</td>
<td>0.948</td>
</tr>
<tr>
<td>2011</td>
<td>642.831</td>
<td>23.1</td>
<td>$&gt;0.001$</td>
<td>0.465</td>
<td>0.92</td>
</tr>
<tr>
<td>All</td>
<td>55.01</td>
<td>77.1</td>
<td>0.009</td>
<td>0.455</td>
<td>0.896</td>
</tr>
</tbody>
</table>
Table 2.3. Results of GLMs using egg sac number as the dependent variable (distribution=poisson, link=log) and female size (PCA1 of mean female size and maximum female size) as the predictor variable

<table>
<thead>
<tr>
<th>Year</th>
<th>$\chi^2$</th>
<th>df</th>
<th>p</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>14.773</td>
<td>212.1</td>
<td>$p &lt; 0.000$</td>
<td>0.279</td>
</tr>
<tr>
<td>2010</td>
<td>19.039</td>
<td>179.1</td>
<td>$p &lt; 0.000$</td>
<td>0.378</td>
</tr>
<tr>
<td>2011</td>
<td>19.830</td>
<td>302.1</td>
<td>$p &lt; 0.000$</td>
<td>0.267</td>
</tr>
</tbody>
</table>
Table 2.4. Correlations between the length of time (in weeks) that a female persisted at a web site and female size (as PCA1 of mean female size and maximum female size), egg sac production (number of egg sacs produced) and web size (in cm$^2$)

<table>
<thead>
<tr>
<th>Year</th>
<th>Size</th>
<th>Egg sac production</th>
<th>Web size</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>$r^2 = 0.361$</td>
<td>$r^2 = 0.460$</td>
<td>$r^2 = 0.330$</td>
</tr>
<tr>
<td></td>
<td>$p &lt; 0.000$</td>
<td>$p &lt; 0.000$</td>
<td>$p &lt; 0.000$</td>
</tr>
<tr>
<td>2010</td>
<td>$r^2 = 0.362$</td>
<td>$r^2 = 0.533$</td>
<td>$r^2 = 0.241$</td>
</tr>
<tr>
<td></td>
<td>$p &lt; 0.000$</td>
<td>$p &lt; 0.000$</td>
<td>$p = 0.001$</td>
</tr>
<tr>
<td>2011</td>
<td>$r^2 = 0.319$</td>
<td>$r^2 = 0.504$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$p &lt; 0.000$</td>
<td>$p &lt; 0.000$</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.5. Results of one-way ANOVA of female size (PCA1 of mean female size and maximum female size) between fields in each year

<table>
<thead>
<tr>
<th>Year</th>
<th>Degrees of freedom (between/within groups)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>5/208</td>
<td>5.910</td>
<td>&gt;0.000</td>
</tr>
<tr>
<td>2010</td>
<td>5/166</td>
<td>6.65</td>
<td>&gt;0.000</td>
</tr>
<tr>
<td>2011</td>
<td>5/298</td>
<td>5.662</td>
<td>&gt;0.000</td>
</tr>
</tbody>
</table>
Table 2.6- Comparison of females that did and did not reproduce using the subgroup of only females present for greater than five weeks during the study.

<table>
<thead>
<tr>
<th>Year</th>
<th>Are females that reproduce found earlier?</th>
<th>Are females that reproduce present for longer?</th>
<th>Are females that reproduce bigger?</th>
<th>Are the webs of females that reproduce bigger?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Absolute</strong></td>
<td><strong>Relative</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>$\chi^2 = 4.973$</td>
<td>$\chi^2 = 4.176$</td>
<td>$\chi^2 = 0.009$</td>
<td>$\chi^2 = 3.158$</td>
</tr>
<tr>
<td></td>
<td>p=0.026</td>
<td>p=0.041</td>
<td>p=0.923</td>
<td>p=0.076</td>
</tr>
<tr>
<td>2010</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>$\chi^2 = 0.170$</td>
<td>$\chi^2 = 2.310$</td>
<td>$\chi^2 = 4.864$</td>
<td>$\chi^2 = 9.391$</td>
</tr>
<tr>
<td></td>
<td>p=0.895</td>
<td>p=0.129</td>
<td>p=0.027</td>
<td>p=0.002</td>
</tr>
<tr>
<td>2011</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>$\chi^2 = 4.454$</td>
<td>$\chi^2 = 12.528$</td>
<td>$\chi^2 = 8.496$</td>
<td>$\chi^2 = 3.772$</td>
</tr>
<tr>
<td></td>
<td>p=0.035</td>
<td>p&lt;0.001</td>
<td>p=0.001</td>
<td>p=0.052</td>
</tr>
</tbody>
</table>
Chapter 3
Genital mutilation need not cause monogyny in spiders

Abstract

Males that mate with polyandrous females may trade off investment in protecting their paternity against investment in seeking future mating opportunities. In some systems, males may achieve higher fitness by trying to monopolize one female’s reproductive output, rather than competing for partial paternity of several females’ offspring. In an apparent example of this, males of many spider species block access to the female’s reproductive tracts with plugs composed of broken parts of the male’s genitalia. This extreme form of paternity protection in spiders is currently thought to have co-evolved with monogyny, and particularly with male sacrifice behaviour. In this study I present evidence suggesting that male genital mutilation and monogyny have instead evolved separately. I focus on the genus *Latrodectus*, one of five taxa known to exhibit both genital mutilation and male self-sacrifice behaviour. Using *L. hesperus*, I investigated the effect of genital mutilation on future reproductive success of males by giving once-mated male spiders three additional opportunities to copulate with virgin females. I show that males are able to mate and inseminate multiple females despite genital mutilation. Furthermore, females mated to non-virgin males did not have reduced fertility, indicating that males are not sperm-depleted after mating once. I review the literature on genital mutilation in light of these results, and suggest (1) the assumption that genital damage during copulation inevitably results in sterility is unfounded and (2) that genital mutilation in members of the genus *Latrodectus* has a different evolutionary history from that proposed for other spiders. Thus *Latrodectus* may be a particularly informative group for comparative analyses of the evolution of monogyny and genital damage.
Introduction

For many animals, the time and energy invested in a single mating generates trade-offs against the time and energy directed towards future matings (Parker 1974; Hasselquist and Bensch 1991; Smith 1995; Pelletier, Hogg et al. 2006; Heubel, Lindstrom et al. 2008). A wide variety of different strategies balance costs and benefits of current investment as a function of the nature of the mating systems and other relevant ecological and social variables (Janetos 1980; Real 1990; Wiegmann, Real et al. 1996). Sexual selection may favour either increased or decreased investment in the current mating depending on the competitive context, with monogamy or polygamy being the ultimate result (Alcock 1994; Schneider and Lesmono 2009; Stoltz, Elias et al. 2009; Barreto and Avise 2010; Cheng and Kam 2010). This fundamental trade-off is particularly important for the fitness of males who mate with polyandrous females as paternity may be shared with every rival subsequently mated to that female, reducing the payoff from the first male’s investment (Emlen, Wrege et al. 1998; Schafer and Uhl 2002; Elgar, Bruce et al. 2003; Schamel, Tracy et al. 2004; Kamimura 2005; Ref snider 2009; Xu and Wang 2010; Yue, Li et al. 2010). Selection on these males thus favours strategies that ensure paternity either by outcompeting rivals at sperm competition games (Parker 1970), or preventing sperm competition from happening altogether (Alcock 1994). Male tactics such as differential allocation of sperm as a function of sperm competition risk or mate guarding may increase male reproductive payoffs (Munehara and Murahana; Hario and Hollmen 2004; Herberstein, Barry et al. 2005; Kokko and Morrell 2005; Cornwallis and Birkhead 2006; Zaldivar-Rae and Drummond 2007; Mass, Heistermann et al. 2009; Ingleby, Lewis et al. 2010) but may have costs that impact the opportunity for future mating (Robinson and Doyle 1985; Alcock 1994; Dickinson 1995; Vahed 2007), and therefore total fitness. Thus, the direction of selection on paternity protection will ultimately affect the resolution of trade-offs between current and future reproduction.

A reduction in the likelihood of successful female remating does not always require the continuous attendance of mate guarding (Bird, Raina et al. 1991; Johnstone and Keller 2000; Vahed 2006; Hosken, Stockley et al. 2009; Uhl, Nessler et al. 2010). Males belonging to taxa ranging from snakes to spiders use some form of plug to block the access of rivals to a female’s reproductive organs (Dickinson and Rutowski 1989; Barker 1994; Shine, Olsson et al. 2000; Dixson and Anderson 2002; Oh and Hankin 2004; Ramm, Parker et al. 2005; Takami, Sasabe et
al. 2008; Althaus, Jacob et al. 2010; Uhl, Nessler et al. 2010). As with other forms of paternity protection, however, the benefits gained by males from plugging females may be tempered by plug production costs and effects on future reproduction potential. In extreme examples of high investment in current mating diminishing future mating opportunities, males of many spider species (across at least 65 genera (Uhl, Nessler et al. 2010)) break off parts of their genitalia during copulation. These parts lodge in females’ reproductive tracts, preventing or reducing the possibility of insemination by rival males (Fromhage and Schneider 2006; Snow, Abdel-Mesih et al. 2006; Foellmer 2008; Uhl, Nessler et al. 2010). However, these methods may exact a high cost as many males are rendered sterile due to genital breakage or sperm depletion (Andrade and Banta 2002; Herberstein, Gaskett et al. 2005; Fromhage and Schneider 2006; Schneider, Herberstein et al. 2008; Michalik, Knoflach et al. 2010). This type of terminal mating investment may be unique to systems in which females can store sperm and plugs do not interfere with fertilization by the male who has just mated (i.e., in spiders, fertilization and insemination ducts are independent, Foelix 1996). In these systems, although males may sacrifice potential benefits from future matings, they ensure high paternity among offspring produced by their first mate over her lifetime.

Plugs derived from damaged genitalia may have evolved under intense sexual selection shaped by conditions such as high mate search mortality (Andrade 2003; Segoli, Harari et al. 2006), or strong inter-male sperm competition arising from a male-biased effective sex ratio (=ratio of males to females that mate at least once) (Fromhage, Elgar et al. 2005; Fromhage, Jacobs et al. 2007; Fromhage, McNamara et al. 2008), or strong inter-sexual conflict over mating duration (Schneider, Gilberg et al. 2006; Kuntner, Coddington et al. 2009), factors also thought to lead to the evolution of monogyny (= males mating with only one female) (Miller 2007). In fact, current hypotheses suggest genital mutilation evolved in concert with monogyny (Miller 2007). The reasoning is simple, since most males are rendered sterile from genital breakage during copulation, they are forced monogynists (Foelix 1996). Support for this hypothesis includes (1) the cessation of sperm production after reaching adulthood in Tidarren argo and various Argiope and Nephila spiders (Michalik, Knoflach et al. 2010; Uhl, Nessler et al. 2010; Michalik and Rittschof 2011), (2) the occurrence of post-copulatory guarding behaviours (as opposed to continued mate-search behaviours) in other araneid spiders (Fromhage and Schneider 2005; Herberstein, Barry et al. 2005), and (3) spontaneous death during copulation (e.g., in Argiope,
Foellmer and Fairbairn 2003)). Comparative analyses also suggest the evolutionary marriage of monogyny and genital mutilation across web-building spiders (Miller 2007) and within the nephiline spiders (Kuntner, Coddington et al. 2009; Kuntner, Kralj-Fiser et al. 2009), in which monogyny linked to sterility or genital damage (‘emasculcation’ in its most extreme form) is the ancestral state and found basally in the clade.

