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Parental effects in orb-web spiders of the genus *Argiope* (Araneae: Araneidae)

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Summary

Non-genetic factors act in parallel to genetic factors to shape offspring phenotype. The magnitude of inherited genetic and non-genetic factors and the interaction between them determines the susceptibility and direction of selection on different life-history traits. In this thesis, I explored the adaptive parental effects mediating reproductive output and offspring quality in the orb-web spider genus, *Argiope* spp. First, I assessed the effect of predation as an environmental factor on the physiology and foraging behaviour of *Argiope* spiders. Then I tested the effects of parental physiological traits, i.e. resting metabolic rate (RMR), age and condition on reproductive output and offspring performance. Finally, I addressed the importance of additive genetic effects and heritability of different life-history traits in offspring. The results revealed that exposure to predation risk altered the foraging behaviour and significantly elevated RMR in the spider. RMR was not correlated with reproductive output or offspring quality, however, female spiders reduced their RMR after mating. This can be an adaptive response for female spiders at the onset of the egg producing process to mediate self-maintenance costs and to allow the allocation of more resources to reproduction. Temporal variation of maternal effects showed that egg sacs laid early in life were larger and offspring emerged with larger body size, however these spiderlings were less tolerant to starvation than spiderlings produced later in the season. This is possibly due to increase in egg size with maternal age. Testing the effect of condition revealed that maternal and paternal condition had asymmetrical effects on egg sac mass. There was also a trade-off between offspring body size and their tolerance to starvation. Analysing half-sib spiderlings showed that egg size and web hub size were heritable traits, suggesting these traits are less plastic and subjected more to selection than other traits. These findings provide insight into the adaptive parental effects, which may have long lasting impact on internal state and behaviour of offspring and hence population dynamics.

Declaration

I certify that this thesis is composed of my original work. I have clearly stated the contribution by the others jointly authored works that are included in this thesis.

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

Mohammad Ameri
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Introduction

Heritable epigenetic and somatic factors act in parallel with genes to induce phenotypic variation in offspring. These factors, which are known as parental effects, do not involve the modification of genetic codes and can alter offspring phenotype (Bonduriansky and Day 2009; Cuzin et al. 2008; Youngson and Whitelaw 2008). Parental effects have been widely assumed to be a source of nuisance variation in early quantitative genetic studies (Falconer 1981), however, since the landmark publication of Mousseau and Fox (1998), extensive empirical evidence has been accumulating, demonstrating that offspring phenotype is altered in response to environmental cues detected by parents (Mousseau and Fox 1998). Since then parental effects have received extensive attention from evolutionary biologists and are now recognized as one of the most important source of offspring phenotype variation with potentially life-long impacts on population life histories (Bernardo 1996; Marshall and Uller 2007; Mousseau et al. 2009; Uller 2008).

Researchers address parental effects from a proximate perspective in which mechanisms that parents use to match offspring phenotype with local condition investigated (Bonduriansky and Day 2009). As such, parental effects may act as a buffer for offspring to minimize the environmental stressors, therefore often considered as adaptive transgenerational plasticity, i.e. adaptive parental effects (Agrawal 2001; Agrawal et al. 1999; Galloway 1995; Mousseau and Dingle 1991). Parental effects have also been investigated from an ultimate perspective in which fitness benefits of these effects were assessed (Bonduriansky et al. 2016; González-Teuber et al. 2008; Jensen et al. 2010; Rotem et al. 2003).

Ultimate mechanisms – are parental effects adaptive?

Marshall and Uller (2007) argued that there are three issues with exploring adaptive significance of parental effects. First, selection may act differently on parents and offspring, however, parental effects have often been assessed based on offspring performance (Mousseau and Fox 1998). Maximizing offspring fitness, selection would favor a small number offspring with large body size. On the other hand, maximizing

parent fitness may favor many offspring with smaller body size (Fox and Czesak 2000; Smith and Fretwell 1974). This is referred to as ‘parent-offspring conflict’ (Godfray 1995; Trivers 2009) and an exclusive focus on either parent or offspring may not reveal a clear picture of the adaptive significance of parental effects.

Second, parental effects may represent temporal reproductive strategies, where a *per capita* decrease in offspring fitness may increase available resources for later reproductive bouts (Marshall and Keough 2004; Najafpour et al. 2018). For example, young soil mites, *Sancassania berlesei*, produce small, low performing offspring, while older females produce larger and higher performing offspring (Plaistow et al. 2007). Therefore, exploring parental effects throughout individual’s reproductive life-time is necessary to assess the relative importance of temporal variation in parental strategies.

Third, parental effects are likely to influence a range of traits simultaneously. This may result in trade-off between traits, producing an entwined network of life-history traits (Roff and Mousseau 1987; Rossiter 1996). For example, in the soil mite *Sancassania berlesei*, protein content of the eggs negatively correlated with fecundity (Benton et al. 2005). Moreover, in lemon sharks there is a trade-off between growth rate and body condition (DiBattista et al. 2007). Thus, assessing a single trait in a classic suitable growth condition versus unsuitable growth condition experimental design may lead to an incomplete or biased interpretation of parental effects. Finally, the strength and the direction of trade-off depends on local condition experienced by parents (Najafpour et al. 2018; Vargas et al. 2012; Vargas et al. 2013), thus caution must be exercised when interpreting the adaptive importance of parental effects on offspring fitness.

Proximate mechanisms of parental effects

Research has explored the mechanisms by which parents mediate their offspring phenotype. These mechanisms, also known as ‘non-genetic inheritance’ are found in

a broad range of taxa and can include epigenetic marks, cytoplasmic factors and manipulation of propagule size (Bonduriansky and Day 2009).

Epigenetics & cytoplasmic factors

Epigenetic effects involve changes in DNA methylation patterns that influence the structure of DNA (Cavalli 2006; Henikoff et al. 2004). The molecular mechanism of DNA methylation is not well understood, but it can affect phenotypic variation that lasts for a few generations without any change in allele frequency (Cuzin et al. 2008; Martin and Zhang 2007; Youngson and Whitelaw 2008). For example, in mice epigenetic factors affect the susceptibility of offspring to obesity and diabetes (Morgan et al. 1999). Eye morphology in *Drosophila* flies changes rapidly in response to artificial selection without change in genetic code (Sollars et al. 2003). Epigenetic effects can be sex specific, with each parent modifying epigenetic marks differently (Martin and Zhang 2007; Spencer 2002; Vigé et al. 2008).

Parental cytoplasmic factors such as protein, hormones and RNA can moderate the phenotype of offspring. This phenomenon is well known in birds where parents manipulate the quantity of hormones of the eggs (Groothuis et al. 2005; Hayward and Wingfield 2004; Okuliarova et al. 2011). Certain proteins transferred from parents to offspring can also alter offspring phenotype (Díaz and Esponda 2004). These proteins are not restricted to oocytes, it has been shown that sperm cells transfer small amounts of proteins that may influence offspring performance (Crean et al. 2016; Wilson et al. 2006). Similarly, RNA found in sperm cells or oocytes can potentially play a significant role as transgenerational signals. For example, in mice, the white tail phenotype induced by RNA molecules can be transferred by both mothers and fathers (Rassoulzadegan et al. 2006). There are also other substances transferred to offspring that alter their phenotype, such as secretions from the oviduct in locusts that induce a shift in morphology and behaviour of offspring (Simpson and Miller 2007), or certain substrates from mammary glands that enhance offspring immunity (Wheeler et al. 2007).

Maternal effects

Studies that explore parental effects have extensively focused on the role of mothers, perhaps because they have a stronger influence on reproductive resource allocation and egg manufacturing (Mousseau and Fox 1998). The effect of various cues experienced by mothers on offspring phenotype have been tested in arthropods, including extrinsic factors such as diet (Vargas et al. 2014), temperature (Huestis and Marshall 2006; Scharf et al. 2010), photoperiod (Giesel 1988), predation (Li 2002), intra-specific competition (Marshall et al. 2006) and intrinsic factors such as condition (Fox and Dingle 1994; Fox 1993), age (Najafpour et al. 2018; Vargas et al. 2014) and metabolic rate in mice (Hayes et al. 1992; Johnson et al. 2001a, 2001b). Shea et al. (2011) characterized factors such as diet or temperature as detection-based maternal effect where the phenotype of the offspring was matched to the environmental cue detected by parents. However, these effects may not have adaptive importance as they may simply reflect the developmental condition of the parents specifically for food deprived treatments. In contrast, factors such as age and metabolic rate are characterized as selection-based maternal effects, which act independently from the immediate environment experienced by parents (Shea et al. 2011).

Egg size

The mother's condition has been shown to be positively correlated with offspring performance (Bonduriansky and Head 2007; Kessler 1971; Najafpour et al. 2018). Since maternal condition and egg size generally have a positive relationship, it is widely assumed that variation in maternal egg size is responsible for offspring phenotype variation (Bernardo 1996; Fox and Czesak 2000; Parker and Begon 1986; Smith and Fretwell 1974). Generally, offspring hatched from larger eggs have larger body size and greater survival rates than their counterparts hatching from small eggs (Fox and Czesak 2000). However, smaller eggs develop quicker than larger eggs, which suggests there is a trade-off between growth rate and body size when

resources are constrained (Fox and Czesak 2000). Offspring hatching from larger eggs can often cope better with environmental stresses such as inter-individual competition (Azevedo et al. 2012), starvation (Carlberg 1991; Gliwicz and Guisande 1992; Mappes et al. 1997; Solbreck et al. 1989; Tessier and Consolatti 1989), desiccation (Sota and Mogi 1992), oxygen stress (Hanazato and Dodson 1995), cold stress (Harvey 1985), nutritional stress (Fox 2000; Fox and Mousseau 1996), and environmental toxins (Enserink et al. 1990). However, some studies have failed to detect fitness advantages of hatching from large eggs; most of these studies have raised progeny in high quality environments (Karlsson and Wiklund 1984; Wiklund 1987; Christer Wiklund et al. 1983), suggesting that selection is generally weak in high quality environments but favours larger eggs in lower quality environments (Fox 2000; Sibly and Calow 1986). Moreover, variation in egg composition can be more influential than variation in egg size (Bernardo 1996; Giron and Casas 2003; Muller et al. 2017), specifically protein content of eggs, which is a better predictor of offspring quality than other components like lipids or sugars (Giron and Casas 2003).

A range of intrinsic and extrinsic factors relate to the size of eggs produced by females (reviewed in Smith and Fretwell 1974; Fox and Czesak 2000). Generally, larger females tend to lay larger eggs, which implies a morphological influence of female on egg size. However, it is a weak relationship, suggesting that egg size is more dependent on the nutritional state of female rather than body size *per se* (Fox and Czesak 2000). Studies manipulating the diet of individuals, with few exceptions (Ernsting and Isaaks 1997; Gwynne 1988; Hard and Bradshaw 1993), have shown that well-fed females lay larger eggs than food-deprived counterparts (Braby and Jones 1995; Fox 1993; Karlsson and Wickman 1990; Kessler 1971; Lynch 1989; Murphy et al. 1983; Steinwascher 1984; Wallin et al. 1992). Theoretical models and empirical evidence support predictions that when food scarcity reduces offspring survival, females increase egg size at the cost of reduced fecundity as an adaptive response to unsuitable condition (Clutton-Brock 1991; Sibly and Calow 1986). Similarly, females reared at high densities tend to increase egg size, possibly by detecting cues released

from other individuals or due to the effect of population density on food availability (Fox et al. 1999; Fox and Savalli 1998; Gwynne 1988; McLain and Mallard 1991). Offspring hatched under these conditions may have higher tolerance to starvation and compete better for food (Cleuvers et al. 1997; Gliwicz and Guisande 1992).

