Dynamics underlying interacting mechanisms of sexual selection

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Abstract

Sexual selection drives the evolution of male morphology, life history, physiology, and behaviour across taxa. Here I examine the mechanisms of sexual selection that arise at various stages in mating interactions to identify congruence or conflict between the traits selected by choice and competition. I first examine plasticity of developing male Australian redback spiders (*Latrodectus hasselti*) and show that male metabolic rates vary adaptively to facilitate the scramble to reach virgins. Next, I show that females cease sex pheromone production after mating and re-advertise receptivity later in their reproductive season effectively creating two windows in which males may compete. I show that females discriminate against males that do not meet a threshold courtship duration suggesting that courtship is the trait selected through choice. However, male-male competition leads to reductions in courtship effort provided to females. During the first window paternity is split equally if rival males mate in quick succession with a virgin female. However, if the second mating is delayed, there is a strong bias in the paternity of the second male. A delay in the second mating is beneficial to females as it reduces longevity costs of polyandry. However, delays in the initial mating decrease female longevity, likely because of elevated metabolic rates of virgins. My research shows that the trait favoured by female choice is in opposition to selection via male-male competition. Females' sex pheromone production yields windows during which mating will optimize female, but not male, fitness. Studies that isolate the mechanisms of sexual selection are valuable in that they can identify the traits under selection. However, my research shows that considering these processes in isolation can lead to incorrect inferences about the net effect of sexual selection.

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Chapter 1
General Introduction

Sexual selection arises due to variation in reproductive success among individuals via male-male competition (Darwin 1871, Andersson 1994) and female choice (Darwin 1871, Kirkpatrick 1982). These agents of sexual selection can extend beyond mating and continue operating after copulation in the form of sperm competition (Birkhead & Møller 1998) and cryptic female choice (Thornhill 1983, Eberhard 1985, Eberhard 1996). There is little debate about how mechanisms of male-male competition operate or fit into the theory of sexual selection. In a wide range of taxa, males that are larger or have superior weapons are more successful in competition, more likely to mate with females (Andersson 1994), and sometimes monopolize mating relative to rivals (e.g. LeBoeuf 1974). Traits favouring success in sperm competition have also been widely documented and include increased sperm production (Parker 1998), ejaculatory proteins that affect female receptivity (Chapman et al. 1995), and deposition of sperm plugs (Thornhill & Alcock 1983). In contrast, from its first suggestion by Darwin (1871), female choice has had a contentious history as a mechanism of sexual selection (Huxley 1938, see Cronin 1993).

Currently there is no debate as to the existence, importance and strength of selection that female choice can exert on male traits (Kirkpatrick 1982, Jennions & Petrie 1997, Andersson & Simmons 2006; e.g. Andersson 1982a, Milinski & Bakker 1990, Hill 1991). However, there is still debate regarding the role and importance of female-mediated variation in sperm use patterns (cryptic choice), partly because of the challenge of disentangling such effects from sperm competition (e.g. Birkhead 1998, Simmons 2002). This debate is linked to the ongoing controversy regarding the evolutionary basis for the maintenance of choice when females do not receive material benefits from their mates (Charlesworth & Hughes 1999, Keightley & Lynch

2003). In such situations, directional mate choice should deplete the additive genetic benefits of choice (Rice 1988, Kirkpatrick & Ryan 1991, Yasui 1998, Andersson & Simmons 2006, Kotiaho et al. 2008). Models suggest that choice for heritable traits may nonetheless be maintained under fluctuating selection or through mutation-selection balance (Tomkins et al. 2004; as well as heterozygote advantage, environmental heterogeneity, sexual antagonism and antagonistic pleiotropy, Hughes & Burleson 2000). Fluctuating selection models argue that optimal phenotypes vary as a function of time or across environments (e.g. Jia et al. 2000, Hamilton & Zuk 1982) whereas mutation-selection balance models suggest that variation can be maintained through the condition dependence of sexually selected traits that reflect genetic quality (Andersson 1982b, Pomiankowski & Møller 1995, Rowe & Houle 1996, Houle 1998).

Recently, there has been a call for more studies that examine how and when competition and choice interact with one another as this interaction will determine the net sexual selection on a given trait (Wong & Candolin 2005, Hunt et al. 2009). It may be important to understand interactive effects of competition and choice as the two rarely act independently and, for example, opposing selection via male-male competition may be sufficient to prevent traits from reaching the values favoured by female choice (Moore & Moore 1999). Although current literature emphasizes the importance of this interaction (Bradbury & Davies 1987, Berglund et al. 1996, Qvarnström & Forsgren 1998, Wong & Candolin 2005, Hunt et al. 2009), the prevalence and conditions under which the two mechanisms act in synergy or in opposition remain unclear. Several studies have demonstrated that traits selected under male-male competition are also favoured through female choice (e.g. Watson 1990, Berglund et al. 1996). Others have argued that this need not be the case and effects favoured by one mechanism could oppose those favoured by the other (e.g. Moore & Moore 1999, Moore et al. 2001; see also Wong & Candolin 2005, Hunt et al. 2009). For example, in the cockroach (*Nauphoeta cinerea*) pheromones influence both male-male competition and female choice, however, the composition of the pheromone most appealing to females differed from that which was related to high dominance status among males leading to conflict (Moore & Moore 1999). Females are able to exercise pre-and post-copulatory choice, suggesting that the resolution of this conflict is in favour of the females (Moore et al. 2001). Similarly, in a set of recent studies on widowbirds, male epaulets were found to be sexually selected through male competition for territories (Pryke & Andersson 2003ab) whereas female choice selects for elongated tail length (Pryke et al. 2001, Pryke & Andersson 2002). In this case, competition can impede choice since long-tailed males are not necessarily the most successful at retaining territories, which females require for breeding (Pryke & Andersson 2003a). Thus traits that are selected through female choice are not necessarily the same traits that are selected through male-male competition. Even in instances where both processes focus on the same trait, the form and strength of sexual selection can differ when the mechanisms are considered in isolation or combination (Hunt et al. 2009). For these reasons, an understanding of how male traits (and benefits of female preferences) are affected by sexual selection requires an approach that integrates insights from studies of competition and choice.

Generally, in systems where traits that affect competitive success are heritable, females can gain indirect benefits by mating with males successful in competition, so traits that increase male competitiveness are likely to be congruent with those selected through female choice (Qvarnström & Forsgren 1998, Hunt et al. 2009, Wong & Candolin 2005). However, this is unlikely in situations where sexual selection has led to the evolution of traits by which individuals maximize their own fitness (Arnqvist & Rowe 2005) at a cost to the other sex (e.g. Fowler & Partridge 1989, Chen 1984, Chapman et al. 1995). Such sexual conflict, arising from a divergence in the reproductive interests of males and females (Bateman 1948), has lead to the evolution of competitive traits that are harmful to females in many systems (Arnqvist & Rowe 2005). For example, outcomes of sexual conflict may include aggression that injures or kills females (Rowe et al. 1994, Clutton-Brock & Parker 1995, Jormalainen et al. 2001), ejaculatory fluids that increase male success at sperm competition at the cost of female longevity (Chapman et al. 1995) and male harassment leading to lower fecundity of females (McLain & Pratt 1999). Selection is likely to lead females to avoid competitively dominant males that express higher values of these traits that result in net reductions in female fitness (Hunt et al. 2009, Reichard et al. 2005).

The effect of male-male competition on female choice can also depend on how the costs and benefits of mating with dominant males vary over time. At each stage of the mating interaction, competition may enhance or decrease the opportunity for choice, and this dynamic will determine whether the two mechanisms act in opposition. Wong & Candolin (2005) suggest three stages where competition can affect female choice: mate detection, mate evaluation and mating outcomes. Even if males are not directly harming females, competition may constrain the female's ability to choose or to mate according to her choice at each stage. During mate detection, competition may allow dominant males to exclude rivals by preventing inferior rivals from establishing territories required to attract mates, or competition can lead to interference in the modality used by females to assess males (e.g. acoustic, chemical; Wong & Candolin 2005). During the evaluation stage, competition generally facilitates choice because it can ensure only suitors with superior morphological or physiological traits (winning males) are considered by females. When females evaluate males based on the same indicators used to mediate fighting, competition may ensure honesty since the consequences of dishonest signaling may be injury or death (Wong & Candolin 2005). In such cases, females may incite agonistic interactions as an aid to evaluation (Watson 1990). However, competition during mate evaluation can also negatively impact choice when competition disrupts or interferes with courtship (Wong & Candolin 2005). At the mating outcome stage, competition may oppose choice when inferior males parasitize dominant rivals or employ competitive strategies that bypass or override choice altogether (Wong & Candolin 2005). Similarly, while females may have mechanisms for biasing sperm use (Eberhard 1996), sperm competition tactics may subvert these by inactivating or flushing out a rival male's sperm (Manier et al. 2010). Thus, conflict can be acute regardless of the stage at which competition and choice occur as there are a range of conditions under which pre- and post-copulatory male-male competition can interfere with choice (Wong & Candolin 2005, Hunt et al. 2009). This can lead to selection for females to suppress competition. Clear evidence for female suppression of competition is rare. One possible example is female ejection of sperm from subordinate males in feral fowl (Pizzari & Birkhead 2000). This decreases the opportunity for competition to occur through sperm competition with dominant rivals, but may also just be a mechanism of choice. Selection on males to mate may be stronger than selection on females to control male mating access however, as the variation in reproductive success of males generally exceeds that of females (Trivers 1972). Depending on the dynamics of a particular system, the opportunity for female manipulation of mating outcomes may vary across the mating sequence, and so female control may evolve despite relatively weak selection. To derive a complete understanding of how the evolution of male sexual traits may be driven by sexual selection requires a thorough approach investigating the effects of male-male competition and female choice in combination and in isolation at different stages, but such comprehensive approaches are rare (Wong & Candolin 2005).

In my thesis, I seek to understand the sexual selection pressures acting on male traits at different stages in the mating sequence, and the congruence and conflict between male-male competition and female choice. I focus on sexual selection in the Australian redback spider

(*Latrodectus hasselti*). Spiders are well suited to investigate questions relating to traits selected through mechanisms of sexual selection and their interaction for a number of reasons (Eberhard 2004, Huber 2005). First, several factors place strong selection on male behavioural, morphological and life history traits that affect interactions with females. In many species (including redbacks, Forster 1992) significant female-biased sexual size dimorphism (Foelix 1996) leads to behaviourally dominant females. Males cannot coerce copulation in these species, and risk cannibalism before, during and after copulation (Elgar 1998, Schneider & Elgar 2004). This also means that spiders are good for studies of sexual selection because female preferences are likely to be reflected in mating outcomes and so can be determined more directly than in many systems. Second, unique aspects of spider genitalia allow manipulations that can isolate post-copulatory sperm competition from cryptic choice (see ‘natural history’ below, reviewed in Eberhard 2004). Third, in web-building spiders, competition and choice can occur independently or simultaneously as a function of the time of arrival of males on females’ webs (Watson 1990, Whitehouse 1991, Henschel et al. 1995). This allows investigation of interactive effects of the two processes. Currently, no work has exploited these features of spiders to build an integrated investigation of how male-male competition, female choice and their interactions operate at the various stages where male traits and female reproductive strategies may have important effects on lifetime reproductive success.

The goal of my thesis was to understand how male-male competition impacts female choice. Further I was interested in determining how different stages of the mating interaction could influence traits under selection and the fitness outcomes. The approach I took in this thesis was to determine traits that are under selection at different stages of the interactions between males and females, and determine how they influence success in male-male competition and/or female choice in isolation and in combination. First I examined physiological shifts in response to cues of the availability of mates and likelihood of competition, as these shifts could support developmental traits leading to tactics that could influence the mating success of adult males (chapter 2). Next I sought to understand mechanisms of female control over the timing of mate attraction as manifested in the timing of sex pheromone production (chapter 3). Males that successfully find females are frequently in competition with rivals who are also attracted to the webs of receptive females. Thus my next step was to detail the traits selected through female choice, and examine how these might be altered depending on the context under which male-male competition is operating (chapter 4). The net effect of female reproductive tactics on male lifetime reproductive success is critically affected by remating and sperm use patterns. Thus, I also determined the influence of females on sperm use (cryptic choice) and how the timing of polyandry can affect female fitness (chapter 5). Finally, I examined in more detail how the timing of mating may affect female lifetime reproductive success by tracking the lifetime reproductive output and longevity of females along with their underlying physiological responses as a function of reproductive status and resource availability (chapter 6).

Below I outline general information about the natural history and reproductive biology of redback spiders before outlining the goals of each chapter in more detail. Some of the natural history information is redundant with material that comes later in the thesis, as chapters were written as manuscripts (so each includes abbreviated natural history information).

Natural History

Redback spiders (*Latrodectus hasselti*) are cobweb weavers in the family Theridiidae which are found throughout Australia, and have been introduced to New Zealand and Japan in recent years (Forster 1984, Nihei et al. 2004). Spiderlings disperse after emerging from their egg sac (e.g. Suter 1991) which may lead to the patchy distribution of sedentary sexually mature females

(Andrade 2002). Upon sexual maturity, males abandon their webs and undergo risky mate searching (>80% mortality) for potential mates (Andrade 2002). In most spiders, including widow spiders (*Latrodectus* spp.), mate localization and the initiation of courtship is triggered by male detection of chemicals released from the webs or body of females (e.g. Ross & Smith 1979, Anava & Lubin 1993, Kasumovic & Andrade 2004).

Male and female life histories are very different. Upon sexual maturity, males cease foraging so are limited with the resources they have acquired during juvenile development to devote to mate searching and courtship. In the laboratory, males have a much shorter lifespan than females living for a maximum of 2 months whereas females can survive for 24 months (Forster 1984, Andrade 1996). Redbacks show extreme female-biased sexual size dimorphism. This arises during development, as females have approximately 2 more instars than males and at adulthood are on average 60x heavier than males (4.4 mg versus 256 mg; Andrade 1996).

Developmental plasticity

The development of penultimate instar male redback spiders is affected by chemical cues indicating factors that could reflect the intensity of male-male competition: the presence of females and density of rival males (Kasumovic & Andrade, 2006, 2009, Kasumovic et al. 2009a). Cues that indicate a high density of virgin females lead to penultimate males accelerating their development and they are of lower mass as adults in comparison to males that slow their development when reared in the absence of females (Kasumovic & Andrade 2006, 2009). Accelerated development has been demonstrated to enhance the ability of males to reach and inseminate females before rivals (Kasumovic & Andrade 2009). Male development is also affected by the presence of rival males. When penultimate instar males detect higher densities of rivals, they mature in higher body condition compared to males that develop with a lower density of rivals (Kasumovic & Andrade 2009). Male body condition is important as, upon sexual maturity, males cease foraging and have a limited energetic budget to devote to mate searching (where >80% of males perish due to depleted energetic reserves or predation; Andrade 2003).

Male-male competition and mating

Since males are monogynous, their reproductive success is limited by the reproductive output of their only mate and their paternity (Andrade & Kasumovic 2005). The timing, order and number of matings are critical to male reproductive success in this species. Females have paired independent sperm storage organs (spermathecae) that are inseminated one at a time by each of a male's paired intromittent organs (palps). If a male is able to inseminate each spermatheca before rivals, there is high first male sperm precedence due to the deposition of a sperm plug in each organ (Snow & Andrade 2005, Snow et al. 2006). However, if a male is limited to a single copulation and a rival inseminates the opposite spermatheca, paternity is shared (Snow & Andrade 2005).

In the field, when a male arrives at a female's web, the competitive context can be highly variable ranging from instances with no competition up to eight rivals on the same web (Andrade 1996). In the absence of competition, courtship is prolonged (approximately 4 hours) and involves the production of vibrational signals on the female's web and abdomen (Andrade 2000). After this prolonged courtship males climb onto the female's abdomen insert one of their paired intromittent organs and perform a copulatory somersault. During the copulatory somersault males twist their bodies above the fangs of their mate (Forster 1992, 1995; Andrade 1996). Cannibalism typically begins when the male inseminates the first storage organ of the female (Forster 1992, 1995, Andrade 1996) with most males that court in the absence of competition surviving that first copulation regardless of the damage imposed by partial cannibalism by the female (Andrade 1998, Andrade et al. 2005). These males return to the web for a second bout of courtship followed by a second copulation with the other palp (Forster 1995). However, females sometimes kill and wrap males in silk after one copulation (= premature lethal cannibalism).

The courtship duration provided by a male prior to a mating attempt is significantly reduced under competition. When two rivals compete for the female, the relative number of copulations achieved and occurrence of premature lethal cannibalism is largely mediated by the duration of courtship provided (Stoltz et al., 2008; 2009). There is an inverse relationship between the courtship duration of the first male to mate and the number of copulations the female accepts from a rival (Snow & Andrade 2005, Snow et al. 2006, Stoltz et al. 2009). Females are also more likely to prevent rapidly-copulating males from achieving a second copulation by killing them after their first copulation (thus blocking sperm precedence, Stoltz et al. 2008, Snow & Andrade 2005).

Reproduction

The sperm plugs deposited at mating are the broken tips of the male's intromittent organs, which remain lodged inside the insemination tubules or spermatheca of the female as the male withdraws his palp. Placement of sperm plugs blocks insemination (but not copulation) by rival males. However, plugs do not interfere with fertilization as the tubules leading from the sperm storage organ to the uterus externus (site of fertilization) are independent of the tubules used for insemination (Bhatnagar & Rempel 1962, Foelix 1996). Plugs remain in place for the lifetime of the female.

After copulation, females use stored sperm to fertilize eggs in one or more egg sacs each month for up to two years following mating (Andrade & Banta 2002). In the field, females may overwinter and produce an average of 9 egg sacs over their lifetime (Andrade & Banta 2002). Despite the physical separation of sperm in separate organs, analysis of morphology has led to the suggestion that females are unable to differentially release sperm from the two storage organs (Berendonck & Greven 2002). This is consistent with the observation that, when rival males each inseminate one of the female's spermathecae, paternity is roughly split between the two males (Snow & Andrade 2005).

Chapter Outlines

The variation in age, size and condition at maturity of last-instar male redbacks as a function of their detection of females' pheromones has been proposed to reflect the importance of scramble competition, i.e. the need to rapidly locate virgin females before rivals, in a particular habitat as indicated by female density (Kasumovic & Andrade 2006, Kasumovic & Andrade 2009). Males also shift their relative investment in morphological traits important to competition (size, body condition) as a function of the density of rivals. In chapter two, I investigated the physiological causes and consequences of this developmental plasticity. I hypothesized that physiological changes associated with different developmental tactics might be adaptive responses to the selective regime that triggered the observed phenotype. Here, resting metabolic rates were measured of males reared in the presence or absence of females' pheromones with variable male density and diet. If physiological responses are plastic, I predicted they would differ in a way that would support optimal performance in the competitive context indicated by cues of the availability of mates and likelihood of competition. In contrast, if variation in resting metabolic rates are caused by variation in the physiological requirements of development time, I predicted correlations only with development time.

Sex pheromones, produced by females and incorporated in their webs, allow males to detect the presence of females but also trigger courtship on arrival at a female's web. In chapter three, I investigated patterns of pheromone production by females as this may allow them to exert control over the timing of male arrival and courtship activity on their webs. I predicted that females would produce sex pheromones as virgins, then cease production once mated to minimize potential costs of polyandry. Redback males dismantle much of their mate's web during courtship and, after mating, females rebuild large portions of the web, making it possible that pheromone profiles could change quickly after mating. Depending on the costs associated with courtship and mating, females may avoid attracting additional males by not adding pheromones to their webs after copulation. I predicted that redback males would determine whether females are sexually mature and discriminate their mating status based on web-borne chemicals and that females would cease pheromone production after mating. I extracted pheromones from the webs of females and predicted that male activity level would be the highest on extracts from webs of virgin females and lowest on extracts from webs of juvenile and mated females. This study was published in *Animal Behaviour* (Stoltz et al. 2007) and is reprinted with permission from the journal.

Once redback males arrive at females' webs, they must satisfy choosy females while competing with rival males. In chapter four I investigated decision rules used by females for mate choice and determined the male trait that was selected through female choice. I hypothesized that female choice is based upon a courtship duration threshold. I used previously published data to determine the female's threshold criterion based on variation in female-mediated reduction in male mating success as a function of courtship duration (Stoltz et al. 2008, 2009). I then investigated the effect of this threshold on male competitive tactics by examining dynamics when 'intruder' males arrive on a web after a 'resident' had satisfied the putative courtship threshold. If females are able to discriminate the source of courtship, I predicted that they should allow males that have approached the threshold to mate while discriminating against intruding males that have not. However, if females are unable or unwilling to discriminate the source of courtship, intruding males should be able to circumvent female choice, mate with little investment in courtship, and not suffer reductions in fitness. This chapter was published in *Proceedings of the Royal Society B: Biological Sciences* (Stoltz & Andrade 2010) and is reprinted with permission of the journal.

The fitness effects of the interaction of male-male competition and female mate choice on mating success will depend on sperm use patterns. Work in this thesis (chapter three) and elsewhere (Perampaladas et al. 2007) showed that female redbacks produce sex pheromones as virgins then again a few months after their first mating. This creates two windows of opportunity for polyandry (shortly after the first copulation and after the production of several egg sacs). In chapter five, I first investigated consequences of polyandry for male and female fitness. I asked whether or not females bias sperm use to favour the paternity of males that mate after a delay by comparing paternity of rival males that mate simultaneously to rivals that mate after a delay. Next, I determined costs associated with polyandry by tracking female longevity and reproductive output as a function of these treatments. I hypothesized that there would be differences in paternity of rivals depending on the interval between matings. When females mate with two males simultaneously, I predicted that paternity will be split and conform to a raffle. After a delay, I predicted a shift with a bias in paternity to males that mated after a delay that would serve to increase the genetic diversity of a female's offspring. Genetic diversity of offspring may be critically important for female fitness in this species where juveniles disperse to new habitats in which traits beneficial to the parents may not be optimal for offspring (e.g. Garant et al. 2005). Polyandry was predicted to have a negative affect on longevity and reproductive output (as found in other systems, see Chapman 2008). I hypothesized that negative effects of mating on female fitness might be dosage-dependent (Radhakrishnan & Taylor 2007, Yamane et al. 2008) with females able to negate the effects only if there was a delay in remating. Thus I predicted costs of polyandry would be strongest when males mated simultaneously relative to when females mated with a second male after a delay.

If females delay their second mating, but polyandry is beneficial, there is a risk that they would not survive long enough to see benefits of having broods sired by multiple males realized. In addition to risks of predation that are found in most systems, these sit-and-wait predators are likely to encounter high variability in food availability that could compromise survival or their ability to reproduce leading to a risk of mortality prior to reproduction or prior to second matings. These effects should shape variation in female choosiness during initial mating or remating. In chapter six, I investigate how female reproductive investment and longevity are affected by periods of fluctuating resource availability. I investigate physiological responses to dietary restriction in females of different reproductive status to determine how females trade off current and future costs of reproduction under varying resource availability. In other taxa, dietary restriction increases longevity (Kirkwood & Austad 2000). I hypothesized that female reproductive status would determine whether or not dietary restriction (DR) increases longevity in redbacks. I measured the effects of dietary restriction on resting energetic rates and longevity as a function of mating status to determine links between physiological responses to DR, longevity and fitness. I subjected virgin and mated females to either a normal diet or a 6 week starvation period and predicted that females should show increased longevity under DR, but only if they were mated and producing fertile eggs. I then resumed feeding and predict that resting energetic rates of mated females would increase immediately, and egg sac production would continue. This chapter is in press at *Functional Ecology* (Stoltz et al. 2010) and is reprinted with permission of the journal.

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Chapter 2
Plasticity in male metabolic rates predicted by cues of sexual selection

Abstract

Environmental cues indicating variation in selection pressures can trigger plasticity including variable development of morphologies and behaviours in a range of taxa. Studies investigating plasticity in physiological responses to these same cues are extremely rare. We examined resting metabolic rates of male redback spiders, (*Latrodectus hasselti* Thorell) reared in the presence of cues indicating variation in the availability of mates and likelihood of encountering rival males [i.e. variation in the intensity of sexual selection]. Juvenile male redbacks that detect female's pheromones accelerate their development relative to males developing in the absence of females. Rapid development increases success at scramble competition for mates when females are nearby while delayed development when females are scarce increases success at arduous mate searching. We ask whether plasticity in metabolic rates accompany this plasticity in life history traits that would increase the success of these phenotypes. We raised males at three diet levels in the presence or absence of females with variation in the density of nearby rival males. As the density of potential rivals increases, this hypothesis predicts a corresponding increase in resting metabolic rates as this would facilitate rapid mate searching and competitive courtship. We show that variation in resting metabolic rates of males is linked to the intensity of perceived competition. However, there was no global change in metabolic rate with changes in total development time, as might be expected if variation simply reflected physiological costs of different developmental trajectories. The metabolic response of males is plastic and fine tuned by environmental cues of the likelihood of competition.

Introduction

Phenotypic plasticity is the ability of a single genotype to produce different morphologies, behaviors and/or physiological states in response to different environmental cues (Bradshaw, 1965; Pigliucci, 2001; Schlichting, 1986; Scheiner, 1993; Sultan, 1987; West-Eberhard, 1989). There are many examples of plasticity across taxa in response to a wide variety of environmental factors such as predation (Bernard 2004; Hagman et al., 2009; Hoverman and Relyea, 2007; Lyytinen et al., 2004; Stamper et al., 2009), variation in temperature and photoperiod (Donaldson-Matasci et al., 2008; Fischer et al., 2003; Geister et al., 2009; Loh et al., 2008; Nijhout, 1999; Stearns, 1989) and demography (Kasumovic and Andrade, 2006; Kasumovic et al., 2009a; Walling et al., 2007). Adaptive plasticity requires reliable and predictable (Ghalambor et al., 2007; Lively, 1986; Scheiner, 1993) cues, typically early in ontogeny, to trigger development of morphology leading to the highest fitness (West-Eberhard, 1989). Plasticity can allow individuals to match their phenotype to the particular competitive challenges they are likely to encounter at maturity (Dudley and Schmitt, 1996; Gotthard and Nylin, 1995; Newman, 1992; Nylin and Gotthard, 1998; Pigliucci, 2005; West-Eberhard, 2005).

Physiological responses may also be adaptively plastic if selection for different physiologies allows individuals to maximize fitness in different environments (e.g. Buchanan et al., 2001; Hawkins et al., 1986). For example, variation in resting metabolic rates may be flexibly linked to nutrient availability (Stoltz et al., in press), will affect basal levels of resource use (Woodring et al., 1979) and allocation to functions critical to fitness such as reproduction and somatic maintenance (Calabi and Porter, 1989; Harvey, 1986; Thompson and Nicoll, 1986) and longevity (Parsons, 2002; 2003). Thus a complete understanding of how selective pressures shape phenotypes in variable environments requires investigations of physiological plasticity, but such studies are extremely rare (Booth and Kiddell, 2007; Garcia-Guerrero et al., 2003). Here we ask whether plasticity in resting metabolic rates is triggered by cues of the intensity of and form of sexual selection. Although recent studies have begun to address the implications that underlying metabolic rates can have on fitness related effects (Fischer et al. 2004, Evenden & Gries 2008, Reim et al. 2009) there is a lack of direct demonstrations relating physiological plasticity to cues of sexual selection. We use males of the Australian redback spider (*Latrodectus hasselti*), which are well suited to this type of study for several reasons. First, two features of redback natural history simplify predictions about how resting metabolic rates are related to fitness: (a) male redbacks have a limited energy budget with which to search for a female, court and mate since they cease foraging after sexual maturity (like many other web-building spiders), and (b) males of this sexually-cannibalistic species are monogynous, so their entire energy budget is devoted to a single mating opportunity with no trade-offs for future expenditures (Andrade, 1996; 1998; 2003; Forster, 1992; Fromhage et al., 2005). Second, redback males show plasticity in development time, adult size and body condition that has been linked to the development of optimal phenotypes for different competitive challenges (Kasumovic and Andrade, 2006; 2009; Kasumovic et al., 2009a). Chemical cues of female presence and male density are detected by last-instar redback males and trigger variation in development (Kasumovic and Andrade, 2006; Kasumovic et al., 2009a). In the presence of virgin females, juvenile males accelerate their development and are relatively small as adults. This rapid development allows small males to reach and inseminate nearby virgin females before rivals (Kasumovic and Andrade, 2009) and deposit sperm plugs which increases their paternity (Snow and Andrade, 2005; Snow et al., 2006). In contrast, males reared in the absence of females mature more slowly and are larger (Kasumovic and Andrade, 2006) with higher metabolic reserves, traits that increase performance in mate searching and competing with rivals

(Kasumovic and Andrade, 2009; Stoltz et al., 2008). Males are also sensitive to cues of the likely intensity of inter-male competition as indicated by male density. When juvenile males detect higher densities of potential rivals, they mature in higher body condition than when the density of rivals is low (whether or not females are also present, Kasumovic and Andrade, 2006). Body condition is likely important for performance in metabolically costly competitive courtship (Stoltz and Andrade, 2010). Finally, metabolic budgets are associated with resource availability prior to sexual maturity. Males placed under more nutrient-rich diets have more rapid development, increased growth and higher body condition across all of these contexts, reflecting higher energy allocation to these traits than is possible for males on low-nutrient diets (Kasumovic and Andrade, 2005).

We hypothesized that plasticity in metabolic rates should be the underlying mechanism that maximizes the likelihood of success of these different phenotypes under the competitive conditions that trigger them (Table 1, Hypothesis 1). If metabolic rates are themselves adaptive responses to the competitive environment however, resting metabolic rates should be linked to cues in the environment that indicate the competitive challenges males are likely to encounter as adults. We tested this hypothesis by measuring resting metabolic rates of adult male redback spiders that were reared through their final instar in the presence or absence of females, with varying numbers of potential rivals under three diet levels (high, medium and low feeding rates, as in Kasumovic and Andrade, 2006). One critical prediction of this hypothesis is that male resting metabolic rates should increase with the number of other males detected during development, as this is an indicator of the intensity of sexual selection. Increased metabolic rates in more competitive situations may increase male fitness for two reasons. More competitors means a heightened advantage for males that are more active during mate searching. Higher metabolic rates would allow males to sustain the elevated levels of activity required to rapidly locate females (Shillington, 2005; Tanaka and Itô, 1982; Watson and Lighton, 1994; but see Kotiaho, 1998) and mate these females before they are reached by rivals (Kasumovic and Andrade, 2009; see also Snow et al., 2005). Male-male competition is largely mediated by the duration of metabolically expensive courtship in these spiders (Stoltz et al., 2008; 2009; Stoltz and Andrade, 2010), and the likelihood of having to engage in competitive courtship would increase with the number of nearby rivals. In addition to predictions about effects of male density, we predicted that male resting metabolic rates should generally increase with the diet quality (e.g. McNab 1986, 1988) as has been found in other systems (Bozinovic et al., 2007; Cruz-Neto and Bozinovic, 2004; Fu et al., 2005; Gonzales-Pacheco et al., 1992; Steyermark et al., 2005). If higher resting metabolic rates generally allow increased male performance (as outlined above), then males that can afford to maintain higher resting rates (i.e., those on high diets) should do so. Finally, this hypothesis predicts that resting metabolic rates should be higher for males reared in the presence of females compared to those reared in the absence of females. In the presence of females the increased metabolic rates of males will allow males to be the first to reach females which is critical in this species due to the high first male sperm precedence (Snow & Andrade 2005). When reaching females requires long-distance mate searching (females-absent treatment), efficient use of energy reserves will be more important than when males scramble to quickly reach females that are nearby (females-present treatment).

We also tested an alternative hypothesis derived from the literature that suggests resting metabolic rates may be heavily dependent on development rate (e.g., Bayne, 2004, Table 1, Hypothesis 2). Several studies suggest the use of resources for growth and maintenance is more efficient at slower development rates, so achieving accelerated development at a given resource level may require increased metabolic efficiency or access to greater resources (e.g. Bayne, 2004; Present and Conover, 1992; Thodesen et al., 1999). For a sit-and-wait predator, lower resting metabolic rates would yield a higher baseline efficiency of energy use. Thus a critical prediction of this hypothesis is that males with accelerated development (females-present treatment, Kasumovic and Andrade, 2006) should have lower resting metabolic rates than males that prolong their development in the absence of females (see Kasumovic and Andrade, 2006). Overall, males on lower diet levels should show relatively lower resting metabolic rates since efficiency is more important than at higher diets. However, this mechanistic correlate of rapid development may constrain plastic shifts in response to competition, so under this hypothesis we expect either no correlation between metabolism and male density, or a positive correlation only for males that have access to ample resources (high diet males). Predictions of both hypotheses are compared in Table 1.

Methods

Spider collection and rearing protocol

Spiders were outbred offspring of individuals collected in Sydney Australia. The general rearing protocols can be found in Andrade and Banta (2002) and Kasumovic and Andrade (2006), but we discuss the protocol here briefly. Spiders were reared within the same rearing room. Each egg sac was kept in an individual 6×4×4 cm cage where spiderlings were initially reared communally to mimic natural conditions. At the third instar, individuals were removed from communal cages and isolated in separate 2×2×3 cm plastic cages prior to being placed into the treatments. We fed all spiders *Drosophila* sp. twice weekly and monitored all individuals daily to identify males, which are distinguishable from females at the fourth (penultimate) instar due to the visible development of intromission organs (Forster and Kingsford, 1983). Penultimate instar males were placed in the presence or absence of virgin females and their webs to mimic extremes of female density in nature and randomly placed on one of three diets (high, mid, or low)

(Kasumovic and Andrade, 2006). High and mid-diet males were fed 3 times per week (6 and 3 *Drosophila*, respectively), while low diet males received 1 *Drosophila* each week. In the female-present treatment, all isolated males within a diet treatment was surrounded by 4 web-building virgin females (each in their own cage). There was no tactile or visual contact between males and females or their webs, but cages were porous to allow the passage of airborne pheromones. Males in the female-absent condition were reared in the absence of females in a separate room held at the same temperature and light cycle. In both treatments, the number of neighbouring males varied throughout the experiment due to the constant addition of newly moulted penultimate males and the immediate removal of sexually mature males from treatments. We thus estimated the average number of sexually mature males an individual could sense per day by counting all the males that were present in the room during the focal male's penultimate instar, and dividing by the number of days a male took to develop.