The genus *Latrodectus* (the widow spiders), contains species with extreme monogynous behaviour. Males of *L. hasselti* and *L. geometricus* sacrifice themselves to the female by somersaulting into her jaws during copulation (Forster 1992; Andrade 1996; Segoli, Arieli et al. 2008). Genital structure is evolutionarily conserved in this genus, and all members studied to date undergo highly stereotyped genital mutilation during copulation (Shulov 1948; Levi 1959; Bhatnagar and Rempel 1962; Abalos and Baez 1963; Kaston 1970; Levy and Amitai 1983; Agnarsson 2004; Agnarsson, Coddington et al. 2007; Neumann and Schneider 2011), and thus traditionally all males were assumed to be sterile post-mating (Bhatnagar and Rempel 1962; Abalos and Baez 1963; Foelix 1996; Berendonck and Greven 2002). As male spiders have no direct connection between their testes (located in the abdomen) and their copulatory organs, (palps: bulb-like modified appendages held anterior to the cephalothorax), it was speculated that genital damage may preclude males from further mating by mechanically preventing copulation or preventing a re-filling of the palps with sperm. Two members of the genus however, *L. pallidus* (Segoli, Lubin et al. 2008) and *L. mactans* (Breene and Sweet 1985), are able to mate with and inseminate multiple females. In these studies it was unclear though whether polygyny arose from variation in the occurrence of copulatory organ damage in males, or variation in the allocation of a single load of sperm among females. The genus *Latrodectus* thus presents an opportunity to examine the costs of genital mutilation across varied male mating tactics, which may lead to further insights into the evolution of monogyny.

My first goal in this study was to examine the cost of genital mutilation in terms of future mating and fertilization success for male *L. hesperus* spiders. My interest was not only in testing whether or not males were able to inseminate multiple females despite copulatory organ damage, but also in comparing sequential mating events to gain a more complete understanding of the relationship between the cost of initial matings and benefits of polygyny in this species.
My second goal was to review the literature on genital plugs among spiders to examine possible links between types of male genital damage, indicators of effective plugging, and obligate monogyny. My preliminary review of the literature suggested that there were disparate types of genital plugs; some of which entail catastrophic damage to male pedipalps, but others which were more consistent with the retention of fertility. In addition to recording available information on mating system (monogyny or polygyny) for species with plugs formed from broken male genitalia, I assessed the degree of and regularity of damage to the male’s genitalia, and the location of placement inside the female’s genitalia. I argue the available evidence suggests separate evolutionary histories for two broad classes of plugs—those that are irregular, potentially variable in terms of blocking access to females’ genitalia, and lead to sterility, and those that have discrete break-points, cause minimal palp damage, and suggest a history of selection for polygyny.

Methods

Study species

*Latrodectus hesperus*, the western black widow spider, is found in western North America and shows female-biased size dimorphism. Males have no direct connection between their palps and gonads, so secrete sperm from the testes onto a web and fill their palps through a long coiled embolus shortly after sexual maturity (e.g., Robinson 1982). The sperm is stored inside the palps until copulation. A typical mating involves two separate copulations, one with each palp, during which one embolus is inserted into one of the female’s paired insemination tubules and sperm is ejaculated into one of the female’s paired sperm storage organs (spermathecae). During copulation the apical sclerite of the embolus breaks off and is often lodged at the entrance to the spermatheca (Kaston 1970). Below, I refer to the insertion of one embolus in the female’s genitalia as a **copulation** whereas a **mating** is any pairing between a male and a particular female that results in one or two copulations.

Study population

I used an outbred population descended from individuals collected in June & July 2007 from San Diego, CA (trials in January - May 2008) and in June & July 2008 from the Hastings Natural
History Reservation, Carmel Valley CA (trials in Sept. – Oct. 2008). Spiders were reared communally with siblings upon hatching and transferred to individual cages (Amacs Plastics Products Ltd) after the fourth instar. Date of hatch and date of adult moult were recorded for all spiders in the Hastings-collected population. All spiders were housed in a temperature and light controlled room (25°C, 12:12 light:dark cycle). Spiderlings and males were fed fruit flies (Drosophila melanogaster) twice per week; females were fed one cricket (Acheta domesticus) per week after they reached approximately the 5th or 6th instar.

Mating trials

Trials were conducted under red lights during the dark cycle as L. hesperus is nocturnal. Mating trials were recorded using Panasonic low light black and white cameras (WV BP330) with macro zoom lenses (Navitar Macro-Zoom 7000) and JVC Professional Super VHS recorders (SR-TS1U)).

Individual females were placed on metal frames within mating arenas (35 × 30 × 15 cm) for 48 h to allow construction of a web prior to the introduction of a male. Males and females were weighed after mating trials with an Ohaus Explorer electronic balance (accurate to 0.01mg), digital images of males’ forelegs were taken on a Zeiss Stemi 2000C dissecting microscope, and male size was estimated as the mean patella + tibia of both front legs (Kasumovic and Andrade 2006; Stoltz and Andrade 2010) from digital images using Image Tool v. 3.00.

To investigate the occurrence and consequences of genital damage for polygyny, I needed to start with males that had copulated once with each palp. Males were chosen randomly from the mature males in the laboratory population, and were paired with a virgin female. Males were placed on webs at the start of a trial, and were left in the arena for 6 hours or until they had copulated twice (once with each palp) and had stopped interacting with the female, whichever came first. In preliminary trials, most L. hesperus males mated within 2.5 hours of introduction to the female. To standardize mating experience, males qualified for the study only if they copulated once with each palp with a single female. Males were given two opportunities to qualify for the study; if they failed to mate during their first pairing they were given a second chance with a different virgin female within 48 hours. Out of 56 initial mating trials, 47 males qualified for entry into my experiment.
The 47 qualifying males were immediately anaesthetized with CO\textsubscript{2} for less than one minute and examined under a dissecting microscope. I visually determined whether the sclerite had been lost from each palp without damaging or uncoiling the embolus to ensure I did not interfere with future mating performance. These once-mated males were paired sequentially with three new virgin females (1) one day after the initial mating, (2) three to five days after the initial mating, and (3) seven to ten days after the initial mating. Individual males were never presented with the same virgin female more than once or with a female from the same maternal line. Mating success was recorded, and the number of copulations, copulation duration, and the order of palp insertions were recorded for all matings.

After matings, females were removed from the arena, returned to their individual cages and fed one cricket weekly. The first three egg sacs produced were collected within 2 days of production and allowed to develop for 14 days after which egg sacs were opened and number of hatchlings and un-hatched eggs were counted to determine fertilization success (see: Snow and Andrade 2005)). Due to the significant correlation of female weight with number of hatchlings in both the first (Pearson correlation = 0.445, p < 0.001) and the second egg sacs (Pearson correlation= 0.470, p < 0.0001), and the propensity for females to lay both unfertilized and unviable eggs in a clutch, I used proportional hatch to compare hatching success between females.

**Literature Review**

I hypothesized that male genital damage evolved independently of monogyny in some lineages because of the benefits of plugging females. I predicted that polygynous species would have experienced selection for minimal functional compromises to the palp, whereas monogynous species would not. If this is so, then traits likely to be correlated with the retention of palp function should arise together in polygynous lineages. For example, while large, irregular portions of broken palps might effectively plug females by taking up large portions of the genital tract, selection on males for retention of fertility should lead to discrete plugs comprising relatively small sections of the palp, and these would need to be placed in locations where they could most effectively block insemination by rivals.

I systematically examined the literature for accounts of male spiders that leave broken genitalia inside of females after mating. I began by examining a recent review of genital plugs by Uhl et
al. (2010) with a focus on those references that categorized plugs as “morphological” (= a broken piece of the male’s genitalia) and embolic in origin. I excluded two taxa (genera *Dolomedes* and *Philodromus*) based on the indicated rarity of genital breakage (Uhl et al. 2010). I scored species for three traits that I hypothesized were integral to whether or not males maintain their ability to mate despite genital damage: (1) whether the male’s palp had a well-defined breaking point rather than breaking in an unpredictable or irregular way. Breakage at a discrete location on the embolus, or the presence of a discrete portion of the palp that is lost at mating, could arise from selection to retain genital function; (2) plug placement in terms of total engagement with insemination ducts and spermathecae (scores based on the combination of positions occupied by the plug as illustrated in Figure 3.1); (3) extent of embolic damage relative to the size of the palp. Selection for post-mating functionality of the palp predicts a decrease in the relative size of the portion that breaks. Thus species with defined break points would also have less overall damage to the palp. I estimated as closely as possible the proportion of the embolus that is lost at mating based on published photographs, diagrams, or written descriptions. I categorized breakage as involving >50%, 5 - 50%, or <5% of the embolus, or as the loss of a ‘scale or cap’. I distinguished ‘scales or caps’ from other forms of breakage because these are discrete sclerites attached loosely to the embolus, and when they are removed during copulation, the embolus is left fully intact (Levi 1971; Levi 1975). Thus ‘scales or caps’ are both discrete and represent a very small proportion of the embolus (<5%). (3) The position of the embolus inside the female.

Subsequent to these examinations, I surveyed the literature for references to either polygynous or monogynous behaviour in all species that I were able to score in the previous three categories using the ‘Web of Science’ search engine, and the species’ scientific and common names (if known).

**Analysis**

Statistical analyses were performed using SPSS v.15.0. Statistical tests were two tailed with mean±SE reported. Male front leg lengths were used as a proxy for overall body size in all analyses (Kasumovic and Andrade 2006; Stoltz and Andrade 2010). Male condition was measured as the residuals of the type II linear regression of the log cubed root of weight plotted against the natural log of front leg length (Kasumovic and Andrade 2006; Kasumovic, Brooks et al. 2009). The distribution of number of hatchlings per egg sac was non-normal. I used non-
parametric statistics to test for differences in hatching success between females that were 1st, 2nd, and 3rd mates of males. I used the total proportion of hatched eggs from the first three egg sacs deposited by each female as the test variable in order to control for natural differences in female fecundity. Sample sizes varied for some tests because not all observations for each trial were recorded, and in some cases I was unable to determine whether the sclerite had been lost by visual examination of qualifying males.

**Results**

**Occurrence of Polygyny**

A little over half of the males (59.5%, n = 28) mated polygynously when given the opportunity. The most common pattern of polygyny was for males to mate with two females (42.5%, n = 20) although eight males (17%) eventually mated with three or more different females (Figure 3.2).

Polygynous males tended to delay remating by several days. Most twice-mated males (n = 20) copulated again 3-5 days (45%, n = 9) or 7-10 days (40%, n = 8) after their initial mating, whereas only three (15%) mated again the day after the initial mating, and significantly fewer males mated on the day after their initial mating than at later opportunities (G-test, G=10.818, p=0.001). Similarly, thrice-mated males showed a significant pattern of polygynous matings occurring disproportionately in the later intervals (G-test, G=15.713, p<0.001), and no males mated three times within five days (Figure 3.3).

Mating durations (sum of both copulations) of the initial male and female pairings were not significantly different between subsequently monogynous or polygynous males (Kruskal-Wallace test, $\chi^2 = 0.608 \ p = 0.738$). However mating duration decreased significantly with increased polygyny. Second matings (Friedman test, $\chi^2 = 6.545, \ p=0.011$), and third matings (Friedman test, $\chi^2 = 5.000, \ p=0.025$) (Figure 3.4) were significantly shorter than the first.

**Predictors of polygyny**

Polygyny was not restricted to males that failed to lose an apical sclerite in their initial mating. There was no significant difference in the pattern of apical sclerite loss the initial matings bout between monogynous and polygynous males (Fisher’s exact test, p=0.6770, Figure 3.3).
Although I did not have data for first-mating sclerite loss for some males (n = 6), and these missing data were not evenly distributed between polygynous (n = 5) and monogynous males (n = 1), the statistical outcome of the Fisher’s exact test would remain unchanged even under the most extreme distributions of hypothetical values of the missing data (Figure 3.2). I used a forward step-wise binary logistic regression to test whether any of the measured male traits (population of origin (Hastings or San Diego), days since hatching (missing 6 values), body condition (missing 3 values), duration of initial mating), and size (missing 2 values) predicted polygyny. I found no significant predictors among the measured variables (Table 3.1).

Polygyny and female fertility

All females mated in both initial and subsequent mating trials produced egg sacs. I used a repeated measures GLM to ask whether fertility in the first three egg sacs was predicted by the female’s position in the mating order (1st, 2nd or 3rd to mate with a given male), including female age and population of origin as covariates. I did not find any difference in proportional hatching success of the first three egg sacs for females in different mating order positions (Kruskal-Wallis test, $\chi^2 = 0.658, p=0.720$, Figure 3.4).