It is well understood that egg size of female arthropods varies throughout the season: most species produce larger eggs at the onset of the season (Avelar 1993; Avelar and Rocha-Pit, 1989; Braby 1994; Brody and Lawlor 1984; Gwynne 1988; McLain and Mallard 1991). However, it is difficult to distinguish adaptive temporal variation in egg size from physiological response to diminishing resources at the end of the season (Green 1966; Lampert 1993). Moreover, egg size and content vary with female age in most arthropods (Fox and Czesak 2000). This decrease in egg size with age is often related to nutritional resource depletion and/or reduced metabolic efficacy with age (Beckman and Ames 1998; Harman 1956; Rose 1991). Similarly, lipid content of eggs reduces with maternal age, which may lower offspring performance (Giron and Casas 2003; Giron et al. 2004; Muller et al. 2017). The reproductive value of clutches laid late in the season could be lower because these offspring are more exposed to predation and fewer resources are available for them. Thus, theoretical models predict females should adaptively reduce resource allocation with age (Begon and Parker 1986; Pianka and Parker 1975).

It has been shown that parental genotype can correlate with egg size. In two seed beetles, the heritability (h^2) of egg size ranged from 0.22 to 0.91 depending on rearing condition of the beetles (Fox 1993; Fox et al. 1999). Similarly, Juliano (1985) showed that egg size in spruce bud-worm *Brachinus lateralis* (Coleoptera: Carabidae) is independent of maternal body size, but it is related to the genotype of the families. Assessing different population of *Daphnia* showed a notable genetic correlation between egg size and parent's genotype across populations (Boersma et al. 1998; Ebert et al. 1993; Glazier 1992). Egg size is also a heritable trait in *Drosophila* (Azevedo et al. 1997; Carlberg 1991; Parsons 1964; Savalli and Fox 1998). Variation in

egg size is affected by some sex-linked genes, however, little is known about the exact alleles that mediate egg size variation in different species (Kawamura 1990). Because egg size is linked to the other life-history traits such as developmental rate or survival, it has been suggested that evolution of egg size is the result of the selection pressure on other traits that are correlated with egg size (Fox and Czesak 2000).

Resting metabolic rate (RMR)

Resting metabolic rate (RMR) is a fundamental trait that determines the baseline rate of energy acquisition and expenditure (Brown et al. 2004). There is a remarkable inter- and intra-individual variation in RMR of different taxa (Biro and Stamps 2010). There is large body of evidence demonstrating that RMR is related to the rate of energy allocation to various life-history traits, such as growth rate, boldness and mating behaviours (Burton et al. 2011; Careau et al. 2008). In terms of maternal effects, RMR of offspring can be mediated by mothers. For example, female birds vary the level of hormones in eggs, which induces changes in RMR in offspring (Chastel et al. 2003; Groothuis et al. 2005; Ros et al. 2004; Tobler et al. 2007). These changes in RMR persist into adulthood in zebra finch offspring (Nilsson et al. 2012).

Predicting the influence of RMR on reproductive investment is often difficult because arguments can be made for an advantage of both high and low RMR. The 'compensation model' predicts that individuals with lower RMR can reduce the energetic costs of self-maintenance, therefore more resources become available to be redirected to reproduction (Bennett and Ruben 1979; Hammond and Diamond 1997; Speakman et al. 2004). But these individuals may not be able to maximize their metabolic rate and energy intake during energy consuming periods such as breeding (Chastel et al. 2003). Conversely, the 'increase intake model' predicts that individuals with higher RMR can accumulate more energy enabling them to allocate more resources to reproduction (Speakman et al. 2004). This come at the cost of producing more induced reactive oxidative species (ROS) that accelerate cellular senescence

and reduce biochemical performance (Janssens and Stoks 2013; Slos and Stoks 2008). This may explain why individuals with higher metabolic rate have lower longevity (Burton et al. 2011).

However, studies that explored the relationship between RMR and reproductive outputs often failed to find a link between RMR and offspring quality and performance (Derting and McClure 1989; Hayes et al. 1992; Johnson et al. 2001a, 2001b; Johnston et al. 2007; but see Sadowska et al. 2012). A major challenge exploring the correlation between metabolic rate and reproductive output is that metabolism is linked with other factors, such as hormones and size of internal organs (Burton et al. 2011), therefore, it is difficult to separate the effect of metabolic rate *per se* from confounding factors. Moreover, most of the studies assess the individual's where *ad libitum* access to food were provided (Burton et al. 2011), a condition which is unlikely to detect trade-offs. The energy budget of an organism that can acquire from environment is limited and this energy budget must be divided among basic physiological processes such as maintenance, growth and reproduction. Therefore, under *ad libitum* condition animals are able to maximize different traits which may diminish trade-offs (Roff 2001; Stearns 1989).

Paternal effects

Early studies of parental effects focused on the role on mother while the effect of father was restricted to the species with parental care (Mousseau and Fox 1998). However, growing evidence suggests that fathers can have a significant contribution to offspring phenotype via mechanisms other than transferring alleles. Such effects are widespread and can influence a wide range of traits. Paternal effects are assumed to induce variation to fitness of offspring, therefore, it is important to distinguish between the variation induced by paternal effects from the other sources of variation (Curley et al. 2011; Rando 2012; Soubry et al. 2014).

Condition-dependent paternal effects

Dietary variation in quantity and quality can influence the condition of males, which can be inherited by offspring (Crean and Bonduriansky 2014; Soubry et al. 2014). For example, when food-deprived and well-fed male rodents mated with control females, food-deprived fathers had offspring with reduced body weight and impaired glucose tolerance (Jimenez-Chillaron et al. 2009). In insects, females lay larger eggs when they mate with larger male or receive larger spermatophores (Fox 1993; Gwynne 1988; Wasserman and Asami 1985). It has been shown that the macronutrients content of paternal diet can alter offspring phenotype. For example, (Bonduriansky et al. 2016) reported that offspring body size in a neriid fly, *Telostylinus angusticollis* (Diptera: Neriidae), is mediated by carbohydrate content and/or carbohydrate-protein interaction in paternal diet. Additionally, medical and epidemiological studies have demonstrated that exposure of fathers to toxin and various chemicals can also induce changes in offspring development (Curley et al. 2011; Rando 2012; Wasserman and Asami 1985).

Seminal fluid

In resource-based mating systems, females take advantage of resources provided by males such as nuptial gift or water via the seminal fluid. In contrast, males in non-resource-based systems do not provide obvious resources for females, and the only contribution of males is a small quantity of ejaculate, which is assumed to be inadequate for transferring nutrition to female (Crean and Bonduriansky 2014). However, recent evidence showed that seminal fluid contains substances that can have non-genetic effects on offspring (Gwynne 1984; Pitnick et al. 1997; Simmons et al. 1999).

Seminal fluids contain various protein, peptides, RNA and some minerals that protect sperm and the female reproductive ducts (Perry et al. 2013) and increase female fecundity and overall reproductive success. Moreover, seminal fluids can have a positive effect on offspring growth and survival in rodents, whereas offspring sired by seminal-vesicle-deficient males were diminished (Jimenez-Chillaron et al. 2009).

However, producing seminal fluid is costly for males, including metabolic costs of protein and non-coding RNA synthesis. The quality of seminal fluid is independent of sperm and genetic quality of male and the volume of seminal fluid stored in accessory glands reduces rapidly with mating frequency, which may have a direct influence on female's reproductive output (Crean et al. 2016; Crean and Bonduriansky 2014; Perry et al. 2013). Yet the environmental factors influencing seminal fluid composition and effects on female mate choice is not well understood (Crean et al. 2016).

Additive genetic effects

Non-genetic paternal effects interact with transferred genes to shape offspring phenotype (Bonduriansky and Day 2009). The relationship between phenotypic variation and inherited alleles from parents and their relationship with fitness has been subjected to great scrutiny by evolutionary biologists (Merilä and Sheldon 1999; Stirling et al. 2002). This can be trace back to Fisher's fundamental theorem arguing that 'the rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time' (Fisher 1930). An inevitable consequence of this theory is reduced genetic variance in traits that are closely related to individual's fitness, because these alleles are more subjected to fixation by selection. Therefore, population genetic theory predicts that traits subjected to high selection pressure should have low genetic additive variance than traits under weak selection pressure (Jones 1987). However, there are assumptions for this prediction, for example number and distribution of loci between comparing traits should be equal (Merilä and Sheldon 1999).

Early studies of quantitative genetics attempted to explore the magnitude of heritability for different life-history traits. Falconer (1996) argued that narrow-sense heritability ($h^2 = V_A/V_P$), where V_A = additive genetic variance, V_P = phenotypic variance of a trait represents its importance in individual's fitness: traits that are closely related to fitness (e.g. sexually selected traits) have higher rates of heritability

than traits with weak contributions to fitness (e.g. morphological traits). Gustafsson (1986) showed that a trait's heritability negatively correlated with life time reproductive success in wild population of collared flycatchers, *Ficedula albicollis*. Roff and Mousseau (1987) reported similar patterns in *Drosophila*. Thus, early evidence supports the idea that traits that are more influential on an individuals' fitness are subject to greater selection pressure and hence have lower genetic variance (Cormack et al. 2006; Mousseau and Roff 1987). However, Price and Schluter (1991) provided an alternative explanation in which the lower heritability rate in some life-history traits may not result from low additive genetic variance (V_A), but due to high environmental variance (V_E). As heritability is the ratio of additive genetic variance to total phenotypic variance ($h^2 = V_A/V_P$), where the effects of dominance and epistasis are excluded, the total phenotypic variance consists of genetic and environmental variance ($V_P = V_A + V_E$). Therefore, the heritability ratio drops with an increase in environmental variance. Price and Schluter (1991) argued that more complex life-history traits and those traits that are affected by other individuals in the population are more subjected to environmental effects, hence these traits could have lower genetic variance and heritability. Houle (1992) provided evidence on different life-history traits of *Drosophila*, which supports this explanation.

Developmental plasticity is the variance in phenotype that is not explained by additive genetic variance and induced by non-genetic factors, i.e. paternal and/or environmental effects (Uller 2008). Highly plastic traits are more influenced by parental effects and the effect of transferred genes can be masked in these traits. While less plastic traits are mediated by genes and are less prone to non-genetic factors (Bonduriansky and Day 2009; Nylin and Gotthard 1998; Uller 2008). Uller (2008) suggested that plasticity should be favoured where (i) there is temporal or spatial fluctuation in environments across generations, (ii) parents can predict offspring condition via reliable cues (iii) the cost of perceiving cues in parents and inducing plasticity in offspring is low. Therefore, by distinguishing the source of

variation on different traits we can explore the relative importance of fitness determinants.

Thesis outline

The overall aim of this thesis is to provide insight into the adaptive parental effects mediating reproductive output and offspring quality. It is hoped that this thesis will reveal the adaptive strategies of the spiders for energy processing and allocation to subsequent generation.

In this thesis, I first assess the effect of predation as an environmental factor on physiology and foraging behaviour in an orb-web spider, *Argiope keyserlingi* (Karsch) (Araneae: Araneidae). Foraging success rate and energy management directly induce changes in parental phenotype (condition) and these changes are very likely to vary phenotype of subsequent generation. I then test the effects of physiological factors, i.e. condition, resting metabolic rate and age of the spiders on reproductive output and offspring performance. Finally, I address the importance of additive genetic effects and heritability of different life-history traits in the offspring.

Chapter one attempts to explore predatory induced variation in foraging behaviour and resting metabolic rate in an orb-web spider, *A. keyserlingi*. Female spiders were exposed to a predatory chemical cue and short-term modification in attack behaviours, web structure and resting metabolic rate were assessed. We hypothesize that under predation pressure, spiders spend less time moving on the web, resulting in delayed responses to prey and reduced handling time under the risk of predation. We also predict that female spiders will invest less energy in foraging, which may result in smaller webs and less frequent rebuilding of damaged webs. Marie E. Herberstein and Kate L. Barry assisted with designing the experiments and Justin McNab assisted with data collection. Marie E. Herberstein, Darrell J. Kemp and Kate L. Barry provided feedbacks on early manuscript. This chapter has been submitted to *Animal Behaviour*.

Chapter two addresses the relationship between resting metabolic rate and offspring performance in the orb-web spider, *A. radon*. To achieve this, temporal variation in resting metabolic rate of male and female spiders were assessed and correlated with reproductive output and offspring quality. We hypothesised that orb-web spiders moderate their energetic costs after mating by changing RMR to facilitate egg sac manufacturing. It is predicted that a lower RMR is favoured in mated compared to unmated spiders thereby reducing self-maintenance energetic costs and enabling greater resource allocation for egg sac manufacture. It is also predicted that parents with higher RMR can convert and allocate more nutrition to offspring resulting in heavier egg sacs, early emergence and larger offspring. Marie E. Herberstein assisted with designing the experiments. Marie E. Herberstein, Jutta Schneider, Kate L. Barry and Darrell J. Kemp provided feedback on early manuscript drafts. This chapter has been submitted to Behavioural Ecology.