Males were monitored daily and date of adult moult noted. Here, we will refer to development time as the total duration of the penultimate instar (in days). For all males, we measured the length of the patella-tibia of their two front legs at the penultimate instar and the adult stage using digital images (Kasumovic and Andrade, 2006) and measurement software (Simple PCI, Compix Inc. Imaging systems, 2002). Male size at each stage was calculated as the average of the two leg measurements. We measured male growth by calculating the change in male size ([5th instar size – 4th instar size] / 4th instar size). All males were also weighed at maturity (Ohaus explorer balance accurate to 0.01 mg). Male body condition was estimated using residuals of log (cubed root of male weight) regressed on log (size) (see Kasumovic and Andrade, 2006 for justification of the use of condition residuals).

Metabolic rate measurements

We used stop flow CO_2 respirometry to estimate resting metabolic rates of males immediately upon sexually maturity (see Lighton, 1991; Vogt and Appel, 1999; Walker and Irwin, 2006). Metabolic measures were taken using a Qubit systems (Ontario, Canada) 8 channel gas controller (G245), a Qubit systems 8 channel gas switcher (GS244) and a Li-Cor (Nebraska, USA) CO_2 analyzer (LI-6252). Prior to recording resting metabolic rate, we took a baseline CO_2 measurement from empty test chambers. Once this was complete, males were placed in the chambers and allowed to settle (approximately 10 minutes). We took repeated measures of CO_2 production (three consecutive 4 minute dwell periods) and subtracted the average baseline CO_2 measurements to yield expired CO_2 estimates for each male. Spiders were placed into cylindrical glass test chambers (10 cm length, 3 cm diameter) plugged at both ends with steel plugs and rubber O rings. CO_2 was removed from incoming air by filtering through soda lime. We ensured males remained motionless during a 4 minute period, during which air and expired CO_2 was kept within the chamber (= dwell period). At the end of the dwell period, the air (with CO_2 produced by the spider) was flushed from the chamber and flowed through a magnesium perchlorate plug to removed moisture before entering the CO_2 analyzer. Flow rate through the chambers was set to 160 ml/min and did not appear to disturb the spiders. All measurements were made at 25°C.

We report metabolic rates as CO_2 umol/h per mg. This is appropriate for our sample since the log resting metabolic rate regressed on log body weight is $y=0.99x-1.59$ (i.e., mass scaling exponent not significantly different from 1.0, see Lighton, 2008, e.g. Tanaka et al., 1985). Figure 1 shows the relationship between male mass and metabolic rates prior to logarithmic transformations. Male mass ranged from 2.0 mg to 8.1 mg and was similar to the mass of field caught males (mean mass ± SE: laboratory: 4.49±0.10 mg; field: 3.70 ± 0.32 mg (Andrade, unpub.)).

Statistical analysis

All data were normally distributed. Analyses were completed using SPSS v13 and JMP v7 as described below. We report results that qualitatively replicate published data on the effects of female presence, male density, and diet on development time, growth and body condition of males (compare to Kasumovic and Andrade, 2006; Kasumovic et al., 2009). We use the same statistical analyses as these reports, and summarize these data, but do not explore interactive effects in detail as these are peripheral to our study (see Kasumovic and Andrade, 2006; Kasumovic et al., 2009 for further discussion). Here we focus on the new data on how these factors affect male resting metabolic rates.

Results

A total of 161 males were placed randomly in the six treatments with similar sample sizes in each treatment, and a total of 108 males reached maturity. As expected if our diet treatments altered resource availability, mortality rates were highest for low diet males, intermediate at medium diets and lowest for males on high diets (contingency table, Wald=15.41, P=0.001; Table 2). However female presence did not affect mortality (Wald=0.003, P=0.96), nor did effects of diet depend on female presence (Wald=0.69, P=0.41).

Variation in development time, growth, adult body condition and adult resting metabolic rates were affected by female presence, diet and the density of males (see Table 3). As in previous studies (Kasumovic and Andrade, 2006; Kasumovic et al., 2009), diet and female presence affected development time, with males on higher diets and in the presence of females maturing more rapidly than those on lower diets or in the absence of females (Figure 2C, Table

4). This confirms our assumption about the link between development time and female presence (see Table 1, Hypothesis 2).

Male growth and adult body condition were affected by the presence of females and diet (Table 4). There was evidence for a trade-off between size and time to maturity as males that developed rapidly in the presence of females generally grew less, although this effect was most clear for high and low-diet males (Figure 2A, Table 4). Growth also increased with diet level, particularly for males developing more slowly in the absence of females (Figure 2A, Table 4). Body condition was higher for males on higher diet levels, and for those reared in the presence of females (particularly when on high or low diets, Figure 2B, Table 4).

These relationships were also affected by male density (Table 4). Within the presence or absence of females treatments, development rate of focal males decreased as rival male density increased (Figure 3). The influence of male density on growth and body condition was more complex (see two and three-way interaction terms, Table 4). In general, body condition tended to decrease as male density increased when females were present ($r^2 = 0.058$, $F_{1,47} = 2.841$, $P = 0.099$) but not when females were absent ($r^2 = 0.004$, $F_{1,60} = 0.217$, $P = 0.643$). Whereas density effects on growth were positive (female present: $r^2 = 0.078$, $F_{1,47} = 3.918$, $P = 0.054$; female absent: $r^2 = 0.056$, $F_{1,60} = 3.513$, $P = 0.066$)

Resting metabolic rates also varied with treatment but, in this case, only diet and male density, but not female presence, were significant factors (Table 4, Figure 2D, Figure 4). Similarly, there was no significant relationship between development time and resting metabolic rate regardless of diet or the presence or absence of females (Regression analysis: $F_{9,109}=0.842$, $P=0.58$). In contrast, males on higher diets had higher resting metabolic rates than males on lower diets (Table 4, Figure 2D). Critically, resting metabolic rates increased with the density of competing males, particularly for males on high or low diets (Figure 4).

Discussion

Our results demonstrate that male redback spiders exhibit plasticity in their resting metabolic rates in response to resource availability and, more intriguingly, in response to environmental cues (Leimar, 2009; Reylea, 2004; Weinig, 2000) indicating the likelihood of male-male competition. Resting metabolic rates increased as the number of potential rivals increased, regardless of whether or not females were present (Table 4), and regardless of development time. To our knowledge, this is the first demonstration that cues reflecting the intensity of competition lead to variation in male metabolic rate. We replicated earlier work (Kasumovic and Andrade, 2006; 2009) showing significant divergence in male development time as a function of whether or not females were detected during development (Figure 2C, Table 4), but in contrast to response to male-male competition found no differences in metabolic rates of these groups of males (Table 4). This is particularly notable because there was no indication that mechanistic requirements of changes in development rates (e.g., Bayne, 2004) demonstrated by males that accelerated development relative to those in which it was prolonged explained variation in resting metabolism rates (Table 4). Increased resting metabolic rates may be critically important to male success, particularly when competition is fierce. Higher resting rates will prime males for higher activity levels (Shillington, 2005; Tanaka and Itô, 1982; Watson and Lighton, 1994, but see Kotiaho, 1998), which would facilitate rapid mate searching and more vigorous courtship once females are found (Stoltz et al. 2008, 2009; Stoltz and Andrade, 2010). Male performance in each of these steps will determine fitness as there is strong first male sperm precedence (Snow and Andrade, 2005), and mating success depends on courtship performance (even when rivals are present, Stoltz et al., 2008; 2009; Stoltz and Andrade, 2010). Our results show that male physiology is fine-tuned by cues of the intensity of sexual competition as well as the availability of resources available to fuel reproductive behaviours.

The temporal advantage of rapid development enables first arrival on a virgin female's web, so smaller males have higher fitness than larger, more slowly developing males (Kasumovic and Andrade, 2009). We considered the possibility that the fitness benefit of rapid development might exert strong selective pressure for rapid development but might entail physiological trade-offs that would be evident in resting metabolic rates (Hypothesis 2, Table 1). Under this hypothesis, we predicted that a physiological correlate of rapid development would be lower resting metabolic rates (Hennemann, 1983). Even though depressed resting rates might decrease reproductive performance (Johnston et al., 2007), the temporal advantage of rapid development (Kasumovic and Andrade, 2009) might be sufficient to favour this developmental trajectory when females are nearby. However, our evidence did not support this hypothesis. We tested whether there was a negative correlation between development time and resting metabolic rates across treatments, which would be consistent with this hypothesis, but there was no relationship between these variables. Thus, there was no indication of a hard link between rapid development and metabolic efficiency. This suggests the correlations between development time and resting metabolic rates that have been found in other systems (e.g., Metcalfe et al., 1995) may not be mechanistic requirements, and require further explanation.

In contrast, we found strong support for the critical prediction of our physiological plasticity hypothesis, because male resting metabolic rates increased with an indicator of the intensity of male-male competition (Hypothesis 1, Table 1). This shift likely reflects increased aerobic capacity, as resting metabolic rates have been argued to be positively related to maximal attainable metabolic rates (see Reinhold, 1999; Shillington, 2005). Higher overall metabolic rates may fuel increased locomotory activity which in turn would increase the chance of female encounters during mate searching. High levels of activity are also required for success in competition and courtship. Female redback spiders' main choice criterion is a courtship duration threshold of approximately 100 minutes (Stoltz and Andrade, 2010). Males that sustain vigorous courtship through this period generally have higher fitness than males that are unable to do so (Stoltz et al., 2008; 2009, Stoltz and Andrade, 2010), and this may require the maintenance of higher metabolic rates. In other species, for example, dominant males maintain higher resting metabolic rates (fishes: Metcalfe et al., 1995; Lahti et al., 2002; birds: Røskaft et al., 1986; Hõgstad, 1987; Bryant and Newton, 1994; mammals: Schradin et al., 2009). The observed shift to higher resting metabolic rates is particularly interesting in sit-and-wait predators like these spiders for which low basal levels of energy use may allow prolonged survivorship, particularly when food resources are limited (e.g., our low diet treatment). These males do not eat as adults, so have limited energy reserves with which to search for mates, court and copulate. In the absence of intense male-male competition, males may thus do better if they have depressed metabolic rates and are able to conserve energy, particularly if food resources are limited.

One particularly interesting result found here is the negative relationship between development time and male density (Figure 3), which was not reported previously by Kasumovic and colleagues (Kasumovic and Andrade, 2006). This suggests that cues of the intensity of competition could also affect development time directly. When virgin females are detected (females present treatment) more rapid development may facilitate scramble competition. However, it is not clear how such a shift might affect fitness when females are absent and males will presumably need to provision for prolonged mate searching. This relationship may be spurious and driven by a few outliers (Figure 4). If the relationship exists however, it suggests an even stronger case for the plasticity hypothesis, as male density itself would then cause both a decrease in development rate and an increase in resting metabolic rate.

Clearly, males cannot maximize all traits given that they have access to a finite pool of resources (e.g., Van Noordwijk and DeJong, 1986; Zera and Harshman, 2001). Well-provisioned males could have such an excess of food that growth and body condition could be maintained even while development is accelerated and basal energy use is increased (e.g., at high male densities). However, this does not seem likely. Instead, there is evidence for a trade-off between body condition and growth (between penultimate and mature molts) for rapidly developing males, including those on a high diet level. Decreased body condition may be the cost paid for increases in other traits, although the effect of this cost may depend on diet. Consistent with this, there was higher mortality rate for mid and low diet males throughout the experiment (Table 1), which suggests resource limitation compromised somatic maintenance. One outstanding puzzle from our data (and also from Kasumovic and Andrade, 2006) is that effects described here are less clear for mid-diet males than for males on high or low diets (Figure 2B, Figure 4). For example, the body condition of mid-diet males did not show a trade off with growth, and there was no significant relationship between male density and resting metabolic rates for these males. While this might suggest some support for the conjecture that resting rates are elevated at the expense of body condition in the other diet treatments, the cause of this difference is as yet unclear.

Studies investigating the adaptive value of plasticity in metabolic rates appear to be rare (DeWitt, 1998; Relyea, 2002, but see Rinke et al., 2008), particularly those examining changes in underlying physiological responses such as metabolic budgets (Booth and Kiddell, 2007; Garcia-Guerrero et al., 2003; Van Handel 1993, but see Fischer et al., 2009), as we have done here. We have shown that males that accelerate their development have similar resting metabolic rates to males that prolong their development in response to cues of social context (Table 4), which challenges the idea that resting metabolic rates constrain development rates. Instead, our results strongly support the hypothesis that male physiological rates, at least for resting metabolic measures, are plastic and fine tuned to the intensity of inter-male competition in redback spiders. We conclude that plasticity can entail shifts in suites of traits, including physiological responses, which are fine tuned to variation in selective regimes encountered in nature.

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Table 2.1- Comparison of predictions from two hypotheses for how the resting metabolic rates of male redback spiders may be affected by indicators of the form and intensity of selection which are detected during their final instar.

Variable	Hypothesis 1: Adaptive shifts support competitive performance	Hypothesis 2: Mechanistic links to development rate
Female presence/absence*	Higher when females present	¥<u>Lower when females present</u>
Diet (high, medium & low)	High diet > medium diet> low diet	High diet > medium diet > low diet
Male density	¥<u>Positive correlation (critical prediction)</u>	No correlation or Positive correlation for high-diet males only

* These predictions assume female presence leads to accelerated development, as in Kasumovic and Andrade 2006, Kasumovic et al. 2009, ¥Critical predictions.

Table 2.2- Sample sizes for a 2x3 rearing design showing the number of males placed in each treatment (brackets indicate percentage that died before reaching maturity).

#males in treatments (#perished)			
		Diet	
Females	**High**	**Mid**	**Low**
Absent	19 (10%)	35 (31%)	31 (39%)
Present	20 (10%)	25 (28%)	31 (61%)

Table 2.3- Results from a MANOVA examining direct and interactive effects of two fixed factors (diet and female presence) and one random factor (rival male density) on variation in male development time, body condition, size and resting metabolic rates (dependent variables) in a laboratory rearing experiment.

Source	**DF**	**F**	**P**
Whole model	11, 96	19.96	**<0.0001**
Female presence	4, 92	3.00	**0.022**
Diet treatment	4, 93	6.36	**<0.001**
Male density	4, 92	5.90	**<0.001**
Female × Diet	4, 93	3.60	**0.009**
Female × Male	4, 92	1.62	0.18
Diet × Male	4, 93	1.30	0.28
Female × Diet × Male	4, 93	3.88	**0.006**

Table 2.4- Results from three-way ANOVAs examining effects of female presence, diet, male density and their interactions on development time, growth, body condition and resting metabolic rates of male spiders.

	Source	DF	F	P
Development time				
	Female presence	1, 95	5.88	**0.02**
	Diet treatment	2, 95	5.45	**0.01**
	Male density	1, 95	5.06	**0.03**
	Female x Diet	2, 95	1.09	0.34
	Female x Male	1, 95	1.69	0.19
	Diet x Male	2, 95	0.05	0.95
	Female x Diet x Male	2, 95	0.83	0.44
Growth				
	Female presence	1, 95	2.81	**0.01**
	Diet treatment	2, 95	0.60	0.55
	Male density	1, 95	5.59	**0.02**
	Female x Diet	2, 95	4.45	**0.01**
	Female x Male	1, 95	0.18	0.67
	Diet x Male	2, 95	0.05	0.95
	Female x Diet x Male	2, 95	4.98	**0.01**

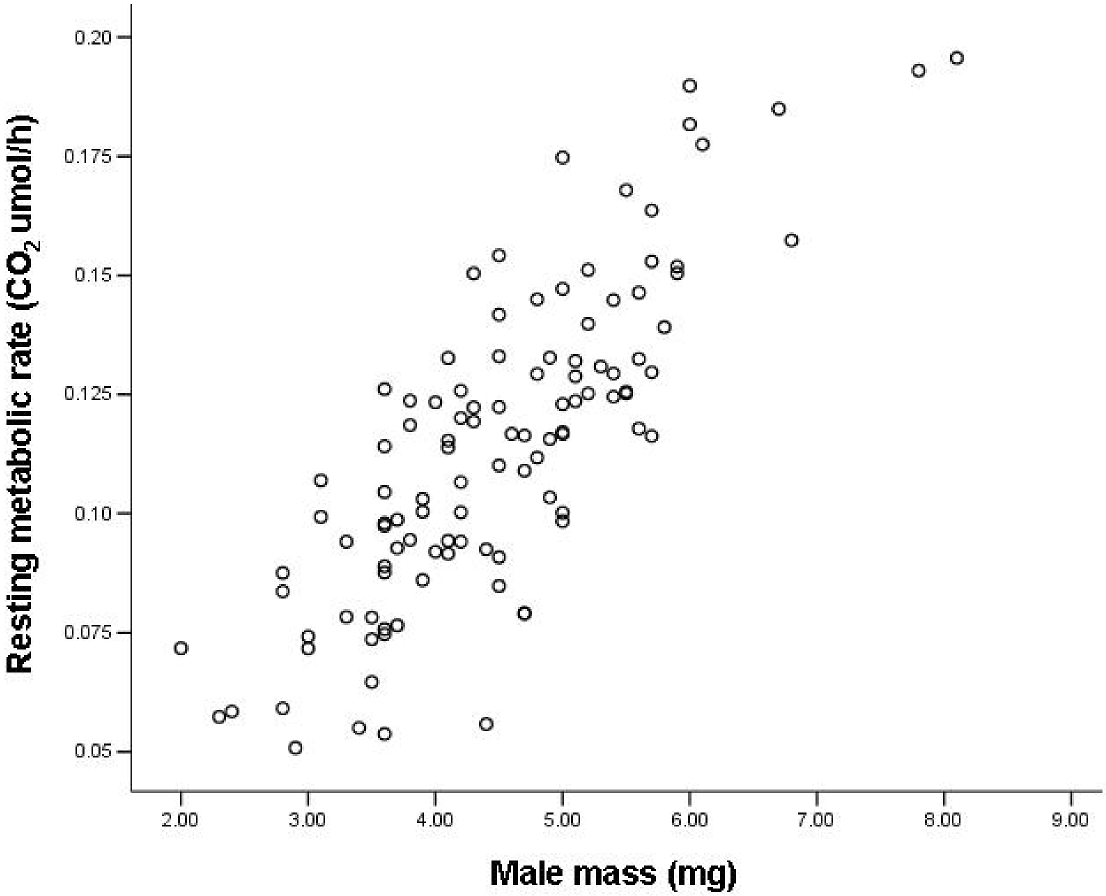
Body condition			
Female presence	1, 95	5.60	**0.02**
Diet treatment	2, 95	3.38	**0.04**
Male density	1, 95	13.50	**<0.001**
Female x Diet	2, 95	5.36	**0.01**
Female x Male	1, 95	4.58	**0.04**
Diet x Male	2, 95	1.01	0.37
Female x Diet x Male	2, 95	4.39	**0.02**
Metabolic Rate			
Female presence	1, 95	0.67	0.42
Diet treatment	2, 95	3.77	**0.03**
Male density	1, 95	8.37	**0.01**
Female x Diet	2, 95	1.81	0.17
Female x Male	1, 95	1.05	0.31
Diet x Male	2, 95	2.04	0.14
Female x Diet x Male	2, 95	1.23	0.30

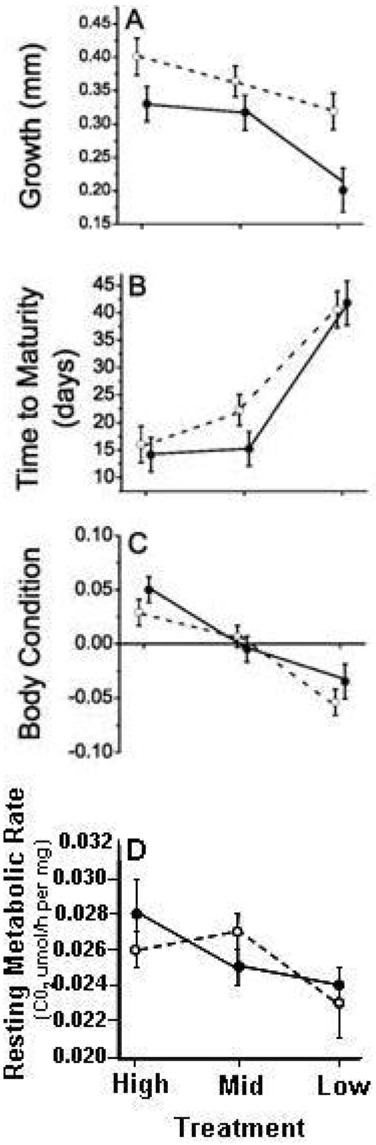
Figure 2.1- Scatter plot of mass and resting metabolic rate of male Australian redback spiders.

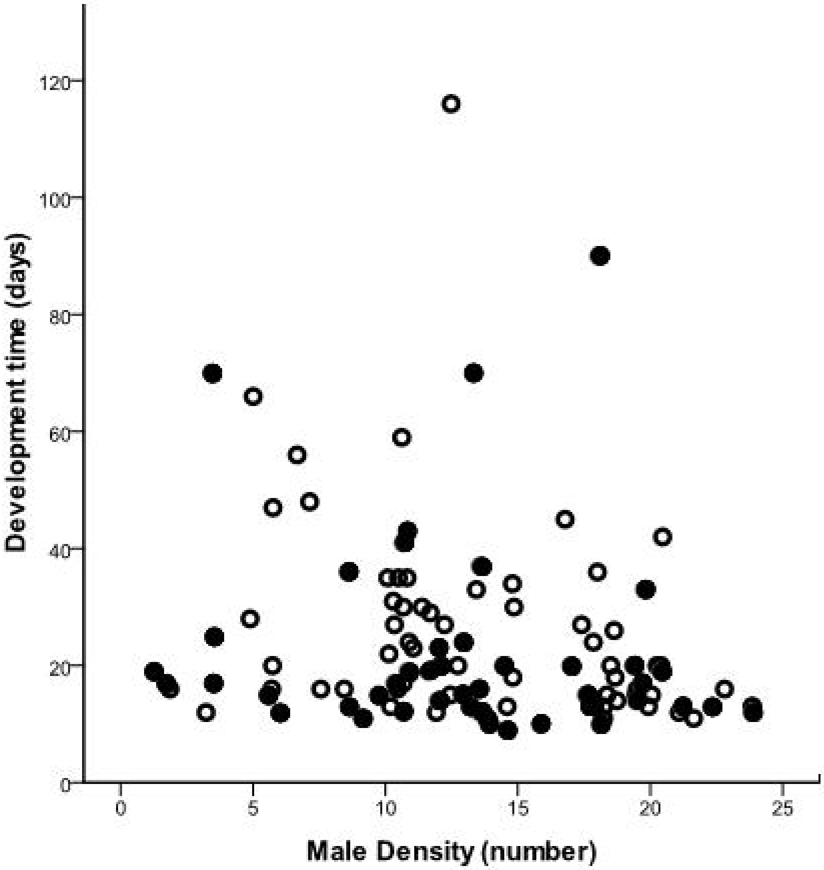
Figure 2.2- Reaction norms of males reared in the absence (open circles) and presence (closed circles) of females in three different diet treatments. (A) growth (size difference between penultimate and maturity), (B) body condition, (C) time to maturity and (D) resting metabolic rates.

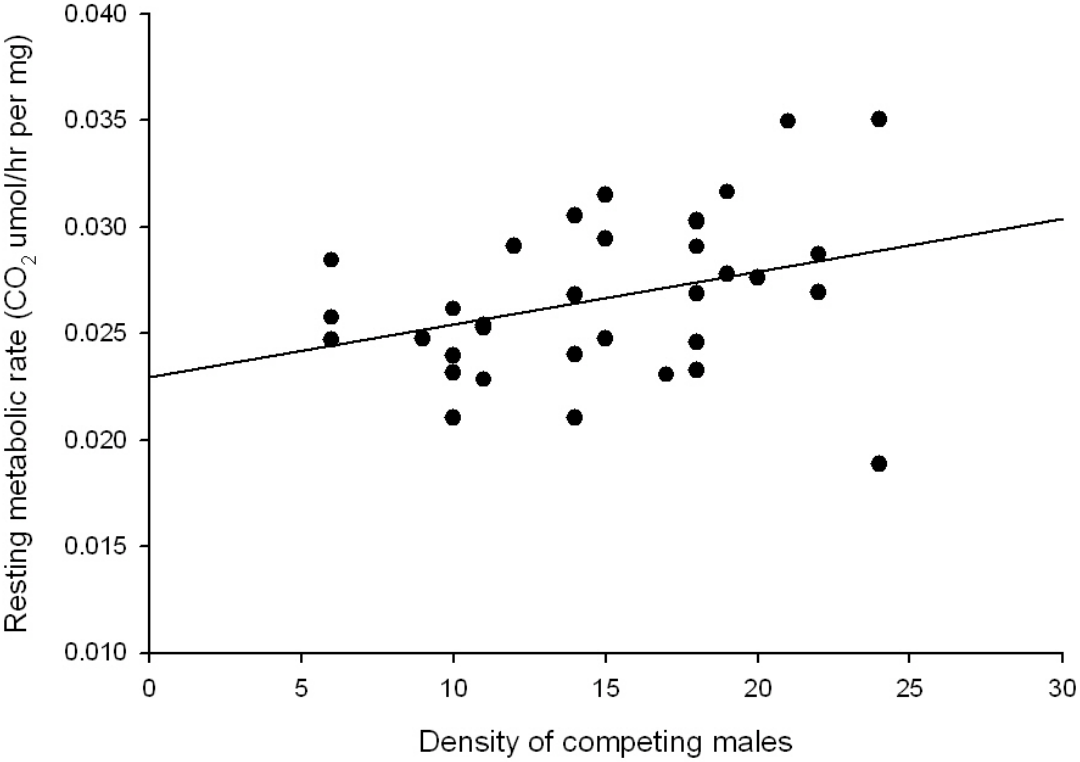
Figure 2.3- Relationship between likelihood of male-male competition and development time (days) as a function of whether females were absent (open circles) or present (closed circles) absent during development (r^2=0.04, $F_{1,107} = 4.491$, $P = 0.04$).

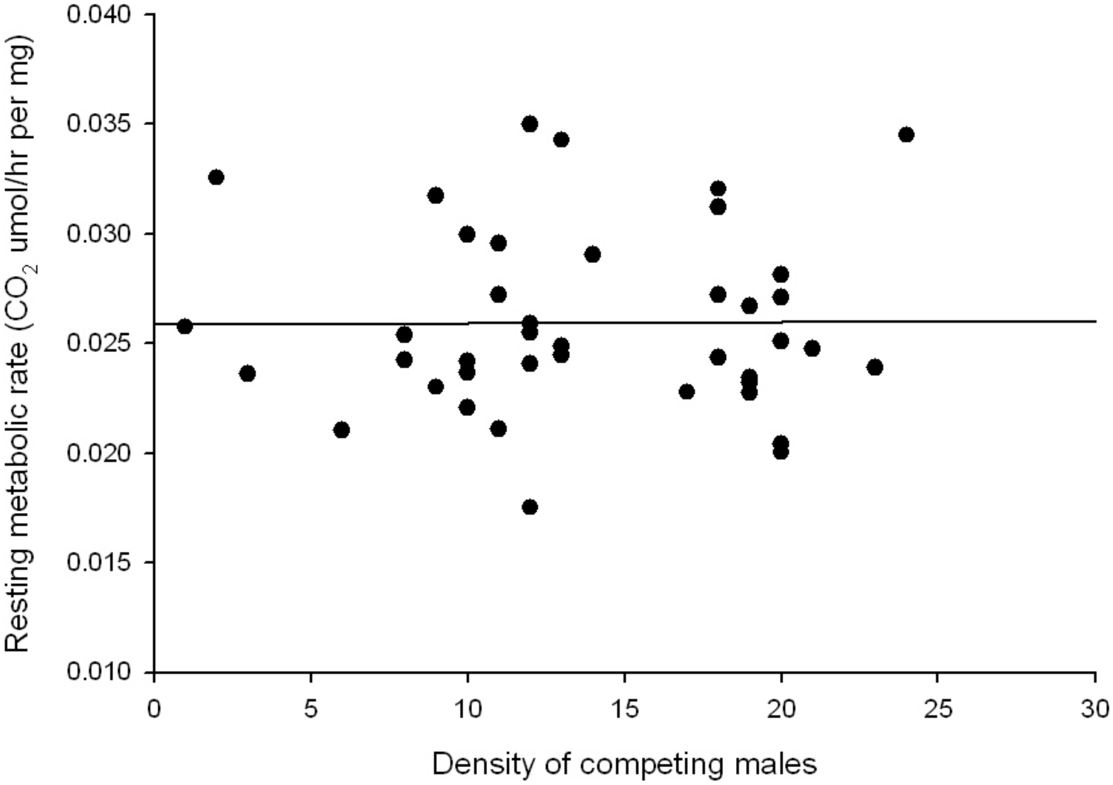
Figure 2.4- Resting metabolic rates of males reared on high (A), medium (B) or low (C) diet levels in the presence of a variable density of male competitors (Regression analyses; high diet: $F_{1,34} = 4.055$, Beta = 0.331, $r^2 = 0.109$, $P = 0.052$; mid-diet: $F_{1,41} = 0.006$, Beta = 0.012, $r^2 = 0.00$, $P = 0.941$; low-diet: $F_{1,30} = 4.951$, Beta = 0.382, $r^2 = 0.146$, $P = 0.034$).

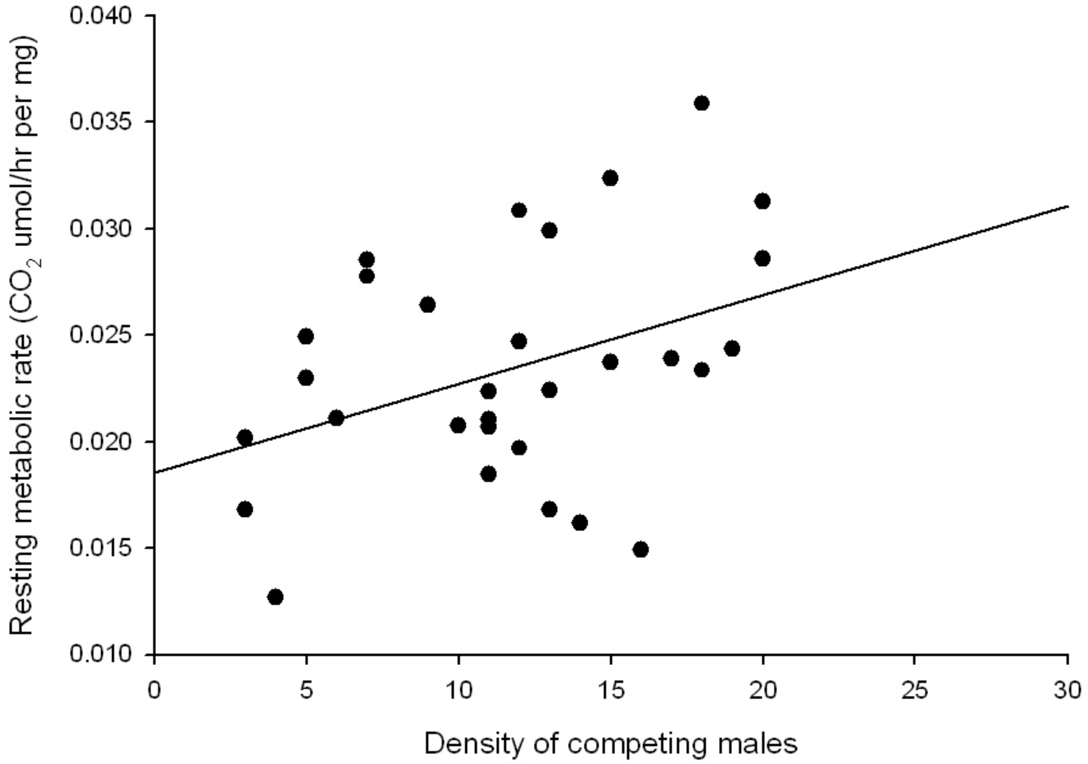

Resting metabolic rate (CO_2 umol/h)
0.20
0.175
0.15
0.125
0.10
0.075
0.05
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
Male mass (mg)


A
Growth (mm)
0.45
0.40
0.35
0.30
0.25
0.20
0.15
B
Time to Maturity (days)
45
40
35
30
25
20
15
10
C
Body Condition
0.10
0.05
0.00
-0.05
-0.10
D
Resting Metabolic Rate
(C0$_2$ umol/h per mg)
0.032
0.030
0.028
0.026
0.024
0.022
0.020
High
Mid
Low
Treatment


Development time (days)
120
100
80
60
40
20
0
0
5
10
15
20
25
Male Density (number)


0.040
0.035
0.030
0.025
0.020
0.015
0.010
Resting metabolic rate (CO_2 umol/hr per mg)
0
5
10
15
20
25
30
Density of competing males


Resting metabolic rate (CO_2 umol/hr per mg)
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0
5
10
15
20
25
30
Density of competing males


Resting metabolic rate (CO2 umol/hr per mg)
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0
5
10
15
20
25
30
Density of competing males

Chapter 3

Males use chemical cues to discriminate just-mated females from virgins in redback spiders

Abstract

In species where virgin females have greater reproductive value to males than non-virgin females, selection will favour males that can discriminate female reproductive status. Similarly, while virgin females may actively seek to attract males, non-virgin females may seek crypticity after mating if copulation is costly. Here we show that Australian redback spider males (*Latrodectus hasselti*) discriminate female maturity and mating status based exclusively on web-borne chemicals. Male activity on extracts from webs of virgin females significantly exceeded activity on extracts of webs of juveniles, non-virgin females and solvent controls. While this level of discernment of female reproductive status has been found in other spiders, here we show that discrimination of web-bound chemicals produced by virgin females disappears rapidly after these females mate. Extracts of webs built by females immediately after copulation elicited no more interest from males than did solvent controls. Redback males dismantle much of their mate's web during courtship, and these results suggest that the web rebuilt by females after mating would not trigger male courtship. We speculate this rapid shift would significantly reduce the opportunity for polyandry in nature.

Introduction

In many species it would be advantageous for males to assess female mating status prior to attempting mating. Particularly if there is first-male sperm precedence, or if female receptivity decreases after copulation, virgin females would be particularly valuable to males and selection may favour males able to detect previously-mated females (Carrière & McNeil 1990). Chemical signals mediate mate attraction and courtship in many invertebrates, and using such signals to discriminate among females of different reproductive value may increase male fitness (Haldane 1955; Shorey 1976; Phelan 1997; Foelix 1996; Huber 2005). In web-building spiders, for example, males may experience high mortality when they abandon their webs in search of mates, and must engage in energetically expensive courtship when they reach a female (Vollrath & Parker 1992; Andrade 2003; Segoli et al. 2006; Kasumovic et al 2007). Thus there is likely to be strong selection on males to discriminate among females at a distance, or to quickly recognize low-value females and abandon them without significant investment in courtship. For example, in desert widows (*Latrodectus revivensis*) males spend greater periods of time with sub-adult females than adult females and also spend great periods of time remaining on their own webs until pheromones are detected (Segev et al. 2003). Similarly, if courtship or mating with multiple males is not advantageous for females, then it may benefit virgin females to advertise receptivity using pheromones, but mated females may seek crypticity after mating (Marcotte et al 2003, 2006).