Literature Review

I surveyed a total of 81 genitally-mutilating species in 29 genera across 12 families (Appendix 2). Although there are 28 families of araneomorphae (true spiders), the majority of species in my sample (75%) were in the family Araneidae. This biased taxonomic distribution (also apparent in Uhl et al’s 2010 review) may reveal a real pattern in genital mutilation, it may also be caused by researcher bias in choosing study animals—there is a dearth of studies on genital mutilation for most groups (Schneider and Andrade 2011).

Occurrence of polygyny

My examination of the literature revealed very little evidence for polygyny among genitally-mutilating spiders, although I could only find information from empirical, systematic studies about mating system for 11 of 67 species. Only two species outside of the genus Latrodectus had any references to multiple mating by males; (1) a theridiid Parasteadoda (formerly Achaearania) tepidariorum was observed mating with two females (Locket and Luczak 1974), however the
authors were unsure as to whether or not the male copulated only once with each palp or re-used a palp in the second mating event: (2) Levi (1971) stated that males of the araneid, *Araneus diadematus* group were polygynous, seemingly based on the evidence that males with and without broken genitalia were found in equal proportions in museum collections. Behavioural research (Elgar and Nash 1988), however, showed that approximately 40% of males are cannibalized at some point during their sexual contact with females, suggesting many males may not have significant opportunity for polygyny. Data on mating systems were insufficient for a test of my primary hypothesis.

‘Plug’ placement

The location of the males’ plugs inside females’ reproductive tracts indicates how rivals are blocked (Figure 3.1) Males may cap the entrance to the insemination ducts (as seen in most *Araneus* species, Appendix 2, Figure 3.1A), they may occupy the insemination ducts, leaving no room for rivals (as seen in *Thalassius*, Appendix 2, Figure 3.1B), they may block the entrance to the spermathecae (as seen in *Latrodectus*, Appendix 2, Figure 3.1C), or some combination of these. Differences in plug location and the ensuing functional implications may indicate differences in the evolutionary origins of male plugs and/or male-female morphological coevolution.

*Latrodectus* males’ apical sclerites penetrate the spermatheca, with only about 1/3 of their length outside in the insemination ducts (Berendonck and Greven 2002; Snow, Abdel-Mesih et al. 2006). Most other species’ broken genitalia were found either at the opening of the insemination ducts or occupying the entire length from the spermathecal entrance outward (See Appendix 2). However, several species appear to have some part of their genitalia in the entrance to the spermathecae (Figure 3.1C or D) in what may be a functionally similar plugging method to *Latrodectus* (See Table 3.2, Figure 3.1B, and C, D).

Embolic break-point

In almost half (39/81) of the reviewed species, male genital mutilation occurs at discrete breaking points (Appendix 2). All cases of discrete breakage in *Araneus* (Araneidae) involved ‘caps’ (Levi 1971; Levi 1973; Levi 1991; Scharff and Coddington 1997), and ‘caps’ were present in only one other species outside this genus (*Acacesia hamata*: Araneidae). In addition to
all *Latrodectus* that have been examined so far, two other theridiid genera have discrete breaking points along their emboli. *Meotipa bitubermaculata* has a whip-shaped embolus similar to *Latrodectus* but the breaking point is basal such that the majority of the embolus is lost, and the broken emboli fill the female genitalia from the spermatheca to the opening of the insemination ducts (Deeleman-Reinhold 2009). In comparison, another Theridiid, *Parasteatoda tepidariorum* has an extremely apical breaking point, with only the very tip of the embolus remaining in the spermatheca of females (<5%) (Abalos and Baez 1963; Locket and Luczak 1974). The incidence of discrete breaking points may be higher than I report in this review because many authors include what appear to be likely fracture points on their illustrations without describing them as such (asterisks beside an entry in the column ‘breaking point’ in Appendix 2 indicate my reasonable inference of the character state).

Although I could not test whether these variables were related to mating system, I could ask whether breaking point, embolic position and amount of embolic damage were correlated as I predicted. I statistically analyzed the data summarized in Appendix 2 and examined the relationships between (1) the presence of a breaking point and the amount of embolic damage using damage scores mimicking the damage rating in Appendix 2. (e.g., Score of 1 = broken embolic cap, through a score of 4 = >50% damage) and (2) the presence of a breaking point and the position/amount of female genitalia occupied by broken emboli using scores related to Appendix 2. (score of 1= occupation of one aspect of female genitalia, either epigynal opening, insemination ducts, entrance to spermathecae, or inner aspect of spermathecae. Score of 2= occupation of two of these positions, through a score of 4 = occupation of 4 of these positions).

Using all the data (both known and from reasonable inference) and each species as an individual case, there was a significant relationship between the existence of a discrete breaking point and both minimal embolic damage (Binary Logistic Regression, Wald statistic = 13.35, p<0.001), and minimal occupation of female genitalia (Binary Logistic Regression, Wald statistic = 14.97, p<0.001). The phylogenetic distribution of the available data precluded a formal comparative test, so instead I collapsed the data to average values for each family, and found these relationships were no longer significant (p=0.998 and p=0.999 respectively).
Discussion

Genital mutilation does not appear to be costly to either male or female black widow spiders. Males are capable of mating with, and inseminating, females regardless of such breakage. Females paired with males copulating for the second or third time do not suffer reduced fertilization success compared with those mated to virgin males. Although a male’s copulation duration was reduced in his second or third mating, the average duration of those matings was never less than the time necessary for sperm transfer in other *Latrodectus* species (Snow and Andrade 2004). My results were consistent across two geographically separated populations, suggesting the capacity for polygyny despite genital damage may be a consistent feature of *L. hesperus* biology.

I also found no evidence for impediments to polygyny that are intrinsic to males. This is in strong contrast to recent work on other species with genital mutilation (including another Theridiid) in which atrophy of the gonads at maturation and subsequent sperm limitation is the rule (Michalik, Knoflach et al. 2010; Michalik and Rittschof 2011). On the contrary, my data show that male black widows have sufficient sperm for multiple matings. This may indicate that males allocate a limited sperm supply across multiple matings, or that males re-fill their palps with sperm after each mating. I have no evidence for variation in male sperm allocation across matings. If polygynous males were those that retain sperm in anticipation of future matings, I would expect variation in egg hatching success as a function of whether females were mated to males that were later polygynous or monogynous. Not only was this not the case, but there was also no relationship between the number of previous matings and female fertility. It is also possible that males that choose to be polygynous are those that naturally had more sperm than their monogynous counterparts. Even if this were the case, it does not invalidate the conclusion that polygyny is not affected by genital damage since polygynous males were as likely to have lost palps on the first mating as were monogynous males (Figure 3.2). I conclude that males are likely inducting more sperm prior to polygynous matings. This is consistent with the temporal pattern of remating—males rarely copulated with females presented the day after a previous mating. Longer inter-copulatory intervals may be required to allow sperm re-induction, something that is seen in other spiders (Bukowski and Christenson 1997).
All *Latrodectus* males studied to date undergo genital mutilation during copulation (Shulov 1948; Levi 1959; Bhatnagar and Rempel 1962; Abalos and Baez 1963; Kaston 1970; Levy and Amitai 1983; Agnarsson 2004; Agnarsson, Coddington et al. 2007). At least four of these species (*L. hasselti, L. hesperus, L. pallidus, L. mactans*) are capable of mating and inseminating females despite such mutilation and three of these are polygynous in the lab (*L. hesperus, L. mactans*) or field (*L. pallidus*) (Breene and Sweet 1985; Snow, Abdel-Mesih et al. 2006; Segoli, Lubin et al. 2008). I hypothesize that the important mechanical factor that enables polygyny in *Latrodectus* is the position of the discrete breakage location on the embolus, which prevents any excess loss of embolic length while still enabling the placement of the apical sclerite at the junction of the copulatory ducts and the spermathecae, where the ducts narrow, effectively blocking rivals’ access (Snow, Abdel-Mesih et al. 2006). In light of this information, I see no reason to continue to assume *a priori* that *Latrodectus* males are sterile after mating. I suggest that, rather than considering genital damage a constraint that may necessitate monogyny (Bhatnagar and Rempel 1962; Foelix 1996; Berendonck and Greven 2002), more fruitful approaches would be to follow theory developed in less extreme systems. Asking whether restrictions in male mating frequency (such as in *L. hasselti* and *L. geometricus*) are an adaptive response to ecological factors that intensify intermale competition and reduce mate encounter rate (Emlen and Oring 1977) may be critical to my understanding of the evolution of genital mutilation. The extreme nature of these challenges in some species leads to the terminal investment seen in several spider species (Andrade 2003; Andrade and Kasumovic 2005; Fromhage, Jacobs et al. 2007; Kasumovic, Bruce et al. 2007; Fromhage, McNamara et al. 2008). Here, sexual selection favours males that gain any competitive reproductive advantage from a terminal investment strategy. Without ecological factors intensifying competition, males remain polygynous and benefit from a genital mutilation which provides a cost-free barrier to sperm competition.

This scenario differs from those proposed for the evolution of genital mutilation and monogyny for all web building spiders (Miller 2007), and for other spider groups that also exhibit these behaviours (Kuntner, Kralj-Fiser et al. 2009). My work suggests that monogyny and genital mutilation need not have a common evolutionary origin in *Latrodectus*. The courtship and copulation behaviours of all studied *Latrodectus* species are remarkably similar, and all males assume the same position on the female’s venter when inserting their palps (Kaston 1970; Ross and Smith 1979; Breene and Sweet 1985; Andrade 1996; Segoli, Harari et al. 2006; Segoli,
Arieli et al. 2008). Unlike polygynists, monogynist males then twist through 180°, a move which places their abdomen onto the female’s mouthparts while still in copula (Andrade 1996; Segoli, Arieli et al. 2008). From their respective positions both polygynous and monogynous males expand and contract their palpal bulbs in a highly similar manner in order to transfer ejaculate (E.C.M. personal observation). The sclerite breaks off only as the male’s palp is removed from the female’s genitalia (Bhatnagar and Rempel 1962), and thus mutilation is temporally separated from self-sacrifice and may be functionally distinct. In other genera with genital mutilation and monogyny there is no such separation as males are emasculated during copulation or die spontaneously after mating (Sasaki and Iwahashi 1995; Knoflach and Van Harten 2000; Knoflach and van Harten 2001; Kuntner 2005; Fromhage and Schneider 2006; Kuntner 2007; Foellmer 2008). This suggests the intriguing hypothesis that these superficially similar mating systems may have evolved by different evolutionary pathways in *Latrodectus* compared to other genital-mutilating monogynists.

Through my examination of the literature for polygyny and genital breakage traits similar to *Latrodectus*, I found no other experimental work on the possibility of polyandry despite genital mutilation in other taxa, even though the extent of genital damage experienced by different taxa is highly variable. Although there are many species in which the assumption of sterility post mating is likely a safe one due to the extent of genital damage, I found that at least three other taxa (members of *Araneus* (Levi 1971; Levi 1973; Levi 1991), some *Argiope bruennichi* (Nessler, Uhl et al. 2007), and *Parasteatoda tepidariorum* (Abalos and Baez 1963; Locket and Luczak 1974)) have minimal genitalic damage after mating, and thus may not be constrained to a monogynous lifestyle.

Differences in genitalic damage and the retention of mating ability may be influenced by differences in sexual conflict among spider taxa. Males with greater amounts of damage and no discrete break points may represent those following an evolutionary trajectory influenced by high inter-sexual conflict over mating, particularly mating duration, in which broken genitalic became secondarily modified as plugs (Fromhage and Schneider 2005; Schneider, Gilberg et al. 2006; Nessler, Uhl et al. 2007; Kuntner, Coddington et al. 2009; Kuntner, Kralj-Fiser et al. 2009). Conversely, selection pressure from inter-male competition and frequent polygyny may have led to the evolution of plugging, with males losing only small and discrete parts of their genitalic which are placed at strategic locations. Although the analyses of my plug data are confounded by
phylogenetic relationships, my results connecting discrete genital breakage to minimal genitalic
damage and minimal occupation of the female genital tract support the validity of these two
separate hypotheses for male genital mutilation. I believe that my work indicates the need for
further investigation into the morphology and behaviour of understudied spider taxa in order to
determine the influence of intra- and inter-sexual selection, on the evolution of this extreme trait.