Chapter three investigates the age-specific variation in maternal resource allocation and offspring performance in the spider, *A. radon*. In this chapter, quantity and quality of the resources allocated to the eggs and their effects on offspring performance were assessed with respect to maternal age. We predicted that maternal investment in reproduction decreases with age, resulting in egg sacs with lower mass, smaller eggs containing less protein. Consequently, performance of progeny is predicted to reduce with maternal allocation reduction. Marie E. Herberstein assisted with designing the experiments. Marie E. Herberstein, Jutta Schneider, Darrell J. Kemp and Kate L. Barry provided feedback on early manuscript drafts. This chapter has been submitted to Evolutionary Biology.

The **forth chapter** assesses the relative importance of additive genetic effects on offspring performance in the spider, *A. radon*. Using half-sib family design, we assessed the narrow-sense heritability and the effect of sires and dams on different life-history traits of early offspring. We hypothesize that additive genetic effects have low influence on egg size and web structure of offspring and these traits are more

influenced by phenotypic parental condition. In contrast, we predict that body size is under greater additive genetic effects as it is closely linked to individual's fitness such as fecundity and longevity. It is also predicted that tolerance to starvation to be less plastic and due to additive genetic effects rather than parental condition. Marie E. Herberstein assisted with designing the experiments. Marie E. Herberstein, Darrell J. Kemp and Kate L. Barry provided feedback on early manuscript drafts. This chapter has been submitted to Behavioural Ecology.

Study system

Spider genus *Argiope* spp. is an orb-web spider of family Araneidae. In Australia, the genus is found in a wide variety of habitats, from rainforest margins to urban gardens, typically found on short long-leaved bushes such as *Lomandra* sp. and *Pandanus* sp (Rao et al. 2007, 2009). After final moulting, male and female spiders live for one to two and two to three months. These spiders, like other orb-web spiders, are sit-and-wait predators, resting at the centre of the web and waiting for a prey, usually an insect, to intercept the web. The size and structure of the web is highly flexible and affected by various physiological factors, such as condition or age, and environmental factors such as prey availability or light intensity (Heiling and Herberstein 2000). A distinct feature of *Argiope* webs is the silk decorations in the form of zigzag deposits of silk stretching outwards from the centre of the web. Juveniles spiders typically build discoid decorations and switch to the zigzag bands form as they grow older. A maximum of four diagonal bands is normally seen in these spiders (Heiling and Herberstein 2000; Herberstein and Tso 2011). Females build webs in the early morning and rebuild the main web daily. Males build webs only until they become adult and then they wander around searching for females, and subsequently cohabit the female web (Schneider and Andrade 2010).

There is sexual dimorphism in this species, with females being 3–4 times larger than the males (Elgar et al. 2000). During mating, males perform courtship shudders once contact with adult female web and continue that sporadically. It has been suggested

that quality of shuddering is linked with female aggressiveness, poor shudders are more prone to the risk of sexual cannibalism. After courtship males cut a hole into the web, next to the female and invite female onto a mating thread for copulation via specific vibratory signals (Wignall et al. 2014; Wignall and Herberstein 2013). Males can mate only two times and at the second mating male dies because of cessation of the heartbeat (Foellmer et al. 2003). Few weeks after copulation females start to lay egg sacs which contain hundreds of eggs packed in a silk protective case. Spiderlings complete embryonic development and moult within the egg sac, then the second stage juvenile emerge from egg sacs. Newly emerged juveniles live together as an aggregation and after a while start to disperse and start to build a web and catch prey independently (Schneider and Andrade 2011).

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Chapter 1

The effect of predatory cues on the foraging behaviour and metabolic rate in an orb-web spider, *Argiope keyserlingi* (Karsch)

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Abstract

Animals are able to assess the risk of predation and respond accordingly via behavioural and physiological changes. Orb-web spiders are in the unique situation where they reside in the middle of their web and are therefore relatively exposed to predators. Thus, these spiders might moderate either their web-building behaviour or their behaviour on the web when exposed to the threat of predation. In this study, we experimentally explored how predatory cues influence the foraging behaviour and metabolic rate in the orb-web spider, *Argiope keyserlingi*. We found that spiders restricted their foraging time budget under predatory cues from a praying mantid: they responded quicker to a vibratory stimulus and spent less time handling the prey. Moreover, spiders were less likely to rebuild the web under predation pressure. Female *A. keyserlingi* exposed to the praying mantid cue significantly elevated their resting metabolic rate compared to the control group. Our findings revealed short-term modifications in foraging behaviour and physiology of female spiders in response to predator cues. Energy deficiency as a result of metabolic rate elevation could explain the reduction in foraging time budget and web investment. In addition to saving energy, reduced foraging would also reduce the spiders' exposure to praying mantid predators.

Keywords: predation, respiration, time allocation, attack behaviour, web structure

Introduction

It is well understood that the risk of predation induces notable changes in the behaviour and physiology of animals (Lima 1998; Lima and Dill 1990). Theoretical (Charnov 1976; MacArthur and Pianka 1966) and empirical evidence (Hawlena and Schmitz 2010) suggests that prey face a trade-off where the benefits of the antipredator response are balanced with other fitness improving activities, such as foraging or mating. For example, Lagos et al. (2014) showed that crickets will continue foraging in a patch right until the last possible second before initiating an escape response from an approaching lizard predator.

Orb-web spiders are sit-and-wait foragers that are relatively exposed to various predators such as birds and mantids. One way of reducing exposure to predators for sit-and-wait foragers is to prevent detection from predators (e.g. background matching in praying mantids: Barry et al. (2015); crab spiders: Heiling et al. (2005)). Orb-web spiders, however, usually reside in the middle of their web and therefore are even more exposed to predators. Thus, these spiders might moderate either their web-building behaviour or their behaviour on the web when exposed to the threat of predation. Our knowledge of antipredator strategies in orb-web spiders is limited to modifying the structure of the web, perhaps because web construction is widely assumed to be the most costly component of their foraging (Peakall and Witt 1976; Tanaka 1989) and directly linked to foraging success (Herberstein et al. 2007; Herberstein and Heiling 1998). Very little is known about how orb-web spiders modify their behaviour on the web, such as attack behaviour, which directly influences foraging success (Nentwig 1982).

While a successful capture of prey in the web will result in an energy gain for the spider, it may also impose several costs. In addition to the energy expenditure of moving, running on the web changes the arrangement of the capture threads, which may affect future prey capture efficiency (Heiling and Herberstein 1999; Herberstein and Heiling 1998; Higgins and Buskirk 1992). More importantly, moving in the web

can attract the attention of visually hunting predators such as praying mantids (Bruce and Herberstein 2006; Herberstein et al. 2002). Considering these potential costs, we expect that spiders moderate their behaviour under the threat of predation based on the relative benefit and costs of prey capture.

A well-established indirect effect of predation risk is an elevated metabolic rate (Beckerman et al. 2007; Lagos and Herberstein 2017) moderated by the release of hormones (Orchard 1982). These physiological changes redirect energy to locomotory organs to prepare for a rapid escape response (Orchard 1993). However, under constant risk, metabolism is reduced presumably to compensate for the loss of foraging opportunities (Barry and Syal 2013; Handelsman et al. 2013; Steiner and Buskirk 2009). The metabolic rate of spiders is generally lower than other ectothermic animals of the same size perhaps because they are sit-and-wait predators (Killen et al. 2016). This may buffer spiders against prey fluctuations and periods of low food availability (Anderson 1970; Greenstone and Bennett 1980). Moreover, considerable temporal variation in resting metabolic rate has been reported between species (Anderson 1970) and individuals (Kasumovic and Seebacher 2013). For more active spiders, temporal variation may correspond to circadian rhythm and diel periods of activity under natural conditions. In web building spiders some of the variation in metabolic rate is associated with constructing or repairing the web (Anderson 1970), but changes in response to predation has not been reported, despite metabolic rates often being reported as an influencing factor on life history (Brown et al. 2004; Biro and Stamps 2010; Auer et al. 2018).

In this study, we explore how predatory cues influence the foraging behaviour and metabolic rate in the orb-web spider, *Argiope keyserlingi*. We hypothesize that under predation pressure, spiders spend less time moving on the web, resulting in delayed responses to prey and reduced handling time under the risk of predation. We also

predict that female spiders will invest less energy in foraging, which may result in smaller webs and less frequent rebuilding of damaged webs.

Materials and Methods

Study Species

Argiope keyserlingi (Araneae: Araneidae) is a medium sized orb-web spider common along the East Coast of Australia where they primarily construct their orb webs in *Lomandra* bushes (Blamires et al. 2007; Rao et al. 2007). A group of 45 juvenile *A. keyserlingi* females were collected from the West Pymble area in Sydney, New South Wales, Australia (33°45'48"S 151°08'10"E) during June and July 2016. A group of 20 juvenile praying mantids, *Archimantis latistylus* were collected from the same area at the same time. All animals were transferred to the laboratory for further tests. Female spiders were transferred into 3D Perspex frames (40 x 40 x 10 cm), where they could build their typical orb-web overnight. A strip of masking tape was attached to the inner side of the frames to aid the attachment of the silk. All animals were sprayed water and fed twice a week with 2-3 small crickets. All animals were housed in a temperature-controlled room (26 ± 2°C; 70% RH) on a 12:12 hour light:dark cycle. All experiments were conducted under the same environmental conditions.

Foraging behaviour under predatory cues

In this experiment, we tested the effect of predatory cues on two aspects of the spiders' foraging behaviour: attack behaviour and web building behaviour. Newly moulted adult females were transferred to a clean Perspex frame and allowed to build a web. Perspex frames were cleaned with 70% ethanol to remove any chemical cues. Spiders were randomly allocated to one of three treatments (N = 15): control, predatory cue and non-predatory cue treatments.

For the control treatment, spiders foraged under normal laboratory conditions. For the predator cue treatment, spiders were allowed to forage while being exposed to

the predator cues. The predator we used was *A. latistylus* (Mantodea: Mantidae), which is known to co-occur with the spider in *Lomandra* bushes and to regularly attack spiders (Blamires et al. 2007; Bruce and Herberstein 2006). The mantids were kept in 200ml ventilated plastic cups and were fed on medium sized crickets, *Acheta sp.*, twice a week. The predator cue was collected on filter paper (7cm diameter) that was placed into the cups with the mantid for 24h. The filter paper along with a mantid exuvia were collected and fixed to the lid inside the spider frame perpendicular to the web hub where the spider resides. The distance from the cue filter paper to the spider was approximately 50 mm. For the non-predatory cue treatment, a young leaf of Sydney blue gum tree, *Eucalyptus saligna*, was crushed on a filter paper and placed in the same position in the frame as the predatory cue. Both predatory and non-predatory filter papers were replaced with new ones every 48 h. Spiders were exposed to cues for 24h before beginning the trials. The trials were conducted from September to October 2016 and spiders were kept food deprived for 4 days prior to commencing the trials to reduce any previous feeding effect.

Attack behaviour

Using an electric toothbrush (Spinbrush Pro Clean Powered Toothbrush), we generated vibrations to simulate prey impact in the web. As we repeatedly exposed the spiders to this stimulus, we rewarded the spider by placing a small cricket ($0.035 \pm 0.003\text{g}$, $N = 15$) next to where the stimulus was applied. This prevented spiders from ignoring the stimulus with repeated exposure. The cricket was narcotized using carbon dioxide gas, and gently placed alternatively along the left and right horizontal radial thread of the web to control for any gravity effect in attack speed (Herberstein and Heiling 1999). The toothbrush was placed next to the radial where the cricket was placed to stimulate an attack from the spider. The response of the spider to the stimulus was recorded using a high-speed camera (CASIO Exilim EX-F1, CASIO Computer Co., Tokyo, Japan) at 300 fps. We used the videos to time the spiders' behaviour: initial response time (time taken to react to the stimulus) and total attack

time (time taken from leaving the web hub to move back to the initial position). Each trial was repeated seven times throughout the experiment every second day allowing spiders to rest and digest prey to avoid over feeding.