We studied male response to pheromones in the Australian redback spider (*Latrodectus hasselti*), a species in which strong first-male sperm precedence reduces the reproductive value of mated females for males. In this study, we examined the potential for polyandry in redbacks by examining whether web-bound pheromones are sufficient for male recognition of virgin females, and the time frame over which male response to web-bound chemicals decreases following female mating. Although redback females will remate in the laboratory (Andrade 1996; Snow and Andrade 2004, 2005), it is not known whether they would frequently have the opportunity to remate in nature. This approach to estimating the opportunity for polyandry may have important implications for other arachnid systems, where the assumption of polyandry is widely used to shape hypotheses about male and female strategies (e.g., Elgar 1998). Although females of some spider species have been shown to be polyandrous in nature (Elgar et al. 2000, 2003; Martyniuk & Jaenicke 1982; Austad 1982; Watson 1990, 1991a,b; Maklakov et al. 2004, 2005), in other species, assumptions of polyandry are based on laboratory observations of once-mated females copulating with additional males presented under laboratory conditions (e.g., Bukowski & Christenson 2000; Herberstein et al. 2002; Suter 1990; Rypstra et al. 2003). Realized levels of polyandry may be considerably reduced if chemically-triggered male attraction or courtship drops rapidly after females mate in nature.

Chemical signals are used in a variety of behavioral contexts across a diverse range of animal taxa (Shorey 1976) and are particularly sophisticated in insects (e.g., moths; Phelan 1997). Although chemical communication is thought to be the ancestral mode of inter-sexual communication for spiders (Foelix 1996; Huber 2005), it has received relatively little attention, and very few compounds have been identified (Schulz 2004; Huber 2005). It is widely accepted, however, that male spiders recognize and respond to chemicals released from the female’s body (Riechert & Singer 1995; Miyashita & Hayashi 1996) or associated with female-produced silk (Tietjen & Rovner 1982; Roberts & Uetz 2004). Silk-bound chemicals are important in mate attraction, initiation of courtship, species discrimination (but see Roberts & Uetz 2004), and assessment of female age or mating status (Kasumovic & Andrade 2004; Andrade & Kasumovic 2005; Papke et al. 2000; Schulz & Toft 1993; Searcy et al. 1999; Tichy et al. 2001; Tietjen 1979;

Trabalon et al. 1997; Papke et al. 2001; Riechert & Singer 1995). Although some of the basic proximate mechanisms involved in pheromone production are understood (Schulz & Toft 1993), it is not yet clear how patterns of pheromone production are related to female reproductive strategies, or how these patterns might affect male mating opportunities.

Male discrimination between virgin and mated females using silk has been demonstrated in many different spider species (e.g., wolf spiders: Roberts & Uetz 2005; black widows: Anava & Lubin 1993; redbacks, Andrade & Kasumovic 2005; funnel web spiders: Riechert & Singer 1995; and jumping spiders: Jackson 1981, 1986). Females may produce chemical signals to reduce the risk that they will remain unmated (Vollrath & Parker 1992; Andrade & Kasumovic 2005; Searcy et al. 1999), or males may detect some cue of female mating status that is produced as a side-effect of physiological processes. Once mating has occurred, as with insects, there could be a period of pheromonostasis (post-mating inhibition of pheromone production by females) of varying duration (Marcotte et al. 2006 submitted; Royer & McNeil 1993). Age-related changes in the composition or concentration of silk-bound compounds may also allow discrimination if females typically mate when young (Roberts & Uetz 2005), but this could lead to discrimination errors for males. In contrast to age-dependent changes, rapid shifts in web-bound chemical profiles may be triggered by mating because pheromones are deposited on or in the silk strands, rather than being an integral part of the silk itself (Pollard et al. 1987; Tietjen & Rovner 1982). This is particularly true in species where the web is constructed anew each day, or must be rebuilt or repaired immediately after mating (e.g., Watson 1986), as this would allow shifts in attractiveness to occur over short time scales.

We examined chemical communication in redback spiders by focusing on the behavior of males exposed to female web pheromones. There is strong selection on redback males to identify virgin females because of strong first-male sperm precedence (Snow & Andrade 2005), frequent cannibalism during mating (Andrade 1996), and a low probability of finding alternative mates (Andrade 2003). Redback males can discriminate between the webs of virgin and previously-mated females in the field and laboratory (Andrade & Kasumovic 2005). Here we focus on whether web-bound chemicals are sufficient to allow discrimination between juvenile, virgin and mated females and ask whether the ability to discriminate is lost immediately after females have mated.

Methods

Spiders

Female spiders were collected in the field (some mothers) or were the F1 offspring (some mothers and all other groups) of field-collected individuals from Sydney, New South Wales, Australia. Spiders were reared in a temperature-controlled room at 25°C (12:12 hour light cycle). Juvenile spiders were reared communally through the first few instars, then isolated in individual cages. Spiders were fed fruit flies, and females were fed crickets from approximately 5th instar onwards. In our experiments, we compared sexually mature virgin male response to extracts of silk produced by four groups: (1) juveniles (< 4th instar), (2) virgin adult females, (3) just-mated females (web collected immediately following mating), and (4) mated females that had already produced at least one egg sac (‘mothers’).

Silk collection and pheromone extraction

Females produced web across clean, inverted, paired U-shaped frames constructed from stainless steel metal rods supported on a Plexiglas or plastic base. Bases were submerged in water inside a 60x40 cm plastic box to confine females to the frames. Approximately 48 hr after being placed on frames, females were removed and webbing was pulled free of the frame using clean glass implements. Between trials all implements and frames were washed in liquid soap, water, then methanol, and air-dried.

To exclude the possibility that tactile cues from the web are involved in discrimination (e.g., Anderson & Morse 2001), we compared male response to filter paper treated with web extracts. We made methanol (low water, ACS, reagent grade, CH3OH, Caledon Laboratories) extracts of webs as this solvent effectively extracts pheromones associated with female silk in other spiders (Ayyagari & Tietjen 1987; Roberts & Uetz 2004). Webbing from each female was placed in a 2.0 ml Teflon-lined sample vial (Fisher Scientific) with just enough methanol to cover the web (0.2-0.4 ml), then placed in the refrigerator for at least two days for extraction. Trials were run within 2 weeks of sample collection.

We compared the behavioural response of males placed on filter paper treated with different methanol extracts. Normally, most courtship activity by male redbacks occurs on the web (see Forster 1995 for a description), so male movements on filter paper are not easily analogized to normal courtship components. Consequently, rather than scoring putative courtship behaviour, we scored the total duration of male movement and considered significant differences in activity level as an indicator of response (Carey & Bull 1986). In preliminary trials, males showed extensive movement on filter paper coated with extracts from the webs of virgin females (known to trigger courtship [Ross & Smith 1979] and web fidelity [Andrade & Kasumovic 2005]), but not on blank filter paper. Behaviours included males moving across the filter paper and laying their own web across the web of the female (= web addition, a major component of web reduction and early courtship; Watson 1986), ‘bouncing walk’ (walking across the filter paper while flexing and extending legs), and exploratory movements (probing filter paper with legs or palpi). In comparison, there was very little movement on blank filter paper.

In our trials, male activity was assessed during contact with extract placed on filter paper inside a glass petri plate (90 mm diameter). Petri plates are sufficiently large to allow unconstrained movement by the diminutive redback males (mean front-leg length of the patella + tibia = 2.99±0.02 mm, n = 212; Kasumovic & Andrade 2006).

Filter paper discs were placed inside glass Petri plates using clean forceps. Each filter paper disc was treated with extract from webs of virgin females (n=17), mothers (n=17), just-mated females (n=12), or juveniles (4th or 5th instar, n=10). There were two controls, a solvent only control (n=15) and a blank filter paper control (n=10). For each disc, three drops of extract were introduced using a glass disposable pipette and discs were allowed to air-dry for 5 minutes. This resulted in a consistent extract volume of 100.59±0.53 µml, (measured independently, n=17). After each trial, the filter paper was discarded and Petri plate was washed in soap, water, then ethanol, and allowed to air dry before reuse.

Males were weighed (body condition might affect activity level) and randomly assigned to a treatment. Each male was allowed to drop from a drag line into the assigned Petri plate arena and the lid was closed. Trials were conducted between 900hr to 1100hr. Arenas were video-taped (using black and white CCD cameras with macro zoom lens) for 60 minutes. As redbacks are nocturnal, all trials were conducted during the dark phase under red lights. Trials were run in blocks of nine (with treatments assigned randomly to blocks), and males were used once only. An observer blind to treatment analyzed video tapes to assess total time spent moving for each male.

Statistical Analysis

Analyses were completed in SPSS Version 13. Male weight was normally distributed (Shapiro-Wilkes $p > 0.05$) but total activity time was not (SW $p < 0.05$). This was driven by a gap in the distribution of activity times of males tested on extracted webs of virgin females relative to other groups (Fig. 1). Transformations did not result in a normal distribution, but a normal probability plot suggested values otherwise followed a normal distribution, and there was little evidence of skew or kurtosis (Sokal & Rohlf 1994). We analyzed data using a General Linear Model with treatment as factor, total duration of activity as the dependent variable, and tested for a treatment*male weight interaction effect. Tukey HSD post-hoc tests were used to examine pairwise differences between treatment levels.

Results

Male activity on treated filter paper depended on the source of the extract (Fig. 1, GLM, Treatment $F_{5,80} = 4.99$, $p = 0.001$). Males tested on extract from webs of virgin females were significantly more active than in any other treatment (all post-hoc tests, $p < 0.05$, Fig. 1). In comparison, males were less active on extract from webs of mated females that had already produced egg sacs, and activity was lowest on extract from webs of females that had just mated, webs of juveniles and controls (Fig 1).

There was a significant interaction effect of treatment and male weight on activity level ($F_{6,80} = 2.50$ $p = 0.030$). Although there was a negative relationship between male weight and activity level in the virgin female treatment ($R^2 = 0.439$, $F_{1,16} = 11.74$, $p = 0.004$), there was no significant relationship between male weight and activity in any other treatment ($p >0.1$ for all analyses).

Discussion

Male redback spiders responded to methanol extractions of webs produced by virgin females and discriminated these from webs of juveniles, from females that had already produced egg sacs, and remarkably, from webs produced just after females mated. Methanol extracts apparently include soluble chemicals that are detected by males through direct contact. These chemicals may be used by males to assess the reproductive status of a female. If polyandry is costly, females may alter the chemicals added to the web after mating, or cease production of these chemicals, to ensure they are cryptic to males. Pheromonostasis could also be a physiological side effect of mating that is not under female control, perhaps resulting from chemicals transferred in the male's ejaculate.

The activity level of males on the extracts of webs produced by virgin females was approximately twice as high as in any other treatment. However, just after females mated, male activity dropped to equal that of males in the mated female (mothers), juvenile and control treatments (Fig 1). Similar results have been reported in fruit flies (*Drosophila melanogaster*) where females elicit less courtship behaviour from males immediately after mating (Tompkins & Hall 1981) and it has been suggested that compounds transferred to females during copulation act as antiaphrodisiacs rendering the female unattractive (Scott 1986). However, it is unlikely the decreased activity by male redbacks in the just-mated female treatment is due to the transfer of antiaphrodisiac compounds during mating, unless these are incorporated in the web by females.

Pheromonostasis may be favoured for females if there are sufficiently high costs associated with multiple mating (e.g., Wing 1988; Herberstein et al. 2002), or may be maladaptive and due to chemical manipulation by males. It is possible that a change in female pheromone production could be triggered by chemicals transferred in the ejaculate (e.g., Chen et al. 1988; Chapman et al. 1995; Liu & Kubli 2003); redbacks require only 5 minutes for sperm transfer but often mate for up to 20 minutes and may transfer non-gametic substances to females (Snow and Andrade 2004). Regardless of the trigger, pheromonostasis is common in insects (Marcotte et al. 2003, 2006), but has been demonstrated in only one other spider *(Linyphia litigiosa*) where web of mated females did not contain active pheromones 1 week after copulation (Schulz & Toft 1993; Watson 1986, 1990). Our study shows that the change in chemical signal production is much more rapid, and given male web reduction behaviour during mating (Watson 1986; Forster 1995), females may be unattractive to males immediately after copulation. Evaluation of competing hypotheses for fitness effects of this change will require analyses of effects of polyandry and post-mating pheromone production on female fitness (e.g., Schneider & Lubin 1998; Maklakov et al. 2005; Maklakov & Lubin 2004).

It is also possible that mated females continue to produce chemicals detectable by males, but males choose not to court mated females due to high first male sperm precedence in this species (Snow & Andrade 2005; see Bonduriansky 2001). This seems unlikely since mate searching is risky for males (Andrade 2003) and male fitness is likely to decrease if any potential mate (regardless of reproductive state) is abandoned. In the field males do not abandon webs of mated females (Andrade & Kasumovic 2005) but in *L revivensis* have been shown to decrease cohabiting time with adult females (Segev et al. 2003). From the male’s perspective, attempting to mate with a non-virgin female may sometimes be a good strategy because some second males achieve high paternity despite sperm precedence (Snow and Andrade 2005; Snow et al. 2006; and see Sims et al. 1987; Svärd & McNeil 1994; Rakitin et al. 1999). Therefore, males might invest in courting and already-mated female since even a small paternity gain from mating is more than would be expected if the male searched for a new female (Andrade 2003; Andrade 1996). These observations suggest that the decreased activity level of males on extracts from webs of just-mated females occurs because these females cease adding pheromones to their webs (pheromonostasis). Ongoing work focuses on identifying these pheromones and assessing how concentrations change as a function of female reproductive status.

In our trials, activity levels of males on extracts from webs of females that had already produced eggsacs tended to be higher than in the juvenile, just-mated and control treatments, although this difference was not significant (Fig 1). This suggests the possibility that females might advertise receptivity while a virgin and then re-advertise receptivity later in the season or the following year (see Andrade & Banta 2002 for data on females overwintering). Consistent with this, males are sometimes attracted to mated females in the field (Gaskett, et al. 2004; Andrade & Kasumovic 2005). However, it is currently unknown whether female redbacks have age-dependent variation in receptivity and pheromone production.

Our study suggests that females of the Australian redback spider may manipulate pheromones on their webs to control mating attempts by males. From the male's perspective, selection is expected to favour detection of female reproductive status, and possibly chemical manipulation of female attractiveness. From the female's perspective, there may also be strong selection to attract males as, in web-building spiders, females may risk remaining unmated at low population densities (Watson 1986). However, if male presence, courtship and/or mating is costly, females should attempt to remain cryptic after mating. The result could be strategic pheromone production by females which changes in response to population density (Watson 1986) as well as sperm stores or mate quality (e.g., Allan & Wang 2001; Ravi & Palaniswami 2002; Raina 1988; Miyashita & Hayashi 1996; Wedell 2001). Given that many pheromones are web-borne, repeated non-destructive sampling of individual pheromone production is possible in spiders. Thus studies of spider pheromone production may be particularly useful for assessing dynamics of female reproductive strategies as a function of ecological and demographic factors.

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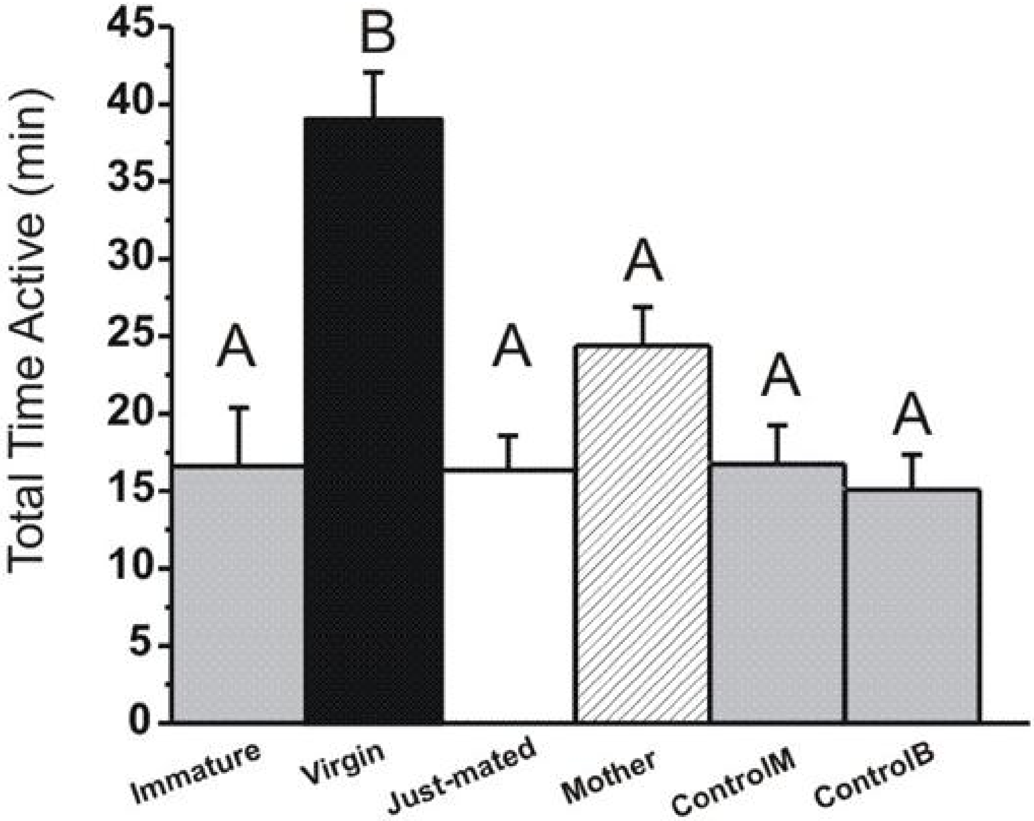
Figure 3.1- Mean (± s.e.) activity levels (min.) of male redback spiders on filter paper treated with methanol extract from webs (first 4 columns), methanol alone (ControlM) or untreated filter paper (ControlB). Different letters above columns denote activity levels that differ significantly according to Tukey HSD post-hoc tests ($p < 0.05$).


Total Time Active (min)
45
40
35
30
25
20
15
10
5
0
A
B
A
A
A
A
Immature
Virgin
Just-mated
Mother
ControlM
ControlB

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Chapter 4

Female's courtship threshold allows intruding males to mate with reduced effort

Abstract

Female decision rules can influence the nature and intensity of sexual selection on males, but empirical demonstrations of rules underlying choice are rare. We hypothesized that female choice is largely based on a courtship duration threshold in the Australian redback spider (*Latrodectus hasselti*) because females kill males before copulation is complete (premature cannibalism) and reduce their paternity if courtship is brief. We used published data to infer the female's threshold is approximately 100 minutes of courtship. We support this hypothesis by showing premature cannibalism is common when the male's courtship duration is below this threshold, but is infrequent and unrelated to duration once courtship exceeds the threshold. We then ask whether females discriminate the source of courtship when rival males compete, as this will determine the effect of the threshold on male competitive tactics. We staged competitions where 'resident' males initially courted females in the absence of competition, exceeding the courtship threshold before 'intruding' males were introduced. Intruding males mated rapidly but were not prematurely cannibalized by females, in contrast to cases where competition starts before the threshold is surpassed. This suggests females do not distinguish which male satisfies the threshold, allowing intruders to parasitize the courtship efforts of residents. To our knowledge, such exploitation of mating efforts by rival males mediated by a female choice threshold has not been demonstrated elsewhere. Ironically, this female choice threshold and the attendant possibility of courtship parasitism may lead to selection for lower quality males to recognize and seek out (rather than avoid) webs in which competitors are already present.

Introduction

Male mating success can depend on traits that increase success in combat or scramble competition such as condition, weaponry, or body size (Huntingford & Turner 1987). However, context may also affect male success, and such effects may be independent of or interact with phenotypic traits such as size. One important contextual determinant of success is residency status; this can be important in systems where ‘intruding’ males may attempt to mate females with which a ‘resident’ male is currently associated (Davies 1978, Krebs 1982, Otronen 1984, Thornhill 1984). In some cases resident males may be more likely to win contests regardless of differences in phenotypic traits such as size (Maynard Smith & Parker 1976, Hammerstein & Parker 1982, Leimar & Enquist 1984, Enquist & Leimar 1987, Grafen 1987). In the majority of studies of residency effects, males defend territories or females directly, but competition is temporally displaced from courtship behaviour and mating. In these systems, residents that win in competition are left with exclusive access to females and their mating success increases (e.g. Kruse & Switzer 2007, reviewed in Kemp & Wiklund 2001). In some systems, however, courtship and competition occur simultaneously (Baker 1983, Uzendoski et al. 1993, Tauber & Eberl 2002, Wong 2004, Stoltz et al. 2008). There may be drastic changes in courtship during competition (e.g., atypical courtship, Baker 1983; alteration of courtship songs, Tauber & Eberl 2002; reduction in courtship effort, Wong 2004, Stoltz et al. 2008) and this can affect female choice when courtship is used to assess males. Female mate choice may thus be influenced by the process of male-male competition. Interactions between choice and competition can have important consequences for the evolution of male traits (reviewed in Hunt et al. 2009). In many systems, understanding how female preferences are affected by the interplay between residency, male phenotypic traits, and courtship effort is critical for understanding selection on male traits in nature (Hunt et al. 2009).

Effects of residency on female mating patterns may depend on decision rules used by females and how these relate to courtship. Two of the most commonly examined decision rules for female choice are sample-based ('best of n') and threshold-based rules (Janetos 1980, Wittenberger 1983, Real 1990, Valone et al. 1996, Wiegmann et al. 1996). Sample-based decisions are comparative and require assessing a number of potential mates, the best of which is then chosen for copulation (Valone et al. 1996). In contrast, under threshold rules, any potential mate that exceeds the female's threshold is acceptable, and this criterion does not depend on whether others of higher quality may be available (Valone et al. 1996). Thus, while best-of-n rules should yield directional selection on male phenotypes, threshold rules may be more likely to give rise to stabilizing selection (Wiegmann et al. 1996). Moreover, male mating success may be more strongly linked to residency when females use thresholds rather than best-of-n rules, as any resident male exceeding the threshold will be an acceptable mate.

We hypothesized that such scenarios may have a different outcome when courtship and competition occur simultaneously (e.g., Stoltz et al. 2008, 2009, Wong & Candolin 2005, Hunt et al. 2009). If females do not discriminate the source of stimulatory courtship signals, threshold criteria may allow males to exploit courtship effort of rivals. Threshold criteria could then lead to reduced selection on male traits that are normally important for mating success. Such exploitation was suggested in red spotted newts (*Notophthalmus viridescens*), where males may intercept receptive females from courting rivals, but this report is anecdotal and females' decision rules are unclear (Verrell 1982). While there are documented examples of cooperative courtship behaviour (e.g., in birds: McDonald & Potts 1994, Lank et al. 2002, Krakauer 2005, Cockburn et al. 2008) and 'sneaker' male tactics with minimal courtship (Parker 1990, Taborsky 1998), threshold-based exploitation would differ in that (1) parasitic males would not necessarily expend courtship effort and would decrease (rather than increase) the fitness of their rival, and

(2) the success of the sneaking tactic would be a step function, dependent on whether or not the threshold had been satisfied by a resident.

Thresholds are either rare in nature or difficult to demonstrate (Valone et al. 1996, but see Ivy & Sakaluk 1997, Parri et al. 1997). However, threshold decision rules of choosy females (e.g., Parri et al. 1997) may allow exploitation by rival males in spiders. In redback spiders (*Latrodectus hasselti*), rival males compete by simultaneously courting the female and several observations suggest a courtship duration threshold may be an important basis for female choice. Courtship duration may reflect male quality (see, Knapp & Kovach 1991, Tregenza et al. 2006, Stapley 2008, Kasumovic et al. 2009) as courtship occurs after a period of mate searching during which almost 90% of males die due to predation and depleted energy reserves (Andrade 2003). Males typically do not contact females or attempt mating until at least 2 hours of courtship are completed, despite the risk that rivals may arrive and usurp their mate (Forster 1995, Andrade 1996). In addition, although some males copulate after relatively brief courtship, females employ two cryptic mechanisms that decrease paternity of these males (e.g., Stoltz et al. 2008, 2009, see Snow & Andrade 2005). First, there is an inverse relationship between the courtship duration of the first male to mate and the number of copulations the female accepts from a rival (Snow & Andrade 2005, Snow et al. 2006, Stoltz et al. 2009). Second, females often kill and cannibalize smaller, rapidly-copulating males prior to completion of a normal mating (= premature lethal cannibalism, Stoltz et al. 2008), which reduces paternity by 50% under sperm competition (Snow & Andrade 2005, see also Prenter et al. 2006). In contrast, larger, longer-courting males mate normally. Thus female choice is apparently linked to courtship effort as well as male size (Stoltz et al. 2008, 2009, see also Prenter et al. 2006), and selection imposed by females may have resulted in males that attempt mating only after satisfying the female's threshold duration of courtship.

In this study, we use published data (Stoltz et al. 2008, 2009) to derive a putative courtship duration threshold for female choice in redback spiders. We then test whether premature lethal cannibalism (i.e., female cryptic choice sensu Eberhard 1996) depends on this threshold by comparing female responses to males that attempt mating before or after the threshold is reached. Next, we investigate whether intruding males can exploit the reproductive effort of resident males that have met the courtship demands of females. We predict (1) whereas courtship duration will determine female responses below the threshold, there will be no relationship between duration and premature cannibalism once courtship exceeds the threshold (e.g., a step function in female discrimination), (2) premature cannibalism of mating males will be less frequent if mating occurs after residents court past the estimated threshold, compared to contexts in which mating is first attempted before the courtship threshold is reached, (3) once a resident meets the courtship duration threshold, intruding males that rapidly ‘sneak’ copulations will not suffer the female-imposed penalties documented when there is no period of resident courtship (Stoltz et al. 2008, 2009).

Methods

Study animals

Spiders were from an outbred laboratory population of *L. hasselti* started with spiders collected from Sydney, Australia (2007). Spiderlings were reared communally until the 4th instar after which they were housed in separate cages (Amacs Plastics Products Ltd) to ensure that they had not mated at the time of trials (males mature at the 5th instar, females at the 7th-8th instar). The spiders were kept in a temperature controlled room at 25ºC 12:12 light:dark cycle. Males were fed fruit flies (*Drosophila* sp.) twice a week and females were fed crickets (*Acheta domesticus*) once per week. All trials were conducted under the dark cycle illuminated by red light as *L. hasselti* are nocturnal.

Mating Trials

Females that had matured within the previous two months (females survive for up to 2 years in the laboratory, Andrade & Banta 2002) were placed in mating arenas (35 cm x 30 cm x 15 cm) for 48 hours and allowed to build webs on metal frames prior to the introduction of males. Trials were 6 hours in length and filmed 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). Male spiders were weighed (Ohaus Explorer electronic balance), leg lengths were measured (mean patella + tibia of both front legs) and males were marked with a small spot of non-toxic paint on the dorsal side of their abdomen (BioQuip Products, Inc) 1 hour prior to a trial. Males were briefly anaesthetized with CO_2 for less than one minute prior to paint marking.

In our trials, one lone male (resident) was allowed to court a female for a period that approached the proposed courtship duration threshold (see below). We then introduced a rival (intruder) that was relatively larger, size matched or relatively smaller than the resident. Crossing relative size with residency status was intended to allow us to determine whether these factors or some interaction affected female choice, since relative size had previously been identified as an important variable (Stoltz et al. 2008, 2009). Pairs of males of similar weight (mean weight difference = 0.17 mg, 3.4% of mean body weight, n=22) or different weight (mean weight difference = 2.97 mg, 67.4% of mean body weight: intruder male larger n=20; intruder male smaller n=20) were randomly chosen from among all males that matured within the previous 14 days.

Estimating and applying the courtship duration threshold

To derive a putative courtship duration threshold for female choice we used a logistic regression on data from a previously published study (Stoltz et al. 2008). In this study, size-mismatched rival males were simultaneously paired with a female and the first male to mate was less likely to be premature cannibalized if he courted for a longer period of time prior until the first copulation (Stoltz et al. 2008). A binary logistic regression relating the occurrence of premature cannibalism (dependent variable) to courtship duration prior to the first copulation (independent variable) was significant (Model $\chi^2 = 5.145$, $n = 27$, $df = 1$, $p= 0.023$, figure 1):

ln(p/1-p) = Bo + Court (equation 1)

where p = probability of premature cannibalism, Bo = 1.002, and Court = courtship duration (min)

To estimate the courtship threshold for choice, we identified the inflection point of the logistic curve (see Emlen 1996, Andrade 1998, Hosmer & Lemeshow 2000), where the outcome predicted by the model shifts between categories (premature cannibalism vs. no premature cannibalism). To do this we set the p value to 0.5 and found Court = 100min. Two standard deviations on either side of this putative threshold value (83-125 minutes) captures 95% of the variance in this estimated courtship duration threshold (figure 1). The model predicts premature cannibalism by females is likely when courtship duration is shorter than this threshold and correctly predicts 88% of the occurrences of premature cannibalism in the published data set (Stoltz et al. 2008).

In our test of this threshold, each trial began when a randomly chosen (resident) male was introduced to the web from a dragline at the furthest point from the female. These males would commence phase one of courtship, which is comprised of exclusively web-based vibrations and no direct contact with the female (Forster 1992, 1995). Phase one typically lasts about 150 minutes when one male courts in isolation (Forster 1995, Stoltz et al. 2008) and ends when the male contacts the female's abdomen (Forster 1995, Stoltz et al. 2008). We allowed residents to complete phase one before introducing a rival as this ensured courtship duration approached or exceeded the proposed threshold before the initiation of competition. Thus the second male (intruder) was placed on the web via a dragline immediately after the resident male contacted the female's abdomen. When single males are courting, this contact marks the start of the second phase of courtship, which usually includes a combination of vibratory behaviour on the female's abdomen and on the web. During the third phase of courtship males typically remain on the female's abdomen and attempt copulation (see Forster 1995 for more details).

We noted total courtship duration prior to mating, the occurrence of premature cannibalism by females, which male(s) mated, and the number of copulations achieved by each male. In redbacks, a normal mating comprises two copulations (Forster 1995, Andrade 1998). During each copulation the male deposits a sperm plug in one of the female's two sperm storage organs (Snow et al 2006). Males that copulate twice can achieve close 100% paternity under sperm competition, but males that mate only once (i.e., due to premature cannibalism) will attain a maximum of 50% paternity when sperm from the two organs mix at fertilization (Snow & Andrade 2005, Snow et al 2006).

Analysis

To test the threshold hypothesis, we compare results of these residency trials to results of previously published trials on the same laboratory population of spiders. In those trials, size matched or size mis-matched rival males were introduced to females' webs simultaneously, and thus the putative courtship threshold was not reached prior to the initiation of competition (Stoltz et al. 2008, 2009). This is justified because procedures for both experiments were identical with the exception of the timing of introduction of the rival males. We also compare our new data to trials in which single males court females (Stoltz et al. 2008) as this provides a baseline for understanding female responses to male courtship in the absence of competition.

Statistical analysis was performed using SPSS version 13.0 or online statistical software (www.graphpad.com for contingency tests). Data that violated the assumption of a normal distribution were log transformed. If the log transformed data did not produce a normal distribution non-parametric statistics were used. Statistical tests are two-tailed with mean ± SE reported. Sample sizes vary for some tests because behavioural details were missed in some trials.

We ensured there was no bias in the females selected for the study. The weight of the females was similar whether the resident was size matched to the intruder (292.17±11.55 mg), was the larger male (279.56±18.55mg), or the smaller male (276.73±13.26 mg; $F_{2,59}=0.33$, $p>0.05$).

Results

The time of first contact with the female's abdomen by the resident (time of introduction of intruder) was after 75±6 min. of courtship regardless of the relative size of the competitors ($F_{2,58}=0.67$, $p=0.52$). In addition, regardless of the relative size of competitors ($F_{2,53} = 0.98$, $p=0.38$), the first copulation occurred approximately 2 hours after the start of the trial, ($F_{2,53} = 0.98$, $p=0.38$), well above the 95% confidence interval for the estimated choice threshold (see figure 1).

Evidence for a courtship duration threshold

To test the threshold hypothesis, we first asked whether premature cannibalism of males depended on the duration of courtship as a function of whether the female experienced a period of exclusive courtship exceeding the threshold (these trials and single-male matings [data from Stoltz et al. 2008]) or whether competition interrupted courtship before the threshold was reached (males introduced simultaneously, data from Stoltz et al. 2008, 2009). We used a separate logistic regression examining premature cannibalism of the first male to mate (dependent variable) as a function of courtship duration, male size and the context of competition (ie. no competition; Stoltz et al. 2008, simultaneous competition; Stoltz et al. 2008, 2009 or competition after a residency delay; current study). Male size had no effect but there was a significant interaction between courtship duration and context of competition (Wald=4.47, p=0.03; figure 2). This arises because the occurrence of premature cannibalism for lone males and simultaneously introduced competing males was negatively related to total pre-copulatory courtship duration (Stoltz et al. 2008, 2009), but, as predicted, there was no effect of total courtship duration when mating occurred after a resident had satisfied the estimated courtship threshold (log t_{53}=0.15, p=0.88; figure 2).

Second, regardless of residency, size, or courtship duration (see figure 3), the first male to mate achieved more copulations than his rival in 64% (36/56) of cases, and equaled his rival in 34% of cases across all trials (compared to only 1 case where the rival copulated more often than the first male to mate; Wilcoxon signed rank test for related samples: $Z = -5.31$, $p < 0.001$). Females typically mated once or twice with the first male to mate (mean ([s.e.] #copulations = 1.39[0.07]) and often did not mate at all with his rival (mean[s.e.] #copulations = 0.43[0.08]). The effect is still strong if the data are separated according to the relative size of competitors (matched: $Z = -2.961$, $p = 0.003$; mismatched: $Z = 4.42$, $p < 0.001$). Overall, residents were more likely to achieve the first copulation but this result was driven by trials in which smaller males were residents (table 1).