Although genital mutilation and monogyny may have often evolved in concert as a response to
intra-sexual competition among several groups of male spiders, I find that in at least one group
of genital mutilating males (*Latrodectus*) this is not a likely scenario. I also find that further
investigation is warranted into the obligate correlation of these two traits. Not only have there
been very few experimental investigations into the effectiveness of broken genitals as barriers to
insemination by rival males, but also the position of broken male genitalia in female reproductive
tracts is unknown for most groups. Although *Latrodectus* males seem unique in their ability to
plug females without paying the cost of reduced polygyny, they may not be unique, as there have
been few investigations into the relationship between the evolution of genital breakage and
monogyny.
References


Figure 3.1. Possible positions for male embolic fragments in female genitalia. Simplified female schematic includes the entrance to the reproductive tract, the epigyne (ep), the sperm storage organ, the spermatheca (sp), and the insemination duct (id) which connects them. Broken aspects of male genitalia are found at one or more of these four locations: blocking the epigyne (A), occupying the insemination duct (B), blocking the entrance to the spermatheca (C) and occupying a position inside the spermatheca (D).
Figure 3.2. Sclerite loss by males during their initial mating. 1 = male did not remate; 2 = male mated with one other female; 3 = male mated with two other females. Males from all groups predominantly lost both of their sclerites during the initial mating (n=33; n=15 for monogynous males, n=18 for polygynous males). However, some males (n = 6) retained a sclerite (n=1 for monogynous males, n=5 for polygynous males) and 1 monogynous male did not lose either sclerite during his initial mating.
Figure 3.3. Distribution of polygynous matings into mating opportunity windows (time since first mating): 1 = one day after initial mating; 2 = 3-5 days after initial mating; 3 = 7-10 days after initial mating. Significant differences are discussed in the text.
Figure 3.4. Average total copulation durations for males that mated once, twice or three times.

The average mating duration (±SE) was 14.28 ± 5.3 for all matings, and 515.47 ± 4.95 mins for the first mating bout, 13.72 ± 6.43 for the second, and 11.33 ± 3.33 for the third. The average total copulation duration of the last mating bout was significantly shorter, with twice mating males spending an average of 11.95 ± 4.72 mins in copula, and thrice mating males spending 11.125 ± 2.85 mins in copula.
Figure 3.5 Percentage of spiderlings that hatched out of the first (1), second (2) and third (3) egg sacs laid by female spiders that were mated to virgin, once-mated, or twice-mated males.

Boxplot with median in dark horizontal line, whiskers extending to points within 1.5*interquartile range, outliers are represented by circles and extreme outliers (> 3*interquartile range) are represented as stars.
Table 3.1. Summary of potential predictors of polygyny and the results of a forward stepwise binary regression model using these variables (overall model statistics here).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Monogynous Males (mean +/- SD)</th>
<th>Polygynous Males (mean +/- SD)</th>
<th>P at step 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days since hatching</td>
<td>114.61 ± 34.48</td>
<td>105.75 ± 41.44</td>
<td>0.99</td>
</tr>
<tr>
<td>Size (average of patella tibial lengths of front legs, mm)</td>
<td>4.61 ± 0.64</td>
<td>4.58 ± 0.70</td>
<td>0.67</td>
</tr>
<tr>
<td>Body condition* (at initial mating)</td>
<td>0.12 ± 1.10</td>
<td>-0.10 ± 0.90</td>
<td>0.35</td>
</tr>
<tr>
<td>First copulation duration (mins)</td>
<td>15.63 ± 5.86</td>
<td>15.36 ± 4.28</td>
<td>0.70</td>
</tr>
<tr>
<td>Population of origin</td>
<td>San Diego, n=7</td>
<td>San Diego, n= 9</td>
<td>0.245</td>
</tr>
<tr>
<td></td>
<td>Hastings, n =12</td>
<td>Hastings, n= 19</td>
<td></td>
</tr>
</tbody>
</table>

*see methods for calculation
Chapter 4

Strong first male sperm precedence despite frequent plug failure in the Western black widow spider *Latrodectus hesperus*

Abstract

In polyandrous species, males may gain fitness benefit through preventing sperm competition. The blocking of rivals’ access to female’s oocytes through copulatory or mating plugs occurs in a variety of taxa. However, plugging is paradoxical as the sperm competition that creates selection pressure on males to plug must remain sufficiently intense to outweigh costs if plugging is to remain an advantageous strategy. Thus the evolutionary maintenance of plugging may entail a significant frequency of plug failure. I examined plugging success and failure in the Western black widow spider, *Latrodectus hesperus*, a species in which males are assumed to plug females using a broken genital fragment. I first show that polyandry occurs in nature; 12 - 27% of wild-caught females had mated with more than one male. In the laboratory, I then used the sterile male technique to estimate plugging frequency and related paternity shares of two consecutively mating males. I found plugging failed frequently when (1) first males did not deposit broken genitalia within females (36.8 % of copulations), or (2) broken pieces were left in ineffective positions within the female’s genitalia by the first male (67.5-88.2% of cases). Males that deposited genital fragments in the correct location prevented second mating males from acquiring paternity. First-mating males that failed to plug also achieved sperm precedence (greater than 80% of available paternity) in 41% of cases. Selection for plugging could be maintained in this species because most males do not deposit effective plugs, ensuring there are fitness advantages for males who do plug successfully. However, there appear to be alternative means of increasing paternity, and these could be related to female responses or morphology.

Introduction

Competition between males for access to females is commonly observed prior to copulation (e.g., Leboeuf 1974; Miyashita 1993; Hovi, Alatalo et al. 1995; Mateos and Carranza 1999;
Zedrosser, Bellemain et al. 2007). In most species however, a male’s fitness depends not only on his ability to achieve matings, but also to ensure that his sperm are used in fertilization. Competition between the ejaculates of rival males for access to oocytes can also occur cryptically, post-copulation, within the reproductive organs of polyandrous females (Parker 1970; Gromko and Pyle 1978; Birkhead 1987; Ginsberg and Huck 1989). Males of polyandrous species may express adaptive phenotypes that promote success in sperm competition, such as increased sperm numbers (e.g., Stoltz and Neff 2006; Boschetto, Gasparini et al. 2011; Kelly and Jennions 2011), or that prevent the occurrence of sperm competition altogether, such as the production of plugs that remain in the female’s genitalia after mating (e.g., Polak, Starmer et al. 1998; Shine, Olsson et al. 2000; Parga 2003; Takami, Sasabe et al. 2008; Althaus, Jacob et al. 2010; Timmermeyer, Gerlach et al. 2010; Uhl, Nessler et al. 2010; Munroe and Koprowski 2012). Such sperm or copulatory plugs are found in diverse taxa such as arthropods (Dickinson and Rutowski 1989; Alcock 1994; Oh and Hankin 2004; Takami, Sasabe et al. 2008; Althaus, Jacob et al. 2010; Uhl, Nessler et al. 2010), reptiles (Shine, Olsson et al. 2000; Moreira, Lopez et al. 2006) and mammals (Dixson and Anderson 2002; Parga 2003; Dunham and Rudolf 2009; Lemaitre, Ramm et al. 2012; Munroe and Koprowski 2012). Plugs vary in their composition: gelatinous secretions, as in nematodes (Barker 1994), coagulated sperm and seminal proteins, as in primates and other mammals (Dixson and Anderson 2002; Ramm, Parker et al. 2005), or male appendages and genital fragments, as in some spiders (Uhl, Nessler et al. 2010). They may also vary in their placement, they may be found externally, covering the genital opening (e.g., insects: Aiken 1992; spiders: Uhl and Busch 2009; nematodes: Timmermeyer, Gerlach et al. 2010), or internally (e.g., flies: Polak, Starmer et al. 1998; lizards: Moreira, Lopez et al. 2006; spiders: Foellmer 2008; squirrels: McCreight, DeWoody et al. 2011). Plugs may also vary in their persistence, with some permanently embedded in the female (e.g., spiders, Snow and Andrade 2005; Foellmer 2008), and others removed shortly after copulation (Koprowski 1992), or during oviposition (Lachmann 2000). Despite the variation among plugs, most are assumed to have evolved as paternity protection in response to sperm competition.

The evolutionary maintenance of plugging is somewhat paradoxical as it both requires and prevents sperm competition. This aspect of plug evolution has received no theoretical attention, but may generate intriguing predictions about plug effectiveness and occurrence across taxa. For example, in a population where most males attempt to plug, if plugging is 100% effective and
persistent enough to ensure complete paternity of the plugging male, males may evolve to avoid previously mated females or will be unable to fertilize them, and sperm competition may eventually cease to exist. Male investment into plugging may then be selected against, as the lack of rival males eliminates any fitness gained by the behaviour (Parker 1984). Thus plugging may be most likely in groups in which plugging success is variable. Such a situation is expected to be the norm since males are both plug depositors and rivals, so selection should favour increased plugging efficiency but also adaptations that enable the displacement or removal of plugs (Fromhage 2012). Sexual conflict between males and females over polyandry may also weaken selection for effective male plugs, if females have adaptations that prevent plug placement or persistence (Kuntner, Coddington et al. 2009; Kuntner, Kralj-Fiser et al. 2009). The likely result of these opposing pressures is a rarity of completely consistent and effective plugging in nature, and indeed, male failure to deposit or place plugs is commonly reported in studies of plugging species (e.g., Dewsbury 1988; Eberle, Perret et al. 2007; Kuntner, Kralj-Fiser et al. 2009; Timmermeyer, Gerlach et al. 2010; Neumann and Schneider 2011). Removal and displacement of rival’s plugs by copulating males have been documented in several species (Parga 2003; Moreira, Nunes et al. 2007; Fromhage 2012), as have female behaviours that influence successful plugging (Aisenberg and Eberhard 2009; Herberstein, Wignall et al. 2012). Variation in plugging success may also be related to male phenotype, with larger or more dominant males achieving higher rates of plugging success (Lemaitre, Ramm et al. 2011; Neumann and Schneider 2011; Herberstein, Wignall et al. 2012). Despite the advantage to males that can monopolize female reproductive output, selection on males and females to counter plugging and natural variation among males in plugging ability may restrict the success of this mating strategy.

Despite the documentation of apparent “plugs” in many species, few studies have actually examined their effectiveness at restricting the paternity of rivals (but see: Dewsbury 1988; Schneider, Thomas et al. 2001; Moreira and Birkhead 2003; Snow, Abdel-Mesih et al. 2006; Eberle, Perret et al. 2007; Nessler, Uhl et al. 2007; Neumann and Schneider 2011). As successful plugging may be inconsistent due to variation in male phenotype or interference from rivals or females, assumptions about plug efficacy and uniform paternity based on morphological descriptions alone are unmerited. For example, within studies that have compared the paternity of the first and second male to mate with the same female, the ability of the first male’s plug to prevent paternity gains by the second male was variable. High levels of plug efficacy were found
in four spider species (Schneider, Thomas et al. 2001; Snow, Abdel-Mesih et al. 2006; Nessler, Uhl et al. 2007; Neumann and Schneider 2011). However, these were also accompanied by frequent plug placement or deposition failure by males. In contrast, no relationship was found between plugging and paternity distribution among competing males in deer mice (Dewsbury 1988; Eberle, Perret et al. 2007). True measures of the ability of plugs to prevent sperm competition are rare, as they require assessment of both first male and rival male’s plugging behaviour and the outcome of possible sperm competition.