Web building behaviour

Spiders were checked daily and we recorded if they built a new web. A photograph of the newly built web was taken to measure the web capture area and capture thread length. Capture thread length (CTL) was estimated using a modified formula (Heiling et al., 1998): $CTL = [(C_{hub} + C_{web}) / 2] \times [(sp_1 + \dots sp_8) / 8]$; where C_{hub} and C_{web} are the circumferences of the hub and web respectively and $sp_{1, 8}$ are the number of spirals in eight sectors of the web, from upper vertical sector moving clockwise every 45 degrees around the entire web. All photographs were measured using ImageJ 1.49v software (U. S. National Institutes of Health, Bethesda, Maryland, USA)

Metabolic rate

In this experiment, we tested for the effect of predatory cues on the spiders' metabolic rate, estimated through CO₂ production. Following the behavioural trials, spiders were allowed to rest for 2-3 weeks under a standard diet (1 medium sized cricket per week). The mass specific metabolic rate was measured under two treatments (N = 15); control and predatory cue. The individuals were allocated to the same control and predatory cue treatments as in the foraging trial, and the cues were obtained in the same way.

To measure the mass specific metabolic rate, spiders were weighted and transferred into a cylindrical container (8 cm height × 6.5 cm diameter) and allowed to habituate for five minutes. A plastic mesh was placed on the inner surface of the container to provide a grip for the spiders, thereby reducing movement and stress. The spiders were not fed 24h before trials to avoid the effect of food digestion and prey capturing and/or rebuilding web. During the pre-experimental period, resting metabolic rate was measured every five seconds for a period of 10 minutes. After 10

minutes, the container was then opened and either the predatory cue or the non-predatory cue (filter paper containing the *Eucalyptus* cue) was immediately placed inside and the container was closed. The metabolic rate was measured for another 10 minutes. The order in which treatments were applied was not randomized for two reasons; first, the focus was to test the effect of a chemical cue on spiders that were initially unaware of the presence of a predator. Second, if the spiders were introduced to the predatory cue first, it would take too long for the metabolic rate to return to normal before applying the control stimulus. Thus, our analysis will compare the change in metabolic rate between the two treatments from the pre-experimental measurements. Also, this method would reveal if the change in metabolism of the spiders is caused by disturbance during changing the chemical cues.

We used open-system respirometry (LI-COR Li-6400XT respirometer, LiCor, Lincoln, NE, USA) with insect respiration configuration pre-installed to measure the amount of released CO₂. This configuration allows for the measurement of μ grams carbon dioxide normalized to the mass of the spider. The final computed metabolic rate was μ grams CO₂ per grams spider per minute. During respirometry measurements, the temperature and humidity were 27.46 ± 0.27 C and $50.49 \pm 3.25\%$, respectively. All measurements were done between 12:00am and 6:00pm.

Statistical analyses

All data were analysed with R 3.3 (R Core Team, 2016). The distribution histograms of the variables were checked for normality before further analysis. The foraging data were analysed with Generalized Linear Model using family Gamma with log link function for attack behaviour data, and Gaussian family with identity link function for web construction data. The different cues (predator, non-predator and control) and trial number were added to the model as fixed factors. For all analyses spider ID was added to the model as a random factor to control the variation between individuals. The package lme4 was used to fit models for each response variable, then models

that included each fixed effect were compared with models that excluded the fixed effects using F-test (Bates et al. 2014).

For metabolic rate analyses, the average CO₂ production for each individual was calculated for the pre-experimental period and the experimental period (predatory cue or non-predatory cue) and then fitted into a Gaussian model with log link function for a pairwise test of significance. Spider ID was added to the model as a random factor to control the individual's effect between pre-experimental and experimental periods.

Results

In the foraging trials, the initial response time to a vibratory cue increased over the seven trials under control treatment, while exposing the spiders to predator or non-predator cues significantly changed their response (Table 1, Figure 1). When exposed to a non-predator cue, the spiders were slower in their response from the third to sixth trials with a sudden drop on seventh trial. However, when exposed to a predator cue, the spiders maintained their quick response over the seven trials, without a noticeable change (Figure 1). Total attack time was similarly influenced by the presence of different cues (Table 1). The spiders did not vary the total attack time over seven trials under the control cue. When experiencing a non-predator cue, total attack time decreased over time. However, under a predator cue the spiders did not vary their behaviour over the seven trials but spent much less time attacking the prey than spiders experiencing the control or non-predator cues (Figure 1). The interaction between the cues and trials was significant for both initial response time and total attacking time (Table 1).

The spiders were able to rebuild their web up to five times (six webs in total) during the 7-day experiment, however, the fifth and the sixth webs were excluded from the analysis due to low sample size to avoid bias and make a balanced experimental design. The number of times the spiders rebuilt the web was significantly less under predatory cues than control or non-predator cues (Table 2, Figure 2). The spiders

doubled the capture area of their web during the experiment, while the length of the capture thread per area remained almost constant. Neither the capture area nor the capture thread length varied between the three treatments (Table 2, Figure 3). There was no significant interaction between different cues and the number of trials for capture area and capture thread length (Table 2).

During the pre-experimental period, there was an initial high rate of CO₂ production ($27 \pm 2 \text{ } \mu\text{gCO}_2\text{min}^{-1}\text{g}^{-1}$), which gradually decreased and stabilized around $11 \text{ } \mu\text{gCO}_2\text{min}^{-1}\text{g}^{-1}$ after 10 minutes (Figure 4). There was no significant difference in the average CO₂ production between the two control groups over the pre-experimental period ($F_{1,29} = 1.54$, $p = 0.225$). Once the cues were added, spiders exposed to predator cue released around two times more CO₂ than non-predatory cue. There were however no significant changes in CO₂ production of spiders between non-predator and pre-experimental control (Figure 4, Table 3).

Discussion

Our findings revealed that the presence of predator cues alters the attack behaviour of the female spiders and elevated their metabolic rate. As predicted, when the risk of predation is elevated, the spiders spent less time foraging— they responded and attacked faster and spent less time with the prey. While the structure of the web was not influenced by the presence of a predator cue, spiders were less likely to rebuild the web under predation pressure.

Argiope keyserlingi females were clearly able to detect and distinguish the chemical cue from praying mantid predators compared to a non-predator cue and adjusted their foraging behaviour accordingly. This response is predicted to be adaptive and has been reported for the congener *Argiope versicolor* (Li and Lee 2004) and other taxa (Amo et al. 2006; Dixon et al. 2012). The ability to distinguish a non-predatory cue from a potential predator avoids the costs of inappropriate anti-predator responses, such as relocating the web (Tanaka 1989). While detection of a true

predator signal initiates an appropriate behaviour that increases fitness (Buxton et al. 2017).

There were not notable differences in initial response time between treatments in early trials, however, in later trials the spiders reacted with a delay to the vibratory stimulus under predation pressure. This may be caused by the food deprivation experienced by the spider before the start of the trials. Animals under an energetically stressed condition usually tolerate higher risk of predation (Lima 1998). It is not clear why spiders under non-predator cue increased their initial response time at third to sixth trials. The significant interaction between the trials and the chemical cues shows that complex decision-making process in the spider where a variety of external and internal factors are involved (Nelson and Jackson 2011).

Overall spiders restricted their foraging time budget under predation pressure as they responded quicker to web vibration and spent less time handling the prey. This is likely to be an adaptive anti-predator tactic specifically against visually oriented hunters like praying mantids. While there is limited direct empirical evidence in spiders, theoretical models predict that orb-web spiders should reduce activity and remain undetected under predation pressure (Venner et al. 2006). For example, Nakata and Mori (2016) showed that two species of orb-web spiders, *Cyclosa argenteoalba* and *Eriophora sagana*, reduce the time they spend building the web under predation pressure.

Overall web architecture was not influenced by the presence of a predatory cue. This is surprising as spider web building behaviour is quite flexible, and they can change their web structure quickly in response to environmental factors (Eberhard 1988; Heiling and Herberstein 2000) including airborne predatory cues (Nakata 2008; Nakata and Mori 2016). In a previous study, focusing on the function of web decorations in *A. keyserlingi* (Bruce and Herberstein 2006) predatory mantid cues similarly did not induce spiders to vary web architecture, including web decorations (stabilimenta). By contrast, the congener *A. versicolor* (Li and Lee 2004) did respond

to the spider predator *Portia labiata*, by trading-off web and decoration size. This may suggest that modifying the structure of the web is not a favoured strategy for spiders to remain undetected from predators.

While our spiders did not change the overall web architecture, they did rebuild their webs less frequently when exposed to predator cues. This is another line of evidence that female *A. keyserlingi* are capable of perceiving and responding to predator cues. The data also suggest that it might be a more effective anti-predatory strategy to hold off rebuilding the web all together rather than to adjust web architecture. This would be beneficial for the spiders as they can save the energy of the web reconstruction and stay undetected from predators. However, not rebuilding a web may entail some costs like drying out of capture silk or reduced prey capture efficiency of damaged web (Heiling and Herberstein 1999).

Female *A. keyserlingi* exposed to predator cues doubled their metabolic rate (estimated through CO₂ production) compared to the control group. This effect has been widely shown in both vertebrate and invertebrate taxa (e.g. Adamo et al. 1995; Lagos and Herberstein, 2017). The proximate mechanism underlying this response is the release of stress hormones, such as octopamine (invertebrates) or norepinephrine (vertebrates). The neurotransmitter octopamine facilitates a quick response by redirecting energy to locomotory organs (Orchard 1993; Orchard 1982).

While modification of metabolic rate under predation stress would be evolutionary advantageous, it may also incur some fitness costs. Janssens and Stoks (2013) showed that damselfly larvae *Enallagma cyathigerum*, produce significantly more reactive oxidative species (ROS) as a by-product of elevated metabolic rates under predation pressure. These oxidative factors redirect energy towards locomotory organs for anti-predator responses, resulting an energy deficiency in other functions such as growth and body maintenance (Benard 2004; Slos and Stoks 2008). Moreover, these damaging effects may be extended to subsequent generations through altering the reproductive strategy of the females, resulting a lower

investment in eggs and reduced offspring quality (Fontaine and Martin 2006; Travers et al. 2010). For example, under predation risk, the females of the egg-carrying spider, *Scytodes pallida*, laid eggs that hatched sooner and spiderlings developed into smaller size (Li 2002).

In conclusion, our study demonstrates that a potential predator affects the foraging behaviour of *A. keyserlingi* females who responded and moved faster, which may have been facilitated by an elevation in metabolic rate. While the web architecture did not vary, the spiders were also less likely to rebuild their web under predation pressure, which may allow them to remain undetected from predators. In addition to the short-term effects on behaviour, predation risk may also have long lasting consequences on the life history of animals (Benard 2004). Therefore, evaluating long term changes of predation risk on individuals and subsequent generation will elucidate the evolutionary importance of the predatory induced responses.

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Tables

Table 1. Generalized linear mixed model analysis of attack behaviour (initial response and total attack time) in female *Argiope keyserlingi* spiders in response to different chemical cues over seven trials.

Response variables	Source of variation	F	df	p
Initial response time	Cue	34.31	16	0.005
	Trial	60.45	21	<0.001
	Cue × trial	30.34	14	0.007
	Random (ID)	Variance = 0.21, SD = 0.46		
	Residual	Variance = 0.74, SD = 0.86		
Total attacking time	Cue	27.86	16	0.033
	Trial	46.06	21	<0.001
	Cue × trial	27.61	14	0.016
	Random	Variance = 0.15, SD = 0.38		
	Residual	Variance = 0.57, SD = 0.76		

Table 2. Generalized linear mixed model analysis of web building behaviour for female *Argiope keyserlingi*, in response to predator and control cues over four consecutive webs.