Exploitation of reproductive effort of residents by intruding males

Although premature cannibalism is unrelated to total courtship duration in the resident-intruder trials, this is not because all males provided an extended courtship. Rather, intruder males invested very little in courtship. Copulation by the first male to mate occurred after an extremely brief courtship if that male was an intruder rather than a resident in the matched-size treatment (log $t_{18}=2.12$, $p=0.05$, figure 3). Similarly, smaller, intruding males in mismatched trials tended to copulate sooner than larger residents (log $t_{15}=1.91$, $p=0.08$, figure 3), although larger intruding males did not show this effect (log $t_{17}=0.32$, $p=0.75$; figure 3).

Given that small intruders mate without significant investment in courtship, we examined how residency and size affected the likelihood of premature cannibalism of first-mating males by females. If females favour the individual male that satisfied the courtship threshold, residents should have lower rates of premature cannibalism. However size-matched males that mated first were no more likely to be cannibalized prematurely when they were residents (2/12 cannibalized) than when they were intruders (3/8 cannibalized, Likelihood ratio = 1.01, df=1, p=0.3). Similarly, in size mismatched trials, there were no significant effects of size, residency, or interactive effects on the likelihood of premature cannibalism of the first-mating male (logistic regression model $\chi^2 = -23.546$; df = 3, $p = 0.058$; interaction t-ratio = -0.022, $p = 0.982$). Moreover, despite the fact that they provided very brief courtship (figure 3), smaller intruding males that mated first were never cannibalized by females (0/7 trials).

Discussion

We show that female redback spiders use threshold-based mate choice decisions based upon the duration of courtship provided, and intruding males can parasitize the courtship efforts of residents once courtship demands of females are met. Intruding males are able to mate with females after providing extremely brief courtship (figure 3) and first-mating males are not penalized by premature cannibalism (figure 2) since the courtship threshold has been surpassed by resident males. Thus females apparently do not distinguish which male is the source of stimulatory courtship. We identify the threshold as approximately 100 minutes of courtship after which the likelihood of premature cannibalism is decreased (figure 1). These results show that, although the ability to provide a prolonged courtship is typically important to male success, the female's mate choice mechanism is susceptible to circumvention of this requirement by smaller males.

In redback spiders, male competition greatly affects courtship, and the context in which competition occurs can influence cryptic female choice. In the absence of competition, female response to males depends on courtship duration and females limit paternity of rapidly-mating males through premature lethal cannibalism (Stoltz et al. 2008). When competition and courtship occur simultaneously, mating occurs well before the lower limit of our predicted courtship threshold for choice (figure 1, see Stoltz et al. 2008, 2009), and females respond by limiting male mating opportunities with a severity that depends on courtship investment (Stoltz et al. 2008, 2009). In contrast, this study shows that when a resident male courts for a period approaching the putative threshold, female penalization of males (premature cannibalism, decreased mating frequency) is no longer predicted by courtship duration (figure 2). Instead, intruding males can mate rapidly, and regardless of which male provided the initial courtship, premature cannibalism is rare (figure 2), and the first male to mate almost always equals (34% of trials) or exceeds (64% of trials) the mating frequency of his rival (total: 98% of trials). This means that intruders can parasitize the courtship effort of residents by mating rapidly without penalty (figures 2, 3). Thus, males unlikely to surpass a threshold may do better if they seek a parasitic association with another male. Effectively, this means there may be selection for smaller males or those in poor body-condition to seek out, rather than avoid, competitive situations in nature. This may explain previous studies that show clustering of males with relatively few females despite the availability of other receptive females that remain uncontested (e.g., Kotiaho et al. 2004, Kasumovic & Andrade 2009).

For a redback male to maximize fitness it is critical to achieve the first copulation in each of a female's paired independent sperm storage organs (Snow & Andrade 2005, Snow et al. 2006). Thus females that cannibalize males after the first copulation and prevent a second copulation will severely limit their paternity (Snow & Andrade 2005, Stoltz et al. 2008). Our results show a threshold close to 100 minutes of courtship reduces the likelihood of such premature cannibalism when rival males compete to copulate (figure 1). The mean rate of premature cannibalism is lowest (10%; Stoltz et al. 2008) when males are not competing and devote considerable effort to courtship, making contact with females after 150 minutes and copulating after 226 minutes (far exceeding threshold demands predicted by our model, figure 1; see Stoltz et al. 2008, 2009). In the competitions staged in this study, males provided 75 minutes of exclusive courtship prior to the introduction of a rival and the first copulation typically occurred after approximately 2 hours (figure 2, high end of the 95% confidence range around the predicted choice threshold, figure 1). This led to intermediate rates of premature cannibalism (mean of 32%) that were not linked to total courtship duration. In comparison, during competitive contexts when rivals were simultaneously introduced, courtship was accelerated

(Stoltz et al. 2008, 2009). Males made first contact with females after about 30 minutes, with the first copulation typically occurring after 93 minutes, but this courtship was interspersed with bouts of competition. This is at the low end of our courtship threshold model (range 83-125 minutes, figure 1), leads to the highest reported rate of premature cannibalism (mean of 44%, Stoltz et al. 2008, 2009), and variation in courtship among males is tightly linked to variation in cannibalism in this case.

It is unlikely that female redback spiders use sample-based mating decisions to determine the success of their first mate. If females had a sample-based decision rule, we would have expected them to discriminate against brief-courting, intruding, or smaller males in this experiment (as intruders and smaller males reduce courtship in the presence of larger rivals; Stoltz et al. 2008, this study). In contrast we provide evidence that females do not discriminate against brief-courting males, as long as at least one male courts prior to the instigation of competition (compare this study to Stoltz et al. 2008, 2009). Moreover, females were never observed to cannibalize smaller intruding males that mated first, even though they provided extremely brief courtship prior to copulation (approximately 20 minutes; figure 3). Our results suggest that female receptivity increases after residents court, which in turn reduces the likelihood of premature cannibalism after the first copulation, regardless of which male copulates (but see Harari et al. 2009). Females thus do not or cannot identify the source of stimulatory courtship. How this affects female fitness is unclear (also see Smith & Reichard 2005, Reichard et al. 2007).

Despite general predictions that sample based rules will yield higher fitness returns when search costs are minimal for the choosy individual (Janetos 1980, Real 1990), many features of spider mating systems suggest sample-based decisions may decrease female fitness if males arrive sequentially in the wild. First, some female spiders apparently remain unmated in nature (Arnqvist & Henriksson 1997, Andrade & Kasumovic 2005), an outcome that may be more likely if females reject early-arriving males. Second, sampling males would require the sustained presence of males on the web. This may be costly because cohabiting males may be kleptoparasites of prey caught by females (Watson 1993), and may engage in conspicuous courtship which can reduce foraging success (Craig 1986, Craig 1988, Herberstein et al. 2002), increase the risk of predation (Herberstein et al. 2002), and result in damage to portions of the web (Watson 1986, Elgar 1991, Anava & Lubin 1993). Consistent with this, there is indirect evidence that redback females minimize the attraction of subsequent males after an initial copulation by turning off sex pheromone production (Stoltz et al. 2007), although they may commence production again later in the reproductive season (Perampaladas et al. 2008).

In addition to allowing us to probe female choice mechanisms in a biologically relevant way, this study allowed examination of how residency status, male size, and courtship duration may interact to affect male success. This is interesting because a large male advantage in competition was documented elsewhere (Stoltz et al. 2008, 2009), but this apparently arises only when rival males encounter a female simultaneously. It seems more likely that one male will arrive prior to rivals in nature (male cohabitants range from 0 to 8 per web, Andrade 2003, pers obs) and thus have a window of uninterrupted courtship as simulated here. If these residents manage to mate prior to the arrival of intruders, premature cannibalism will be low and relative mating success high (e.g., Kasumovic & Andrade 2009). Regardless of the relative size of the resident however, an intruder will have a significant opportunity for success under this scenario if he arrives before the resident mates. Thus, rather than selection for a particular phenotype, female choice rules may favour flexibility in male behaviour as a function of context (e.g., Kasumovic & Andrade 2009).

In this study we show that females use threshold based decisions and the context of competition determines whether or not the threshold value is reached. Very few examples of choice via thresholds exist in the literature. This may be because this decision rule is rare, but it may also be because thresholds are plastic, or female tactics shift under different situations. Thus there may be advantages to examining dynamics of female choice criteria in species such as redback spiders, where mating interactions strongly reflect the decision rules of physically dominant females (due to female-biased size dimorphism). Here, use of this system has revealed an unexpected, major effect of a choice threshold on the success of male mating tactics.

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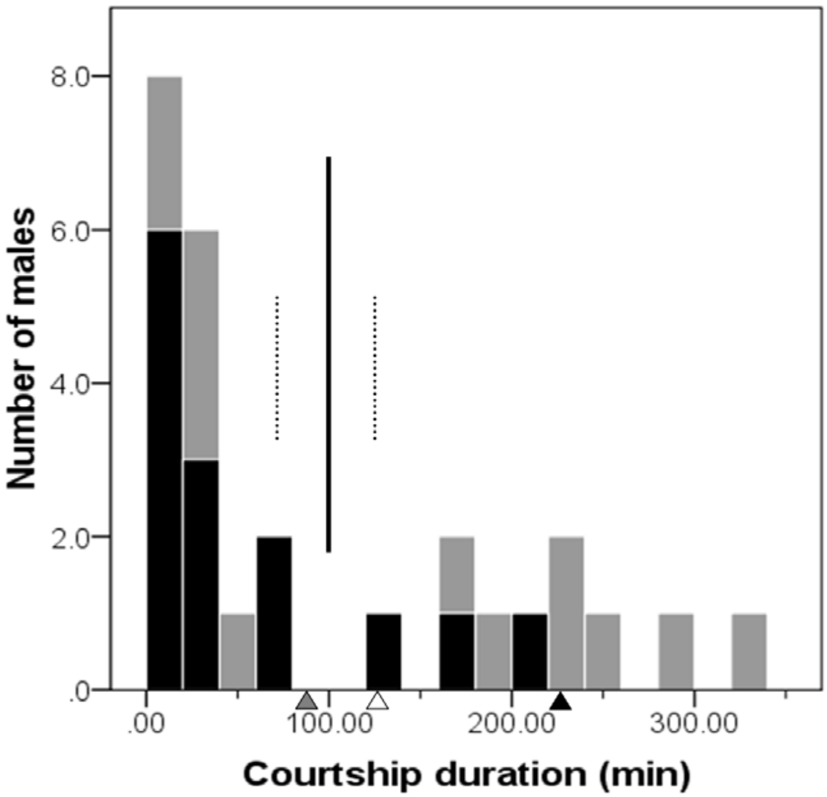
Table 4.1- Proportion of male redback spiders that obtained the first and second copulations in competitive trials as a function of their residency status and relative size.

Relative Size of Resident	Copulation	Status of copulating male			Statistic	P
		Resident	Intruder	Copulation did not occur		
All pooled	First	64% (N=36)	36% (N=20)	6	χ^2=4.57	0.03
	Second	58% (N=21)	42% (N=15)	26	χ^2=1.00	0.32
Smaller	First	74% (N=14)	26% (N=5)	1	χ^2=4.26	0.04
	Second	64% (N=7)	36% (N=4)	9	χ^2=0.82	0.37
Larger	First	59% (N=10)	41% (N=7)	3	χ^2=0.53	0.47
	Second	45% (N=5)	55% (N=6)	9	χ^2=0.09	0.76
Matched	First	60% (N=12)	40% (N=8)	2	χ^2=0.8	0.37
	Second	64% (N=9)	36% (N=5)	8	χ^2=1.14	0.29

Figure 4.1- Frequency histogram showing the relationship between premature cannibalism (yes = black, no = grey) and total courtship duration in redback spiders (data from Stoltz et al. 2008, n=27). A logistic regression predicts females are unlikely to prematurely cannibalize males that attempt mating if total courtship exceeds 100 minutes (bold vertical line; dotted lines = 95% confidence interval). Average total courtship duration prior to the first copulation is also indicated on the X-axis for single males (black triangle, from Stoltz et al. 2008), competing males introduced simultaneously (grey triangle, from Stoltz et al. 2009) and competing males in which a resident courts prior to introduction of a rival (white triangle, this study).

Figure 4.2- Relationship between total courtship duration prior to the first copulation by either male and whether males were prematurely cannibalized (black bars) or not (grey bars) across different competitive contexts (no competition, Stoltz et al. 2008; when a competitor is simultaneously introduced, Stoltz et al. 2008, 2009; or if the rival was introduced after a delay, current study).

Figure 4.3- Duration of courtship following rival introduction as a function of the size and residency status of the first male to mate. First-mating males were smaller residents (S.R.) or intruders (S.I.), larger residents (L.R.) or intruders (L.I.) or size-matched residents (M.R.) or intruders (M.I.).


Number of males
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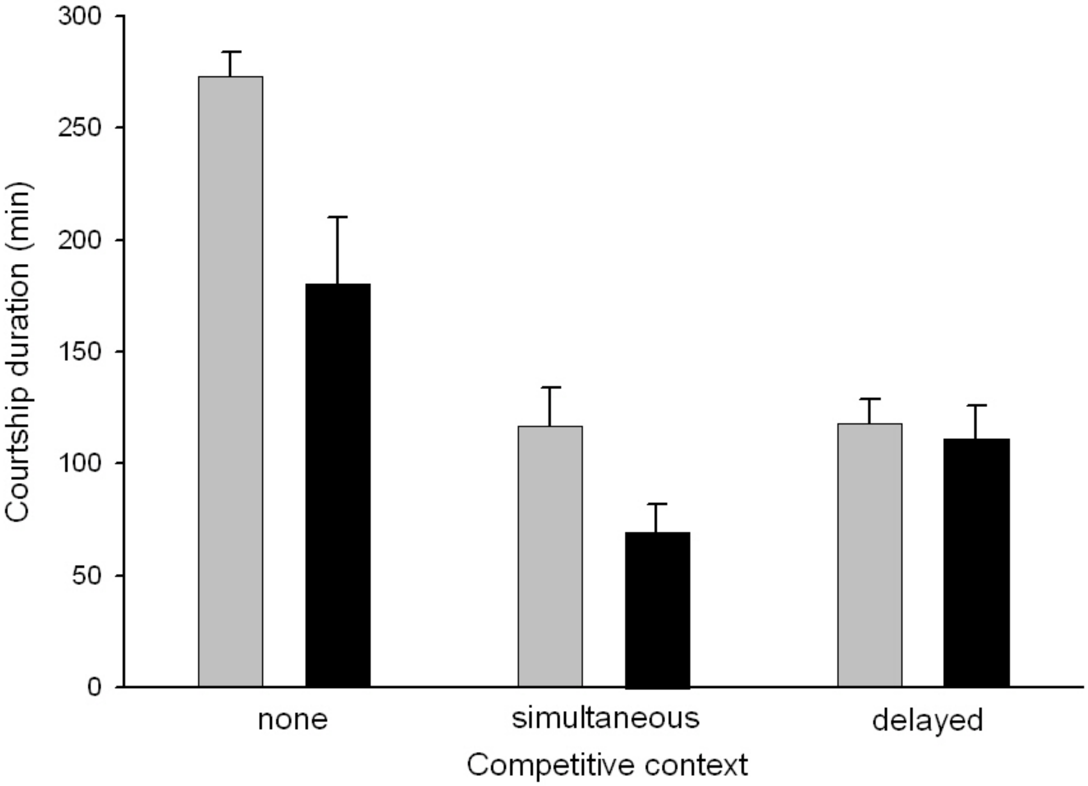
6.0
4.0
2.0
.0
.00
100.00
200.00
300.00
Courtship duration (min)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250
200
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100
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Courtship duration (min)
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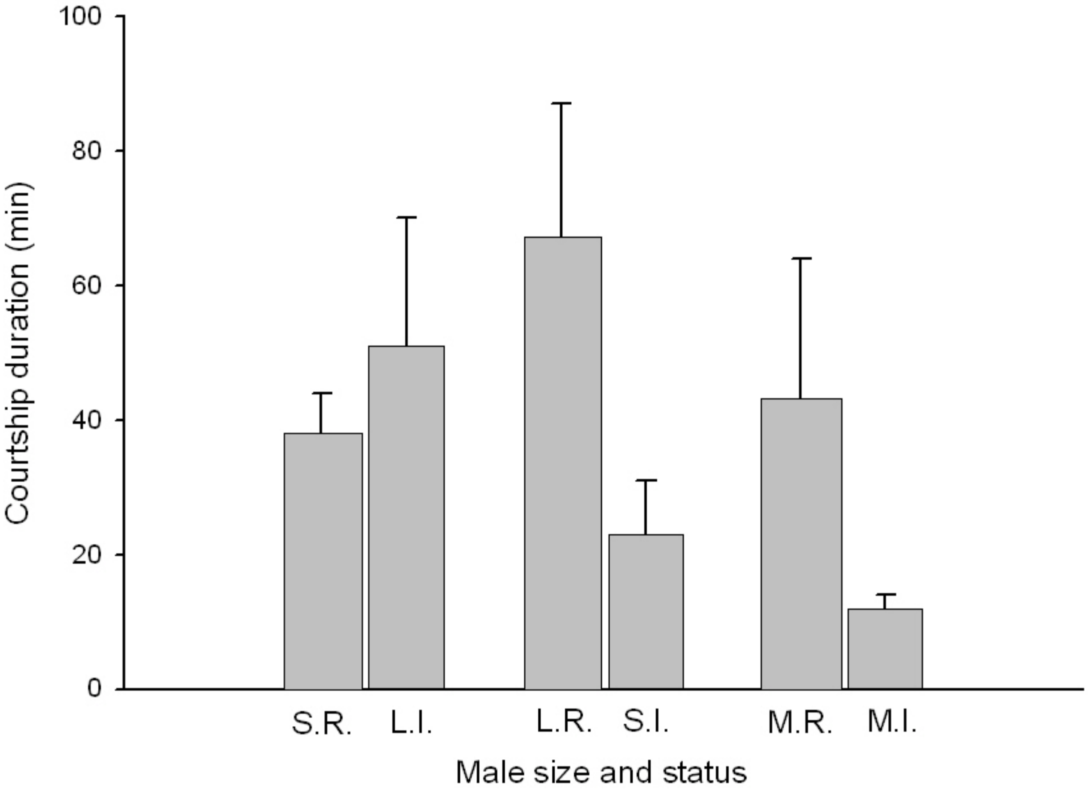
simultaneous
delayed
Competitive context


100
80
60
40
20
0
Courtship duration (min)
S.R.
L.I.
L.R.
S.I.
M.R.
M.I.
Male size and status

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Chapter 5
Female cryptic choice and a cost of simultaneous polyandry

Abstract

Genetic benefits have been proposed as one explanation for the widespread prevalence of female multiple mating. The importance of genetic benefits can be revealed in systems where females exert a strong post-copulatory influence on sperm use to bias paternity in favour of males with particular traits. Alternatively, if paternity is balanced across all mates, benefits to females can derive from genetic diversity of offspring. In this study we examine consequences of polyandry in Australian redback spiders (*Latrodectus hasselti*), in which independent storage of rival male's ejaculates by females (1) allows control of paternity, and (2) eliminates confounding effects of direct sperm competition. In our laboratory study, male phenotype was not correlated with paternity, but the remating intervals, which mirror two natural periods of female receptivity, determined the pattern of sperm use. After simultaneous mating, paternity was roughly split between rivals, but after a delay in remating, there was a paternity bias favouring the second male. However, there were also costs of polyandry-- decreased female reproductive output and longevity--and these costs were most severe with simultaneous mating. Our findings suggest that there are time-dependent costs of multiple mating which females need to balance with the benefits of polyandry to maximize fitness.

Introduction

In many species, females mate with more than one male within a single reproductive cycle (= polyandry, Thornhill & Alcock 1983; Ridley 1988; Keller & Reeve 1995; Birkhead & Møller 1998; Arnqvist & Nilsson 2000; Jennions & Petrie 2000; Simmons 2001, 2005), and this influences the evolution of male anatomy, behaviour and physiology (Smith 1984; Birkhead & Møller 1992). Understanding patterns of polyandry is critical to understanding the evolution of mating strategies and reproductive behaviour (Parker 1970; Smith 1984; Simmons 2001). The timing (e.g. Dewsbury 1988) and order of mating with different males (Voss 1979; Barker & Bellis 1988; Lorch et al. 1993; Barker 1994; Shine et al. 2000; Sauter et al. 2001; Snow & Andrade 2005; Nessler et al. 2007) can affect paternity and impose strong selective pressures on male mating behaviour and adaptations for sperm competition (but see Schwartz et al. 1989). Similarly, if variable paternity of offspring affects females' fitness, selection can act on females to shape paternity by establishing conditions for competition (Wiley & Poston 1996) and constraining effects of male adaptations to sperm competition (Jennions & Petrie 2000). For example, female sexual refractory periods after mating are well documented and can affect the timing and likelihood of polyandry (Thornhill & Alcock 1983). It has been suggested this can occur due to female responses to the physical stimulation of mating or sperm transfer (Sugawara 1979) or chemicals transferred by males (Chen 1984, Leopold 1976, Simmons & Gwynne 1991) among other explanations (Simmons & Gwynne 1991). Whether refractory periods represent male or female interests remains difficult to discern (Simmons & Gwynne 1991).

There are numerous costs associated with polyandry for females (Chapman et al. 1995; Stockley 1997; Prout & Clark 2000) including reduced time for foraging, increased exposure to predation and disease, injury and harassment by males (Daly 1978; Thornhill & Alcock 1983;

Watson et al. 1998). However, female fitness can increase with polyandry through the increased acquisition of resources (Thornhill & Alcock 1983; Dussourd et al. 1988; Gwynne 1988; Arnqvist & Nilsson 2000) as well as reduced harassment and protection from other males (Rowe 1992; Weigensberg & Fairbairn 1994). Polyandrous females can also indirectly benefit by producing offspring of higher genetic quality (Yasui 1998; Jennions & Petrie 2000; Tregenza & Wedell 2000; Zeh & Zeh 2001; Neff & Pitcher 2005) which may have higher reproductive success and viability (Baer & Schmid-Hempel 1999; Fedorka & Mousseau 2002; Simmons 2005) than the offspring of monandrous females. Polyandry may also be beneficial because it diversifies offspring genotypes and phenotypes. Genetic diversity may be particularly important if offspring disperse to new habitats so that traits that are beneficial for parents are not necessarily so for offspring (Garant et al. 2005).

The literature is replete with examples of male mechanisms that maximize paternity under sperm competition (see Birkhead & Møller 1992), and some of these also reduce female fitness. A classic example is the accessory gland proteins found in seminal fluids (see Eberhard & Cordero 1995; Eberhard 1996; Arnqvist & Nilsson 2000), which stimulate egg production and reduce female receptivity, but also reduce female longevity in *Drosophila* (Fowler & Partridge 1989; Chapman et al. 1995, 1998). In addition, accessory substances can act as anti-aphrodisiacs that reduce attraction of rivals to females (Andersson et al. 2000), modulate sperm storage (Wolfner 1997; Gillott 2003), or degrade the sperm of competitors (Clark et al. 1995). Males may also exploit female mechanisms for monitoring insemination. For example, male butterflies (*Pieris napi*) exploit links between female receptivity and the number of stored sperm when they transfer anucleate sperm that are not able to fertilize eggs but nonetheless delay female remating (Cook & Wedell 1999).

Post-copulatory sexual selection may also derive from female mechanisms that bias paternity in favour of a particular male (cryptic choice, Eberhard 1996). Cryptic choice can occur by numerous mechanisms (Eberhard 1996), including selective sperm use (Birkhead 1998; Pitnick et al. 1999), diversion of resources away from zygotes fertilized by inferior mates (see Thornhill 1983; Sheldon 2000), or alteration of patterns of remating as a function of characteristics of first mates (Stoltz et al. 2009). Walker (1980) suggests that patterns of sperm precedence in many insects are the result of female utilization strategies, although this is often difficult to distinguish from the effects of variation in the competitiveness of sperm or biochemical effects of seminal fluids (LaMunyon & Ward 1998; Chapman 2001; Cornwallis & O'Connor 2009). Dissection of whether cryptic choice or sperm competition leads to variation in sperm use patterns is possible in taxa where ejaculates of different males may be stored separately by females or where matings by rival males are temporally separated (e.g., some insects, Ward 1993; see Simmons 2002, many spiders, Welke & Schneider 2009; for reviews see Elgar 1998; Eberhard 2004). Such species provide an opportunity to test for evidence of biases in sperm use by females (Hellreigel & Ward 1998; Simmons 2001; Eberhard 2004) and intersexual conflict (Schneider & Lubin 1998).

Here, we staged matings between females and pairs of males of the Australian redback spider (*Latrodectus hasselti*), with remating intervals, mimicking expected timings in nature (two matings in quick succession or the second mating following a 2 month delay, Perampaladas et al. 2008). The timing of female advertisement may be considered a mechanism of indirect female choice as this affects the subset of males that may mate and achieve paternity (Wiley & Poston 1996). Our goal was to determine patterns of paternity and costs of polyandry as a function of the timing of remating (e.g. Radhakrishnan & Taylor 2007; McNamara et al. 2008a; Yamane et al. 2008), while removing possible effects of sperm competition. Female redback spiders store sperm in two separate sperm storage organs for their entire life (Andrade & Banta 2002) and suffer little decline in reproductive output during this time (below-average yield has been shown to occur only after females produce 15 egg sacs; Andrade & Banta 2002), suggesting a single copulation confers ample sperm to fertilize all their eggs (Andrade & Banta 2002). Nevertheless, some females are polyandrous in nature (Andrade 1996) and the frequency and order of mating influences paternity. There is strong first male sperm precedence if a male is able to achieve two copulations (Snow & Andrade 2005) due to the deposition of sperm plugs in each spermatheca (Snow et al. 2006). However females can ensure males inseminate only one spermatheca by killing them after the first copulation (Stoltz et al. 2008; Stoltz & Andrade 2010). If a rival inseminates the other spermatheca within 24 hours (hereafter called 'simultaneous' mating), paternity is shared (P2 ~ 50±28%, Snow & Andrade 2004, 2005). This is one likely scenario for polyandry in nature in this species, as in the period shortly after a female's first copulation, active sex pheromones in the web-silk may lead to the attraction of rival males and possible remating (Stoltz et al. 2007; Jerhot et al. 2010). During courtship, the webs of virgin females are partially dismantled by males and rebuilt by females after copulation. When rebuilt by females they no longer contain pheromones (Stoltz et al. 2007) and do not attract males (Andrade & Kasumovic 2004; Kasumovic & Andrade 2009). Indirect female choice (Wiley & Poston 1996) has been suggested to provide a second window for female remating months later when females resume pheromone production (Perampaladas et al. 2008, hereafter called 'delayed' mating). The fact that females turn off pheromone emission after an initial mating, halting mate attraction (Stoltz et al. 2007), and re-advertise receptivity later in the season (Perampaladas et al. 2008) leads us to assume similar processes may underlie mechanisms of refractory periods observed in many insects (Chen 1984, Leopold 1976, Simmons & Gwynne 1991). Whether the outcome of sperm competition differs during these windows of receptivity is unknown. If sperm competition depends on female-based variation in mate attraction, this would suggest indirect female choice (Wiley & Poston 1996) may have very broad implications for sexual selection.

We used the sterile male technique (Boorman & Parker 1976; Snow & Andrade 2005) to assess patterns of paternity when the ejaculates of rival males were in separate spermathecae and mating was' simultaneous' (inter-mating interval of 24 hours) or 'delayed' (inter-mating interval of two months). If paternity is determined by a 'raffle' process, we predict similar patterns of paternity between simultaneous and delayed competitions (Parker 1990; Parker et al. 1997; Parker 1998, as suggested in Snow & Andrade 2004 for insemination of separate spermathecae). In contrast, if females are able to bias paternity (cryptic choice), we have different predictions depending on the function of polyandry for female redbacks. First, if polyandrous females benefit from genetic diversity of their offspring, (reviewed in Jennions & Petrie 2000) we expect females to bias paternity towards the second male in the 'delayed' treatment to hedge bets against the genetic similarity of offspring produced earlier. Second, if females bias paternity towards males with good genes or 'sexy' genes, paternity should be related to relative measures of male phenotype or performance (e.g. weight: Stoltz et al. 2008, 2009, courtship duration: Stoltz & Andrade 2010) regardless of the remating interval. Finally, although sperm are stored separately they may interact briefly when released to fertilize eggs (Foelix 1996). If sperm use patterns depend on features of the sperm themselves, this may produce unexplained variation in paternity, or may be indistinguishable from female choice for good genes (e.g., if male mating traits correlate with sperm competitiveness or some other unmeasured trait, Keller & Reeve 1995).

We also examined whether or not polyandry had direct effects on female fitness, and whether such effects depended on remating interval. In redbacks, all sperm transfer occurs in the first five minutes of mating, but copulations last between 11 and 25 minutes (Andrade 1996; Snow & Andrade 2004), suggesting males have substantial time to transfer seminal fluids or proteins. Seminal proteins are little-studied in spiders but there is considerable variability in the type and characteristics of seminal proteins within and across species (see Michalik 2009). In other taxa, seminal proteins can affect female reproductive output or longevity (positively, e.g., Pivnick et al. 1987; Schrempf et al. 2005 or negatively e.g., Chapman 2008). Male-mediated effects on female fitness could be incurred after mating with a single male, but could also accumulate in intensity with polyandry, and/or show time-dependent effects (Radhakrishnan & Taylor 2007; McNamara et al. 2008a; Yamane et al. 2008). Therefore we tracked female longevity and lifetime reproductive output in our two mating treatments. If seminal products transferred to females during mating are harmful, and such effects are cumulative, we expect a direct cost of polyandry in the survivorship and/or reproductive output of ‘simultaneous’ females relative to ‘delayed’ females prior to their second mating (when delayed females have mated only once). If such effects are time-dependent (Radhakrishnan & Taylor 2007; McNamara et al. 2008b; Yamane et al. 2008), lifespan and reproductive output of ‘simultaneous’ females (single mating by each of two rivals within 24 hours) should also be reduced relative to ’delayed’ females after all females have mated twice (where the second copulation by a rival was after a two month interval). Alternatively, if seminal products are beneficial to females, then longevity and reproductive output of ‘simultaneous’ females should exceed that of ‘delayed’ females prior to their second mating. After all females have mated twice, longevity and reproductive output of females in each treatment may be similar, or, with time-dependent effects, females in the simultaneous treatment may have higher reproductive output or longevity.

Methods

Study animals

Spiders were from an outbred laboratory population of *L. hasselti* originally collected from Sydney, Australia (2007). Spiderlings were reared communally until the 4th instar after which they were housed individually to ensure that they had not mated (males mature at the 5th instar, females at the 7th-8th instar). The spiders were kept in a temperature controlled room at 25ºC 12:12 light:dark cycle. Males were fed fruit flies (*Drosophila* sp.) twice a week and females were fed crickets (*Acheta domesticus*) once per week.

Mating Trials

Prior to trials, adult females were placed in mating arenas (35 cm x 30 cm x 15 cm) for 24 hours where they built webs on inverted U-shaped metal frames. All trials were conducted under the dark cycle illuminated by red light since L. hasselti are nocturnal.

Treatments

Virgin females that had matured within the last two months were randomly assigned to either a simultaneous or delayed mating treatment. Each female mated with a total of two males, each of which was allowed a single copulation (matings with single copulations are not uncommon in the field and laboratory; Andrade 1996; Snow & Andrade 2005; Stoltz et al. 2008). In the simultaneous treatment, the rival was allowed to mate within 24 hours of the first copulation, in the delayed treatment, rivals mated approximately 2 months after the first copulation. Variation in the availability of mature male spiders sometimes necessitated that delayed trials were run a few days prior to or after the 2 month target (mean ± s.e., inter-copulatory interval: 62±3 days).

Females were fed crickets once per week throughout their adult lives. There was no difference in the weights of females in the simultaneous (287±15) and delayed treatments (275±14; independent t39= -0.63, p=0.53) at the beginning of the experiment. The weight of females in the delayed treatment was similar prior to the first (275±15) and second mating trials (2 months later, 236±15; paired t21=1.85, p=0.08).

Males were chosen randomly from among males that matured within the 7-14 days prior to a trial, as long as they were unrelated to experimental females and to their rival. Males were not fed as adults, since adult males do not capture prey in this species (Foelix 1996). Male mass did not differ between treatments (simultaneous: 4.34 ± 0.17 mg; delayed: 4.10±0.18 mg; Kruskal Wallis chi-square =2.65, df=3, p=0.45).

Palp manipulation

All males were manipulated to ensure rival males inseminated opposite sperm storage organs (e.g., Snow & Andrade 2005) and to allow assessment of paternity using a sterile male technique (Boorman & Parker 1976, see below). Males were briefly anaesthetized with CO_2 and either the left or right embolus was removed (apical portion of the palp inserted in the female at copulation; see Bhatnagar & Rempel 1962; Snow & Andrade 2005) using micro-dissection scissors. Removal of the sclerotized embolus does not affect male behaviour, and redback males show no overall pattern of 'handedness' in initial inseminations (Snow & Andrade 2005). Embolus-amputated males perform all normal courtship behaviours but are unable to copulate using the manipulated palp (Snow & Andrade 2005). Since palp insertion is ipsilateral, removing the right embolus ensures insertion in the female's left spermatheca and vice versa. For each pair of males then, we randomly assigned one male to left and one to right embolus amputation. Males were returned to their cages to recover from the amputation, which was completed approximately 12 hour before the start of mating trials.

Paternity

To assess paternity we used a sterile male technique (Boorman & Parker 1976) where females copulated with a normal (N) and an irradiated (R) male. Prior to the palp manipulation, one male from each competing pair was randomly assigned to the R treatment. R males were exposed to 9 krad of gamma irradiation at 0.75 krad min-1 from a Cs 137 source (Snow & Andrade 2005), 1-5 days prior to mating trials. As a result of deleterious mutations induced in R males' sperm, eggs that are fertilized by R sperm do not develop, whereas eggs fertilized by N males develop normally (Boorman & Parker 1976). This method does not appear to affect male behaviour or sperm phenotype (Henneberry & McGovern 1963; Boorman & Parker 1976) and has been used successfully to assess paternity in redbacks (see Andrade 1996; Snow & Andrade 2005). Males were mated to females in sequence and mating order is varied (NR or RN mating order) to allow tests of the assumption that R and N sperm are equally competitive (e.g. Boorman & Parker 1976). We confirmed that eggs fertilized by irradiated males never develop by mating control females to pairs of R males (RR control, n = 8, 100% irradiation success, consistent with Snow & Andrade 2005). In experimental pairings (NR or RN mating order), paternity of the N male was estimated as the proportion of eggs that hatch, where we controlled for the proportion of eggs that do not develop when females mate with two normal males (hatching failure rate of NN control: 1.4±0.004%, range 0-4%, n=13, see Boorman & Parker 1976 for calculations). Paternity is reported as proportion of spiderlings fathered by the second male to mate (P_2), by convention (Boorman & Parker 1976).