Spiders are an excellent model system for studying the evolution of plugging because a variety of potential plugs have been documented (reviewed in Uhl, Nessler et al. 2010). Spiders have paired genitalia, with two separate copulatory ducts and sperm storage organs in females complimenting paired intromittent organs in males. This facilitates manipulative experimentation as the success of males at inseminating plugged and unplugged tracts can be conducted on one mating pair. Also, the paradox of plugging may be of particular interest in spiders because plugs may be permanently embedded in females (as determined by plug presence after natural death in captive (Fromhage and Schneider 2006; Nessler, Uhl et al. 2009) and wild-caught females (e.g., Levi 1959; Levi 1971; Levi 1975; Davies 2003; Marusik, Ovchinnikov et al. 2006; Deeleman-Reinhold 2009; Kuntner, Kralj-Fiser et al. 2009; Ramirez, Wight et al. 2009).

Moreover, these long-lasting plugs may secure a male’s paternity for a female’s lifetime reproductive output in species in which females have separate ducts for fertilization and insemination, and so do not require plug removal prior to oviposition (Foelix 1996). For some species of spiders, plugs are also particularly valuable because they come at a great cost; the genital breakage that causes plugging renders males sterile (Herberstein, Gaskett et al. 2005; Fromhage and Schneider 2006; Schneider, Herberstein et al. 2008). Plugs in spiders may be the product of sexual selection pressure resulting from sperm competition (Chapter 1, Ghione and Costa 2011; Izquierdo and Rubio 2011), or the result of sexual conflict over mating in cannibalistic species (Fromhage and Schneider 2005; Schneider, Gilberg et al. 2006; Miller 2007), but are generally assumed to be used for paternity protection (Uhl, Nessler et al. 2010). Failure in the process of plug deposition has been demonstrated in several species (Schneider, Thomas et al. 2001; Nessler, Uhl et al. 2007; Aisenberg 2009; Kuntner, Kralj-Fiser et al. 2009; Uhl and Busch 2009; Kralj-Fiser and Kuntner 2012), as has female complicity in plugging (Aisenberg and Eberhard 2009), and variation in plugging success, and plug persistence, due to
male phenotype (Herberstein, Schneider et al. 2002; Schafer and Uhl 2002; Aisenberg 2009; Neumann and Schneider 2011; Herberstein, Wignall et al. 2012). The majority of investigations into plug efficacy and paternity, based on manipulated double mating experiments involving a sterile male technique, have found that the paternity of second males is highly variable and often dependent upon the correct positioning of first male’s plugs (Snow, Abdel-Mesih et al. 2006; Nessler, Uhl et al. 2007; Neumann and Schneider 2011). These studies all involved males whose plugs are composed of broken genital fragments, and as these are sclerotized structures, their positioning within the female insemination duct may be more vital to their efficacy than plugs composed of amorphous secretions. All these studies involved the manipulation of male genitalia in order to force males to insert into a plugged or unplugged insemination duct. While this is valuable for establishing effects of plugging, it is less helpful for identifying natural patterns of plug loss and placement, and correlated paternity effects. To gather those data we need to perform paternity experiments that combine un-manipulated double mating tests with detailed post-mating examination of males and post-reproduction examination of female’s genitalia. Such an experimental protocol is possible using the Western black widow spider, *Latrodectus hesperus* as the test species.

*Latrodectus hesperus* males are significantly more attracted to virgin over previously mated females in the field (Chapter 1), and for males, the genital breakage that creates plugs does not limit male mating ability or frequency (Chapter 3). These aspects of *L. hesperus* natural history may result in low selection on males to maintain effective plugging, as sperm competition may be rare and male fitness may not be solely dependent on plugging success, as in other spiders (e.g., Herberstein, Gaskett et al. 2005; Fromhage and Schneider 2006; Foellmer 2008). However, evidence from manipulative double mating studies using other *Latrodectus* species that deposit plugs shows that there is opportunity for sperm competition in polyandrous females, and outcomes of this competition are variable (Snow and Andrade 2005; Neumann and Schneider 2011). Second-mating males copulating into previously used insemination ducts were able to achieve an average of 66.8% ± 30.5% paternity in *L. tredecimguttatus* (Neumann and Schneider 2011), and in *L. hasselti* last male sperm precedence (80% to 100% paternity) was achieved in 18% of copulations (Snow and Andrade 2005). The lack of complete first male sperm precedence was attributed to variation in males’ ability to position their broken genitalia at the entrance to the sperm storage organs although this was only demonstrated in *L. hasselti* (Snow et
al 2006). The goal of my study is to gain insight into how plugging is maintained through examining the success of broken genitalia as mating plugs in *L. hesperus*, the factors influencing plugging success and the potential rates of polyandry in the wild.

Reproductive natural history

The reproductive organs of *L. hesperus* spiders are represented in Figures 4.1 and 4.2. The reproductive organs of male spiders, called palps (Figure 4.2), are modified forelegs used to store sperm (Foelix 1996). The female reproductive tract consists of two openings (gonopores) leading to paired insemination ducts (Figure 4.1). Each duct terminates in a separate sperm storage organ (spermatheca). A separate fertilization duct connects the spermatheca to the canal through which eggs pass to be fertilized then deposited (the uterus internus) (Berendonck and Greven 2002).

Prior to mating, male *L. hesperus* engage in pre-copulatory courtship behaviours that include initial plucking and strumming of the female’s web, followed by mounting the female’s venter and palpal drumming on the female’s abdomen (ECM Pers. Obs). Copulation occurs when the male inserts the sclerotized, coiled embolus of one palp into one of the female’s insemination ducts. During copulation, the end of the embolus, the apical sclerite, breaks off inside the female (Foelix 1996). Apical sclerites are usually positioned at the entrance to the spermatheca protruding into the spermathecal lumen, although they can also be found in the insemination duct (as seen in congeners, Berendonck and Greven 2002; Snow, Abdel-Mesih et al. 2006). Once a male has copulated with one palp, he resumes courtship until he commences copulating with the unused palp, whose embolus is then inserted in the female’s other insemination duct. *Latrodectus hesperus* females are rarely sexually cannibalistic (ECM Pers. Obs.), although this is more likely if they are hungry (Johnson, Trubl et al. 2011), and males typically survive matings (Chapter 3).

Methods

Estimating polyandry in the field

Females were collected from grassy fields at Hastings Natural History Reservation, in Carmel Valley, California, USA, between 2008 and 2011. Collection of females (n = 108) occurred at times which were near to (2008) or at the end (2009 - 2011) of the breeding season (~May – July, Table 4.1). Females were removed to the laboratory at the University of Toronto
Scarborough, Ontario, Canada where they were housed in light and temperature-controlled rearing facilities. After their natural death, females were preserved by freezing. Subsequently, female internal reproductive organs were removed by dissection and were cleared by placing them in a 10% aq KOH solution for one week (as in Snow, Abdel-Mesih et al. 2006). Male’s apical sclerites were visible within either the spermathecae or insemination ducts upon clearing (Figure 4.1B,C).

**Sperm competition and plug loss/positioning**

Double mating experiments using a sterile male technique (Parker 1970) with laboratory-reared spiders were conducted during three periods (October 2009 - April 2010, January 2011 - April 2011, and July - August 2011). Through the sterile male technique the paternity shares of two males that mate with the same female can be assessed. Females are mated to both a control male and a male treated with a dose of radiation (Irradiated) that does not affect sperm phenotype, but results in early embryo mortality (Parker 1970; Schneider and Andrade 2011). Paternity can be assessed through the comparison of hatched and unhatched eggs in these experimental treatments in which the order of presentation of irradiated and control males if varied randomly (Control-Irradiated and Irradiated-Control). Unhatched eggs are assumed to have been fertilized by irradiated males after correcting for natural levels of inviable offspring, and the success of the irradiation procedure tested by using a formula that estimates these quantities from controls in which females are mated to two control males, or two irradiated males (Boorman and Parker 1976).

Spiders were either first or second generation descendents of field-collected females (see above for collection location). Hatched spiderlings were reared communally with egg-sac-mates on a diet of *Drosophila melanogaster* flies twice per week. At approximately the fourth instar they were transferred into individual cages (4.12cm² x 4.76cm Amac Plastic Products Ltd) where they continued to be fed flies twice per week. After reaching an appropriate size, usually in their 6th instar, females were fed a diet of one cricket, once per week. Adult male spiders and males in their penultimate instar (those with visibly enlarged but incompletely developed palps) were also given one drop of water in their cages once per week. All rearing occurred at ~25°C and under a 12:12 light cycle.
Prior to trials, spiders in their penultimate instars were transferred to a room on the experimental light cycle (lights off: 8:30 am, lights on 8:30pm). Females were used for experiments within 6-20 days of their final moult, and males no earlier than 7 days after their final moult. Forty-eight hours prior to an experimental trial females were weighed using an Ohaus Explorer electronic balance and were placed on inverted U shaped wire frames fixed in plastic blocks within plastic tubs (44cm x 29cm x 17cm) to build webs. All adult males to be used in the subsequent 3-4 days of experiments were removed to another location at the University of Toronto, where a subset were randomly selected to be treated with 9000 Krads of gamma radiation delivered at a rate of approximately 780 krads/min (2010) and 690 Krads/min (2011) from a Cs 137 source.

All experimental trials began between 9am and 11am, and lasted for eight hours if males were actively courting, and six hours if they were not. Mating experiments were performed under a dark cycle and were digitally recorded under red light using Panasonic WW CP484 SDIII cameras with Navitar 7000 zoom lenses. An experimental trial began when a male was introduced to a female’s web using forceps. I subsequently recorded the times of (1) first touch of male to any part of the female’s body (typically the legs), (2) first mount of the female’s abdomen, and (3) start and end times of copulations. Both males and females were given a second opportunity to mate if they failed to do so with their first partner, and individuals that failed to mate in their second pairing were excluded from the experiment. After an initial mating, females were given three further opportunities to mate a second time. Second mating opportunities followed the same procedure as first matings, always began approximately 24 hours after the beginning of the initial mating trial and were completed within the following five days.

Following trials, males were weighed, euthanized with CO2, and examined for apical sclerite loss. Male size was estimated by the mean length of the patella + tibia of both front legs. After their second mating females were removed from the wire frames, weighed, and returned to their original containers in their original rearing space. I collected the first seven egg sacs laid by experimental females for paternity assessment. Egg sacs were cut open to monitor development, and all eggs were euthanized by freezing either one to two days after eggs hatched or one month after deposition (if no eggs hatched). Hatched spiderlings and unhatched eggs within egg sacs were subsequently counted. After their natural death females were preserved by freezing until dissection of their spermathecae (described above). Females were also photographed and patella
+ tibia of both front legs were measured. Spider body condition was calculated for all individuals using the residuals of a type II linear regression of the log10 of the cubed root of weight on ln size (as in Kasumovic, Brooks et al. 2009).

In 2010 mating trials were conducted using 151 individual females, which yielded 23 females that mated completely (both males using both palps) to both control and irradiated males (11 control-irradiated (CI), 12 irradiated-control (IC)) and 7 double mated control females (5 mated to two control males (CC), 2 to two irradiated males (II)). However, due to unforeseen problems with my rearing practice only 12 experimental females deposited egg sacs post-mating. Females used in trials in 2011 were reared under expanded spectrum fluorescent lights and were given crickets whose diet had been supplemented with fish flakes. In 2011 I conducted mating trials with 157 individual females; 34 of them mated with both irradiated and control males (17 CI, 17 IC) and 15 of them mated to two males of the same treatment (9 CC, 6 II). Females that failed to deposit egg sacs in 2010 were excluded from my analysis. Paternity was assessed using the equation from Boorman and Parker (1976), which uses the proportion of hatched versus unhatched eggs corrected for natural levels of inviability and imperfect irradiation effects. The equation

\[ P_i = \left[ 1 - \left( \frac{x}{p} \right) \right] + \left[ \frac{z}{p} \times \frac{1 - \left( \frac{x}{p} \right)}{1 - \left( \frac{z}{p} \right)} \right] \]

determines the paternity shares of an irradiated male when \( p \) = the proportion of eggs fertilized after a normal mating, \( z \) = the proportion of egg fertilized after a female mates with an irradiated male, and \( x \) is the proportion of eggs that hatched in the experimental egg sac. In neither 2010, nor in 2011 did any female mated to two irradiated males lay eggs that hatched, so the second term of the equation is zero in all my calculations. Paternity estimates were adjusted for an average hatch rate in normal-normal matings \( (p) \) of 72.63% among CC females in 2010, and 90.5% in 2011.