Response variables	Source of variation	F	df	p
Web reconstruction frequency	Cue	2.13	40	0.039
Capture thread area	Cue	7.71	8	0.463
	Web no.	75.79	9	<0.001
	Cue × web no.	6.15	6	0.407
	Random	Variance = 173303, SD = 416		
	Residual	Variance = 199253, SD = 446		
Capture thread length per area	Cue	2.86	8	0.943
	Web no.	14.34	9	0.111
	Cue × web no.	2.60	6	0.857
	Random	Variance = 0.46, SD = 0.67		
	Residual	Variance = 0.69, SD = 0.83		

Table 3. Generalized linear mixed model analysis of resting metabolic rate of female *Argiope keyserlingi*, in response to predator and control cues.

Pairwise comparisons	F	df	p
Control vs. predation	4.94	1	0.026
Control vs. non-predation	1.41	1	0.183

Figures

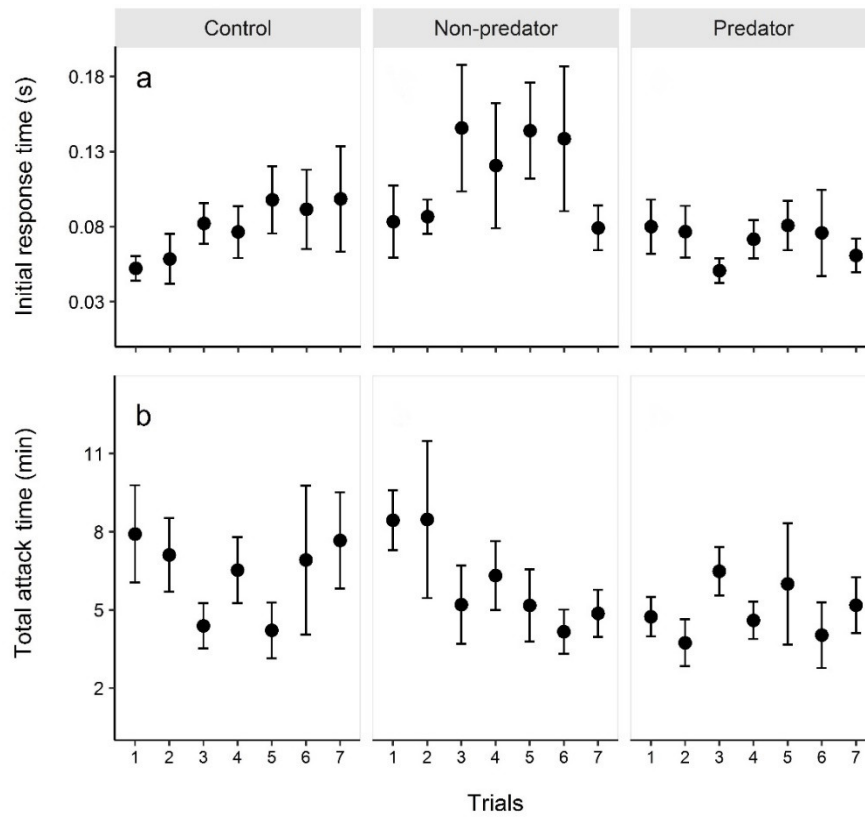


Figure 1. Mean (\pm SE) attack behaviour (a: response time; b: total attack time) of female *Argiope keyserlingi*, in response to predator and control cues over seven trials.

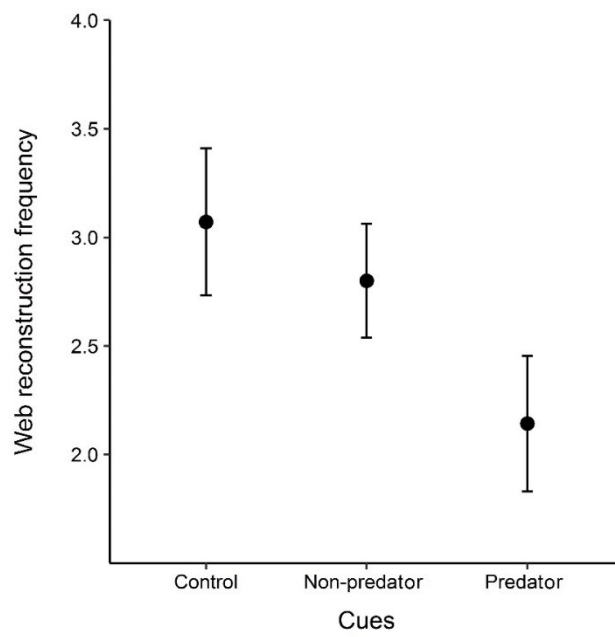


Figure 2. Mean (\pm SE) numbers of times female *Argiope keyserlingi*, rebuilt the web over 14 days in response to predator and control cues.

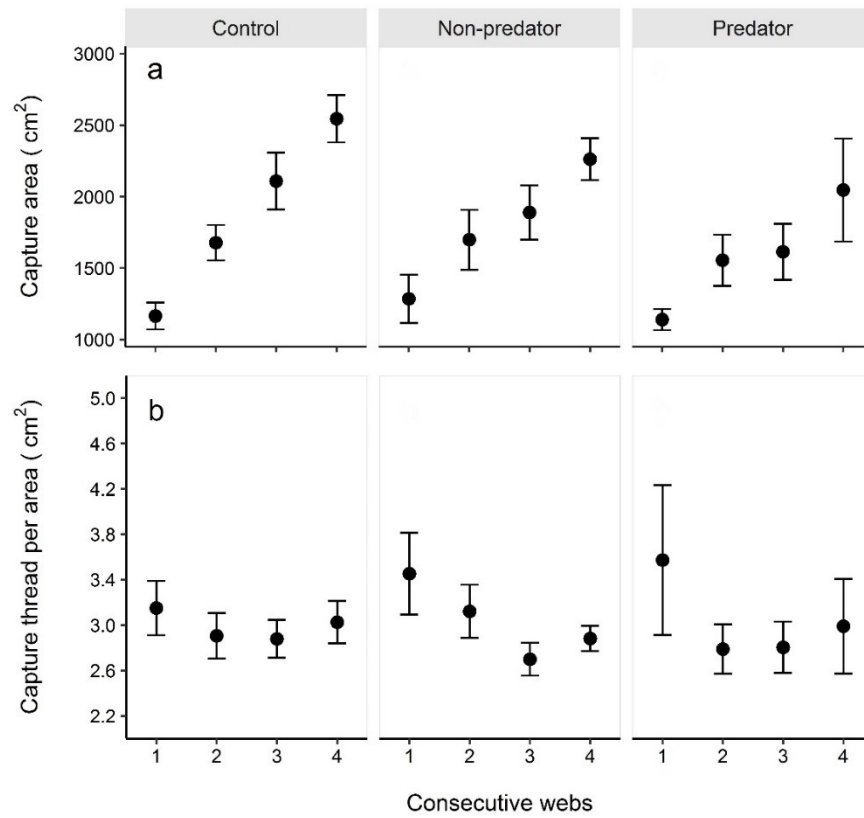


Figure 3. Mean (\pm SE) web architecture (capture area and capture thread length/capture area) of female *Argiope keyserlingi*, in response to predator and control cues across four consecutive webs.

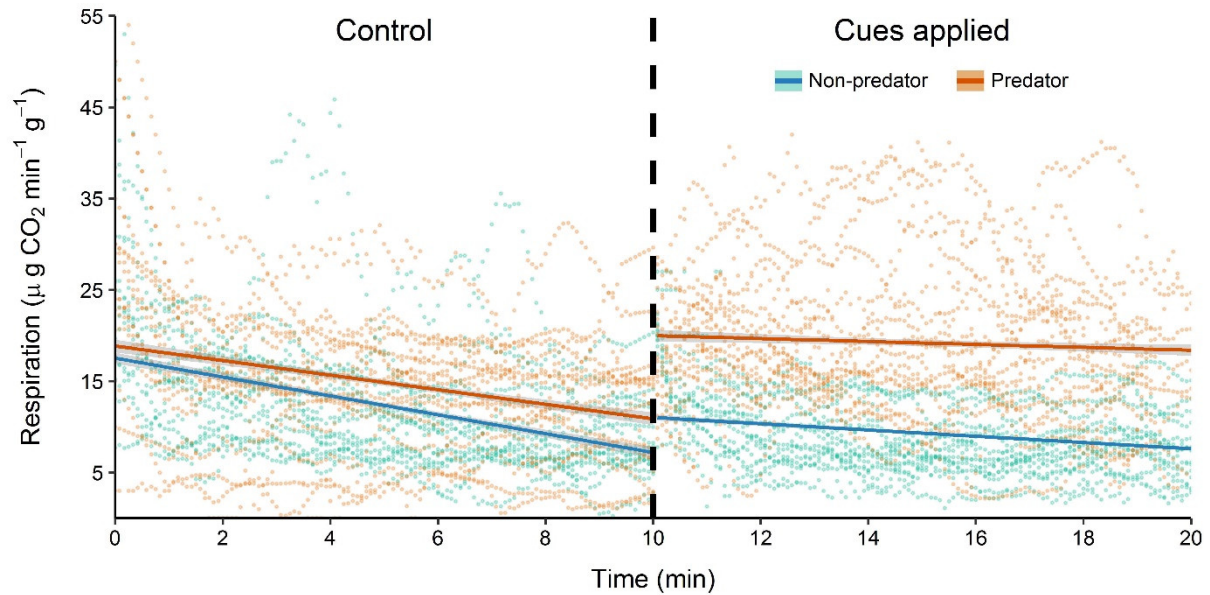


Figure 4. Mass specific CO_2 production of female *Argiope keyserlingi*, in response to predator and control cues over the course of 20 minute. Measurements every 5 seconds for each individual (dots) and regression line (control: $y = -1284x + 20.16$, $R^2 = 0.22$ (non-predator), $y = -0.033x + 18.19$, $R^2 = 0.02$ (predator); cues applied: $y = -469.14x + 17.39$, $R^2 = 0.14$ (non-predator), $y = -0.015x + 21.85$, $R^2 = 0.0053$ (predator)) are illustrated. At minute 10 the container lid was opened to put in the cues.

Chapter 2

Temporal variation in resting metabolic rate and its consequence in progeny performance in an orb-web spider, *Argiope radon*

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Abstract

Resting metabolic rate (RMR) is a fundamental feature of animal biology that reflects the baseline level of energy expenditure. Arthropods display notable inter-individual variation in RMR (standardised for body temperature), however the consistency of RMR through ontogeny and its consequence for reproductive fitness has rarely been investigated. Orb-web spiders are sit-and-wait foragers that typically reside at the centre of their web waiting to intercept prey. Given their sedentary resource acquisition strategy, the “compensation” model predicts that lower RMR would be favoured to reduce self-maintenance energetic costs and allow higher allocation to oogenesis (i.e., egg sac development). In this study, we tested temporal variation in RMR of the female spiders *Argiope radon* (Araneae: Araneidae) in response to mating status. Then we tested the degree to which inter-individual variation in the parents RMR relate to reproductive output and spiderlings performance. Despite notable inter-individual variation, a temporal consistency of RMR was found in the female spiders. However, mated females significantly reduced their RMR by around 35 percent compared to their unmated stage. We did not detect significant correlation between male and female RMR with reproductive output and offspring quality in the spiders. The temporal consistency in RMR is possibly due to the conditions experienced by individuals early in their life. The significant reduction of RMR in the mated females led to support for compensation model in which lower RMR is a favourable strategy during energy consuming process.

Keywords: metabolism, energy, reproduction, parental effects, offspring performance

Introduction

Metabolism is a complex chain of biochemical reactions, responsible for energy processing within the body. Metabolism regulates the rate at which an animal must gain resources from the environment, transform them into usable forms, and allocate them to biological functions such as body maintenance, growth and reproduction (Brown et al. 2004; Careau et al. 2008; Speakman et al. 2004). Therefore, metabolic rate directly associates with the rate of biosynthesis and hence the 'pace of life' (Le Galliard et al. 2013; Reale et al. 2010).

Basal or resting metabolic rate (hereafter: RMR) refers to the minimal energy expenditure of an individual during periods of inactivity (Kleiber 1961). Individual RMR is highly repeatable and is linked to other physiological and behavioural traits such as growth, survival and reproductive output (Biro and Stamps 2010; Burton et al. 2011). This trait has received intensive attention in comparative studies aimed at the differences between populations and species. By contrast, our knowledge of inter-individual variation and its physiological and behavioural consequences is limited, even despite evidence that such variation may be large (e.g. crickets: Ketola and Kotiaho 2010).