Mating Trials

On the day of a mating trial, males were weighed (Ohaus electronic balance) and measured (mean patella + tibia length of front legs using Nikon Simple PCI measurement software on digital images from a Nikon camera mounted on a Zeiss Stemi dissecting microscope). Male body condition was calculated using residuals of log (cubed root of male weight) regressed on log (size) (see Kasumovic & Andrade 2006 for justification of the use of a residual condition index in this species).

At the start of a trial, each male was allowed to dangle from forceps on a dragline and was introduced to a female's web at the furthest point from the female. Trials were filmed using Panasonic low light black and white cameras (WV-BP330) with macro zoom lenses and Sony Professional Super VHS recorders (SR-TS1U) for a maximum of 8 hours or until one copulation occurred after which he was removed. For each mating we recorded the time and duration of the copulation (Stoltz et al. 2008, 2009; Stoltz & Andrade 2010).

For the second mating, the rival had the opposite radiation exposure and embolus amputation manipulation. Trials followed the same procedure. For the simultaneous treatment females were left on mating frames overnight but for the delayed treatment, females were returned to rearing cages until 24 hours prior to the second mating. Each male spider was used in only one trial.

Twenty females were mated in the simultaneous mating treatment (11 RN, 9NR), however one trial was removed as one male copulated for less than the 5 minutes required to transfer all sperm (Snow & Andrade 2004). Seventy-six females (48 RN, 28NR) were placed in the delayed treatment as we expected mortality (see Andrade & Banta 2002) to reduce our sample size in this treatment. During the two month delay some females did not produce any eggs (8 R, 7N), suggesting they had not been successfully inseminated in the first copulation (females usually produce fertile eggs within 2 weeks of insemination when on our laboratory diets). In addition, many females died (29 R, 10 N) before their second copulation. This left twenty-two 'delayed' females that mated with two rival males and produced at least one eggsac before and at least one egg sac after the second mating (12 RN, 10 NR). After mating, females were checked daily and eggsacs were removed after they had been constructed. Sacs were opened after 15 days, and the eggs counted and classified as developed (spiderlings visible) or undeveloped (see Snow & Andrade 2005).

Analysis

Statistical analysis was performed using SPSS version 13.0. If log transformation data did not produce a normal distribution non-parametric statistics were used (rival trait comparisons in the delayed treatment). Statistical tests are two-tailed with mean ± SE reported. Sample sizes vary for some tests because not all observations for each trial were recorded.

Results

As found elsewhere (Snow & Andrade 2005), paternity of the second male (P_2) did not change in sequential egg sacs for females in a given treatment (repeated measures ANOVA: simultaneous $F_{2,20}=0.45$, $p=0.64$; delayed $F_{2,20}=0.41$, $p=0.67$), so below we report average paternity, either across all egg sacs (simultaneous treatment) or only across those egg sacs produced after 60 days (comparisons of simultaneous and delayed polyandry). Results were qualitatively similar (analyses not shown) when we completed these same analyses using only P_2 in the first egg sac (commonly used in sperm competition studies, see Snow & Andrade 2005).

There were substantial paternity differences between treatments (figure 1). We examined variation in average paternity (P_2) across all egg sacs produced after the second mating in a two-factor ANOVA (first factor: remating interval (simultaneous or delayed); second factor: mating order (NR/RN)). There was a significant effect of remating interval ($F_{1,37}$ =9.53, p<0.001) but not of mating order ($F_{1,37}$=1.404, p=0.24, which confirms that sperm competitiveness was not impaired by irradiation, see Snow & Andrade 2005), and no significant interaction effects ($F_{1,37}$=0.26, p=0.61). Similar results were obtained if we restrict this analysis to include only egg sacs produced after 60 days in the simultaneous treatment, so the time frame matches that for which data were collected in the delayed treatment (remating interval: $F_{1,26}$=5.06, p=0.03; mating order, $F_{1,26}$=0.53, p=0.47; interval x order, $F_{1,26=}$1.55, p=0.22).

In the simultaneous treatment, average paternity was diversified in most cases (mean P_2=49%, 95% CI: 37-61%, figure 1) with P_2 values between 20-80% in 79% (15/19) of the trials, and in only three cases (16% of trials) did the second male father more than 80% of offspring (figure 1). In strong contrast, after the second copulation in the delayed treatment there was significant second male sperm precedence (mean P_2=71%, 95% CI: 62-82% figure 1), with the number of second males achieving over 80% paternity almost tripling to 45% (10/22 trials), a significant increase relative to the simultaneous treatment (Fisher's exact test, p = 0.05).

In the delayed treatment, we also assessed total paternity of the first male across all of the egg sacs produced by a female in her lifetime using this expression:

$$\frac{MP_M}{M+D} + \frac{D(1-P_2)}{M+D} \qquad (1)$$

Where M and D are the number of egg sacs produced by females prior to (M) and after (D) the delayed second mating, PM is paternity in the M sacs, and P2 is paternity of the second male in the D sacs. Since PM is 1.0 (all eggs in M sacs are fertilized by the first male), this reduces to:

$$\frac{M + D(1 - P_2)}{(M + D)} \quad (2)$$

Although the first male fathered all offspring produced in the first 2 months (prior to the second mating), the 71% paternity of second males after this time reduced the overall first male paternity to 47% (by equation 2). The overall paternity of the first male was not significantly different from 0.5 (t_{22}=-0.37, p=0.71).

We analyzed possible correlates of paternity within each treatment separately. Variation in P_2 was not explained by the relative value (second male – first male) of any measured male traits (weight, body condition) or differences in features of the mating (courtship or copulation duration, table 1) in either treatment.

We determined whether our treatments lead to unintended variation in male mating behaviour using two separate ANCOVA models with duration of courtship or copulation as the dependent variable and with the independent variable a composite of mating interval and mating order (i.e., 4 levels, with males categorized as the first or second to copulate with a given female in the simultaneous or delayed treatment). Male weight was used as a covariate in both analyses, as male size may affect courtship or copulation duration (see Elgar et al. 2000; Schneider et al. 2000). There was not a significant interaction between male mass and treatment (p>0.29) in either analysis, so the interaction was dropped from the model. Copulation occurred on average after 3 hours of courtship (ANCOVA $F_{3,78}$=0.49, p=0.69), and lasted around 20 minutes, regardless of treatment (ANCOVA $F_{3,75}$ =0.70, p=0.55).

Lifetime reproductive output and longevity

We looked for the global effect of simultaneous versus delayed polyandry by testing for treatment effects on female lifetime reproductive output. Then we examined direct effects of polyandry alone on female reproduction by assessing variation in reproductive output within the 60 days following the first mating trials for (polyandrous) simultaneous females compared to delayed females (which were monandrous at that point). Finally, we examined whether direct effects of polyandry depended on the remating interval by testing for treatment effects on reproduction that occurred after 60 days (when all females are polyandrous; table 2). We performed six separate ANCOVA's which differed in our measure of reproductive output (number of eggs sacs or number of spiderlings per egg sac) and the interval we were examining (entire life, first 60 days after initial mating trials, post-60 days). Female mass was a covariate in each analysis. We found no significant interactions between treatment and female mass in any of the analyses so in each case the interaction was dropped from the model and the ANCOVA re-run (table 2). Lifetime reproductive output (number of eggs and spiderlings per egg sac) was greater for females that became polyandrous after a delay compared to females that mated with two males simultaneously (table 2, figure 2A); that is, the lifetime reproductive output depended on the timing of polyandry. In the first 60 days, when females in the delayed treatment were still monandrous, these females had a greater number of eggsacs containing on average more spiderlings compared to females in the simultaneous polyandry treatment. During the first 60 days, when controlling for female mass, individuals in the delayed treatment had more spiderlings in an egg sac than those in the simultaneous treatment (table 2, figure 2B). However, after 60 days, when females in the delayed treatment were now polyandrous, there was no longer a statistically significant difference in spiderlings per egg sac or the number of egg sacs subsequently produced (table 2, figure 2C).

We examined direct effects of polyandry on female survivorship by comparing the proportion of females that survived the 60 days following the first mating trial in each treatment. We used a logistic regression with spiderlings/sac as a covariate (to control for possible negative effects of reproductive output on longevity, Stoltz unpublished data). Monandrous delayed females had higher survivorship (77% of 56 survived) than polyandrous simultaneous females (68% of 19 survived) in this time period (logistic regression Wald=4.82, d.f.=1, n=75, p=0.03) with no interaction between treatment and reproductive output (logistic regression Wald=0.69, d.f.=1, n=75, p=0.41).

We next examined the effect of remating interval on longevity by using data only for those females that survived through the first 60 days of the experiment (and mated with a rival in the delayed treatment). We used ANCOVA with treatment as a factor (simultaneous or delayed), survival (days after the first mating) as an independent variable, and the average number of spiderlings per egg sac as a covariate. We did not find a significant treatment by covariate interaction so this term was dropped and the model reanalyzed (table 2). Females in the delayed treatment lived significantly longer than females in the simultaneous polyandry treatment and longevity was unrelated to reproductive investment (table 2, figure 3).

Discussion

In redback spiders, polyandrous females suffer direct decreases in longevity and reproductive output relative to females that were initially monandrous, and these effects were less acute when monandrous females obtained their second matings after a delay. For males, the timing of second matings also affects fertilization patterns such that paternity of new males is enhanced after a delay, but not when they copulate immediately after the first mate. When rival males mate in quick succession (simultaneous treatment), paternity is, on average, shared equally (replicating

Snow & Andrade 2005), conforming to predictions from a fair raffle model for sperm use (Parker 1990). In contrast, when there is a delay before the rival mates, there is strong second male sperm priority. This is unlikely to be the result of the depletion of sperm transferred by the first male, as single copulations provide sufficient sperm to fertilize the female's lifetime egg production (Andrade & Banta 2002). Neither is it likely that this effect is mediated by increased competitiveness of sperm from the second male, as in this study paternity does not correlate with male phenotype, and ejaculates of rival males are stored independently. Rather, this bias is likely the result of females cryptically favouring sperm from a second male that mates after a delay. Intriguingly, the overall effect of this bias is that average paternity of the two males is roughly equal across the total number of offspring produced by the female. Our results also show that polyandry is costly to females however, and the timing of remating can affect female fitness. An initial reproductive advantage of delaying polyandry translated into higher lifetime reproductive output and higher survivorship. These results suggest that males transfer toxic substances in their ejaculates with additive effects on reproduction, and time-sensitive effects on female longevity. Our findings also suggest the widespread occurrence of delays in female remating in many species (e.g. refractory periods) could be a result of selection on females to maximize the benefits of polyandry while reducing the costs.

It seems likely that the negative effects of polyandry for females result from physiological responses to proteins in the male's ejaculate (e.g., Chapman et al. 1995). Extensive work on *Drosophila* has demonstrated that seminal proteins can have profound effects on female reproductive output and longevity (reviewed in Chapman 2008). Although little is known about seminal proteins in spiders, recent work has shown diverse proteins are present in spider ejaculates (Michalik 2009), and in one species in the same family as redbacks (Theridiidae), the female's spermatheca is largely filled with seminal proteins after copulation (Michalik et al.

2010). In redbacks, copulation typically lasts approximately 20 minutes but all of the sperm is transferred within the first 5 minutes (Snow & Andrade 2005), suggesting that males may be transferring other substances with their ejaculate (Eberhard & Cordero 1995; Eberhard 1996). Why females allow copulation to extend beyond the time needed to transfer sperm is unknown but may arise from sexual conflict over copulation duration. In redbacks, females may be able to negate the effects of a single copulation or two copulations separated by a period of time (i.e. in our delayed treatment), but the volume of proteins introduced by two rivals that mate simultaneously may adversely affect a female's ability to neutralize harmful chemicals contained within the ejaculate. The effect of the dosage of seminal products received on female fitness has been investigated directly only recently (Radhakrishnan & Taylor 2007; McNamara et al. 2008a; Yamane et al. 2008). In a striking study on almond moths (*Cadra cautella*), an increased dosage of seminal products increased egg production and longevity (McNamara et al. 2008a). In this case, the effect of seminal products was positive for females. Products transferred within the ejaculate may contain compounds critical to enhance female longevity under certain environmental conditions (Ivy et al. 1999) or may act to decrease it in others (McNamara et al. 2008b). Understanding the impact of seminal products on female longevity and fitness requires consideration of both the conflict of interests between the sexes in a given species and the environment in which reproduction takes place.

Our results show a strong bias towards use of sperm from second males when there is a long delay (60 days) between copulations relative to when matings by rival males occur within 24 hours (figure 1). Such an outcome could be the result of sperm competition or cryptic choice, but our results suggest that latter mechanism is responsible for a number of reasons. First, since our methodology ensures independent storage of ejaculates, any sperm competition effects are restricted to a brief period when sperm mix with eggs at fertilization immediately before they are laid (Foelix 1996), and this eliminates many possible mechanisms related to inter-ejaculate interactions (e.g., ‘sexually selected sperm’ advantage for particular males, Keller & Reeve 1995, or inactivation of rival sperm, Kura & Nakashima 2000). Second, although the observed time-related effects could arise if older sperm are less successful when sperm are released for fertilization (e.g. Brown 1985), this is unlikely in the time frame used in this study. In spiders each sperm cell is encapsulated in a protein sheath until activated just before fertilization (Foelix 1996). In redbacks, inactive stored sperm can be used for up to two years after mating (the maximum lifespan of the female, Andrade & Banta 2002). Third, the shift in paternity could arise if sperm of the first male is depleted, resulting in a disadvantage in raffle sperm competition (Parker 1970). In the delayed treatment, females produced an average of 4 egg sacs using the first male’s sperm prior to the second mating. However, this is unlikely to cause the large-scale shift in paternity seen here (figure 1). If sperm depletion explained these results, P_2 should have increased over subsequent egg sacs in the delayed treatment (but this was not the case). In redbacks, sufficient sperm are transferred in a single copulation to fertilize the female’s lifetime egg production. There is no decrease in fertilization success of these sacs until females have produced 15 egg sacs (more than three times as many as were produced during the inter-copulatory interval in this experiment), and even this decline is minor and more likely due to female senescence rather than sperm depletion (Andrade & Banta 2002). Thus males clearly transfer sperm far in excess of the number required by females in normal copulations, and since all copulations in this experiment lasted longer than 5 min, all males would have had sufficient time for total sperm transfer (Snow & Andrade 2004, see Schneider et al. 2000). Together, this suggests that the observed shift in paternity with mating interval likely arises from female mechanisms. Although it is unclear how females could differentially use sperm from the two storage organs (see Berendonck & Graven 2002), the physical separation of the ejaculates makes differential release at fertilization a possibility (also see Schneider & Lesmono 2009).

The female's apparent active adjustment of the paternity of novel males in the delayed treatment suggests remating might yield genetic benefits to females, and our data best fit the 'genetic diversity' hypothesis for polyandry. We found no support for the alternative hypotheses. First if polyandry is favoured because it allows the acquisition of heritable benefits for offspring (Jennions & Petrie 2000) through 'trading up' to higher quality fathers (Pitcher et al. 2003), we should have found relationships between indicators of relative male quality or attractiveness and paternity (table 1). This was not the case as paternity was not related to measured traits that reflect male quality in redbacks (body condition, Kasumovic et al. 2009), or that affected mating decisions of redback females in other studies (e.g., weight, courtship duration, Stoltz et al. 2009; Stoltz & Andrade 2010, table 1).

Although previous work shows that redback females assess males using a courtship duration threshold, in our experiment, all males exceeded the threshold (~100 minutes, Stoltz & Andrade 2010), so variation in this aspect of male attractiveness could not explain our results. In this experiment, paternity was on average evenly split between males across the female's lifetime, and this is contrary to expectations if females were biasing paternity towards particular males. However, this pattern would maximize genetic variability of offspring (Yasui 1998; Neff & Pitcher 2005). The ability for females to control the paternity of their offspring may be beneficial for females if offspring must perform under conditions that are unrelated to those experienced by parents. Redback spiderlings disperse by ballooning to unpredictable habitats, so traits that increase success of parents may not be the same traits that will maximize success of offspring. In our simultaneous mating trials, paternity was split between the males, a result consistent with a genetic diversity hypothesis. It is tempting to speculate that the paired independent spermathecae of many spider species could be an evolutionary result of selection for mixed paternity of offspring. However, given the paired spermathecae, partitioning paternity in our simultaneous mating treatment requires only equal sperm release from each spermatheca after mating twice, with no evidence for conditional sperm use. Strong support for a genetic diversity benefit is provided by the delayed re-mating treatment, where females produced a number of offspring with one male before mating with a new male. Here, balanced paternity from both males would require an increase in sperm use favouring the second male. The shift to 71% paternity of the second male at this point functionally equalized paternity across the two males (overall paternity of the first male in the delayed treatment across all egg sacs equaled 47%). Determining whether this tactic increases female fitness would require measuring the success of offspring sired from singly and multiply mated females at different times of the reproductive season under (simulated or actual) field conditions.

If genetic diversity is the favoured outcome for a female's offspring, females should seek matings with multiple males, but our results suggest females should delay second matings, effectively leading to indirect female choice (Wiley and Poston 1996). Laboratory trials show females often mate with only one of two simultaneously competing males (Stoltz et al. 2008, 2009; Stoltz & Andrade 2010), and, after the initial mating, cease advertising for additional mates until after they have produced several egg sacs (Stoltz et al. 2007, Perampaladas et al. 2008). This is consistent with our data on costs of immediate polyandry. We show that immediate multiple mating would decrease female reproductive output (fewer eggs and spiderlings per egg) and longevity relative to females that mate only once (figure 2). Even though the reproductive output of twice-mated females in our delayed polyandry treatment was similar to females in the simultaneous polyandry treatment (figure 2), the initial advantage of higher offspring production of females during the inter-mating interval led to a marked increase in total lifetime reproductive output of females that delay polyandry (figure 2). Similarly, Maklakov and colleagues (2009) demonstrated that multiple mating can lead to reductions in offspring production in seed beetles (*Callosobruchus maculates*). A second, even stronger benefit of delaying polyandry is clear in female survivorship patterns. Females in the delayed treatment had higher survivorship even after mating a second time. Although survivorship was lower in the simultaneous treatment it appeared as though increased reproductive investment prolonged their lives whereas in the delayed treatment reproductive investment was unrelated to longevity. Thus, if there is a benefit to polyandry, females should mate more than once, but should seek to do so after a delay of months. Consistent with this prediction, other work shows that mated female redbacks resume producing sex pheromones approximately 2 months after their initial mating (Perampladas et al. 2008). Thus, studies of sex pheromone production suggest that, in nature, females attract courting males when they are virgins, then become invisible to males while they begin producing egg sacs. A few months later, they again advertise receptivity. If females remate in this second window, costs of polyandry will be significantly reduced (figures 2, 3). Of course, while this may minimize costs of polyandry, it will also affect when potential benefits of polyandry are realized. For females using this tactic, cohorts of spiderlings produced during the start of the mating season will be fathered by a single male, and only at the end of the season (approximately 3 to 4 months later), would diverse offspring be produced. It may be that genetic diversity is most critical at the end of the season, as these spiderlings will overwinter and compete as adults under conditions in the following year.

Conflict between the sexes over mating frequency, duration and fertilization (Parker 1979) is thought to place strong selection pressures on males as failure means they may not produce offspring at all, whereas for females, the outcome of conflict affects offspring quality

(Parker 1984). However, when fertilization is internal, females may have significant control over the movements of sperm and eggs (Ward 1993). Predicting how these issues are balanced to produce a resolution of conflict between the sexes is complicated (Knowlton & Greenwell 1984; Parker 1984) and effects are obscured by the ongoing inter-sexual arms race in a given species (Arqvist & Rowe 2002). In redback spiders a female's independent sperm storage organs provide an opportunity for females to directly block male control of fertilization. Males inseminate each sperm storage organ in a separate copulation, and females can kill males to prevent a second copulation (Stoltz et al. 2008). Here, we provide evidence that the resolution of this conflict is typically in favour of females (also see Elgar et al. 2000) and they are able to bias paternity to favour new males that mate after a delay (figure 1).

The effects of inter-mating intervals and mating order on paternity remain a fundamental question in the study of sperm competition (Dewsbury 1984). Here, we provide evidence that redback females exercise control over the outcome of paternity by biasing paternity if favour of novel males. However, polyandry can have significant costs in the form of reduced longevity when multiple mating occurs over acute periods, whereas a delay in re-mating apparently allows females time to recover from the harmful effects of substances transferred during copulation. Such considerations have important implications for female mating strategies and will dictate the timing of mate attraction that optimizes fitness.

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Table 5.1- Relationship between P_2 and the relative traits of rival males, or relative features of first and second matings (values for second male – values for first male) within two mating treatments where paternity of the second male is measured as the average value across all egg sacs produced in the female's lifetime or only across egg sacs produced after 60 days (values within brackets).

Factor	r^2	N	P
	Simultaneous Treatment		
Weight	0.07 (0.32)	20 (8)	0.28 (0.14)
body condition	0.002 (0.03)	19 (8)	0.84 (0.60)
Courtship duration	0.02 (0.11)	19 (8)	0.56 (0.42)
copulation duration	0.04 (0.25)	19 (8)	0.43 (0.21)
	Delayed Treatment		
	spearman rho*		
Weight	(-0.18)	(22)	(0.43)
body condition	(0.13)	(22)	(0.58)
courtship duration	(-0.41)	(22)	(0.06)
copulation duration	(0.29)	(22)	(0.19)

*paternity was not normally distributed in this treatment

Table 5.2- Reproductive output of female redback spiders throughout their lives, during the first 60 days (when females in the simultaneous treatment were polyandrous relative to females in the delayed treatment that were monandrous) and after 60 days (delayed treatment females were now polyandrous) (A), and the longevity of females after 60 days since their initial mating (B) in redback spiders.

ANCOVA

(A) REPRODUCTIVE OUTPUT	F	d.f.	p
Lifetime			
Eggs			
Treatment x mass	0.08	1,41	0.77
Treatment	9.69	1,41	**0.004**
Mass	0.002	1,41	0.97
Spiderlings			
Treatment x mass	0.004	1,41	0.95
Treatment	6.48	1,41	**0.02**
Mass	3.20	1,41	0.08
Between 0 – 60 days			
Eggs			

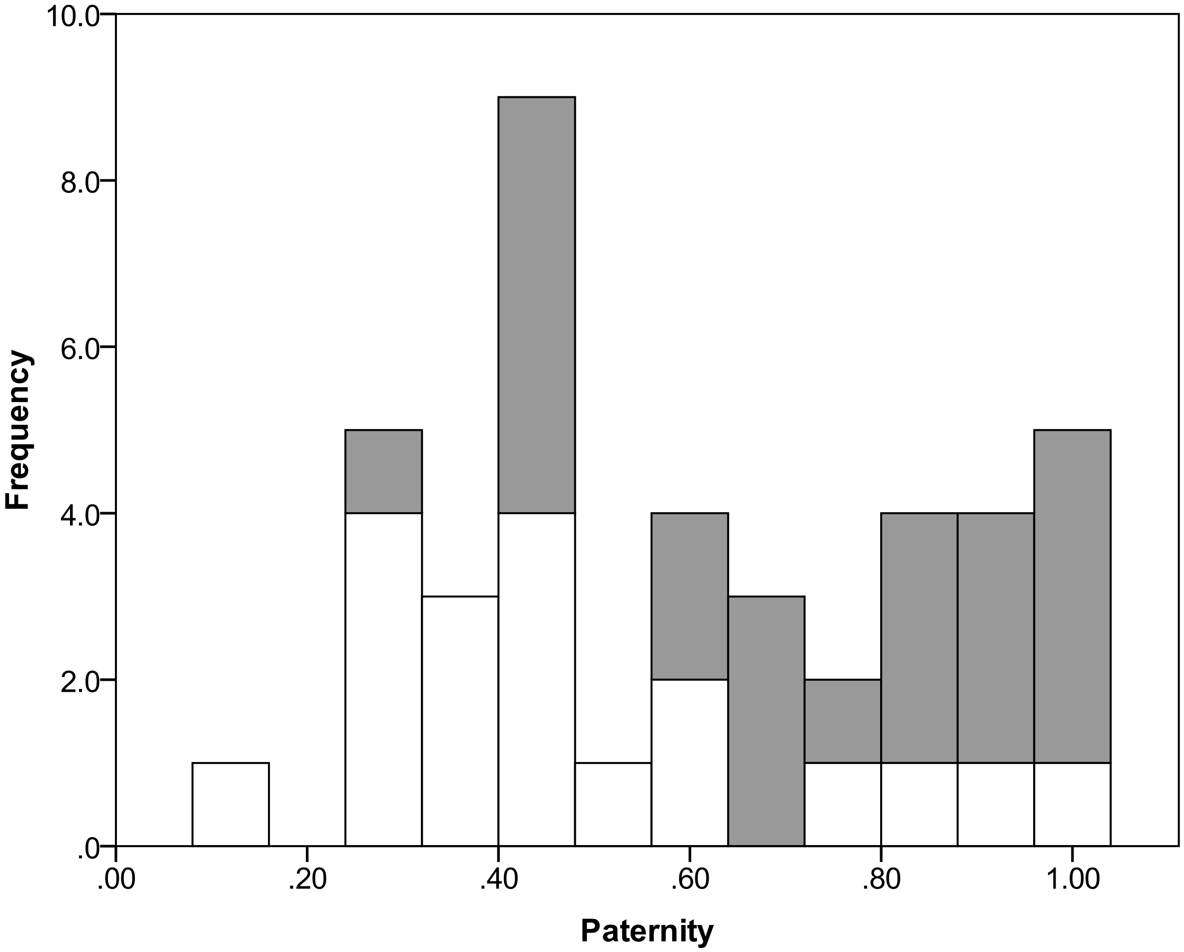
Treatment x mass	0.003	1,41	0.96
Treatment	11.87	1,41	**0.001**
Mass	0.21	1,41	0.65
Spiderlings			
Treatment x mass	0.55	1,41	0.47
Treatment	17.82	1,41	**<0.001**
Mass	6.66	1,41	**0.01**
After 60 days			
Eggs			
Treatment x mass	0.02	1,30	0.88
Treatment	0.19	1,30	0.67
Mass	<0.01	1,30	0.99
Spiderlings			
Treatment x mass	0.32	1,30	0.58
Treatment	1.25	1,30	0.27
Mass	1.41	1,30	0.25
(B) LONGEVITY			
Treatment x spiderlings per egg	0.002	1,35	0.96
Treatment	5.21	1,35	**0.03**

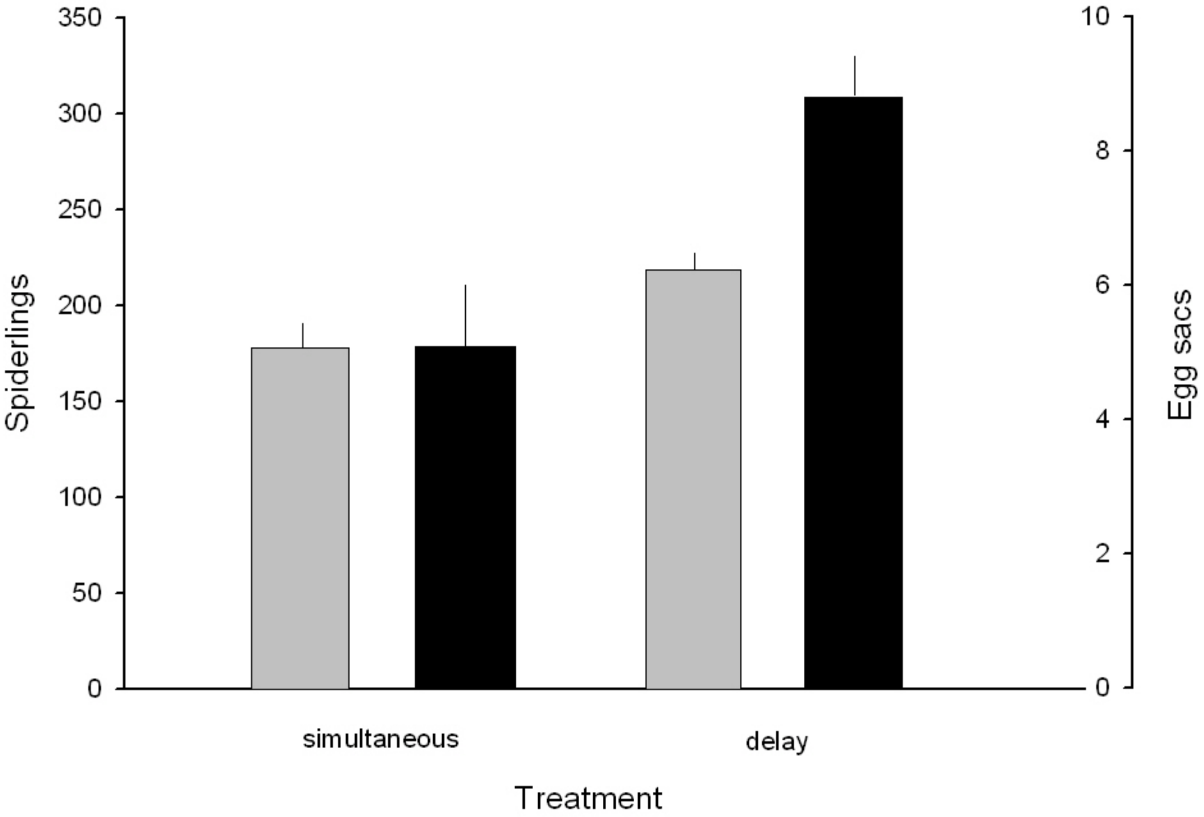
Spiderlings per egg	0.02	1,35	0.90

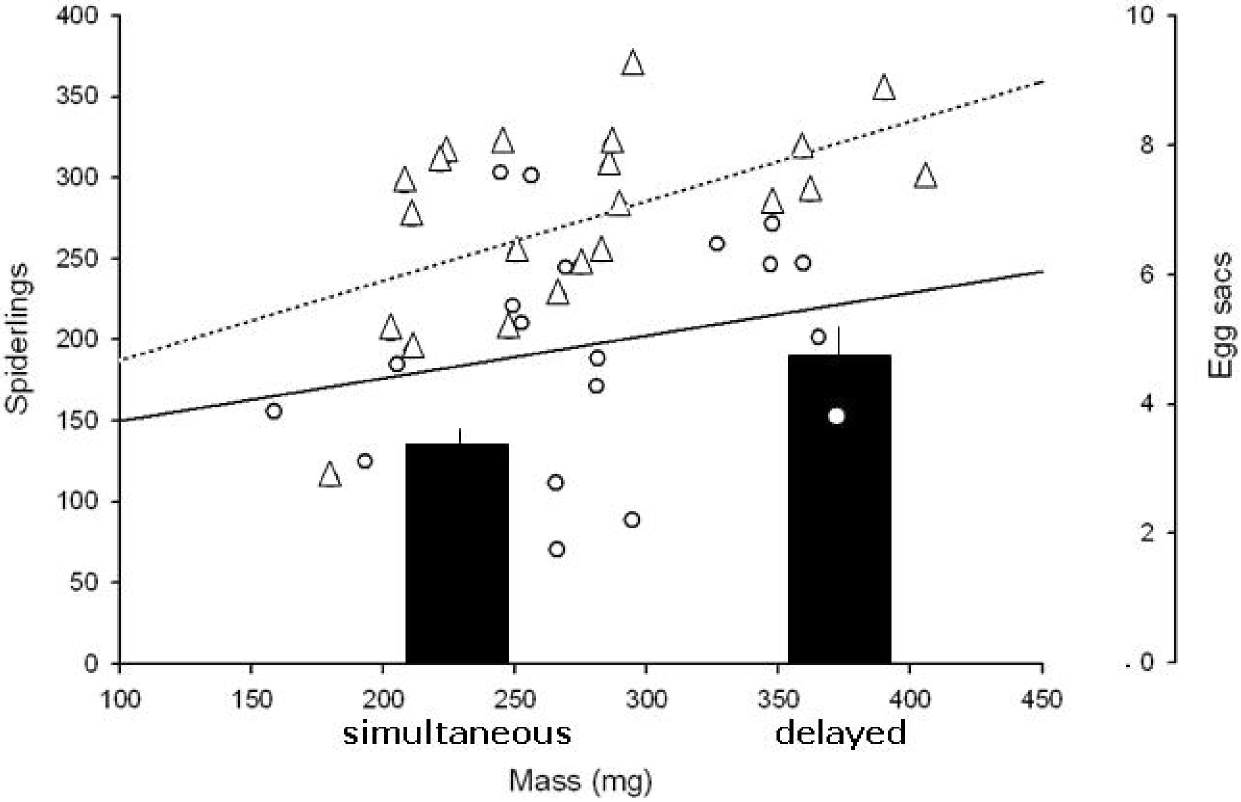
Figure 5.1- Histogram of paternity of the second male to mate when two rival males copulated within 24 hours of each other (‘simultaneous’, open bars) or after a delay of two months ‘delayed’, filled bars).