Results

Evidence for Polyandry in the field

One hundred of the females collected from the field subsequently reproduced in the lab, and thus had been inseminated in the field. There was no significant difference between years in the
average number of plugs found within females (Kruskal-Wallis test, $\chi^2 = 3.694, df=3, p=0.269$), which ranged from 0 to 6 in a single female, so data were pooled for additional analysis. Of these mated females, 12 individuals (12%) had more than two apical sclerites within their reproductive organs (Figure 4.3); this is an estimate of the frequency of polyandry since each male has two sclerites. Although all these females had all been mated in nature (confirmed by their production of offspring in the lab), 30% of them had no apical sclerites in their genitalia (Figure 4.3). This suggests almost one third of matings do not include sclerite placement. Thus my estimate of polyandry based on number of sclerites found is very conservative. For example, if we assume 30% of the females with two sclerites in their genitalia ($n = 50$) were actually mated by two males, my estimate of polyandry increases to 27%.

There was morphological evidence of plug failure in these dissections. Three females had two apical sclerites occupying the entrance to a single spermatheca, and in one of those females, both spermathecae showed this pattern.

**Double-mating trials**

**Apical Sclerite Loss**

There was no significant difference in apical sclerite loss between 2010 and 2011 (Kruskal-Wallis test: $\chi^2=2.595, p=0.273$), nor was there any effect of male treatment (I or C) on sclerite loss (Kruskal-Wallis test: $\chi^2=1.315, p=0.251$), so the following analyses involve pooled data from both years ($n = 116$ males). First-mating males ($n= 58$) in double mating trials had variable apical sclerite loss (Figure 4.4). Although the most common outcome was to lose both sclerites, approximately half of first mating males (50.8%) failed to do so (Figure 4.4). The number of sclerites lost by these males increased with male weight (Pearson correlation: $r = 0.273, p=0.032$) and size (Pearson correlation: $r=0.377, p=0.032$). Sclerite loss was also related to the progress of the mating with shorter total elapsed time from the beginning of the trial until the first copulation (Pearson correlation: $r=-0.328, p=0.009$). Post-hoc tests showed that most of these differences arise between males that lose no sclerites and those that lose 1 or 2. Males that lost two apical sclerites were significantly larger (Kruskal-Wallis test: $\chi^2=4.477, p=0.034$, Figure 4.5a) and had significantly shorter latencies to first copulation than males that failed to lose any sclerites (Kruskal-Wallis test: $\chi^2=4.418, p=0.036$, Figure 4.5b), and males that lost no sclerites spent
significantly longer amounts of time in the courtship stage between first mount and first copulation than males that lost one or two apical sclerites (Kruskal-Wallis test: $\chi^2=3.835$, $p=0.050$, Figure 4.5c). Male size and courtship duration were not correlated (Pearson correlation: $r^2=0.031$, $p=0.870$). None of my measures of female morphology and age were correlated with male apical sclerite loss (size, weight, age, all $p > 0.05$).

Second-mating males had a different pattern of apical sclerite loss (Figure 4.6). Again, data were pooled as there was no difference in apical sclerite loss of second-mating males between years (Kruskal-Wallis test, $\chi^2=1.669$, $p=0.434$), or between male treatments (C or I, Kruskal-Wallis test, $\chi^2<0.000$, $p=0.998$). The least common outcome of second-matings was double apical sclerite loss, occurring 30% of the time, whereas 70% of second-mating males lost either one or no apical sclerites (Figure 4.6). Apical sclerite loss of second-mating males was not significantly correlated with any of my measured variables (all $p > 0.05$), nor was it correlated with the number of apical sclerites lost by the rival, first-mating male in each trial ($r=-0.082$, $p=0.532$).

The most common outcome of these trials was for double-mated females to have two apical sclerites within their reproductive organs. Thus the total number of apical sclerites found in a female accurately reflects the history of polyandry in only 37.5% of cases (21 females with more than 2 sclerites out of 56 females) (Figure 4.7). This is close to the estimated error rate from field-dissected females (30%, as above).

### Apical Sclerite positioning

Of 79 sclerites found in 36 females after these trials, 78.5% were in a position where they are likely to block insemination by rival males, 21.5% were in the insemination tubules and so not likely to be effective. I also found 8 incidents of two plugs occupying a single entrance to a spermatheca, two instances of which were within one female.

In these double mating trials I cannot determine the origins of apical sclerites (either male 1 or male 2) since females can only be dissected after the second mating. Determination of the frequency of correct placement of apical sclerites at the entrance to the spermathecae by each male is therefore impossible. However, I can assign maximum and minimum rates of placement success to males by recognizing how many sclerites were lost by each male and by making different assumptions about which male left which plug. First, if I assume that when both males
lost apical sclerites, male 1 is more likely to have left his in the correct position, then first males place plugs in the correct location in 88.5% of cases (54 plugs in good position out of 61 total plugs left by male 1). Second, if I assume the opposite, that male 1 is more likely to have deposited any plugs found in incorrect positions, then first males deposited apical sclerites correctly in 67.2% of cases (41 good plugs out of 61). If I do the same for second males, I find that they deposited a minimum of 66.7% and maximum of 93.7% apical sclerites in the correct location (32 and 45 out of 48 plugs left by second males respectively).

Paternity

There was no significant difference between paternity shares of males who had lost zero, one or two apical sclerites during mating ($\chi^2 = 2.588$, p=0.274) (Figure 4.8). However, as the likelihood of correct plug placement among first-mating males is not 100%, some second-mating males may have had access to females’ spermathecae even when first-mating males lost both sclerites. I thus examined separately cases in which I could unambiguously assign sclerites to particular males.

First, there were four cases in which the first-mating male lost both apical sclerites, the second male none, and the dissected female spermathecae contained two correctly placed plugs. In these cases, there was always high first male sperm priority (mean $P_1 = 95.75\% \pm 7.85$). Paternity of these males is not significantly different from those of first-mating males that left one or zero apical sclerites (n= 22, mean $P_1=52.05\% \pm 43.24$, Kruskal Wallis test: $\chi^2=2.644$, p=0.104), or first-mating males that left two apical sclerites but failed to deposit them in the correct location, or had more than one apical sclerite within the entrance to the spermathecae (n=8, mean $P_1=58.5\% \pm 37.92\%$, Kruskal-Wallis test: $\chi^2= 3.540$, p= 0.060). In this analysis, high variance in paternity in the latter two groups arises from a bimodal distribution of paternities, and so the ~40% differences seen here are not significant.

Second, although I excluded from my general estimate of paternity all cases in which one male copulated only once with a female, these data are useful to discern paternity patterns. For example, I asked whether or not first-mating males that leave two correctly positioned apical sclerites (as determined by a lack of apical sclerite loss by male 2) are better able to prevent paternity gains from second-mating males than are first-mating males that lose <two sclerites. To
do this, I included in the analysis cases in which the first male left two sclerites but the second male mated with one palp, and did not break a sclerite (n=5, Case 1, Figure 4.9). In these cases, first-mating males that deposit 2 correctly placed sclerites (n = 5) have significantly higher paternity than those that deposited one or zero apical sclerites (n= 25, Kruskal-Wallis test: $\chi^2=4.251$, p=0.039, Case 2 in Figure 4.9) and those that deposited 2 sclerites but without correct positioning (n=8, Kruskal-Wallis test: $\chi^2=4.633$, p=0.031, Case 3 in Figure 4.9).

The distribution of paternity among males appears bimodal, with the most common outcome of double matings being first male sperm priority (P1 > 80% paternity), and the second most common being second male sperm priority (P1< 20% paternity) (Figure 4.10a). This bimodal pattern persists regardless of whether or not first males lost zero, one or two apical sclerites (Figure 4.10). I asked whether the incidence of second male sperm priority is different between first males that lost zero, one or two apical sclerites. I found that paternity of second males was independent of first male sclerite loss (Kruskal-Wallis test: $\chi^2=1.832$, p=0.400). In 40.9% (9/22) of cases in which males deposited zero or only one apical sclerite, first-male sperm priority was maintained (Figure 4.10b and c). As the number of sclerites lost by the first-mating male increases (0, 1 or 2), the proportion of the paternity shares attributable to the first male also tends to increase (Figures 4.10b – d), although this trend is not significant (p >0.05). I also found that sperm mixing was not common, occurring in only 16.25% of cases (7/43, Figure 4.10a), and was not more likely to occur in cases in which both males deposited apical sclerites within the entrance to a spermatheca. Finally, I asked whether or not differences between the paternity of first-and second-mating males were related to differences in male courtship or copulation times, or with differences in male sizes, conditions or ages. I found no significant correlations between these factors that predicted the male more likely to gain sperm precedence. The difference in paternity between the first and second male to mate was significantly negatively correlated with differences in male weight ($r=-0.339$, p=0.032). However, this was driven by relatively rare cases of sperm mixing--when examining only trials with either first or second male sperm priority, this relationship no longer existed ($r=-0.155$, p=0.382).

**Discussion**

These results indicate that *L. hesperus* males are capable of depositing plugs in females which restrict or prevent paternity gains by rivals. Nevertheless the opportunities for sperm
competition are high within this system as 12 – 27% of females are polyandrous and there is frequent plug deposition and positioning failure. Furthermore, I find that plugging success of a first-mating male is related to his phenotype; larger males were more likely to lose sclerites. Moreover, it appeared that males that eventually lost sclerites in mating were also those that secured copulations more quickly—having spent less time on courtship, and less time copulating with females than males that did not lose sclerites at mating. Finally there is little evidence for sperm mixing. Instead in most cases paternity was dominated by one male, most frequently the first male to mate (although there were reversals, Figure 4.10).

For laboratory tests of sperm competition to be meaningful, the frequency of polyandry among wild females should first be established. I found direct evidence of polyandry, the presence of more than two apical sclerites, in approximately 12% of females. But polyandry may be more common, as males frequently fail to deposit their apical sclerites within females during mating. Although all of my experimental females mated with two males, the proportion of females that would be scored as polyandrous due to the presence of three or more apical sclerites was only 37.5%. Additionally, about 30% of field-captured females did not have apical sclerites present in their genitalia (Figure 4.2) even though they were inseminated. Either these females mated with non-virgin males who had already lost their apical sclerites in previous matings or with males who failed to lose an apical sclerite. In both cases, the interaction left no immediate evidence that mating had occurred. Thus, field-caught females with two sclerites or fewer may well have mated with two different males. A comparison of experimental and wild-caught females with one apical sclerite in their spermathecae provides further support for polyandry in the wild. The proportion of wild-caught females with one sclerite is approximately 10%, which is lower than expected under monandry (~ 30% of first-mating males lose only one sclerite: Figure 4.4) and under experimentally induced polyandry (found in ~20% of cases). This under-representation among field-caught females may be due to the acquisition of other apical sclerites through polyandry by females whose first mate lost only one sclerite. However, this same under-representation is not seen in wild-caught females that contained no apical sclerites, which may indicate that the under-representation of wild females with one sclerite is caused by factors other than polyandry, such as a) lower levels of losing one sclerite among wild males or b) that the proportion of one-plug females is indicative of polygyny, where females mated with males that had already lost sclerites, or c) females have adaptations that prevent apical sclerite deposition in
nature, rendering them less likely to acquire any despite multiple matings. Despite the complications of determining natural levels of polyandry using this method, this study provides irrefutable evidence that multiple males mate can and do mate with *L. hesperus* females, enabling the persistence of sperm competition and the resulting selection pressures in this species.