How RMR relates to life history traits is still unclear as arguments can be made for negative and positive correlations. The 'increased intake model' predicts a positive relationship between RMR and life history traits. Individuals with a high RMR have larger internal organs and this larger 'metabolic machinery' facilitates a greater rate of food intake and assimilation (Biro and Stamps 2010), consequently, more resources will be available for biological functions such as foraging and reproduction. However, larger metabolic organs burn resources at a higher rate during inactivity periods, which is known as idling cost (Bennett and Ruben 1979; Hammond and Diamond 1997; Speakman et al. 2004). Moreover, higher metabolic rates are more likely to produce reactive oxygen species (ROS), which accelerate cellular senescence and reduce performance (Janssens and Stoks 2013; Slos and Stoks 2008). The

‘compensation model’ predicts that individuals with lower RMR require less energy for self-maintenance, enabling the allocation of surplus resources to the growth and reproduction (Speakman et al. 2004). However, individuals with a lower RMR may face an energy deficiency during energy consuming processes, such as breeding or producing acoustic signals, because they cannot maximise their metabolism and energy intake (Chastel et al. 2003). Selection is likely to favour individuals who can adjust metabolic rate for different life history stages. Marine bryozoans, for example, are known to adjust their metabolism across fine time-scales in relation to physiological needs (Pettersen et al. 2016).

The relationship between RMR and life history traits has been examined in various taxa using proxies such as growth rate, survival and number of surviving offspring (fish: McCarthy (2000); birds: Mathot et al. (2009); rat: Derting et al. (2012); snail: Artacho and Nespolo (2009)). This body of work has however generated little evidence for a general association between reproductive output and RMR. For example, laboratory tests failed to find significant correlation between mass-specific RMR with litter size or litter mass in *Mus domesticus* (Johnson et al. 2001b; Johnston et al. 2007). However, Chastel et al. (2003) showed that in house sparrows, *Passer domesticus*, RMR increases during breeding season through hormonal changes and individuals with higher RMR produced more offspring early in the season. To our knowledge, the current study is the first attempt to explore the relationship between RMR and aspects of reproductive strategy in arthropods.

The female reproductive system of arthropods develops during the juvenile stages alongside with oocytes until they reach adulthood when a fraction of oocytes is matured (Chapman 1998). Oocytes continue to mature at maturity and once mating occurred, females allocate significant amount of energy to the eggs (Kessler 1971). Spiders pack their eggs in a protective silk case, which entails additional energetic costs as egg sac manufacturing an energy demanding process (Anderson 1978, 1990; Kessler 1971). We hypothesised that orb-web spiders moderate their energetic costs

after mating by changing RMR to facilitate egg sac manufacturing. We tested this hypothesis in an orb-web spider, *Argiope radon* (Araneae: Araneidae) by first examining intra-individual temporal variation in RMR of the female spiders in response to mating status. Orb-web spiders are sit-and-wait foragers and usually reside at the centre of their webs waiting for prey to intercept the web (Herberstein et al. 1998). Therefore, we predict that a lower RMR is favoured in mated compared to unmated spiders thereby reducing self-maintenance energetic costs and enabling greater resource allocation for egg sac manufacture (Angilletta et al. 2003). Second, we tested the degree to which inter-individual variation in male and female RMR relate to female reproductive allocation and spiderling performance. We predicted that parents with higher RMR can convert and allocate more nutrition to offspring resulting in heavier egg sacs, early emergence and larger offspring.

Materials and Methods

Study Species

Argiope radon is a common Australian orb-web spider distributed in the Northern Territory and Queensland where they construct their orb webs on trees and shrubs overhanging river banks (Rao et al. 2009). A group of subadult females (N=20) and males (N=20) were collected from the banks of the Katherine River in the Northern Territory, Australia, during October 2016. All animals were transferred to the laboratory for further tests (Zschokke and Herberstein 2005). Female spiders were transferred into 3D Perspex frames (50 x 50 x 15 cm), where they could build their typical orb-web over the night. A strip of masking tape was attached to the inner side of the frames to aid the attachment of the silk. Male spiders were kept in 300ml ventilated plastic cups. The female spiders were fed on one large cricket (0.368 ± 0.02 g) per week and the males were fed on *Drosophila* flies twice a week, 10-15 flies each time. All animals were housed in a temperature-controlled room ($26 \pm 2^\circ\text{C}$; 70 % RH) on a 12:12 hour light:dark cycle and sprayed water twice a week. All experiments were conducted under the same environmental condition.

Experimental procedure

Once individuals moulted to the adult stage a photograph of the patella-tibia length of the right fore leg in females and carapace width in males were taken using a digital camera (CASIO Exilim EX-F1, CASIO Computer Co., Tokyo, Japan) as proxy for body size. Mass-specific resting metabolic rate of the unmated females were measured at the age of 8.03 ± 0.91 days following final molt. Adult females and males were crossed randomly. Males at the time of mating were 15 ± 0.6 days following final moult. Mass and mass-specific resting metabolic rate of the males were measured after mating. For the females, metabolic rate measurements were taken weekly until they laid an egg sac. All photographs were measured using ImageJ 1.49v software (U. S. National Institutes of Health, Bethesda, Maryland, USA).

To measure mass specific metabolic rate, spiders were transferred into a cylindrical container (8 cm height \times 6.5 cm diameter). The spiders were not fed 24h before trials to avoid the effect of food digestion. After a habituation period of five minutes, resting metabolic rate was measured every five seconds for a period of five minutes. This generated 60 measurements which were subsequently averaged for each individual. A plastic mesh was placed on inner surface of the container where spiders could hang from, to reduce stress and movement. If the measurements were too high indicating that the spider was not in a resting state, the trial was repeated to ensure that the lowest measurements were recorded. An open-system respirometry (LI-COR Li-6400XT respirometer, LiCor, Lincoln, NE, USA) with insect respiration configuration pre-installed was used to measure the amount of released CO₂. This configuration allows the measurement of $\mu\text{gCO}_2\text{min}^{-1}\text{g}^{-1}$ for each individual. During respirometry measurements, the temperature and humidity were $27.46 \pm 0.27^\circ\text{C}$ and $50.49 \pm 5\%$, respectively. All measurements were conducted between 12:00am to 8:00pm.

Once the females laid egg sacs, the time from mating to laying egg sac was recorded and the Mass of the egg sacs was measured to the nearest 1 mg using a

microbalance (Mettler Toledo PB303-S). The egg sacs were kept in 300 ml ventilated plastic cup and sprayed water twice a week until the spiderlings hatched. Once the spiderlings hatched, the emerging time was recorded and a group of 15 spiderlings were randomly selected from each egg sac. Spiderlings were killed via exposure to -80°C for 5 minutes then air dried for 30 minutes under a fume hood to avoid the effect of condensed moisture left on their body. They were weighted individually to the nearest 0.01 mg using a microbalance (Mettler Toledo New Classic MS) and average spiderling mass was calculated for each egg sac.

Statistical analyses

All data were analyzed with R 3.3 (R Development Core Team). The distribution histograms of the response variables (egg sac mass, egg sac lay timing, offspring emerging time and body mass) were checked for normality prior to analysis. The package lme4 (Douglas M Bates et al. 2014) was used to fit a generalized linear mixed model, testing the significance of the fixed factors and the estimation of the random factors. First, we tested the temporal variation RMR in the females where age and mating status were the fixed factor and individual's ID was random factor. The relationship between mated male and mated female RMR with reproductive output was also tested using ANCOVA. RMR of the mated females before laying egg sac was included in the models. Because condition (mass divided by carapace width) is widely reported as an influential factor on reproductive output in various taxa (James H. Brown et al. 1993; Honek 1993), female condition was added in the all models as a covariation factor.

Results

(a) Lifetime variation in RMR

The mixed model analysis showed that RMR of female spiders did not vary with age *per se* (Table 1, Figure 1), but it did vary significantly according to mating status (Table 1, Figure 2). Whereas the RMR of unmated females ranged from 5.16 to 31.79

$\mu\text{gCO}_2\text{min}^{-1}\text{g}^{-1}$, it ranged from 5.24 to 12.58 $\mu\text{gCO}_2\text{min}^{-1}\text{g}^{-1}$ for mated females, representing a significant post-mating decrease of around 35 % (Figure 2).

(b) Fitness correlates of parental RMR

We found no evidence that parental RMR is related to time it took females to lay the egg sac after mating (Table 2). Spiderlings of parents with higher RMR emerged earlier from the egg sacs. However, when the effect of female condition was controlled, no significant correlation between male or female RMR and spiderling emerging time was evident. Similarly, male and female RMR correlated positively with offspring body size, but these relationships were not significant (Table 2, Figure 3). However, female RMR was positively correlated with the mass of the egg sac. Female condition also positively covaried with the mass of egg sac (Table 2, Figure 3). No interaction between male and female RMR were found in reproductive output of the spider (Table 2).

Discussion

Our findings showed a temporal consistency of RMR in the female *Argiope radon* spiders: age *per se* did not affect RMR. However, mated females significantly reduced their RMR by around 29%-35% compared to their unmated stage. We did not detect significant correlations between male and female RMR with reproductive output and offspring quality in these spiders.

Exploring the range of RMR measurements, a 6-fold inter-individual difference in RMR of *A. radon* females were seen. However, this variation dropped to a 2.5-fold difference for the mated females. This is consistent with other studies where reported a difference of 2 to 3-fold inter-individual variation in RMR (Careau et al. 2008). Despite the inter-individual variations, RMR in the females appeared to be temporally consistent within individuals, with no overt effect of age. Investigating the repeatability at individual level has shown a consistency in RMR for several species and ranged from few days to even months (Burton et al. 2011; Careau et al. 2008).

One possible explanation for this relatively short-term consistency is that RMR is restricted by the time required to change the sizes of organs associated with metabolism (Chappell et al. 2007). For example, a cold temperature experienced by mice immediately changed the rate of food consumption, while increase of the size of internal organs like heart, kidney and liver was delayed by around four to six days (Książek et al. 2009). Similarly, changes in cellular components and biochemical pathways may also take time and thus constrain rapid changes in RMR (Haggerty et al. 2008; Speakman et al. 2004). Genetic factors may also play a role in intra-individual consistency of RMR (Gębczyński and Konarzewski 2009; Nilsson et al. 2009), as may environmental conditioning; that is, conditions experienced early in ontogeny may induce inter-individual differences in RMR that persist into adulthood (Criscuolo et al. 2008).

Female *A. radon* spiders showed a significant reduction of around 29%- 35% after mating. We predicted that a reduction of RMR after mating in the female spiders would allow the allocation of additional resources to the egg sac manufacturing process. These findings support the compensation model in which lower RMR is favoured during energy demanding activities. Similar results have been reported for Zebra finches, *Taeniopygia guttate*, and European starlings, *Sturnus vulgaris* (Bautista et al. 1998; Deerenberg et al. 1998). Schmitz (2004) showed that RMR of mated *Pardosa lugubris* (Lycosidae) females without egg sacks were significantly higher than *Marpissa muscosa* (Salticidae) females that have recently laid egg sac. In general, when individuals are faced with an unpredictable energetic demanding activity, they reduce their RMR thereby saving energy (birds: Bautista et al. 1998; Deerenberg et al. 1998; insect: Lagos and Herberstein 2017). However, unlike active foragers, a lower metabolic rate is favoured for sit-and-wait predators such as orb-web spiders to reduce the idling cost. In addition to re-directing energy to reproduction, there are other possible benefits for the adaptive control of RMR. By reducing their energy requirements through a lower RMR, spiders can reduce their exposure to predators by reducing physical attacks on prey or overall web size (Bruce and Herberstein 2006;

Nakata and Mori 2016; Venner et al. 2000). Moreover, when food is scarce or unpredictable, lower RMR can buffer the risk of exhausting current energetic reserves (Alvarez and Nicieza 2005; Killen et al. 2011). Finally, a reduced RMR may also decrease the reactive oxygen species (ROS) factors, as performance deteriorative agents are less likely to be produced (Jensen et al. 2010; Slos and Stoks 2008), which may be conducive to a longer adult lifespan.