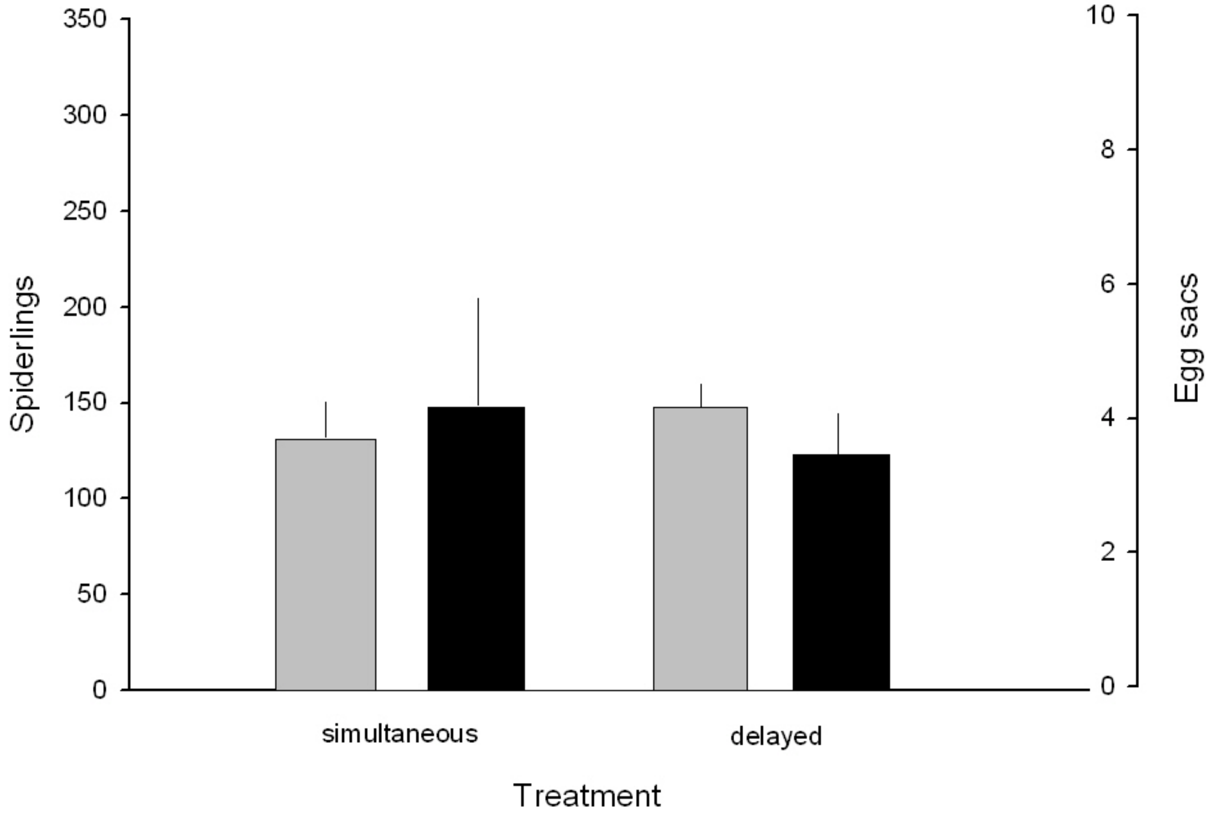
Figure 5.2- Mean (± S.E.) reproductive output of females measured as number of egg sacs (black bars) or average spiderlings per egg sac (grey bars) throughout a female’s lifetime (A), during only the first 60 days. Spiderling data of delayed (triangles, broken line) and simultaneous treatments (circles, solid line) are presented as a scatter plot since the female mass covariate was significant (B), and after 60 days (C).

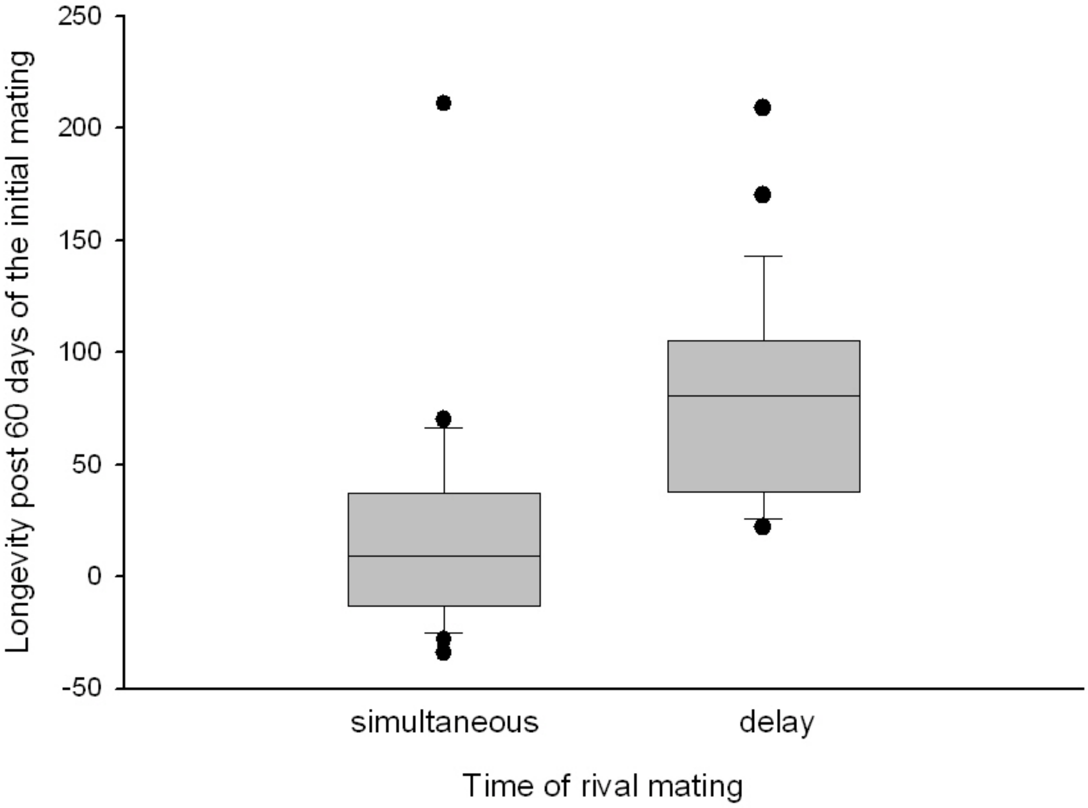
Figure 5.3- Lifespan (days following initial mating) of females that copulated with two males in delayed (closed circles, solid line) or simultaneous (open circles, dashed line) polyandry mating treatments as a function of their reproductive investment.


10.0
8.0
6.0
4.0
2.0
.0
Frequency
.00
.20
.40
.60
.80
1.00
Paternity


350
300
250
200
150
100
50
0
Spiderlings
10
8
6
4
2
0
Egg sacs
simultaneous
delay
Treatment


Spiderlings
Egg sacs
400
350
300
250
200
150
100
50
0
10
8
6
4
2
0
100
150
200
250
300
350
400
450
simultaneous
delayed
Mass (mg)


Spiderlings
350
300
250
200
150
100
50
0
Egg sacs
10
8
6
4
2
0
simultaneous
delayed
Treatment


Longevity post 60 days of the initial mating
250
200
150
100
50
0
-50
simultaneous
delay
Time of rival mating

Chapter 6
Longevity cost of remaining unmated under dietary restriction

Abstract

1. Increases in longevity from dietary restriction (DR) have been linked to the evolution of physiological strategies for maintaining lifetime reproductive output despite periods of nutritional deficiency. If this is the case, then reproductive life history and mating status should determine whether or not dietary restriction increases longevity.

2. We investigate the effects of DR and mating status (mated or unmated) on longevity and lifetime reproductive output and explore mechanisms underlying these patterns by measuring resting energetic rates and changes in mass over time. We study iteroparous female Australian redback spiders (*Latrodectus hasselti*), which are known to tolerate periods of starvation (ecologically relevant DR) in nature.

3. Mated DR females had the highest longevity of any experimental group, in strong contrast to results for semelparous males of this species (Kasumovic, Andrade & Brooks 2009). Most strikingly, mated females (whether DR or not) had higher longevity than unmated females.

4. Mated females rapidly decreased their resting energetic rates and stopped egg production under DR. Once feeding returned to normal, resting energetic rates of mated females increased immediately, egg sac production continued and there was no net decrease in lifetime reproductive output relative to mated females without DR.

5. This shows DR increases longevity for mated females, but there is a longevity cost of remaining unmated, which may arise because unmated females (regardless of diet) maintained high energetic rates. This may be an evolved pattern of physiological support for reproductive

readiness which exerts a fitness cost when mating is delayed. Taken together, these results suggest positive effects of DR on longevity critically depend on reproductive life history (iteroparity or semelparity) and individual mating status.

Introduction

One principal determinant of the evolution of longevity in all current evolutionary theories is the relative rate of extrinsic mortality (Williams 1957, Kirkwood 1977, Kirkwood 1996, Martin, Austad & Johnson 1996, Stearns et al. 2000). In species with high extrinsic mortality there is decreased selection for investment into somatic maintenance; however, prolonged life expectancy requires a relative increase in somatic maintenance (Williams 1957, Hamilton 1966, Charlesworth 1980, Rose 1991, Kirkwood & Austad 2000). Linked to the importance of extrinsic mortality is reproductive life history (timing and frequency of reproduction), as together these dictate the optimal allocation of metabolic resources between somatic maintenance and reproduction at different times in an individual's life (Lack 1966, Williams 1966, Hirshfield & Tinkle 1975, Calow 1979, Reznick 1985, Bell & Koufopanou 1986, Sibly & Calow 1986, Partridge 1989, Stearns 1989, Roff 1992, Kirkwood & Austad 2000). Dietary restriction (DR) has been shown to extend longevity in a variety of taxa suggesting a conserved mechanism linking energy intake to ageing (Kirkwood & Austad 2000). One mechanism through which dietary restriction has been proposed to prolong lifespan is a reduction in metabolic rate (Sacher 1977, Sohal & Weindruch 1996) due to the relationship between oxygen consumption and the generation of damaging metabolic by-products (Harman 1955, Sohal et al. 1994, Finkel & Holbrook 2000). Since life history responses to dietary restriction may shift along with shifts in allocation priorities, links between reproduction and somatic maintenance may be of critical importance to understanding variation in longevity (Shanley & Kirkwood 2000). However, it is not yet clear whether the extended lifespan effects of dietary restriction are global, or whether they are dependent on reproductive life history and mating status.

Mating status may have particularly strong effects on allocation decisions of females that must provide costly investment in offspring. Direct demonstrations of adaptive female responses to DR remain elusive (but see Partridge, Gems & Withers 2005, Lee et al. 2006, Carey et al. 2008). However, delayed or diminished reproduction under DR has been demonstrated in a variety of species (Austad 1989, Ernsting & Isaaks 1991, Kaitala 1991, Boggs & Ross 1993, Chippindale et al. 1993, Schwarzkopf 1993, Ohgushi 1996, Langley & Clutton-Brock 1998, Miles, Sinervo & Frankino 2000, Stelzer 2001, Weithoff 2007, Carey et al. 2008), likely because resources are shifted to somatic maintenance during DR, or there are insufficient resources to invest into reproduction (Shanely & Kirkwood 2000). Responses to DR may be adaptive if deferring reproductive output increases the likelihood of survival until resources are available (Partridge et al. 2005). However, studies in fruit flies demonstrate that delays in reproduction increase longevity but decrease fecundity and viability of larval offspring (Rose & Charlesworth 1980, Partridge, Prowse & Pignatelli 1999, Buck et al. 2000), supporting the notion of a trade-off between fertility and survival (Kirkwood & Austad 2000). Adaptive responses to DR may be most likely in iteroparous species as survival until resources become available again may allow multiple future reproductive bouts. When starved, females have been shown to rapidly reduce the amount of energy allocated to reproduction (e.g. Bradley, Baird & Calow 1990), and reduced reproduction when food is limited appears to be a common feature of most taxa (Boggs 1994). Such a strategy may increase lifetime reproductive success, particularly if food shortages are brief relative to lifespan and animals that survive can resume reproduction once food is available again (Clutton-Brock 1991, Mauck & Grubb 1995, Ohgushi 1996, Festa-Bianchet & Jorgenson 1998, Siems & Sikes 1998, Loison & Strand 2005, Grange, Duncan & Gaillard 2009, but see, Jordan & Snell 2002, Descamps et al. 2009). In concert with this hypothesis, DR has been shown to increase longevity across taxa (Ball, Barnes & Wisscher 1947, Weindrich & Walford, 1988, Masoro 2000), although such effects are not necessarily restricted to individuals that have mated and are producing offspring (Partridge et al. 2005).

Despite changes in longevity with DR, the net effect of resource availability on lifetime reproductive output is not yet clear (see varying results in: Reznick 1985, 1992, Bell & Koufopanou 1986, Austad 1989, Roff 1992, Boggs & Ross 1993, Ohgushi 1996, Zera & Harshman 2001, Obeso 2002, Bauerfeind & Fischer 2005). For example, although DR may decrease damage from metabolic by-products (Harman 1981, Sohal & Weindruch 1996, Yu 1996, Merry 2002), this may be balanced in mated DR females by an increased susceptibility to the oxidative stress associated with metabolic processes (Salmon, Marx & Harshman 2001). Nevertheless, in at least one study, mated females survived DR better than unmated ones, although effects on reproduction were not measured (Ball et al. 1947). In addition, recent studies suggest that the longevity benefits of DR may depend on a number of factors including reproductive status, diet levels, the age at which DR occurs and the duration of the DR (e.g., Carey et al. 2008). This complexity could reflect a dependence of DR effects on shifting allocation priorities. General conclusions are difficult because most studies of DR, reproduction and longevity are on model systems such as fruit flies, nematodes or laboratory rodents. More work on a wider range of animals, particularly those facing regular extremes in food availability, is critical. These may allow elucidation of general principles governing how physiology and life history interact to influence resource allocation to survival and reproduction in a particular nutrient environment.

One group in which physiological responses of individuals are adapted to an evolutionary history of extreme variation in food are the arachnids (Itô 1964, Miyashita 1969, Anderson 1970, Anderson 1974, Nakamura 1972, Seymour & Vinegar 1973, Humphreys 1977, Greenstone &

Bennett 1980, McQueen 1980, Kotiaho 1998). The low metabolic rates of arachnids may have evolved as an adaptation to inconsistent food availability (Anderson 1970, Seymour & Vinegar 1973, Greenstone & Bennett 1980, Anderson 1994). In fact, it has been argued that the ability of spiders to depress metabolic rates during periods of food limitation has contributed to their evolutionary success as a group (Anderson & Preswitch 1982). Web-building spiders in particular are sit-and-wait predators that often eat infrequently, so periods of starvation during which food is unavailable would present ecologically relevant DR. As this is typical of spider natural history, starvation is expected to trigger responses to DR seen in other species, but would not necessarily be classified as a period of malnutrition (as it would for species adapted to constant food availability).

Here we examine how reproduction and longevity are affected by a period of DR during adulthood for mated and unmated females. We examine the effects of DR on fitness in the laboratory by comparing survivorship of females that experience DR to those that were fed continuously, and by comparing lifetime offspring production for mated females under DR or normal diets. We measure resting energetic rates to infer underlying physiological expenditure on somatic maintenance. Finally, we compare data on body mass and reproductive output of females in our laboratory study to that of females in their natural habitat at the beginning, middle, and end of the reproductive season to assess how our treatments relate to natural conditions.

We focus on female Australian redback spiders (*Latrodectus hasselti*, Theridiidae, Fig. 1). Previous research has demonstrated that short-lived male redback spiders survive approximately 20 days after becoming sexually mature (Andrade 2003) and do not increase their longevity under DR (Kasumovic, Brooks & Andrade 2009). It was suggested that this occurs because these males mate only once in their lifetime and die shortly afterwards (Andrade 1996, 2003), so DR should not be associated with reallocation of resources to somatic maintenance (Kasumovic et al. 2009). This is consistent with the hypothesis that longevity effects of DR depend on allocation priorities shaped by the pattern and frequency of reproduction by adults. The allocation hypothesis predicts that, in contrast to semelparous males, female redbacks (which can produce numerous clutches of offspring for over 2 years after mating, Andrade & Banta 2002), should show increased longevity under DR. Moreover, if allocation shifts with reproductive context within this life history, this link should exist only for mated females.

Prior to mating, adult female spiders begin to develop eggs, and must maintain them until fertilization (Foelix 1992). Virgin females of many species (including redbacks) also continuously produce sex pheromones used for mate attraction and courtship initiation (Gaskett 2009, Jerhod et al. 2010) which are laid down in silk that is added to the web each night (Andrade & Kasumovic 2005). These processes may require elevated metabolic rates (e.g., Johansson & Jones 2007) and represent physiologically costly allocation to future reproductive function for unmated females. In contrast, mated redback females cease sex pheromone production after copulation (Stoltz, McNeil & Andrade 2007, Jerhod et al. 2010), and typically fertilize and lay already-formed eggs in their first egg sac. Under DR, female spiders then typically restrict egg production (e.g. Kreiter & Wise 2001). Resources can thus be directed solely to somatic maintenance for mated females (with positive effects on longevity), but this is not the case for unmated females. Thus consistent with other literature on DR, we predict that starved, mated females will cease reproduction (e.g., Partridge et al. 2005) and have increased longevity relative to well-fed (mated) females but also relative to unmated females. The mechanistic underpinning of this change should be a depression in resting energetic rates of starved females relative to well-fed females, and this should be particularly acute for females that have mated.

Methods

Field data

To obtain data on natural body mass (which should reflect variation in food availability) and reproductive output, female redback spiders were collected from a natural population on the grounds of Macquarie University, Sydney, Australia in 2007. Spiders were located by identifying their webs and were used either for assessment of body mass or to measure natural rates of egg production. To assess how female mass changes over time, a subset of mated females were collected at the beginning (Dec 15-Jan 15; n=7), middle (Jan15-Feb15, n=14) or end (Feb 15-Feb 23, n=22) of the mating season and weighed within 5 hours of capture. Field-caught females were determined to be mated if an egg sac with viable eggs was present in their web in the field, or if they produced a viable egg sac while being held in the lab over the course of the following 60 days. Females were kept in the laboratory for the remainder of their lives and fed one adult cricket per week.

To assess rates of reproductive output in nature, a subset of mated females (n=18) was monitored throughout the reproductive season (Dec15-Feb 23) at marked locations in the field. Every week, each female’s web was checked for the presence of egg sacs, which were collected as they were produced. Field-collected egg sacs were classified according to date of production as beginning (collected December 15-January 15; n=16), middle (January 16- Feb15; n=14) or end of season (Feb 15-Feb 23; n=2).

Laboratory rearing

Experimental spiders were from an outbred laboratory population of *L. hasselti* initiated and maintained with individuals collected from Sydney, New South Wales, Australia (2002) and transported to the University of Toronto Scarborough. Spiderlings were reared communally in a temperature controlled room at 25ºC 12:12 light:dark cycle until the 4th instar after which they were housed in separate cages (4x4x6 cm; Amacs Plastics products Ltd) to ensure that they had not mated at the time of trials (males mature at the 5th instar, females at the 7th-8th instar). Spiderlings and males were fed fruit flies (*Drosophila melanogaster*) twice a week and females were fed adult crickets (*Acheta domesticus*) once per week.

Females of the Australian redback spider are sedentary, remaining on their webs throughout their lives, and employ a sit-and-wait predation strategy. They are iteroparous, and if well fed, produce an egg sac with roughly 120 eggs approximately every 11 days (Andrade & Banta 2002). Virgin females are able to survive long periods of DR (mean of 120 days at 10°C), which occur periodically in the field, and usually regain normal mass and function if feeding resumes after 7 to 12 weeks of DR (Forster & Kavale 1989), suggesting a period of DR is a naturalistic way of imposing dietary restriction.

Experimental design

Females that had become sexually mature within the previous 3 months (females live up to 2 years in the lab, Andrade & Banta 2002) were fed 1 adult cricket one day prior to the beginning of the experiment and then randomly placed into one of four treatments defined by mating status and diet: virgin DR (n=13), virgin fed (1 adult cricket once per week; n=7), mated DR (n=15) and mated fed (same diet as virgin fed, n=15). Females in the mated treatments were randomly paired with an unrelated male and allowed to mate one day after feeding, prior to assignment to a DR or fed treatment. Virgin DR females were starved and survivorship and metabolic measurements taken for six weeks. Six weeks of DR was chosen since females normally recover from this treatment, suggesting physiological adaptation (Forster & Kavale 1989). In contrast, to allow measurement of how lifetime reproductive output of mated females is affected by DR, it was necessary to eventually resume feeding starved mated females (which cease egg production during DR, see results). For this reason, after the first 6 weeks, starved mated females were switched to the ‘fed’ diet and data were collected for all mated females for a further 4 weeks. Females were monitored daily throughout the experiment, egg sacs were removed within a day of being deposited and the date of a female’s death was recorded. Adult longevity was calculated as the interval between their adult moult and death.

Energetic rate measurements

Since *L. hasselti* are nocturnal all energetic rate measurements were conducted in darkness illuminated by red light. Energetic rates were estimated using CO_2 production of spiders at rest (see Watson & Lighton 1994, Kotiaho 1998). We measured resting energetic rates of all females in the laboratory study once every 2 weeks throughout the experiment. Females were weighed (Ohaus Explorer electronic balance) prior to each resting energetic rate measurement. All energetic rates are reported in umol/h. Energetic measures were taken using a Qubit systems (Ontario, Canada) 8 channel gas controller (G245) using stop-flow CO_2 respirometry (see Lighton 1991, Vogt & Appel 1999), a Qubit systems 8 channel gas switcher (GS244) and a Li-Cor (Nebraska, USA) CO_2 analyzer (LI-6252). Spiders were placed into cylindrical, air-tight glass chambers (10 cm length, 3 cm diameter) plugged at both ends with steel plugs and sealed with rubber O rings. CO_2 was removed from incoming air by filtering the air through soda lime. Flow rate through the chambers was set to 160 ml/min and did not appear to disturb the spiders. Air from the test chamber was passed through a magnesium perchlorate filter to remove moisture then passed into the CO_2 analyzer which measured respired CO_2 in umol/hr. To control for leakage of CO_2 into the system, we measured the CO_2 output of chambers with no animals to get a baseline measurement of CO_2. We took repeated control measures (three 4 minute dwell periods for resting rates) on each trial morning before measuring female resting energetic rates. The average baseline energetic rates were subsequently subtracted from the average of three 4 minute dwell periods of females.

Female resting energetic rates were taken during their inactive period (between 8:00-18:00) when spiders have been shown to reach their lowest metabolic rates (Anderson 1970). Females were acclimated in chambers for approximately 30 minutes prior to energetic rate measurements. Briefly, in stop-flow respirometry the gas controller seals off the sample chamber for a set period of time (dwell time) allowing the spider to build up CO_2. The built up CO_2 is then flushed out of the sample chambers and the umol/hr CO_2 is calculated (see Lighton 1991). All measurements were made at temperatures of 25 °C as small variations in temperature can substantially perturb arthropod metabolic rates (see Anderson 1970, Lighton et al. 2001).

Reproductive output

Egg sac production by laboratory-mated and field-mated females was monitored for their entire lives. Egg sacs collected from laboratory and field-females were held at 25°C for fifteen days after which they were opened and the number of spiderlings was counted. Spiderlings hatch within 15 days at this temperature and normally spend the first instar inside the egg sac; (see Snow & Andrade 2005).

Analysis

First, we examined sources of variation in female survivorship (adult longevity) using an ANCOVA with Poisson error distribution as female deaths were not uniformly distributed over time (Kingman 1993). In this model, diet and mating status were the factors, adult longevity (days) was the independent variable, female mass (prior to the start of diet treatment) was a covariate (the latter because preliminary results suggested mass predicted survivorship of mated females).

Second, we make comparisons of reproductive output within the mated treatment to see how DR affected female reproductive output and how differences may be affected by resumption of feeding in the starved group. We also compare reproductive output from experimental females to that of females collected in nature.

Third, to determine whether energetic expenditure may underlie any treatment effects on longevity and reproduction, we ask how mating status and diet affect resting energetic rates (in CO_2 umol/h). We use repeated measures ANCOVA with female mass at each measurement as a covariate (essential to control for the allometric relationship between mass and energetic rates, see Lighton 2008).

Fourth, to examine whether our treatments are ecologically relevant, we compared the mass of mated females found in the field at different periods to mated females in our treatments after DR and after feeding resumed using ANOVA.

Statistical analyses were performed using SPSS version 13.0 or R. Data that violated the assumption of a normal distribution were log transformed where necessary. Statistical tests are two-tailed with mean ± SE reported.

Results

Longevity

Overall, virgin females had a shorter lifespan (63±4 days) than mated females (103±13 days; $F_{1,45}$=7.74, p=0.04, Fig 2, Table 1). For virgin females, there was no significant effect of diet (t_{14}=0.21, p=0.83) or initial body mass (fed: r^2 = 0.002, d.f. = 1, p = 0.90, DR: r^2 = 0.53, d.f. =1, p = 0.16) on longevity. However, for mated females, longevity was negatively associated with initial body mass and there was a steeper slope for mated, DR females (r^2 = 0.33, d.f. = 1, p = 0.03, slope = -0.51) than for mated, fed females (r^2 = 0.02, d.f. = 1, p = 0.58, slope = -0.16, Fig. 3). DR increased the mass-adjusted longevity of mated females (108±20) relative to their fed counterparts (99±17, ANCOVA $F_{1,26}$ = 4.89, p= 0.04; Fig. 3).

Reproductive output

Mated females typically produced their first egg sac within 7 days after commencement of the feeding treatment. Regardless of diet, females produced a similar number of spiderlings within this first egg sac (fed: 230.13±27.25; DR: 216±24.55; t_{14}=0.39, p=0.71, Fig. 4). During the 6 week diet treatment, fed females typically produced one egg sac every two weeks (3.77±0.40 sacs) whereas starved females rarely produced any sacs after the first one produced at the onset of DR (1.10±0.10 sacs; t_{20}= 5.79, p<0.001). Within the fed group, there was a decrease in the number of spiderlings in successive egg sacs over the six week period (repeated measures ANOVA: F_5 = 8.66, p=0.02; Fig. 4). In comparison, the number of spiderlings produced by females in the field was constant across the season (F_2 = 0.91, p=0.42; Fig. 5A) and was less than the number produced in the first egg sac of experimental females (t_{47}=-3.51, p=0.001).

After we resumed feeding the starved females, they typically produced an egg sac within 18 days. The number of spiderlings in this first egg sac produced after feeding recommenced

(98±9.54) was similar to the number of spiderlings from the egg sacs produced by fed females after 6 weeks on a normal diet (105.75±38.60; t_9 =0.25, p = 0.81). Fed females produced more egg sacs in their lifetime (7.23±1.32) than those that were starved then re-fed (3.92±1.28; log t_{23} = 2.48, p = 0.02). However, starved females tended to produce more spiderlings per egg sac on average after feeding resumed (80±13) compared to females fed throughout the period (68±13; log t_9 = 0.81, p = 0.44) but this difference was not significant. Nevertheless, there was no significant difference in the lifetime total number of spiderlings produced by fed (587.08±124.19) compared to starved then re-fed females (525.00±133.6; log t_{19} = 0.43, p = 0.67; Fig 6). This may reflect the fact that most offspring production occurred in the first 4 egg sacs regardless of female diet (Fig 4).

Metabolic rate

Female reproductive status and diet both affected female resting metabolic rates. Overall, resting energetic rates of fed mated females were higher than those of starved mated females ($F_{3,53}$=11.31, p<0.001) but diet did not appear to affect resting energetic rates of virgin females ($F_{3,41}$=0.46, p=0.71; Table 2). A precipitous drop in energetic rates of starved mated females relative to fed mated females was clear in the first measurement after initiation of DR ($F_{1,25}$=20.34, p<0.001, Fig 7). In contrast, energetic rates of starved virgins remained high and equal to that of fed mated females even after 2 weeks of treatment ($F_{1,24}$=2.71, p=0.11, Fig. 7). It was not until the measurement at 4 weeks that energetic rates of starved virgins declined below that of fed mated females ($F_{1,22}$= 26.72, p<0.001, Fig. 7).

After feeding recommenced (week 6), energetic rates of starved, mated females quickly increased and matched those of fed, mated females by week 8 ($F_{1,13}$=1.14, p=0.31) but over subsequent measurements both groups showed a decrease ($F_{2,27}$=9.68, p=0.001, Fig. 7).

Female Mass

Female masses were similar in the four treatments at the beginning of the laboratory experiment ($F_{3,46}$=1.23, p=0.31, see Appendix S1 in Supporting Information). Temporal variation in female mass was related to reproductive status and diet. Virgin females under both diets lost mass over the 6 week period (F_3=13.25, p=0.001; see Appendix S1, S2). In mated females, there was a significant interactive effect of diet and time on mass (2 way repeated ANOVA: F_3=9.3, p=0.001, see Appendix S1, S2). This interaction arises because fed, mated females lost mass initially but there was little change in their mass for weeks 2 – 6, whereas starved, mated females experienced continued mass loss throughout that period.

Variation in the mass of mated females was within the range of variation seen in the field (Fig 5B, see Appendix S1 for additional comparison). Mated females fed for 6 weeks in the laboratory were comparable in mass to field-caught females caught at the start of the mating season (Mann-Whitney U=31.00, n1=10, n2=7, p=0.70). Mated females lost mass during the 6 weeks of DR (log repeated measures ANOVA: $F_{3,24}$=15.07, p<0.001), but after 6 weeks of DR, were comparable to females found at the end of the season in nature (t_{26}=0.57, p=0.58). Mated females that were starved quickly recovered after feeding resumed, as they were similar in mass to mated females fed continuously by week 8 (2 weeks after the resumption of feeding: log t_{16}=0.60, p=0.56 , see Appendix S1, S2).

Discussion

The responses of female redback spiders to a period of DR were strongly affected by mating status and, apparently, also by their reproductive life history. Consistent with other taxa mated females largely ceased egg laying during DR, but resumed egg production when given access to food as predicted (for recent examples see: Stelzer, 2001, Weithoff 2007, Carey et al. 2008). This effect was linked to an increase in their longevity (Table 1, Fig 2, 3) with no net change in their lifetime reproductive output (Fig. 4). This is particularly striking because relative to mated females subject to DR, there was no comparable increase in longevity for mated females that were fed (i.e., no effect of mating status alone) nor for virgin females subjected to DR (i.e., no effect of DR alone, Fig. 3). These data support the hypothesis that DR triggers adaptive mechanisms that increase longevity if reproduction can be delayed (Partridge et al. 2005). These results are in contrast to a recent study of male redback spiders, in which adult condition and energy expenditure were the strongest predictors of longevity, with no direct effect of DR (Kasumovic et al. 2009). This sex difference is consistent with life history hypotheses that posit effects of DR depend on how DR shapes the resource allocation trade-offs inherent to reproduction. Redback spiders have sexually divergent reproductive life histories. Males are short-lived (approximately 20 days past sexual maturity; Andrade 2003), cease foraging upon sexual maturity and are semelparous (they mate only once, Andrade 1996), so are unlikely to reap fitness benefits from cessation of reproductive activity during DR. In contrast, the relatively long lived females (minimal average survival past maturity: virgins 63 days; mated 103 days, Fig. 2) produce eggs in numerous bouts throughout their adult life, and, as we show here, can maintain their lifetime reproductive output by prolonging their reproductive lives, even if egg production ceases during periods of DR.

Patterns of energy use (resting metabolic rates) suggest links between costs of reproduction, longevity and underlying physiological shifts in these sit-and-wait predators. Whereas mated females responded to DR by rapidly decreasing their resting energetic rates relative to fed mated females, energy expenditure of virgin females remained relatively high throughout the experiment, regardless of diet. This constant, elevated energy expenditure may explain one unexpected result of our study --the overall decreased longevity of virgin relative to mated females (Fig. 3, Table 1). The costly maintenance of a moderate level of metabolic activity, regardless of food availability, suggests some underlying physiological activity in virgins that is not present for mated females. The cost for virgins may be due to females having to maintain their eggs in an initial stage of yolk accumulation, as is common for spiders (Foelix 1982). In contrast, mated females fertilize and lay their stored eggs prior to shutting down egg production during DR. Although physiological mechanisms underlying the cost of reproduction remain largely unknown (Zera & Harshman 2001, Harshman & Zera 2007), mechanistic costs related to trade-offs in reproduction are thought to involve hormones that may have positive or negative pleiotropic effects on physiological systems (Williams et al. 2005, Zera, Harshman & Williams 2007) and may thus be linked to an individual's metabolic output.

Differences in longevity due to DR between virgin and mated females may also be related to costs associated with the allocation of resources to mate attraction for virgin but not mated females. Males are attracted to pheromones deposited in virgin females' webs (Stoltz et al. 2007). New pheromone-bearing silk must be added to the web each night to maintain its attractiveness (Andrade & Kasumovic 2005) and virgin females held in the absence of males increase their pheromone production (Watson 1986). Although pheromones have historically been considered to be relatively trivial to produce, there are recent suggestions that they may be costly in some taxa (for review see; Johansson & Jones 2007). Moreover, the recent demonstration that a sex pheromone of female redbacks is a serine derivative suggests costs in this species may derive from trade-offs when molecules important in other physiological functions are diverted to pheromone production (Jerhod et al 2010). Investment in mate attraction may be very important in the field, despite energetic challenges, as virgin females sometimes remain unmated (Andrade & Kasumovic 2005). Thus the higher energetic rates and decreased longevity of virgin females relative to mated females may arise from continued investment in mate attraction by virgins. In comparison, mated females shut off pheromone production rapidly after copulating (Stoltz et al. 2007).

One alternative explanation for these results that is unrelated to allocation differences is that mated females may show higher longevity because males transfer beneficial products within the seminal fluids (Ivy, Johnson, & Sakaluk 1999). This is unlikely however, as no such effects have been found in any spider, and other evidence suggests that seminal products transferred at copulation may decrease longevity of females (e.g. Chapman et al. 1995) and this may also occur in redbacks (Stoltz & Andrade unpub.). Moreover, if mating itself yields longevity benefits, all mated females should show this effect. However, mass-adjusted longevity was higher for DR than non DR mated females (Fig. 3). Thus the most likely explanation for our results is that virgin females are unable to devote as much energy to somatic maintenance as are mated females, and the result is a decrease in longevity.

Despite six weeks without food, when mated females were finally fed, their resting energetic rates immediately increased almost 3-fold to match that of mated females fed throughout the experiment (and their body mass also quickly recovered, see Appendix S1). This rapid recovery suggests our use of a period of DR as ecologically relevant DR is reasonable, as females are apparently able to withstand periods of DR with little permanent negative effect. The increase in resting energetic rates is also consistent with a need to increase metabolism to support renewed egg production. Although spiders have lower metabolic rates overall than expected based on their body size, theridiid spiders (like *L. hasselti*) appear to be a special case, with metabolic rates 17% higher than expected based on Hemmingsen's (1960) poikilotherm equation, and rates 1.3-2.9 times higher than those reported for spiders in general (Anderson

1970, Greenstone & Bennett 1980, Anderson & Prestwich 1982). Anderson & Preswitch (1982) argue that these higher metabolic rates are a prerequisite for higher reproductive rates, and this suggestion is supported by the finding that theridiid spiders have larger clutch sizes for their body size than do most other spiders (Enders 1976). High energetic rates of theridiids may also allow them to rapidly convert the energy in ingested food into growth and reproductive efforts, thus accounting for the relatively high population densities and widespread distribution of many of these species (suggested by Anderson 1994). These observations suggest there may be a substantial energetic cost of reproduction for female theridiids. Energetic requirements of egg production have been found in many species of birds where the energetic rates of females producing eggs increase up to 26% relative to those that are not (Chappell, Bech & Buttemer 1999, Nilsson & Raberg 2001, Vézina & Williams 2002, 2005). However, our data also clearly show that such costs do not handicap physiological mechanisms for surviving DR (Fig. 3).