Understanding the maintenance of sperm competition may also require consideration of plug deposition and positioning error. Although the documentation of successful plugging establishes functionality, documentation of male failure to plug is integral to understanding the evolutionary maintenance of this strategy. Approximately half of first-mating males in my trials failed to deposit both apical sclerites within the female, and among the males that deposited both, placement success was not guaranteed. Frequent failure among males to monopolize females enables the persistence of sperm competition. The frequencies of plug deposition and placement failure determined by this experiment may be artificially high as I required that females mate with two males to be included in my study. If males are less likely to mate with a previously-mated female with two correctly positioned plugs, or conversely if females are less likely to accept second mates under the same conditions, then the frequency of plugging failure in my experiments will be inflated. Male *L. hasselti* and *L. tredecimguttatus* have both been found to discriminate against previously used insemination tracts (Snow and Andrade 2005; Neumann and Schneider 2011), but the mechanism by which this occurs is unknown. *Latrodectus* males may be able to infer plug presence when sensing females, potentially through different vibratory properties of plugged and unplugged spermathecae during palpal drumming. If so, then *L. hesperus* males in my experiment may have also been less inclined to pursue copulations with plugged females. Determination of natural rates of plug loss and placement among first-mating males is necessary in order to understand the contribution of this aspect of plug failure towards the maintenance of polygyny, however my results suggest that among females that are polyandrous in the lab, plugging failure is frequent.

The relationship between plugging success and male phenotype may be integral to maintaining a balance between plugging and sperm competition. My data show males that deposit both apical sclerites are larger and heavier, and have shorter courtship durations than those that fail to
deposit any apical sclerites. A similar relationship between male size and plugging success was found in a congener with almost identical male genitalia and plugging method, *L. tredecimguttatus* (Neumann and Schneider 2011). The restriction of plugging to certain males that express phenotypes which are determined partially by variables related to the environment, enables the coexistence of sexual selection for the plugging phenotype and sperm competition. If, as in *L. hesperus*, plugging success is related to male size, this phenotype may be dependent on the resources accrued by males in their juvenile stages (as in other spiders, Uhl, Schmitt et al. 2004; Fernandez-Montraveta and Moya-Larano 2007; Kleinteich and Schneider 2011). Thus even if significant additive genetic variation underlies body size, the offspring of larger, successful-plugging-males may be limited in their expression of the same advantageous phenotype as their sire by the distribution of food resources. Plugging may then be favoured by sexual selection, but fail to become a common strategy within the population.

Larger males may achieve higher levels of plug deposition success due to male actions, female actions or conflict between the sexes. Whether genitalia of males or females scale with body size, or are of a relatively consistent size regardless of body size has not been determined for any *Latrodectus* species. The mechanism behind larger male plug deposition success may be simply an advantage in embolic or apical sclerite size (but see: Eberhard, Huber et al. 1998). Longer emboli or larger apical sclerites may facilitate the lodging and breakage of genitalia. This may be of particular importance if the female’s genitalia also vary in size, as large males may be able to plug a larger proportion of females than small males. Similarly, the size or length of the female’s insemination ducts and spermathecal entry point may have adapted to promote plugging by larger males, if male genitalia are scaled with male size. Berendonck and Greven (2002) also suggested that females may be able to exert muscular control over the entrance to the spermatheca, tightening the entrance to the spermatheca and facilitating genital breakage in some males. Finally, increased plug deposition success by larger males may also be indicative of sexual conflict over plugging. Selection on females to avoid plugging may also lead to adaptations that increase the width of the spermathecal entrance, preventing males from effectively blocking the sperm or apical sclerites of other males. Counter-selection on males for increased apical sclerite size and width may be particularly effective in larger males, especially if the size of male genitalia is related to individual size. Although males that have shorter courtship durations also experience greater levels of plug deposition success, I find it more
difficult to speculate on a plausible mechanism for this occurrence. Reduced investment in courtship should be advantageous to males, particularly in this potentially polygynous species. If this trait is heritable, females may benefit by having low-investing sons and so may facilitate plugging by males with this trait. Further insights into the mechanism of plugging may be gained by morphological studies on *L. hesperus* males and females.

The patterns of paternity seen in my study indicate that plugging males gain fitness benefits, but surprisingly, that failure to plug does not necessarily result in the loss of paternity shares. The ability of males to prevent or restrict sperm competition by depositing both apical sclerites at the entrance to females’ spermathecae is supported in my study by the first male sperm precedence, without reversals or shared paternity, within these males. This pattern of paternity was significantly different from those of first-mating males that deposited zero or one apical sclerite, and for males that deposited both apical sclerites but failed to position both correctly. However, first-mating males that did not deposit both sclerites maintained first male sperm precedence (80% -100% fertilization) in approximately 41% of cases. My measured variables failed to predict whether or not the first male would lose or share paternity with the second male, as differences in paternity were not predicted by differences between first and second males in their phenotypes, their courtship durations, or their copulation durations. These results have important implications for the paradox of plugging. Although selection may favour plugging males as they are able to gain fitness benefits through monopolizing paternity, the strength of selection on plugging behaviour is weakened by the ability of first males to gain paternity regardless of their phenotype or ability to leave plugs. This weakened selection pressure may be vital to maintaining the balance of sperm competition and plugging. If males that failed to plug females were selected against through considerable fitness losses, then plugging as a strategy may become fixed in the population.

The patterns of paternity generated by my double mating experiment suggest that, when plugs fail, the resulting sperm competition and the underlying mechanics of how sperm are stored and used in fertilization are more complex than a fair raffle model (where sperm mixes and paternity is determined by the relative contributions of sperm from each male, Parker 1990). The bimodal distribution of paternity and the relatively few incidents of shared paternity (16.29% or 7/43 cases), even among males that both inserted apical sclerites into the spermathecal opening (4/6 cases), indicate biases in sperm use which were not accounted for by differences in male traits.
The paternity shares of different males may instead be influenced by spermathecal morphology (e.g., Austad 1982), the position of ejaculates, and the degree of sperm mixing (Harvey and Parker 2000). In spiders, sperm are transferred in encapsulated clumps, not as discrete, swimming spermatozoa (Foelix 1996; Huber 2005). In *L. hesperus*, spermathecae are dumbbell shaped, with rounded anterior and posterior lobes connected by a narrow median canal (as in a congener, Berendonck and Greven 2002), which may facilitate the effective compartmentalization of ejaculates from separate males. The fertilization ducts exit the spermathecae from the apical ends of the posterior lobes (Berendonck and Greven 2002), and this may create a bias as to which sperm mass is more likely to move or be moved into the fertilization duct, resulting in the dominant paternity of one male. The spermathecal morphology and the clumped nature of sperm within the spermathecae may thus both prohibit sperm mixing and generate instead the bimodal pattern that I observed. Sperm competition in this instance may be indirect, with males competing for, and displacing each other from, the favoured position for fertilization within the spermathecae.

Female control over sperm storage and usage may also influence paternity patterns, however our research failed to identify any indication that females were biasing paternity towards a particular male. Although male weight, size and copulation duration all influenced apical sclerite deposition, there is no indication that females were selectively using sperm from larger, heavier or shorter courting males. There was no difference in these traits between males that did and did not father offspring in cases in which both males had access to at least one spermatheca. It has been argued that female cryptic choice within *Latrodectus* is unlikely. Investigations into a congener failed to locate any receptors within the spermathecae or surrounding tissue which could trigger a response to sperm deposition that might bias paternity towards a particular male (Berendonck and Greven 2002). However, the potential for female control through spermathecal morphology and secretions that compartmentalize ejaculates (as in Burger, Michalik et al. 2006), the activation of sperm through targeted glandular secretions (suggested in Uhl 2002) and the potential for females to preferentially draw sperm from one spermatheca remain uninvestigated and could provide insight into the occurrence of reversals of first male sperm priority in *L. hesperus*. Investigations into pre-copulatory aspects of female choice and inter-male competition may also reveal benefits to both polyandry and female control over mating that were not evident from my study.
Paternity may be influenced by a myriad of factors. My work emphasizes how post-copulatory competition between males may be influenced by multiple aspects of male behaviour and that sperm competition may be multi-faceted. I find that although plugging may be advantageous for males, its prevalence as a reproductive strategy is constantly forestalled by adaptations to sperm competition occurring on different competitive fronts, such as those that may enable second mating males to insert their apical sclerite into a plugged spermathecae or those that maintain first male sperm precedence despite plug absence, and potentially by adaptations in females that increase control over paternity. These insights into the evolutionary maintenance of plugs have been gained through examining both the success and failure of plugging, which is a novel approach despite the widespread use of plugs by animals.

References


Figure 4.1. Female reproductive organs. (A) line drawing of female genitalia, (B) cleared female reproductive organs, 1- plugs placed correctly at the entrance to the spermathecae, 2- plugs deposited within the insemination ducts, (C) cleared spermatheca, 3- approximate exit of fertilization duct, 4- two plugs within spermthecal entrance.
Figure 4.2. Male genitalia, dotted arrow points to the absence of an apical sclerite attached to the embolus on the palp on the right
Figure 4.3. Distribution of the number of apical sclerites found in the reproductive organs of wild-caught females (n = 100) that had been inseminated in nature.
Figure 4.4. Distribution of apical sclerite loss among the first males to mate in the double mating trial (n = 57 males)
A

Number of apical sclerites lost by the first mating male

Error Bars: 95% CI

B

Number of apical sclerites lost by the first mating male

Error Bars: 95% CI
Figure 4.5. Comparison of first-mating males that lost 0, 1, or 2 apical sclerites in their (A) mean body size, (B) mean latency to first copulation and (C) mean duration of the courtship interval between the first mount and first copulation, where significant differences are indicated by different letters above each column. Error bars represent 95% confidence intervals. Significant differences are illustrated by different letter labels above columns.
Figure 4.6. Distribution of apical sclerite loss among the second-mating males (n = 56) in the double mating trials.
Figure 4.7. Distribution of the total number of apical sclerites found within females used in double mating trials ($n = 56$)
Figure 4.8. Variation in the paternity of first-mating males that lost 0, 1, or 2 apical sclerites during copulation. Boxes represent interquartile ranges, whiskers extend to 1.5 times the interquartile range, and medians are marked with a black horizontal line.
Figure 4.9. Proportion of paternity gained by first mating males who deposited 2 apical sclerites and positioned them correctly (CASE 1, n = 5), deposited either 0, or 1 sclerite (CASE 2, n = 25), or deposited 2 sclerites but failed to position them correctly (CASE 3, n = 8) for only those cases where the second-mating male did not break off sclerites, so those found in the female can be unambiguously assigned to the first-mating male. Boxes represent interquartile ranges, whiskers extend to 1.5 times the interquartile range, and medians are marked with a black horizontal line.
Figure 4.10. Frequency distribution of the paternity attributed to the first-mating male (P1) in double-mating trials, including all experimental trials (A), cases in which the first-mating male: lost 0 apical sclerites during copulation (B), lost 1 apical sclerite during copulation (C), or lost 2 apical sclerites during copulation (D).
Table 4.1. Number of wild-caught females that were collected and dissected each year and their collection dates.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of females collected/dissected</th>
<th>Collection dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>34</td>
<td>June 5-6</td>
</tr>
<tr>
<td>2009</td>
<td>37</td>
<td>July 11-12</td>
</tr>
<tr>
<td>2010</td>
<td>30</td>
<td>June 29-30</td>
</tr>
<tr>
<td>2011</td>
<td>7</td>
<td>July 1-2</td>
</tr>
</tbody>
</table>
General conclusions

I find male mate choice for both virgin females and larger/well-fed females exists in *Latrodectus hesperus*, and that this choice is strong and virtually unanimous in the field despite natural costs of being choosy (Chapter 1). Male choice behaviour occurs despite the relatively low cost of male investment in paternity protection (males can mate with multiple females despite genital mutilation, Chapter 3). Moreover, choice occurs despite inconsistencies in (1) links between preferred female traits and reproductive output, and in (2) the amount of variation in female quality and thus the magnitude of the benefit of choice across years (Chapter 2). However, male mate choice for virginity may be favoured by sexual selection since first male sperm precedence is common, and this is true regardless of plugging success (Chapter 4). Male choice for large female size may be favoured by natural selection due to the advantage to avoiding smaller females that are less likely to reproduce successfully during the breeding season. These results show male mate preferences are closely aligned with qualities of females that are associated with increased fitness in nature, and that male choice may be driven by the benefits of choice more than by restrictions in male mating opportunities.