McNab (1980) showed a positive correlation between RMR and the rate of biosynthesis, therefore we predicted that female spiders with higher RMR can produce heavier egg sacs and offspring with larger body size. Similarly, we found that female with higher RMR produced heavier egg sacs. However, we did not detect a significant correlation between *A. radon* male and female RMR with time it took females to lay the egg sac after mating and offspring quality (emerging time and body mass). There are numerous studies that similarly failed to find a significant relationship between inter-individual variation in RMR and reproductive traits in rodents (Derting and McClure 1989; Earle and Lavigne 1990; Hayes et al. 1992; Johnson et al. 2001a; Stephenson and Racey 1993; Symonds 1999). However, these rodent studies measured RMR at pre-reproductive stages (Johnston et al. 2007), while we measured RMR at the mating stage, and still we did not find any correlations. Another reason could be the limitation in the available food for the tested animals, in fact higher RMR may be beneficial where *ad libitum* food is available (Killen et al. 2011). For example, Alvarez and Nicieza (2005) showed that brown trout with high RMR had higher growth rates when fed *ad libitum* in captivity, but not when they were released in natural streams.

To conclude, we found notable inter-individual variation in RMR of female *A. radon* that appeared temporally consistent early in life yet reduced by around 35% after mating. This supports the compensation model, in which lower RMR is favoured for reducing the energetic cost of self-maintenance, thereby allowing greater allocation to egg provisioning. Variation in RMR across parents was however unrelated to

reproductive output and offspring performance. RMR is often linked to hormonal changes, which also influence the other life history traits (Burton et al. 2011). Thus, it is difficult to investigate the effect of metabolic rate alone and its contribution to the population dynamics. The obvious next step is to isolate the contribution of genetic and/or environmental factors to inter-individual RMR variation, and to explore the long-term consequence of such variation to individual life histories.

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Tables

Table 1. General linear mixed models of the effect of age and mating status on RMR of female spiders *Argiope radon*.

Source of variation	Coefficient \pm SE	
Fixed effects		
Age	0.02 \pm 0.01	t = 1.06, df = 54, p = 0.287
Mating status	0.39 \pm 0.14	t = 2.78, df = 54, p = 0.005
Age \times mating status	-0.006 \pm 0.02	t = 0.32, df = 54, p = 0.751
Random effects		
Random factor (ID)	Variance = 0.07, SD = 0.26	
Residual	Variance = 0.07, SD = 0.27	

Table 2. General linear mixed models of the effect of parental RMR on the reproductive output and progeny quality of female spiders *Argiope radon*.

Traits	source of variation	F	df	P value
Egg sac lay timing				
	Female	0.01	1, 15	0.949
	Male	0.37	1, 15	0.549
	Female × male	0.895	1, 15	0.359
	Covariation (female condition)	5.43	1, 15	0.034
Egg sac mass				
	Female	6.25	1, 15	0.024
	Male	0.41	1, 15	0.532
	Female × male	0.04	1, 15	0.845
	Covariation (female condition)	23.77	1, 15	<0.001
Progeny developmental time				
	Female	0.52	1, 15	0.482
	Male	0.11	1, 15	0.744
	Female × male	0.002	1, 15	0.977
	Covariation (female condition)	0.32	1, 15	0.584
Progeny mass				
	Female	1.49	1, 15	0.246
	Male	2.22	1, 15	0.163
	Female × male	2.18	1, 15	0.167
	Covariation (female condition)	0.58	1, 15	0.461

Figures

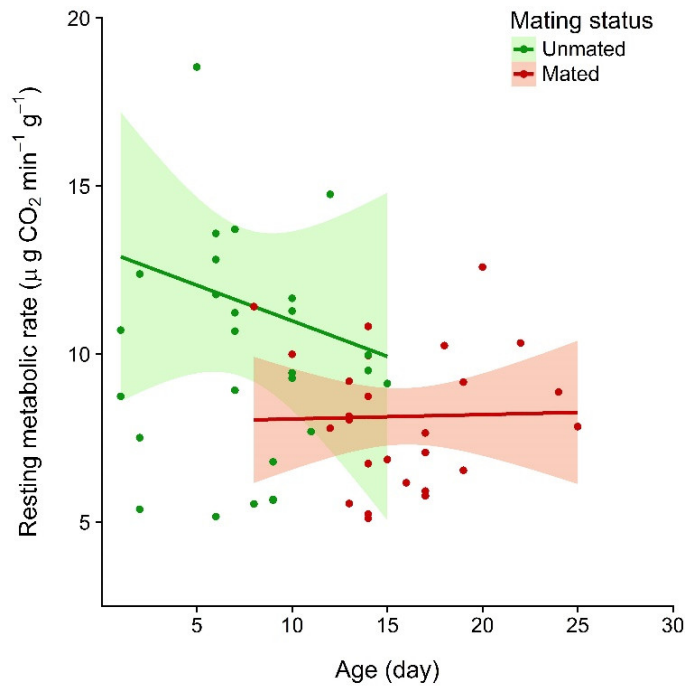


Figure 1. Temporal variation in mean \pm SE (shaded area) resting metabolic rate in female *Argiope radon* spiders (N=18), grouped here according to mating status.

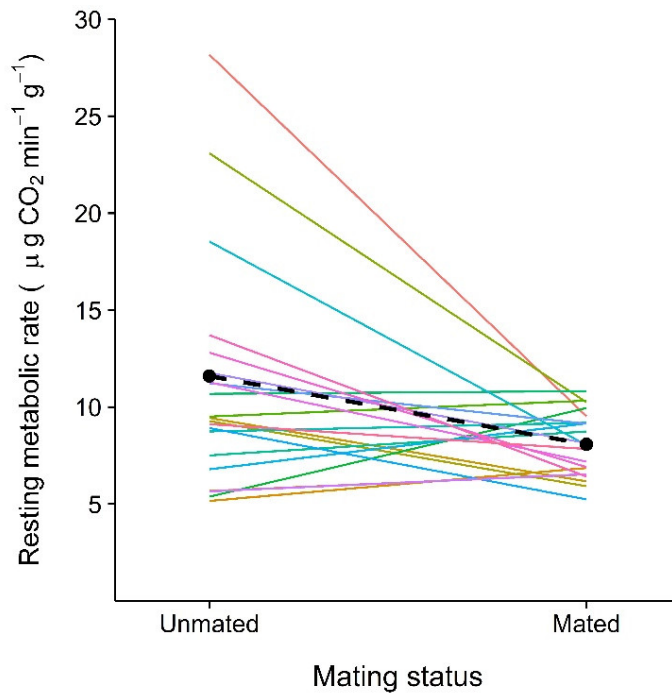


Figure 2. Mean \pm SE resting metabolic rate of unmated versus mated female *Argiope radon* (N=18). The solid lines demonstrate variation in individual RMR for different mating status and the dashed line with dots demonstrate average of the data.

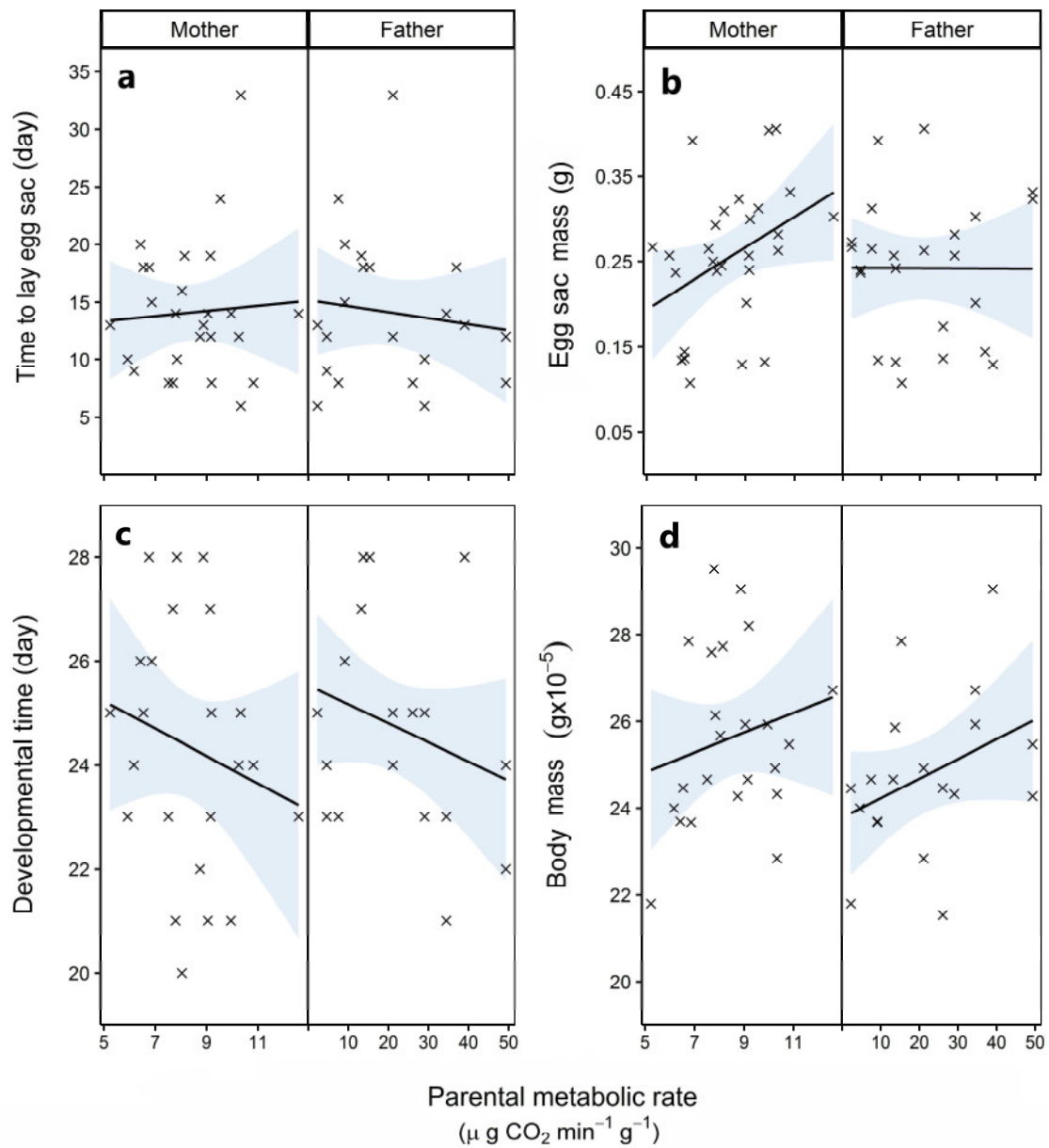


Figure 3. Relationship between metabolic rate of males and females with reproductive strategy components for *Argiope radon* (N=30) (a: time to lay egg sac; b: egg sac mass; c: offspring emerging time; d: offspring body mass).

Chapter 3

Age-specific reproductive investment and offspring performance in an orb-web spider, *Argiope radon*

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Abstract

Temporal variation in reproductive investment, e.g. maternal egg provisioning, has a substantial effect on offspring fitness therefore has received great attention by evolutionary biologists. Maternal allocation into egg size and egg content directly influences performance of offspring in many taxa, but spiders have rarely been investigated in this regard. In this study, we investigate the temporal changes in maternal reproductive investment and offspring performance in an orb-web, *Argiope radon*. A group of male and female spiders were mated randomly in the laboratory. Female spiders were kept under standard condition until they laid egg sacs. For each egg sac, egg sac mass, egg size and egg protein content were measured across all egg sacs. Once the spiderlings emerged, emerging time and toleration to starvation of the spiderlings were recorded. Egg sacs laid early in life were heavier, have larger eggs and offspring emerge sooner than the egg sacs laid by the old females. The spiderlings from the early egg sacs were more likely to emerge, however, these offspring had less toleration to starvation than the egg sacs laid by old females. There was no significant correlation between egg size and egg protein content with offspring performance. The results showed how the female spiders strategically allocate resources to egg sacs and its consequences on the offspring emerging time and toleration to starvation. This study suggests an adaptive maternal reproductive investment strategy which allows the female spiders to gain maximum fitness in each reproductive bout.