Our field data on female body mass and reproductive output suggest the laboratory diet treatments are reasonable mimics of natural conditions, suggesting these inferences would apply to females in nature. The mass of field-caught females suggest females may have been food stressed in nature and that DR of the type simulated here may not be unusual (Fig. 5B). This tentative conclusion awaits experimental confirmation in the field, since a wide range of factors other than food availability may affect female mass and reproduction in nature. Since redback females are able to store sperm for up to 2 years, ceasing reproduction during a period of DR will not compromise the ability to resume reproduction using the stored sperm as soon as food levels increase (Andrade & Banta 2002). Females can store reserves of carbohydrates, lipids and proteins to be used for egg production during periods of high food availability in a variety of taxa including crabs (Gibson & Barker 1979, Chang & O'Conner 1983, Spaargoren & Haefner 1994), fish (Fishelson, Montgomery & Myrberg 1985, Montgomery, Myrberg & Fishelson 1989), geese

(Prop & Deerenberg 1991) and lizards (Marken Lichtenbelt 1993). Spiderling production per egg sac was also comparable in the field and laboratory (Fig. 5A), which is consistent with our finding that offspring number per egg sac is similar regardless of female diet history. Similar results are reported in brine shrimp where females that have a reproductive delay produce larger broods with shorter interbrood intervals to have equal number of offspring as controls (Browne 1982). Another example is seen in rotifers where restoration of food levels causes previously starved females to respond with high fecundities (Robertson & Salt 1981). While redback offspring number is not affected by DR overall, it is not clear whether the condition of offspring produced by food-stressed females differs from that of offspring of females that are well fed, or whether there are other consequences of DR and reproductive cessation that may affect female fitness. For example, if a constant predation risk in the field is assumed, increased longevity may not always translate into more time to make eggs.

Our results highlight a fundamental life history trade off in resource allocation. Energetic states of females can affect future reproductive potential as well as investment in a current reproductive bout, and ultimately, how energy is allocated (Trivers 1972, Boggs 1992, Roff 1992, Stearns 1992, Williams & Cooch 1996). An individual's reproductive investment may be constrained by low energy reserves at the beginning of reproduction (Jönsson 1997, Glazier 1999, Fox & Czesak 2000). We provide evidence for this assumption as female redback spiders in the starved group generally produced a single egg sac at the onset of DR, which leads to a substantial loss in mass (see Appendix S1, S2), and then ceased further reproductive output during DR. These results are not unusual as most animals have discrete periods of breeding and non-breeding that are dictated by environmental cues such as prey availability (e.g. Wingfield et al. 1992, Wingfield, Hahn & Doak 1993). Food availability has been shown to affect the timing of reproduction in many species including birds (Perrins 1970, Drent & Daan 1980), reptiles

(Rand & Green 1982, Wiewandt 1982, Marken Lichtenbelt 1993), mammals (Bodenheimar & Sulman 1946, Tabot & Talbot 1963, Bunnell 1980), fishes (Clifton 1995, Caceres, Fuentes & Ojeda 1994) and invertebrates (Kennish 1997). In redbacks, the energetic demand of producing egg sacs (approximately 37% of female body mass, Stoltz unpub.) is likely an important cause of the decreased energetic rates of mated females when food is restricted. Although females may have sufficient resources to lay additional eggs, retaining those nutrients for somatic maintenance allows prolonged survival and maintains the ability to rapidly capitalize on food availability with little long-term physiological cost (Fig. 7).

We conclude that adaptive responses to DR are related to reproductive life history (i.e., iteroparity versus semelparity) and the mating status of individuals, as this shapes trade-offs in the allocation of resources during periods of DR. Although the energetic challenges for unmated redback females (maintenance of eggs and attractiveness) may be specific to animals with similar natural history, comparable variation as a function of mating status may exist in other taxa. Costs associated with remaining in a state of reproductive ‘readiness’ are rarely considered but may thus have important implications for resource allocation decisions. Critically, variation in such costs may explain why links between DR and longevity are proving to be variable within and across taxa (e.g., Carey et al. 2008).

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SUPPORTING INFORMATION

The following Supporting Information is available for this article:

Figure S1 Change in the mass of female redback spiders over time.

Table S2 Effect of mating status, diet and time on female mass.

This material is available as part of the online article from:

http://www.blackwell-synergy.com/doi/full10.1111/j.1365-2745.XXXX.XXXXX.x

Table 6.1- ANCOVA for effects of diet, mating status (Mate), and initial mass on female longevity.

	Coefficient (S.E.)	Z-score	p
All females			
Diet	0.636 (0.2871)	2.206	<0.001
Status	1.983 (0.1574)	12.602	<0.001
Mass	-0.0001 (0.0005)	-0.264	0.7921
Mass x Status	-0.005 (0.0006)	-8.531	<0.001
Mass x Diet	-0.003 (0.0011)	-2.309	0.0209
Mate x Diet	-1.72 (0.3303)	-5.209	<0.001
Mass x Mate x Diet	0.006 (0.0012)	4.914	< 0.001

Table 6.2- Effects of (A) diet, and time on resting energetic rates of mated or virgin female redback spiders and (B) mating status and time on resting energetic rates of starved or fed female redback spiders.

Mixed model repeated-measures ANCOVA	F	d.f.	p
WITHIN MATING STATUS			
Virgin			
Diet	2.34	1,13	0.15
Time	6.98	3,41	<0.01
Time x diet	0.46	3,41	0.71
Mated			
Diet	38.1	1,17	<0.001
Time	2.03	3,53	0.12
Time x diet	11.31	3,53	<0.001
Mated (after resumption of feeding)			
Diet	1.14	1,13	0.31
Time	9.68	2,27	<0.01
Time x diet	0.65	2,27	0.53

WITHIN DIET TREATMENTS			
Starved			
Status	24.76	1,17	<0.001
Time	1.22	3,53	0.31
Time x status	2.59	3,53	0.06
Fed			
Status	6.82	1,13	0.02
Time	12.2	3,41	<0.001
Time x status	4.37	3,41	<0.01

Figure 6.1- Female Australian redback spider on her web with an egg sac.

Figure 6.2- Histogram of female longevity as a function of mating status and resource availability (DR = diet restriction).

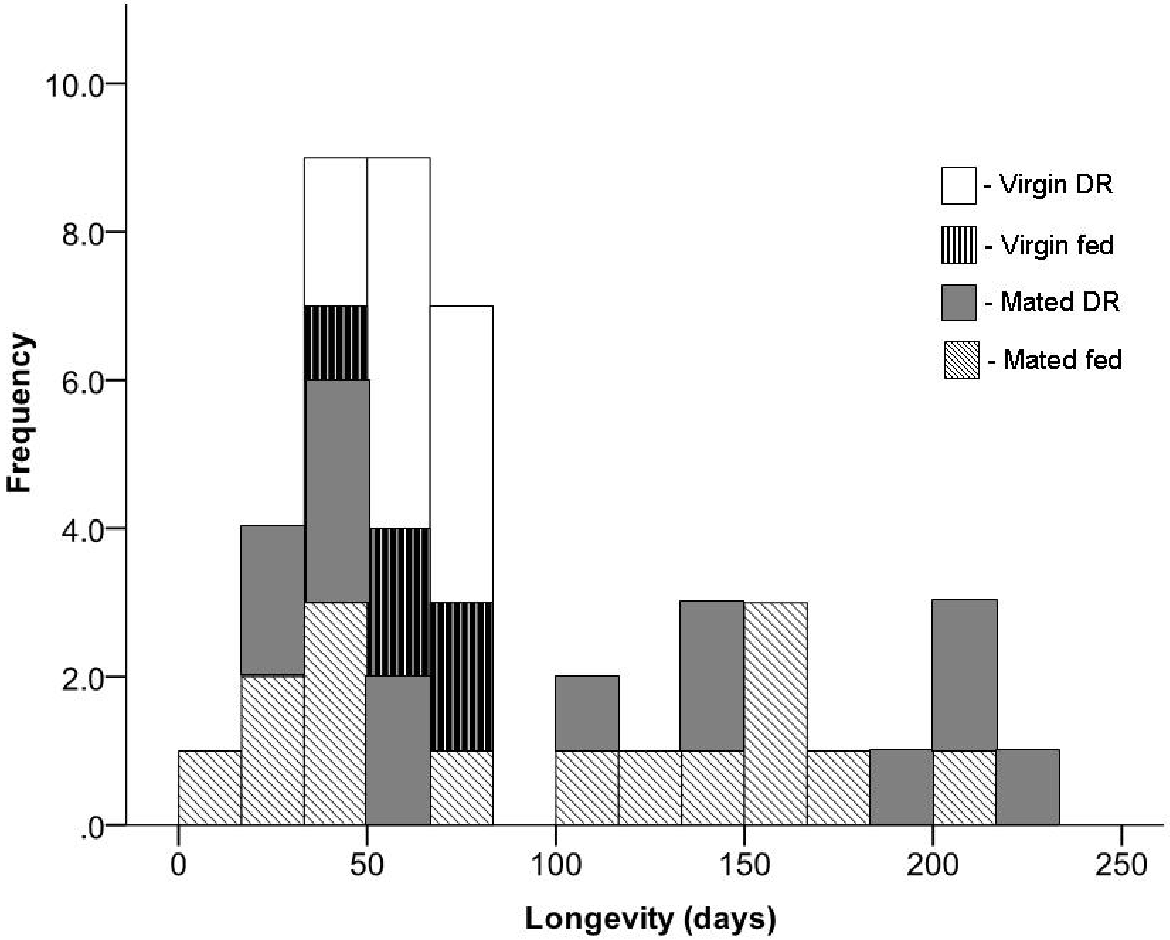
Figure 6.3- Relationship between initial mass (mg) of female redback spiders prior to experimental treatments and adult survivorship (days). Longevity decreased with initial mass for mated females, particularly those that were starved. Lines show trends for visual comparison only.

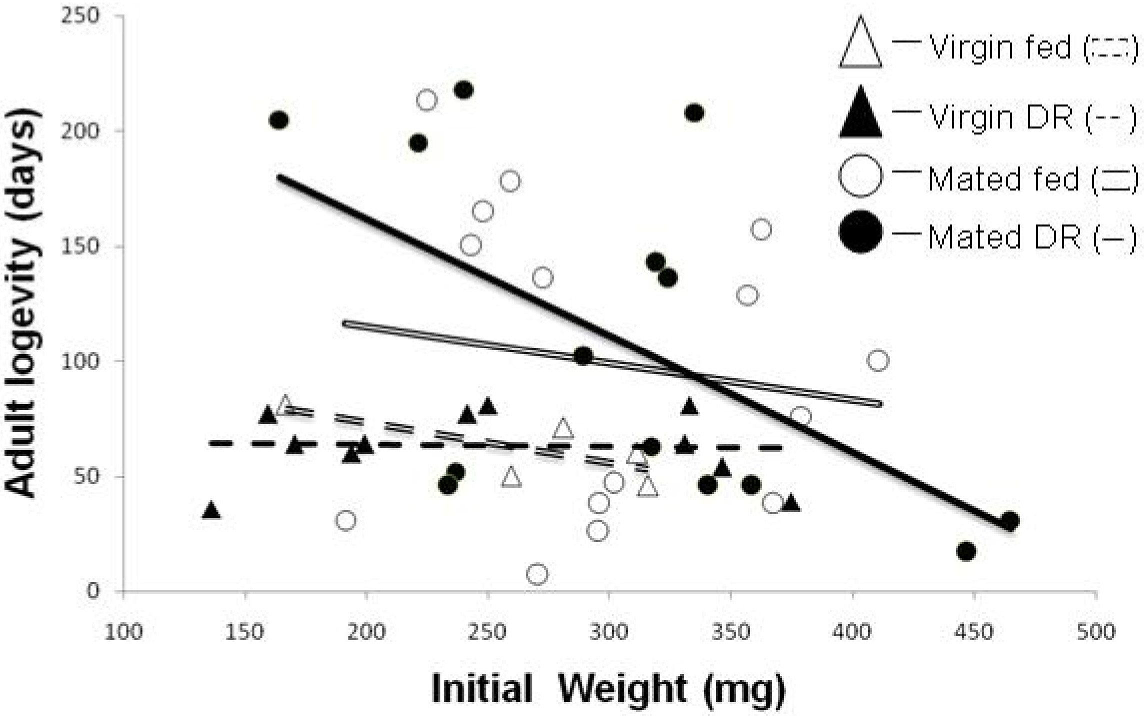
Figure 6.4- The number of spiderlings produced in the first six successive egg sacs by mated females that were in the DR treatment to those that were not. Note that, for starved females, the second egg sac was produced only after resumption of feeding at week 6, whereas for fed females, egg sac production occurred continuously through the experiment.

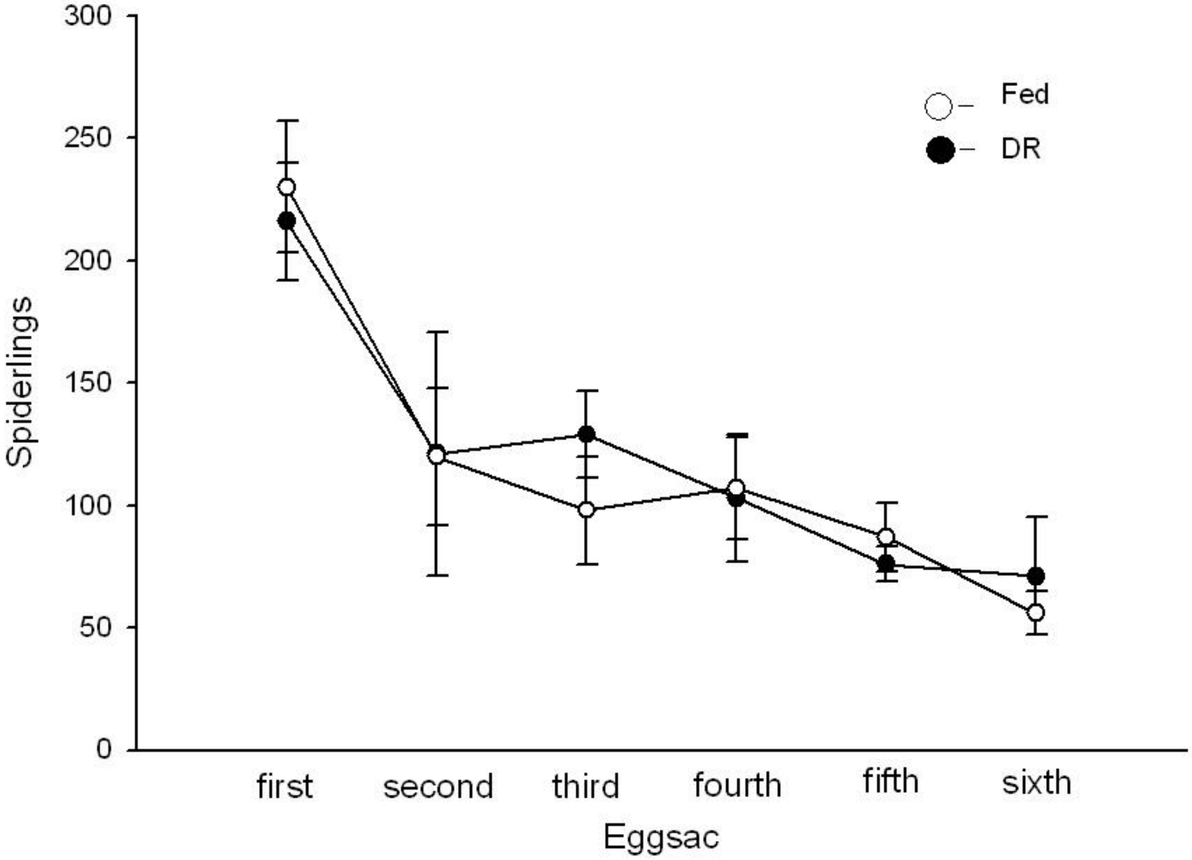
Figure 6.5- The number of offspring produced per egg sac (A) and body mass of mated female redback spiders (B) for spiders and sacs collected in the field (Sydney, Australia) at the beginning (Dec 15 – Jan 15), middle (Jan 16 - Feb 15) or end (Feb 16 - Feb 23) of the reproductive season. Horizontal lines denote (A) the average # of spiderlings in the first egg sac of laboratory mated experimental females (fed and starved combined); or (B) the average mass of laboratory reared, mated females at the beginning of the experiment (solid line), and during weeks 2-6 of the experiment when females were fed normally (broken line) or starved (dashed line). See text for sample sizes and analysis of laboratory data. Letters denote significantly different groups (scheffe $p<0.05$) and numbers are sample sizes at each collection point.

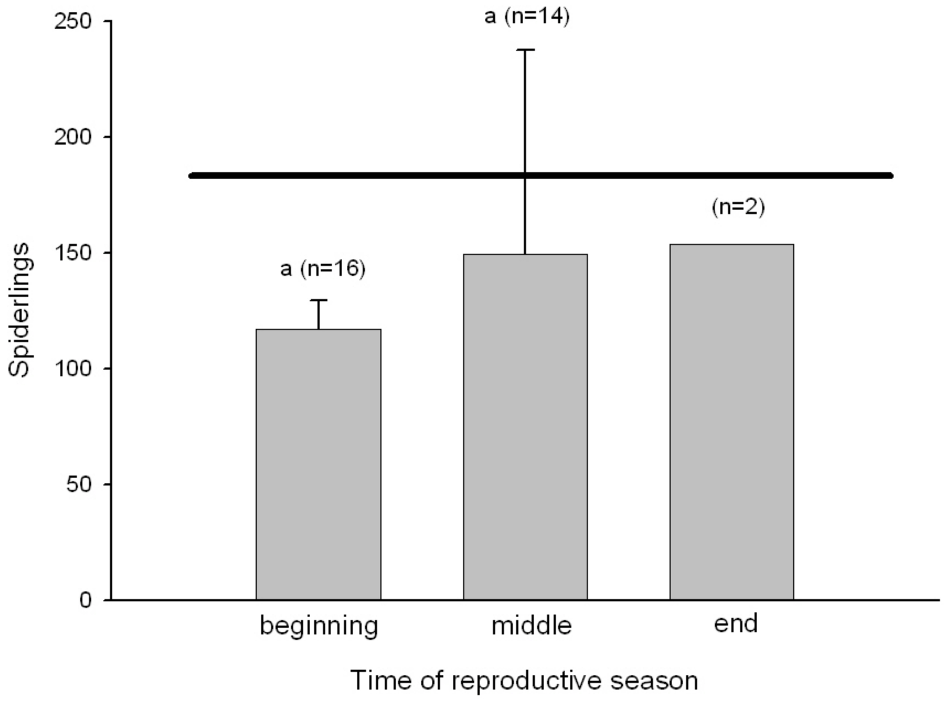
Figure 6.6- Total reproductive output of mated females that were either fed normally or placed in the DR treatment.

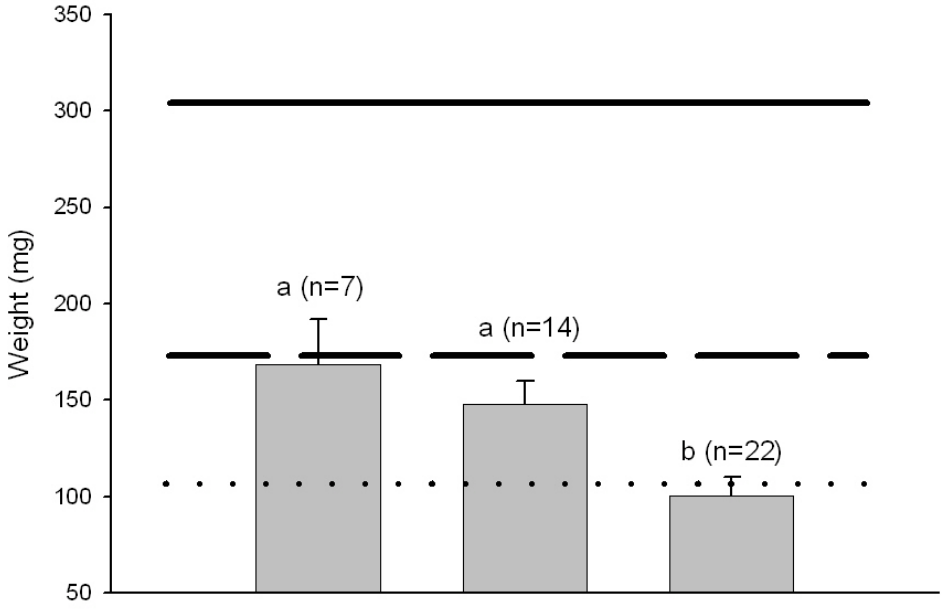
Figure 6.7- Resting energetic rates (estimated by µmol CO_2 expired per hr) of females with different reproductive status and diets as a function of time since the start of the experiment. After the six week DR period, mated females that were in the DR treatment were returned to a normal diet (weeks 8 – 12).

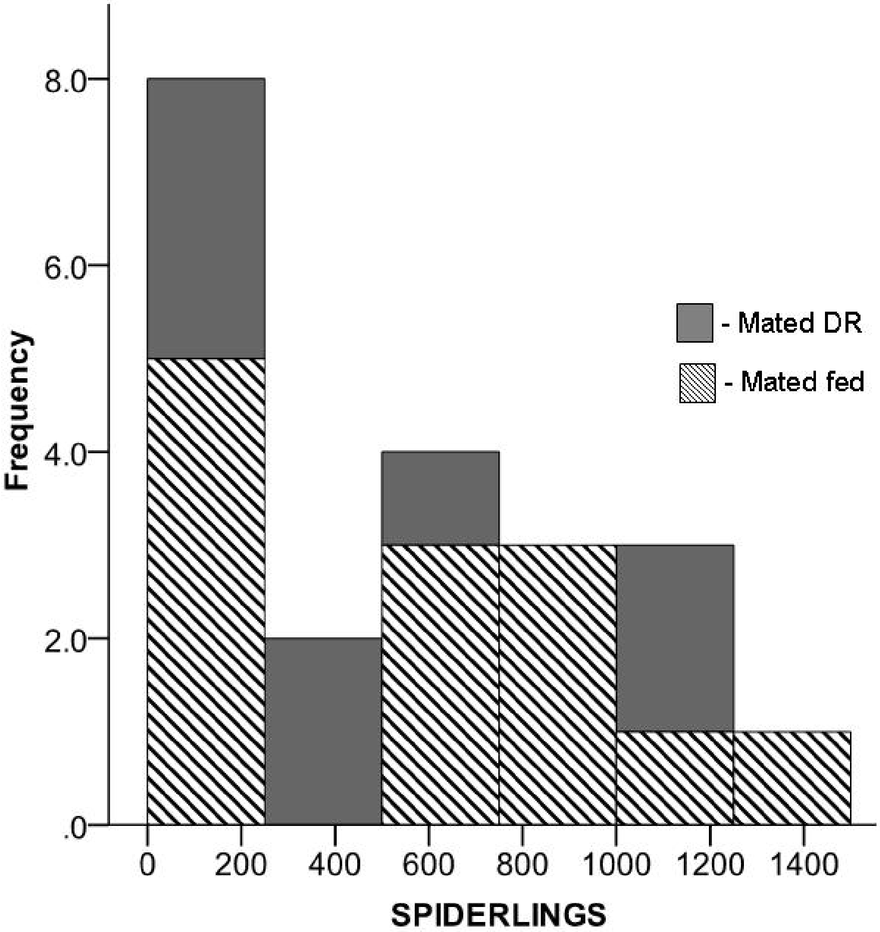

- Virgin DR
- Virgin fed
- Mated DR
- Mated fed
Frequency
10.0
8.0
6.0
4.0
2.0
.0
0
50
100
150
200
250
Longevity (days)

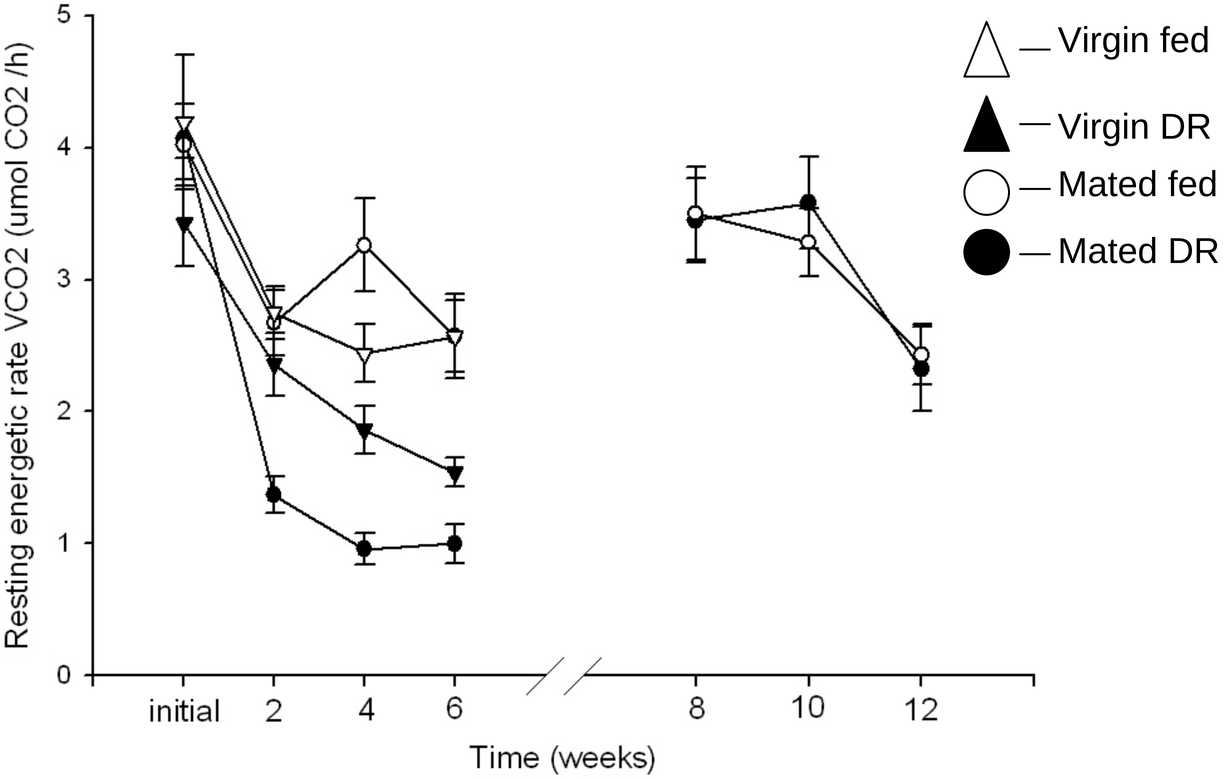

250
200
150
100
50
0
100
150
200
250
300
350
400
450
500
Adult logevity (days)
Initial Weight (mg)
△ — Virgin fed (==)
▲ — Virgin DR (--)
○ — Mated fed (=)
● — Mated DR (—)


300
250
200
150
100
50
0
Spiderlings
first
second
third
fourth
fifth
sixth
Eggsac
○– Fed
●– DR


a (n=14)
(n=2)
a (n=16)
Spiderlings
250
200
150
100
50
0
beginning
middle
end
Time of reproductive season


Weight (mg)
350
300
250
200
150
100
50
a (n=7)
a (n=14)
b (n=22)


8.0
6.0
4.0
2.0
.0
Frequency
0
200
400
600
800
1000
1200
1400
SPIDERLINGS
- Mated DR
- Mated fed


Resting energetic rate VCO2 (umol CO2 /h)
0
1
2
3
4
5
initial
2
4
6
8
10
12
Time (weeks)
△ — Virgin fed
▲ — Virgin DR
○ — Mated fed
● — Mated DR

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Chapter 7
General Discussion

A complete understanding of how sexual selection (Darwin 1871) affects the evolution of male traits requires a thorough understanding of how male-male competition (Darwin 1871, Andersson 1994, Birkhead & Møller 1998) and female choice (Kirkpatrick 1982, Eberhard 1996) interact with one another to result in net sexual selection (see Hunt et al. 2009, Wong & Candolin 2005, e.g. Moore & Moore 1999). Studies isolating one or both mechanisms are of importance to identify traits under selection, but may exaggerate or underestimate their importance, and ultimately obscure how certain traits have evolved (Kodric-Brown & Brown 1984). In my thesis I investigated mechanisms of sexual selection that arise at various stages of the interaction between males and females and determine their impact at each stage of mating. Understanding interactive effects of competition and choice may be particularly important if sexual conflict is intense.

Australian redback spiders (*Latrodectus hasselti*) provide a unique opportunity to investigate interacting mechanisms of sexual selection. Pre-and post-copulatory sexual selection on males are intense because (1) males achieve only a single mating opportunity during their lifetime because they are cannibalized during copulation (Andrade 1998), (2) male residual reproductive value is extremely low due to the extremely high (approximately 80%) mortality rate during mate search (Andrade 2003), (3) strong first male sperm precedence makes it important that males mate with virgin females (Snow & Andrade 2005), leading to adaptations to control paternity (Snow et al. 2006) and developmental shifts that can yield optimal competitive phenotypes under a given mate distribution (Kasumovic & Andrade 2006, see Kasumovic & Andrade 2009), and (4) males are often subjected to intense intra-sexual selection with 2 to 7 rivals per web (Andrade 2003, Andrade 1996). Moreover, despite strong selection on males, mating outcomes are strongly influenced by female preferences because females are physically dominant (males are only 1-2% the size of females, Andrade 1996). This female-biased size dimorphism, together with the different demands of alternative competitive contexts (Stoltz et al. 2008, 2009), may constrain male competitive strategies by suppressing agonistic interactions between males. It is likely, therefore, that females 'set the rules' by which males must compete and female reproductive strategies may have large effects on male traits in redbacks. Further, this well-studied system allows opportunities to consider the impacts of traits selected by male-male competition and female choice at the various stages necessary for reproduction and the impact that competition can have on choice at various stages of the mating interaction (i.e., developing an optimal competitive phenotype, mate search, courtship and competition, mating, sperm use).

When environmental conditions change, there may be selection for the evolution of plasticity in the phenotype that increases lifetime reproductive success in a given context. In chapter two I asked whether altering cues indicating the availability of mates and likelihood of competition would alter traits that would be adaptive affecting their reproductive success. Male redback spiders, reared in the presence of females, have been shown to accelerate their development relative to males reared in the absence of females (Kasumovic & Andrade 2006). I measured physiological responses of male redback spiders reared with varying numbers of rivals in the presence or absence of females to see if energetic rates were related to cues of the availability of mates and likelihood of competition. Strikingly, I provide evidence that physiological variation (in resting metabolic rates) is linked to the number of rivals but unrelated to changes in development in response to cues of the availability of mates. Increased resting energetic rates with increasing number of rivals may reflect energetic requirements for mating success as a function of the likelihood of competition and lead to advantages during scramble competition to arrive on female webs prior to rival suitors. The physiological responses of males are plastic and fine-tuned to environmental cues of the likelihood of competition. Studies investigating physiological plasticity in response to cues of the availability of mates and likelihood of competition are rare but in this chapter I have demonstrated how cues indicating the likelihood of competition can alter male physiological responses important for maximizing fitness under male-male competition independent of female choice.

Regardless of conspecific density, males of species with sedentary females must actively seek mates. Sex pheromones enable this process for many species, and can also trigger courtship (fruit flies: Shorey & Bartell 1970; crayfish: Aweyaw-Akumfi & Hazlett 1975; moths: Baker & Carde 1979; snakes: Garstka & Crews 1981; crickets: Tregenza & Wedell 1997; salamanders: Rollmann et al. 2000; spiders: Papke et al. 2001). When females are widely or unpredictably distributed, variation in female production of sex pheromones can limit male mating opportunities and allow females control over the opportunity for polyandry. In chapter three I determined that male redback spiders discriminate female mating status based exclusively on web-borne chemicals. I showed that males exposed to extracts from webs of virgin females were more active than males on extracts of webs of juveniles, non-virgin females and solvent controls. Male activity level to extracts of webs produced by virgin females disappeared rapidly after these females mated, suggesting that females cease pheromone production immediately after mating. During courtship, male redbacks dismantle much of the female's web, and these results suggest that the web rebuilt by females after mating does not trigger male courtship. Although male discrimination based upon female reproductive status is common in many species, the rapid shift demonstrated in this work suggests that the timing of mate attraction is under female control. Together with collaborators I subsequently identified and synthesized the pheromone, and confirmed my conclusion that females cease production of this sex pheromone after mating (Jerhod et al. 2010).

More than one male is frequently attracted to the pheromone-emitting web of a virgin female, but females often mate with only one or two males. This suggests the importance of choice in male mating success. Choosy females may use a variety of decision rules, which roughly fall into threshold or best-of-n categories. Identifying such rules is challenging, and decision rules have been determined in only a handful of taxa (Moore & Moore 1988, Zuk et al. 1990, Parri et al. 2007, Ivy & Sakaluk 2007). In chapter four I hypothesized that females use a threshold criterion for choice based on the male's courtship duration, and argued that this could have a significant effect on competitive interactions. I used published data (Stoltz et al. 2008) to infer a threshold of approximately 100 minutes of courtship. Data presented within this chapter demonstrated that premature cannibalism was common if the male's courtship duration was below this threshold, but was unlikely once the duration was satisfied. Next I examined how this might affect competitive outcomes by testing whether females discriminate the source of courtship when rival males compete. I staged competitions where one male (the resident) courted to the female's threshold before a rival was introduced. I determined that males that were introduced after the threshold had already been met by the resident were able to mate rapidly with minimal courtship effort. These 'parasitic' males were not prematurely cannibalized by females, contrary to the situation when males mate quickly with no resident courtship. This work suggests that although courtship duration is the trait that is selected through female choice, the apparent lack of discrimination by females among males may favour parasitic tactics and minimal courtship from males in some contexts. This is a clear example of a situation in which a trait that is selected through female choice (prolonged courtship) is in opposition to that favoured by male-male competition (minimal courtship to ensure first mating), and this drives the selected trait away from the female optimum that can maximize male fitness.