Chapter summaries

Chapter 1

This study shows that male *L. hesperus* are differentially attracted to well-fed, virgin females over females that were poorly fed and/or previously mated. These preferences were demonstrated among laboratory-reared males in a field enclosure, but also among wild males. The latter is notable as wild males would have had natural individual differences in body condition and in proximity to preferred females. The unanimity of choice is also notable—there is little evidence for variation in male choice, and none for self-assessment shaping male choice. This study also indicates that both aspects of female quality (mating status and diet) are discernible to males through airborne chemical cues. This is, to our knowledge, the first study to show that female spiders produce volatile chemical cues that allow discrimination of diet (or size). The expression of male mate choice along both of the lines of preference commonly found in males in other taxa, for fecundity (e.g., Olsson 1993; Hoefler 2007; Bel-Venner, Dray et al.
Chapter 2

In my survey of female phenotypes and reproductive success I show that in each year a significant number of females failed to lay an egg sac during the breeding season. The proportion of these non-reproductive females, as well as the proportions of females laying one or multiple egg sacs, varied across years. We found that reproductive output was related to female phenotype, and in all years females that deposited egg sacs were larger than females that did not, although the threshold size for reproduction varied. Female persistence at one web location was also related to the likelihood that she would produce an egg sac. Finally, in two of the years of this 3-year survey, reproductive failure of some females may have been due to rejection by males. These females failed to reproduce although they were present on their webs long enough to allow time for sac production, and were theoretically large enough to produce egg sacs, but were significantly smaller on average than females that produced offspring that year. This suggests the potential for male mate choice to exert sexual selection on females, but this pressure may not be particularly meaningful as selection for increase fecundity also promotes increases in female size. Our results suggest the advantage for choosy males arises from avoiding the high cost of investing in females that are unlikely to reproduce during the breeding season because they lie below a threshold size which marks the point at which reproductive failure becomes common. This study is one of the first to document natural variation in the quality of females in terms of their phenotype and reproductive output in a system with male mate choice.

Chapter 3

In this Chapter I definitively documented the co-occurrence of male genital mutilation and polyandry. Data show that males were capable of inseminating multiple females, and pairing with a previously mated male did not result in any fecundity loss to females. Polygyny in L. hesperus, and likely other members of the genus that leave plugs, may be mechanically possible
due to the relatively small amount of damage to genitalia incurred during mating. In most other spider species where genital mutilation during copulation has been documented, males undergo more substantial damage that results in sterility (see appendix 1). This may suggest an evolutionary history of sexual conflict over mating (e.g., Schneider, Gilberg et al. 2006) and plugging as a response to attempted sexual cannibalism by females (Miller 2007) with broken fragments then co-opted to function as plugs in some taxa. In contrast, broken genitalia in _Latrodectus_ may have evolved via selection pressure on males to protect paternity. This idea arises from several observations, including: the lack of sterility-inducing damage, the positioning of the lost apical sclerites at locations that are efficient for plugging, the limited distribution of cannibalism risk in _Latrodectus_, as well as the characteristic male self-sacrifice behaviour of two _Latrodectus_ species (Forster 1992; Andrade 1996; Segoli, Arieli et al. 2008).

Chapter 4

I examined the potential for females to mate polyandrously in the wild and found direct evidence of polyandry in 12% of cases. Data suggest that, if correctly positioned, the male’s apical sclerites are effective at blocking a rival male’s ejaculate from entering the female’s sperm storage organs. However, males frequently failed to lose one or both of their apical sclerites during copulation, and often fail to position lost sclerites in the optimal position for effective plugging. Male weight was predictive of male propensity to deposit apical sclerites within females. Despite frequent plugging failure, first male sperm precedence was found to be common, although reversals in paternity increased as the first male lost fewer apical sclerites. Paternity was rarely shared between two males, even in cases where two plugs were found in the opening to the female’s spermatheca. In cases where both males contribute sperm to the spermatheca, the observed paternity pattern could be due to the lack of sperm mixing combined with the position of ejaculates within the spermatheca. Alternatively, the pattern may be due to female morphology or cryptic physiological actions, which bias paternity in favour of one male.

Thematic conclusions

Male mate choice
The expression of male mate choice in a species is determined by the relative strength of selection pressure resulting from three factors (Bonduriansky 2001), (1) the cost of each mating to a male in terms of time and energy investment, which determines the contribution that each pairing contributes towards a male’s total lifetime fitness (Gwynne 1991; Kokko and Monaghan 2001), (2) the variation in the quality of females, which determines the potential fitness benefits from mating selectively (Parker 1983; Owens and Thompson 1994), and (3) the constraints to choosiness, which include the ease (Barry and Kokko 2010) and accuracy of assessment (Parker 1983). Integrating the results of my studies on *L. hesperus*, I can summarize the insights gained into each of these factors that influence male mate choice.

**Male investment**

The investment of male *L. hesperus*, and likely most *Latrodectus* males, into mating and paternity protection is likely lower than other males employing a plugging strategy as *Latrodectus* males retain the function of their copulatory organs post mating, allowing for the possibility of polygyny. Although I do not have an estimate of the likelihood of polygyny in the wild, examination of plugs in field-collected females (Chapter 4) suggests polygyny in nature. The high proportion of wild-caught females that reproduced in the laboratory but had no apical sclerites within them (Figure 4.3) suggests that some of them mated with males that had previously lost their apical sclerites (i.e., mated previously). Low levels of male investment in each mating and a polygynous mating system do not necessarily promote male mate choice. However, the competitive advantage to plugging females in terms of male fitness may result in selection for choosy males. Despite the low cost of genital mutilation among *L. hesperus*, *L. hasselti*, and likely other *Latrodectus* males, the potential for a male to monopolize the paternity of all the offspring produced by a female through plugging (Chapter 4), or first male sperm priority, may result in fitness advantages to males who prefer females with indicators of high fecundity and of virginity, and ultimately selection for male mate choice. That is, for males, choice could be driven more by possible payoffs rather than by trade-offs caused by high investment.

Male mate choice is influenced by other forms of male investment into mating effort. In *L. hesperus* this may include time and energy spent on travelling to females, and post-mating recovery time (Chapter 3), as these actions likely increase the time associated with each mating
and reduce the total number of mates a male can acquire in his lifetime ((Clutton-Brock and Parker 1992). Our investigation into polygyny in *L. hesperus* revealed a time period, approximately one day after mating, where males were less likely to mate with another female. This time may represent recovery from energetic courtship, or more likely, the time required for males to refill their palps with new sperm from the testes. This time period may not be of consequence in long lived species, but *L. hesperus* males usually survive for only 46.5 ± 34.6 days post maturity in captivity (Kaston 1970), and likely for less time under natural circumstances. We also expect that time and energy required to travel to sedentary females may also constitute mating effort on the part of these relatively short-lived males. High costs associated with mate searching are typically expected to restrict the evolution of mate choice (Real 1990; Barry and Kokko 2010). However, search costs may be tempered in *L. hesperus* as females may be found in close proximity to each other within their preferred habitat (E.C.M pers. Obs). These behavioural and physiological aspects of *L. hesperus* life-history promote male mate choice through increasing mate cycling time and reducing the expected number of mates a male is likely to acquire. Future work addressing the persistence of male mate choice after apical sclerite loss, which would remove the influence of the potential benefits of plugging on male behaviour, may clarify the contribution of male mating effort towards male mate choice. The reduced mating rate associated with the necessity of providing mating effort may promote consistent male choice behaviour.

**Variation in female quality**

My studies show that *L. hesperus* males benefit from mating selectively due to the potential fitness gains from mating with virgin females and larger females. Pairings with virgin females offer males the potential for high paternity gains, either through plugging – if apical sclerites are still intact- or through the high likelihood of first male sperm precedence. Avoidance of previously mated females may be particularly important for males still in possession of their apical sclerites, as they may lose these valuable plugs without gaining any paternity from the mating. Mating with smaller females may also be costly for males, as these females are less likely to produce offspring over the breeding season. The low levels of shared paternity among mates of polyandrous females, and the consistently high proportion of females that fail to produce an egg sac in the wild may exert strong selection pressure on males to be choosy.
Indiscriminate mating can result in no paternity gains whatsoever, rather than marginally reduced fitness benefits. Male mate choice for increased female fecundity may not be driven by broad distributions in female reproductive output, or an increasing function of mate quality variance as suggested by Parker (1983), but instead by a threshold-like divide between females that are more and less likely to reproduce. This distinction may be particularly important in the field, but will not be captured in most laboratory studies. Future investigations into natural variation in female reproductive output in other species that show variable preferences for female size may allow determination of whether male mate choice is more common where there is a consistently large non-reproductive class of females.

Constraints on Choice behaviour

While I did not directly study constraints on choosiness in *L. hesperus*, my results suggest male use of airborne chemical cues released from females’ webs may reduce constraints on mate location. A model by Barry and Kokko (2010) demonstrated that mate choice was less likely to evolve when the choosy sex must evaluate potential mates sequentially. Results from Chapter 1 indicated that male *L. hesperus* are able to assess potential mates without sequential visitation, as males travelled directly to their chosen female. Although we have yet to examine the properties of female volatile chemical cues, their use by males may enable simultaneous comparisons between the mating status and diet-status of females in a given area (as seen in mantids: Lelito and Brown 2008), and facilitate mate choice through reducing the costs associated with searching and assessing females. The accuracy of the mode of mate assessment may also constrain the evolution of male mate choice through reducing the benefits of choosy behaviour. Assessment through chemical means may be particularly accurate, as female chemical profiles change with mating status (Schulz and Toft 1993; Baruffaldi, Costa et al. 2010; Jerhot, Stoltz et al. 2010). As female pheromone production has been linked to metabolism (Gaskett 2007) changes in female chemical profile may be highly reflective of her dietary state. The reduction of costs to male mate choice through the use of chemical cues may be integral to the evolution of male mate choice on a mechanistic level. Future work comparing the potential for male mate choice in spider species with and without volatile chemical cues could elucidate this relationship.

Summary
The expression of male mate choice in *L. hesperus* is promoted by the potential for fitness gains through plugging, the costs of random mating, and likely by the possibility of an accurate mate assessment at a distance. Through unprecedented examinations of female reproductive output in a male choice system, I identified the potential for male choice to be a response to the absence of fitness gains from pairing with low quality females, and therefore to the costs of random mating rather than the benefits of selectively mating with the highest quality individuals. The insights from my work are particularly important as they are either based in, or compliment to, studies of *L. hesperus* under natural circumstances, and they suggest that field observations are vital to future insights into male mate choice.


Appendices

Appendix 1- Results from GLMs using number of weeks present as the dependent variable, two measures of presence (absolute, relative), and field ID, week of initial discovery, female size and web size as predictor variables. Models either used all observed females in each year, or restricted observations to only those females found prior to the fourth week of the study in each year. Dots indicate the absence of the variable from the final model.

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Appendix 2. Compilation of information available on the mating system (monogyny or polygyny), the presence of a discrete embolic breakage location (Break point Y/N), embolic damage as an estimate of percent missing post mating, and plug position as illustrated in Figure 5 for spiders known from (Uhl, Nessler et al. 2010) to leave plugs of embolic origin. Asterisks indicate a conclusion from reasonable inference, whereas dots indicate factual knowledge.

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<td>hasselti</td>
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<td>M</td>
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<td>* * *</td>
<td>(Andrade 1996; Snow, Abdel-Mesih et al. 2006)</td>
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<td></td>
<td>hesperus</td>
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<td>P</td>
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<td>* * *</td>
<td>(Kaston 1970)</td>
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<td>(Kaston 1970; Breene and Sweet 1985)</td>
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<td>pallidus</td>
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<td>(Segoli, Harari et al. 2006;</td>
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<td>revivensis</td>
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<td>(Segoli, Lubin et al. 2008)</td>
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<td>Thomisidae</td>
<td>Hedana</td>
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<td>(Chrysanthus 1964)</td>
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Appendix 1


Evans, M. R. and B. J. Hatchwell (1992). "An Experimental-Study of Male Adornment in the Scarlet-Tufted Malachite Sunbird .2. the Role of the Elongated Tail in Mate Choice and