Lifetime reproductive success of males and females may depend on patterns of remating as well as the outcome of the first mating interaction. Variation in mate attraction patterns by sedentary females will set the natural windows during which polyandry may occur. I capitalized on my own earlier work (chapter three, Perampaladas et al. 2007, Jerhod et al 2010) to identify two natural widows of opportunity for polyandry in redback spiders—one when females are virgins (chapter 3, Jerhod et al. 2010) and one after females recommence pheromone production after producing several egg sacs (Perampaladas et al. 2007). In chapter five I examined polyandry within these two windows and compared how this affected male and female lifetime reproductive success. I mated females to pairs of males within 24 hours, or with a two month delay between matings. I compared paternity of rival males in these treatments, and also assessed the reproductive output and longevity of females to determine if there is a cost associated with the timing of polyandry. With simultaneous polyandry paternity was roughly split conforming to a ‘raffle’ process. However, when there was a delay of two months between rival matings there was a shift to bias paternity in favour of the novel male. The net effect of this pattern was equal paternity for each mate across the lifetime of the female, which would increase the genetic diversity of the female’s offspring. However, polyandry came at a cost for females related to when the second mating occurred. In the first 60 days (when females in the delayed treatment were still monandrous) females in the simultaneous polyandry treatment had lower reproductive output relative to females in the delayed treatment where their output increased with mass. However, comparing reproductive output after 60 days (delayed treatment females were now polyandrous) there was no difference in reproductive output as a function of the timing of the second mating. Nevertheless, the initial reproductive gain that females in the delayed treatment had while monandrous (during the first 60 days) led to increased lifetime reproductive output relative to females where multiple mating occurred simultaneously. This work clearly demonstrates that the timing of polyandry places strong selective pressure on females to control the timing of mate attraction. This study, to my knowledge, is one of the first demonstrations of how costs of polyandry can be reduced through female control of mate attraction cues.

The effects of delayed polyandry on female reproduction would also depend on factors such as food availability during the inter-mating period. Web-building spiders experience wide variation in food intake, and periodic starvation is likely to be a normal part of their natural history. However, as in other taxa, decreased food intake (up to a point) could increase longevity (Kirkwood & Austad 2000) at the cost of decreased reproduction (Carey et al. 2008). In chapter six I investigated the effects of dietary restriction on female longevity and reproductive output while tracking resting energetic rates to determine links between physiological responses, reproductive output, and variable food availability in females. I found that starved mated females rapidly decreased their resting energetic rates and stopped egg production. Once resource availability resumed, the resting energetic rates of mated females increased immediately and egg sac production continued. The result was no net decrease in lifetime reproductive output relative to mated females fed a normal diet. I discovered longevity was negatively correlated with reproductive effort, particularly for females operating under a period of dietary restriction. Despite this however, starved mated females had the highest longevity of any experimental group. Further, mated females (whether fed or starved) had higher longevity than virgins, suggesting virgins pay longevity costs of remaining unmated, perhaps due to the maintenance of high energetic rates. This result, and in particular the link to a possible underlying mechanism (elevated metabolic rates) may lead to new areas of study in the physiology of mating and in the assessment of costs and benefits of mating patterns.

Although there are costs of polyandry dependent on when multiple mating occurs (chapter five), my data suggest that there is also a cost of remaining unmated for redback females, apart from the loss of reproduction. The solicitation of males by pheromones (chapter 2) is thus necessary, but may lead to inter-sexual conflict depending on the number of suitors that arrive. Females are selected to minimize the costs of polyandry and maximize the benefits by controlling the timing of rival male arrival. However, males are selected to seek out any receptive female leading to instances where multiple males arrive simultaneously, although this can be costly to females (chapter 4, chapter 5).

In my thesis, the approach I have taken is to detail how traits that are selected through male-male competition and female choice interact, and how they change depending on the context under which the sexes are interacting. Studies investigating sexual selection often consider either only male-male competition or female choice and rarely integrate their interactions (Hunt et al. 2009). Whether male traits selected through male-male competition and female choice are in conflict or congruent varies depending on the species considered (Watson 1990, Berglund et al. 1996, Wiley & Poston 1996, Moore & Moore 1999, Moore et al. 2001) and can depend on the timing of the mating interaction considered (Wong & Candolin 2005, Hunt et al. 2009). The effects of male–male competition on female choice can depend on how the outcome of competition affects the direct and indirect benefits for females that mate with the victor (Wong & Candolin 2005). This effect may vary depending on which stage (during detection, evaluation and mating outcomes) is considered (Wong & Candolin 2005). To maximize total fitness a female may have to trade off direct and indirect benefits of choice and polyandry. For example, Wong & Candolin (2005) suggest that in many species of birds the most attractive males provide indirect benefits but also direct costs due to their low levels of provisioning that must be compensated by females. Critically, understanding how interacting mechanisms of sexual selection will affect trait evolution requires thorough knowledge of the life-history of the species of interest (Hunt et al. 2009, Wong & Candolin 2005).

Female reproductive decisions can be shaped by the potential direct or indirect benefits of mating with particular males at particular times. Wagner & Basolo (2008) suggest that past costs and benefits of mating can lead to conditional mating tactics of females over time. The impact of indirect benefits of timing of mating can be exemplified in systems where females ‘trade up’ if higher quality individuals become available (Pitcher et al. 2003) or if females re-advertise receptivity later in the reproductive season. The research I present on female control over mate attraction (chapter three) reflects the ability of female redback spiders to incite male-male competition (e.g. Cox & Le Boeuf 1977, Oda & Masataka 1996, Wiley & Poston 1996, Berglund & Rosenqvist 2001) at two separate times during their life history—while a virgin, and months post-mating. The incitation of competition by advertising receptivity through sex pheromones (chapter 3) may benefit choice by ensuring honesty in the signals used by males under competition (Wong & Candolin 2005) when rivals simultaneously arrive on a female’s web. Alternatively, during mate detection, competition may hamper choice if it limits the ability of preferred males to locate females (Wong & Candolin 2005). During the first window of mate attraction, competition could interfere with mate detection if males that detect an increased likelihood of competition rapidly locate females (chapter 2) and limit the ability of preferred rivals to localize females. Competition can also interfere with choice during mating outcomes if males can parasitize the attractiveness of higher quality suitors and bypass choice (Wong & Candolin 2005). Although females appear to incite competition (chapter 2), and ‘set the rules’ through which competition occurs, during mating outcomes, intruding male redback spiders appear able to bypass or circumvent female choice (chapter 4) providing a clear demonstration of a negative impact that competition can have on choice. While the specific direct and indirect benefits related to the timing of mating in redbacks remain unknown, mating in these two intervals has very different implications for the paternity of competing males as well as for female longevity and reproductive output (chapter five).

An additional important factor that may shape female reproductive decisions is the reliability and volume of food availability in a given habitat. Female fitness following a given pattern of mating and remating will depend heavily on whether food intake is sufficient to allow the continued production of offspring. Since food scarcity can lead to rapid cessation of egg production for mated females, (reviewed in: Kirkwood & Austad 2000, chapter six) any delay in remating could entail the risk that possible benefits of polyandry may never be realized. However, for mated redback females, since longevity is increased by a period of scarcity (dietary restriction of 6 weeks) and lifetime reproductive output is unaffected, relative to the output of females in which dietary restriction did not occur (chapter six), the tactic of delaying polyandry may be optimal if the sources of extrinsic mortality (e.g. predation) are low. The importance of a life history perspective is further demonstrated in my study detailing how female longevity is affected by the timing of polyandry (chapter five) along with underlying physiological responses as a function of reproductive status and resource availability (chapter six).

Clearly, male behaviour can be shaped by choice and competition. I show here that these processes can have impacts on physiological function (chapter 2) as well as behaviour. Moreover, the success of male competitive tactics (behavioural and physiological) can depend on the type and implementation of female choice decision rules (chapter 4). My study on male developmental plasticity showed how physiological responses (chapter 2) as well as phenotypic traits of males are tuned to cues of the likelihood of competition. In the presence of rivals, redback males accelerate development (Kasumovic & Andrade 2006). The cost of accelerated development is smaller body size, which could reduce success in direct competition (Kasumovic & Andrade 2009, Stoltz et al. 2008). This developmental tactic can nevertheless be favoured because it gives males a time advantage so they arrive at a female's web before larger rivals (Kasumovic & Andrade 2009). I show that this alteration of male developmental trajectories extends to the level of physiological support for this 'scramble' competition tactic. The elevated metabolic rates of scrambling males may allow males to sustain the levels of activity required to rapidly locate females (e.g. Shillington 2005) and can thus be selected through male-male competition for finding virgin females (chapter two). Elevated resting metabolism however, will also deplete the reserves available for energetically costly courtship (Stoltz, unpub.). Thus this trait favoured through competition may decrease mating success with females since courtship duration is the main focus of female choice (chapter four). Males that are unable to meet the demands of prolonged courtship (chapter four) are killed by females before achieving a second copulation (Stoltz et al. 2009) which would lead to a decrease in total expected paternity if females remate (chapter six). Thus my work provides a new example of how traits selected through female choice (prolonged courtship duration; chapter four) may be in opposition to those selected through male-male competition (elevated metabolic rates; chapter two).

The tension between traits favoured by choice and competition is particularly evident if one considers the opposing selection imposed by the need to (1) satisfy the female's courtship duration threshold (chapter four) versus (2) achieve mating before the female's sex pheromone (chapter two) attracts a rival male. This tension is particularly important for males that arrive first on the web of an uncontested virgin female. If a rival arrives before the mating occurs, the first male's courtship effort could be usurped and that male may be excluded from paternity (chapter four). Thus, particularly when the density of rival males is high, net selection may essentially drive courtship duration away from the value that is selected through female choice. Under this context, when male-male competition is intense we see that traits selected through female choice appear to be in opposition to those selected through male-male competition (Hunt et al. 2009). This effect would be reinforced by the absence of discrimination of females among males (chapter four) since this favours sneaking behaviour, particularly by males in poor body condition who may not be able to sustain courtship. In contrast, when the density of rivals is low, the low risk of usurpation may increase the extent to which courtship duration can increase male fitness through female discrimination. This shift in net selection, linked to density, may be a second, unexplored reason for the developmental plasticity documented by Kasumovic & Andrade (2006). This work highlights the importance of how traits selected through competition can be in opposition to those selected through choice, and how the net effect on selection may shift with context. Studying one mechanism in isolation would not capture the complexities of these interactions, or allow accurate predictions about how net selection may operate under different demographic conditions.

Future Directions

My thesis has successfully identified how mechanisms of sexual selection interact with one another at various stages of the interaction between the sexes. However, such a broad approach addressing sexual selection at various levels is likely to leave many questions at each stage unanswered. Here, I outline what I consider to be some of the most outstanding questions that would benefit from future consideration.

Plasticity underlies much of the wide variation seen in phenotypic diversity (Tomkins & Moczek 2009, Tomkins et al. 2005, Emlen & Nijhout 2000, West-Eberhard 2003), and is a critical evolutionary force (Gotthard & Nylin 1995, West-Eberhard 2005). In chapter two my results demonstrated that male redback spiders exhibited plasticity in their physiological response to the availability of mates and the likelihood of competition. While I used published data on other taxa to make arguments regarding the effects of variation in resting metabolic rates on male performance, an important next step would involve collecting field data on how such physiological responses translate to outcomes during male-male competition and female choice. This research could highlight relationships between development, energetic rates, processes such as mate searching, and sexual performance which remain largely unknown (but see Kasumovic & Andrade 2009).

My results from chapter three demonstrated that web-bound chemicals produced by virgin females disappears rapidly after these females mate. This is particularly interesting as it is likely to be the result of females' strategy to reduce male harassment. Although my research suggests the timing of mate attraction is under female control it may be the case that chemicals transferred with the ejaculate of the first mate prevent females from producing pheromones for a period after mating. Sexual refractory periods of females have been investigated in other invertebrates (Thornhill & Alcock 1983) and whether they represent male or female interest remains unclear (Simmons & Gwynne 1991). The cessation of pheromone production demonstrated in redbacks (chapter two, Perampaladas et al. 2007) is a functional refractory period. Here, there is great potential to determine whether the refractory period represents male or female interests. Studies investigating the inhibiting effects of male ejaculate on female remating suggest that short term inhibition can be caused by accessory gland fluids (Hihara 1981), however, long term inhibition is caused by sperm that have bound sex peptides (Peng et al. 2005). Studies separating the transfer of sperm from seminal products would likely elucidate which sex has control over the timing of the re-advertisement of female receptivity.

In chapter four I identified how female choice selects for prolonged courtship and females used a courtship duration threshold rule. I further explore the idea that females are unable to discriminate the source of courtship allowing intruding males to mate with no penalty after the threshold has been reached. Further research should address why courtship is selected through female choice by measuring energetic rates needed for courtship and investigating the relationship between courtship, male quality, and offspring fitness. Energetic costs of courtship have been investigated in several taxa (Prestwich & Walker 1981, Bennett & Houck 1983, Watson & Lighton 1994, Kotiaho et al. 1998) and links between signal attractiveness and energy expenditure have been explored (Watson & Lighton 1994, Taigen & Wells 1985, Wells & Taigen 1989, Reinhold et al. 1998) but concrete links between energy expenditure and fitness are lacking. Work at this stage should also investigate the intruding ‘sneaker male’ strategy in nature to see if low quality males do indeed seek out competitive situations with rivals since this may increase success through usurpation. Although alternative male mating strategies, such as sneaking, arise when males differ in competitive ability (Gross 1996), in redbacks, the sneaking strategy may represent the exploitative ability of males to circumvent female choice rather than differences in competitive abilities among males. Studies are desperately needed to determine whether such a strategy is related to male quality or the inability of females to discriminate among males. Moreover, such work could examine whether females in fact benefit from mating with such males, which may help illuminate the tendency of females to mate with ‘sneaker’ males across taxa (see Reichard et al. 2007). It is highly unlikely that male redbacks benefit from the presence of sneakers through mechanisms such as costs of aggression reducing mating success or their presence being beneficial in attracting females (Candolin & Reynolds 2002). Such benefits are unlikely due to the critical importance of first male sperm precedence (Snow & Andrade 2005) ensuring aggression is warranted to achieve first mating and the fact that females attract males due to sex pheromones deposited on their webs rather than males attracting females (Jerhod et al. 2010). Due to the large sexual size dimorphism it is unlikely that males can coerce females suggesting that sneaker males may not be low quality males making the ‘best of a bad job’. If females are unable or unwilling to discriminate the source of courtship then males of low or high quality should be equally likely to employ the sneaking strategy when the opportunity is available to ensure first copulation. Alternatively, females may benefit from mating with sneaker males by increasing their additive genetic quality (Kokko et al. 2002), non-additive genetic quality (Zeh & Zeh 1996), enhanced genetic diversity (Watson 1991) or fertility insurance (Wedell et al. 2002). An understanding of the extent to which each of these mechanisms contributes to the benefits of mating with males employing a sneaker strategy remains unknown.

In chapter five my results suggest that cryptic female choice can bias paternity to novel males and polyandry has a cost of reduced longevity dependent on the timing of the matings. Here it would be of great interest to measure offspring survivability for females that mate singly or multiply to determine the benefits of polyandry in a variety of environmental conditions (e.g. different resource availabilities, temperatures etc) (Zeh & Zeh 1996, Tregenza & Wedell 1998, Arnqvist & Nilsson 2000, Jennions & Petrie 2000, Simmons 2001, Forster et al. 2003). An investigation of the mechanism underlying decreased longevity with simultaneous polyandry would be interesting, particularly because this is a common but poorly understood effect across taxa (Arnqvist & Nilsson 2000).

Finally in chapter six I demonstrated life history trade-offs between mating history and resource availability on female longevity (see Stearns 1989). Although I showed no decrease in offspring number with starvation, it is not clear whether these offspring differ in condition from those produced by females that were well fed (e.g. Sinervo 1990, Allen et al. 2008). Such data are necessary to determine how food scarcity would affect female fitness in nature. Similarly, it would be interesting to track rates of predation of females in the field to determine if the longevity acquired by depressing energetic rates translates into time to make more eggs and thus increased fitness (see Shine 1980). One of the more intriguing results of this study was the elevated resting metabolic rates of virgin females and correlated survival cost. Investigation of the mechanistic underpinning of these effects may reveal important aspects of the female's reproductive tactic with respect to the timing of mating.

Conclusion

Sexual selection arises due to variation in reproductive success among individuals via the mechanisms of male-male competition and female choice. Until recently, many studies isolate and report one mechanism which may over/under estimate the importance of the trait investigated. More realistic, but complicated outcomes are obtained by studies that incorporate the simultaneous impact of male-male competition and female choice. Studies that isolate one mechanism are still vital as they can determine which traits are selected, but conclusions about global effects on trait evolution that extrapolate from such studies should be interpreted with caution. My thesis has investigated the simultaneous influence of male-male competition and female choice operating at various levels of the interaction between the sexes on several traits across their lifetime. Due to differences in the reproductive interests of males and females, the mechanisms of sexual selection will not always act in concert. Comprehensive accounts of sexual selection should include studies that show how traits selected through male-male competition can act in congruence or conflict with those selected through female choice at different stages of the mating interaction.

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Appendix
Male competition and female mass

In this appendix I include additional results (including tables and figures) from the studies outlined in chapters four and six. These were not included in the published paper (Stoltz & Andrade 2010) to focus the paper and reduce its overall length in accordance with reviewer and editorial suggestions or included as part of the supplementary material (Stoltz et al. 2010). Additional results are provided for Figures and Tables 1-3. The results of Figure and Table 4 appear as part of the results in Chapter six.

Additional results:

Resident males did not achieve significantly more copulations than intruders when all treatments were pooled, when larger males were residents, when smaller males were residents, or when residents were size matched with intruders (Table 1). Although previous research has suggested that larger males typically win during competition when they compete simultaneously for females (Stoltz et al. 2008) I show that residency can reverse the small male size disadvantage in direct interactions on the female's web (Table 1).

There was no significant difference in the total time prior to the first copulation (i.e., time from the start of trial, including the period of exclusive access) whether that first copulation was achieved by a resident (which had courted in isolation) or an intruder. This was true regardless of whether the resident was smaller, larger or matched in size to the intruder (Table 2).

I compared my results to previously published data from similar trials in which males were introduced simultaneously to females' webs (i.e., size matched or mismatched males, but no residency treatment; Stoltz et al. 2008, 2009). Whether males were introduced simultaneously or one male was given a residency advantage did not affect the total number of times a female copulated in a trial (Table 3). For size mismatched trials, the total number of copulations a smaller male achieved was not significantly different whether he was the resident, intruder or simultaneously introduced with a relatively larger rival (Table 3). Similarly, there was no significant difference in the total number of copulations a larger male obtained regardless of whether he was the resident, intruder or simultaneously introduced with a relatively smaller rival (Table 3).

Figure one depicts the weight distribution of males used within the experiment.

I examined whether residency status, relative male size, or some combination affected courtship progress or the timing of mating. Regardless of the size of the resident male relative to the intruder, the first contact with the female's abdomen (phase 2, time of introduction of intruder) occurred after 75 ±6 min. of courtship (log $F_{2,58}$=0.67, p=0.52; Fig. 2). The first copulation occurred approximately 2 hours after the start of the trial regardless of treatment (log $F_{2,53} = 0.98$, p=0.38; Fig. 2), well above the 95% confidence interval for the estimated choice threshold.

Finally, I asked whether the relative mating success of rivals was related to premature cannibalism of first-mating males. Regardless of whether the first-mating male was prematurely cannibalized, females mated relatively infrequently with rivals (Fig. 3). There was no significant relationship between premature cannibalism of the first male and number of copulations achieved by the second male (0, 1 or 2) in mismatched-size trials (Logistic model $\chi^2 = 2.95$, df = 2, p = 0.229) or matched-size trials (Logistic model $\chi^2 = 2.96$, df = 2, p = 0.228).

Table 1- Comparison of the proportion of resident and intruder male redback spiders (*Latrodectus hasselti*) achieving 1, 2 or 3 copulations in competitive trials as a function of their relative size.

Relative Size of Resident	Male	Number of Copulations				Statistic	P
		0	1	2	3		
Smaller	Resident	23% (N=3)	31% (N=4)	46% (N=6)		χ^2=3.6	0.17
	Intruder	54% (N=7)	31% (N=4)	15% (N=2)			
Larger	Resident	41% (N=9)	36% (N=8)	23% (N=5)		χ^2=0.47	0.79
	Intruder	31% (N=7)	46% (N=10)	23% (N=5)			
Matched	Resident	14% (N=3)	73% (N=16)	9% (N=2)	4% (N=1)	χ^2=2.36	0.5
	Intruder	27% (N=6)	68% (N=15)	5% (N=1)			
All pooled	Resident	23% (N=13)	53% (N=30)	23% (N=13)	1% (N=1)	χ^2=4.66	0.2
	Intruder	39% (N=22)	47% (N=27)	14% (N=8)			

Table 2- Total latency to the first copulation by either male as a function of the size of resident males relative to intruders in competitive mating trials.

Relative Size of Resident	Male	Total time to first copulation (min.)	Statistic	P
Rival delayed introduction				
Smaller	Resident	122±18	log t_{17}=-0.72	0.48
	Intruder	134±24		
Larger	Resident	120±24	log t_{15}=-0.32	0.75
	Intruder	116±17		
Matched	Resident	119±24	log t_{18}=-0.94	0.94
	Intruder	116±17		
Simultaneous rival introduction (from Stoltz et al. 2008, in press)				
Smaller		85±14		
Larger		208±26		
matched		83±11		

Table 3- The total number of copulations a male obtained as a function of his residency status and size relative to his rival.

Relative Size of Male	Number of copulations	Timing of male introduction			Test	P
		Resident	Intruder	Simultaneous		
Smaller	0	20% (N=4)	41% (N=7)	31% (N=16)	χ^2=3.22	0.78
	1	45% (N=9)	41% (N=7)	39% (N=20)		
	2	35% (N=7)	18% (N=3)	28% (N=14)		
	3	0% (N=0)	0% (N=0)	2% (N=1)		
Larger	0	40% (N=8)	45% (N=9)	33% (N=17)	χ^2=3.27	0.51
	1	45% (N=9)	45% (N=9)	39% (N=20)		
	2	15% (N=3)	10% (N=2)	28% (N=14)		
	3	0%	0%	0%		

Table 4- Effect of (A) female mating status (virgin or mated), diet and time (initial measure, 2, 4, or 6 weeks) on mass of female redback spiders and (B) diet and time on mass for mated females only in weeks 8-12 when previously starved females were fed again.

2 factor repeated mixed model ANOVA

A. Weeks 1 - 6

	F	d.f.	p
Between subjects			
Diet x status	25.19	1,31	**<0.001**
Diet	9.78	1,31	**<0.01**
Status	0.51	1,31	0.48
Within subjects			
Time x status x diet	5.4	3,95	**<0.01**
Time x status	0.46	3,95	0.71
Time x diet	8.91	3,95	**<0.001**
Time	5.87	3,95	**<0.01**

B. Weeks 8 – 12 (mated females only)

	F	d.f.	p
Between subjects			
Diet	1.14	1,13	0.31

Within subjects			
Time x diet	0.65	2,27	0.53
Time	9.68	2,27	**0.001**

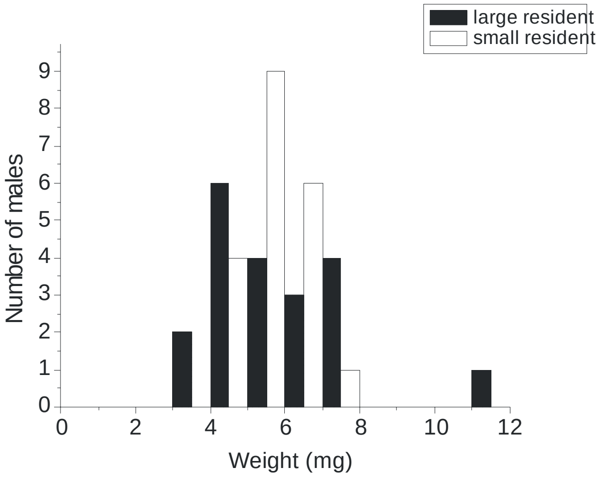
Figure 1- Weights of male Australian redback spiders which were larger (A), smaller (B) or matched in size (C) to their rival in staged competition trials. For (A) and (B), black boxes represent trials where larger males were the resident and open boxes where the smaller male was the resident.

Figure 2- Latency to first abdomen contact by the resident male (black bars) and latency to the first copulation by either male (grey bars) as a function of the size of the resident male.

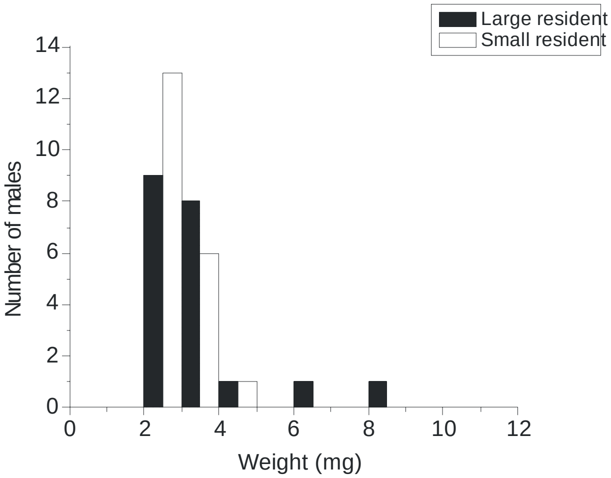
Figure 3- Percent of rival males achieving 0, 1 or 2 copulations as a function of whether the first male to mate was prematurely cannibalized (black bars) or not (grey bars).

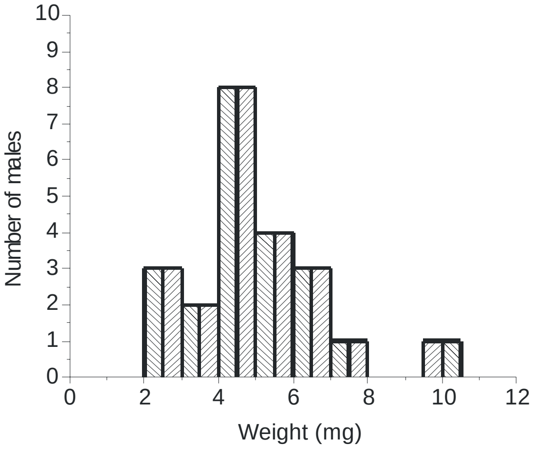
Figure 4- Mass of virgin females (triangles) over a six week period during which they were fed a normal diet (open symbols) or starved (closed symbols). Mated females (circles) are shown over the same six week period during which they also fed (open) or starved (closed). After the six week starvation period, mated females that had been starved were returned to a normal diet (week's 8 – 12, closed circles) and compared to mated females that had been fed throughout the period (open circles,).

A

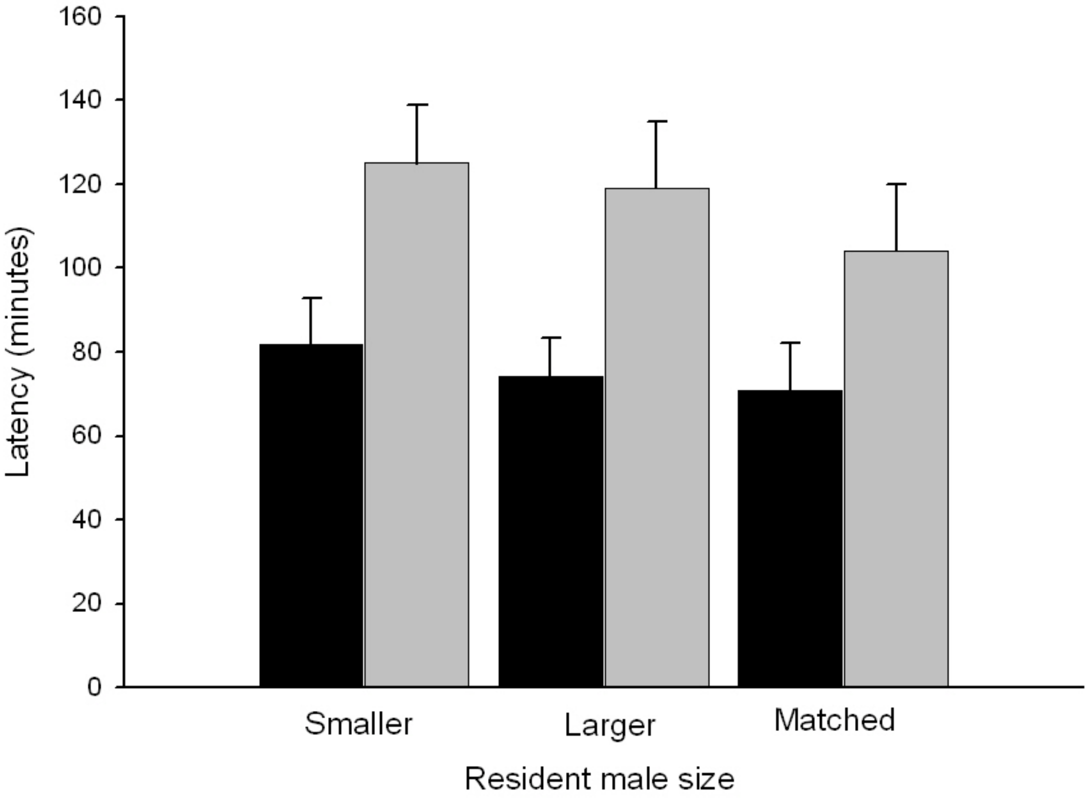

large resident
small resident
Number of males
0
1
2
3
4
5
6
7
8
9
0
2
4
6
8
10
12
Weight (mg)

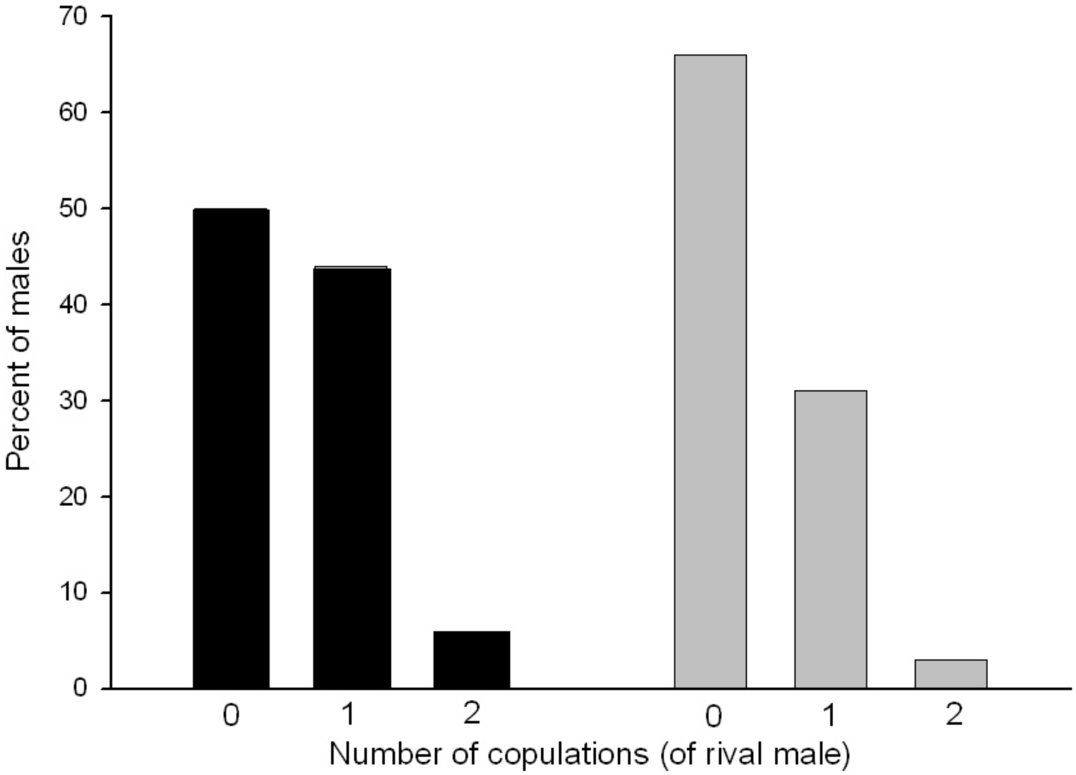
B

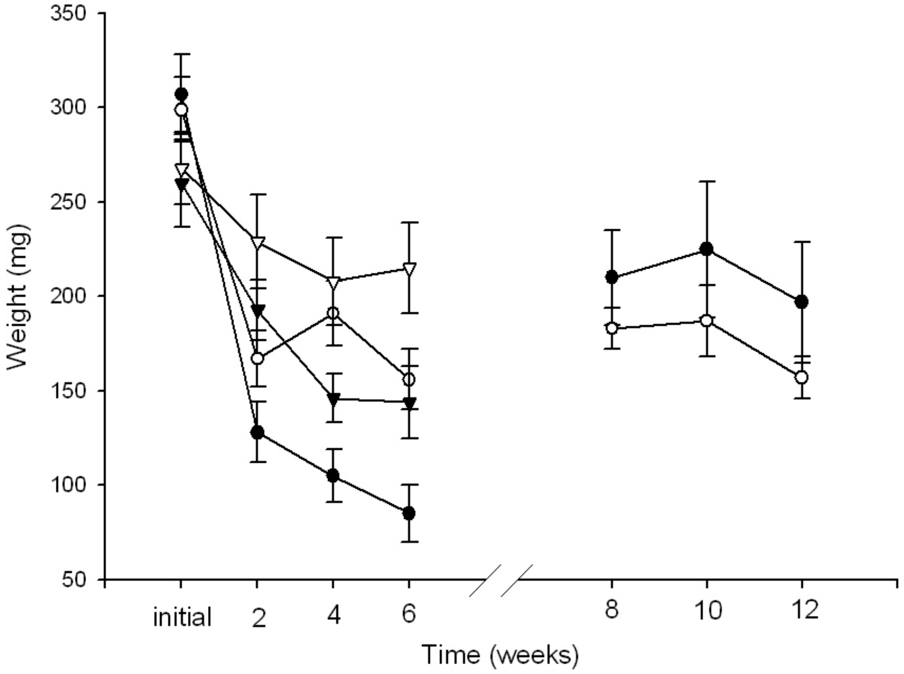

Large resident
Small resident
Number of males
0
2
4
6
8
10
12
14
0
2
4
6
8
10
12
Weight (mg)


Number of males
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
Weight (mg)

C


160
140
120
100
80
60
40
20
0
Latency (minutes)
Smaller
Larger
Matched
Resident male size


70
60
50
40
30
20
10
0
Percent of males
0
1
2
0
1
2
Number of copulations (of rival male)


350
300
250
200
150
100
50
Weight (mg)
initial
2
4
6
8
10
12
Time (weeks)