Keywords: maternal effects, egg size, egg provision, offspring performance

Introduction

Maternal effects are an important source of phenotype variation in offspring by transmission of non-genetic factors such as hormones, RNA and epigenetic marks (Bonduriansky and Day 2009; Mousseau et al. 2009). Maternal effects act in parallel to genetic additive factors, so that the degree to which a trait responds to maternal effects is reflected in plasticity. Assessing this plasticity is particularly important for exploring the direction of selection on the traits and, more broadly the dynamics of natural populations (Mousseau 1998; Uller 2008).

Numerous studies have been examined the effect of the environment experienced by parents in arthropods (e.g. diet: Vargas et al. 2014; temperature: Huestis and Marshall 2006; Scharf et al. 2010; photoperiod: Giesel 1988; predation: Li 2002; intra-specific competition: Marshall et al. 2006) on the life history of offspring. Shea et al. (2011) characterized these as detection-based maternal effect where the phenotype of the offspring was matched to the environmental cue experienced by parents. However, these effects may not have adaptive importance as they may simply reflect the developmental condition of the parents, specifically for food deprived treatments (Vargas et al. 2012; Vargas et al. 2014). In contrast, selection-based maternal effects are independent of the immediate environment experienced by parents. Temporal variations in progeny phenotype within a female's reproductive life time is an example of selection-based maternal effects (Shea et al. 2011). However, assessing the maternal effects in a single reproductive bout may bias how we interpret the adaptive significance of these effects (Marshall and Uller 2007). For example, in soil mite *Sancassania berlesei* (Acaridae) maternal reproductive strategy shifts from making more eggs early in life to making larger eggs later in life, regardless of the prevailing environmental conditions (Plaistow et al. 2007). Predation risk experienced by *Bugula neritina* bryozoan colonies resulted in smaller body size in offspring. However, this allows mothers to save resources until they recover from predation, thereby increasing resource allocation in later reproduction (Marshall and Keough 2004). Therefore, it is crucial to examine the

fitness consequences of a maternal effect across the reproductive life-span before interpreting their adaptive value.

Offspring performance varies with maternal age in arthropods (Fox and Czesak 2000). Offspring born early in the mother's life may have higher hatching success rate (Vargas et al. 2013), higher competitive capabilities (Marshall and Keough 2004) and higher longevity (Singh and Omkar 2009; Reid et al. 2010; but see Plaistow et al. 2007). Maternal egg size has been widely considered as a primary source of the temporal variation in offspring phenotype (Begon and Parker 1986; Bernardo 1996; Fox and Czesak 2000; Smith and Fretwell 1974). Egg size declines with maternal age in most arthropods (Fox and Czesak 2000) and offspring performance is reduced as a result of reduced maternal egg provision (Richards and Myers 1980; Sinervo and McEdward 1988; Sinervo 1990; Steinwascher 1984). But the positive relationship between egg size and offspring performance does not always hold (Michaud, and Nechols 2013; Ameri et al. 2014). This raises the suggestion that variation in egg composition can be more influential than variation in egg size (Bernardo 1996; Fox and Czesak 2000; Muller et al. 2017), specifically the protein content of eggs, which is a better predictor of offspring fitness than other components such as lipids or sugars (Giron and Casas 2003). Nevertheless, little or no information exists regarding the relationship between egg composition and offspring performance in spiders.

In this study, we assessed the temporal variation in maternal resource allocation and offspring performance of *Argiope radon* (Araneae: Araneidae) spiders. We tested how the maternal resource allocation influence offspring emerging time and toleration to starvation. We predicted that maternal investment in reproduction decreases with age, resulting in egg sacs with lower mass, smaller eggs containing less protein. Consequently, performance of progeny is predicted to reduce with maternal allocation reduction.

Materials and Methods

Study Species

Argiope radon is a common Australian orb-web spider distributed in the Northern Territory and Queensland where they construct their orb webs on trees and shrubs overhanging river banks (Rao et al. 2009). A group of subadult females (N = 40) and males (N = 20) were collected from the banks of the Katherine River in the Northern Territory, Australia, during October 2016. All animals were transferred to the laboratory for further tests (Zschokke and Herberstein 2005). Female spiders were transferred into 3D Perspex frames (50 x 50 x 15 cm), where they could build an orb web overnight. A strip of masking tape was attached to the inner side of the frames to aid the attachment of the silk. Male spiders were kept in 300ml ventilated plastic cups. Female spiders were fed on one large cricket (0.368 ± 0.02 g) per week and the males were fed on *Drosophila* flies twice a week, 10-15 flies each time. All animals were housed in a temperature-controlled room ($26 \pm 2^\circ\text{C}$; 70% RH) on a 12:12 hour light:dark cycle and sprayed water two time a week. All experiments were conducted under the same environmental condition.

Experimental procedure

Once individuals moulted to the adult stage, males and females were mated randomly. The females were kept on a standard diet until they start laying egg sac. Once the females laid egg sacs, the mass of the egg sacs was measured to the nearest 0.001g using a microbalance (Mettler Toledo PB303-S). Each egg sac was opened gently with sterilized forceps and a random group of 15 eggs was taken with a fine paint brush. The eggs were photographed under a stereo-microscope (Motic SMZ-171) equipped with a camera (Moticam 3.0MP). The diameter of each egg was measured using ImageJ 1.49v software (U. S. National Institutes of Health, Bethesda, Maryland, USA). The eggs are packed in a very dense batch within egg sac and there is a pressure from the surrounding eggs. Therefore, the eggs might not be perfectly spherical. We ignored these small changes and assumed that eggs are spherical and

egg volume, as a proxy of egg size, was estimated, using formula $\frac{4}{3}\pi(R/2)^3$ where R is the egg diameter. This method results in more accurate measurements than weighting the eggs and produce a less biased measurements.

Then the eggs were used to estimate egg volume were frozen at -80 for protein quantification. The rest of the eggs remained in the egg sac and were kept in 100 ml ventilated plastic cups and sprayed with water twice a week.

The group of 15 eggs selected from each egg sac was analyzed for protein content. Protein content of eggs was quantified using the Bradford assay modified for use in 96-well microplates (Bradford 1976). The eggs were dried at 60°C for 48 h and then weighed to the nearest 0.00001 g using a microbalance (Mettler Toledo New Classic MS). The dried eggs were crushed with a polypropylene pestle in a 2 ml Eppendorf tube. Soluble proteins were extracted with 0.1 M NaOH, 1 ml per mg of dry mass. The solution was sonicated for 30 minutes at room temperature and heated at 90°C for 15 minutes. The undigested tissue formed into a pellet after 10 min centrifuging at 13000 rpm. We plated 250 µl of Coomassie Blue dye into a polypropylene microplate along with 5 µl of the supernatant. Each sample had three replications and each plate had a separate standard curve, in which a predetermined amount of Bovine Gamma Globulin (BGG) was used as a protein standard. The plate was read at 595 nm with a spectrophotometer (PHERAstar FSX).

Once the spiderlings emerged from the egg sacs the time from laying the egg sac to spiderlings emergence was recorded. A group of 30 newly emerged spiderlings were randomly selected from each egg sac and were kept in a 100 ml ventilated plastic cup. The cups were checked daily and the tolerance to starvation of the spiderlings was recorded by noting the number of days the spiderlings survived from their emergence. In this treatment, the spiderlings did not receive any food or water.

Statistical analyses

All data were analyzed using R 3.3 (R Development Core Team). The distribution histograms of the response variables were checked for normality before further analysis. The package lme4 (Bates et al. 2014) was used to fit general linear mixed model, testing the significance of the fixed factors and the estimation of the random factors. Egg sac order and maternal age at the time of laying the egg sac were added as the fixed factors and female ID as the random factor in the models with independent slope for each individual. We started with the full model and tested for the significance of parameters by excluding them in turn, using maximum likelihood to compare the models. The correlation between maternal resource allocation (egg size and egg protein content) with offspring emerging time and toleration to starvation was tested using glm models (Crawley 2014).

Results

Out of 36 mated females, 33 of the individuals laid an egg sac, followed by 23 and 13 that laid a second and third egg sac, respectively. Due to low sample size, the fourth (N=4) and the fifth egg sacs (N=1) were excluded from the analysis. Egg sac emerging success rate (number of emerged egg sac divided by total number of egg sacs) declined from 90% for the first egg sac to 78% and 54% for the second and the third egg sacs, respectively.

The first egg sac was around 37% heavier than the second and the third egg sacs, while there was no notable difference between the second and the third egg sac (Table 1, Figure 1A). The mass of the egg sacs declined with female age at the time of laying the egg sac, regardless of the egg sac order (Table 1, Figure 2A). The size of the eggs in the first egg sac was significantly greater than those in the second and the third egg sacs (Table 1, Figure 1B). The size of the eggs also tended to increase with the maternal age across the egg sac order (Table 1, Figure 2B).

Egg sacs lost around 80% of their weight during drying process. Neither egg sac order nor maternal age had an influence on the protein content of the eggs (Table 1, Figure 1C, 2C). No significant interaction was detected between egg sac order and maternal age for egg sac mass, egg size and protein content (Table 1).

Emerging time of the offspring showed an increasing trend where spiderlings from the first egg sac emerged significantly earlier followed by the second and the third egg sacs (Table 2, Figure 3A). The effect of maternal age was not constant across different egg sacs: in the first and the third egg sac emerging time increased with the maternal age while in the second egg sac it was on the decline (Figure 4A). Maternal age significantly influenced spiderlings toleration to starvation (Table 2), Spiderlings emerged from the first egg sac survived 12 days less than spiderlings emerging from the second and the third egg sacs (Figure 3B). However, the toleration to starvation did not vary with the egg sac order (Table 2, Figure 4B). The interaction between egg sac order and maternal age was significant for emerging time but it was not significant for offspring toleration to starvation (Table 2).

There was no significant correlation between offspring emerging time with egg size ($t_{38}=0.98$; $p=0.331$) and egg protein content ($t_{38}=-0.11$; $p=0.919$). Similarly, we found no relationship between offspring toleration to starvation, egg size ($t_{31}=-0.57$; $p=0.573$) and egg protein content ($t_{31}=-0.45$; $p=0.657$) and their interaction was also not significant for offspring emerging time ($t_{38}=0.28$; $p=0.781$) and toleration to starvation ($t_{38}=0.41$; $p=0.689$).

Discussion

This study tested how temporal variation in maternal resource allocation affects offspring performance in the orb-web spider *A. radon*. The first egg sac laid by the female spiders was significantly heavier along with larger eggs compare to the second and the third egg sacs. Moreover, the mass of the egg sac correlated negatively with maternal age across all the egg sacs. The protein content of the eggs, however, did not vary with egg sac order or maternal age. The spiderlings emerged

significantly earlier from first egg sacs, followed by the second and the third egg sacs, however, spiderlings from early egg sacs had lower toleration to starvation.

The reduction in maternal reproductive allocation in older females is often linked to the depletion of the resources (Fox and Czesak 2000). This might be the consequence of a decline in metabolic efficacy with age due to accumulating of damaging reactive oxygen species (ROS) (Beckman and Ames 1998; Harman 1956; Rose 1991). Moreover, metabolic costs of the self-maintenances increase with the age as individuals accumulate more resources over the time (Bartholomew and Casey 1977; Bartholomew and Casey 1978; Coelho and Moore 1989). However, spiders like other arthropods have abrupt pattern of growth at pre-mature stages, and during maturity they store fats which may not essentially has metabolic cost. However, they continue developing oocytes at maturity which is a highly protein demanding process. Prey availability also varies during the season, prey become scarcer at the end of the season specifically for insectivorous predators (Crouch and Lubin 2000; Halaj et al. 1998; Riechert and Tracy 1975). Thus, at the end of the season females may already have used reserves for their early clutches, while low prey availability precludes them from regaining enough resources to maximize their clutch size. However, there might be some adaptive aspects in the age-specific reduction in reproductive investment. Egg sacs laid by older females at the end of the season are less likely to develop probably because of sperm depletion (Andrade and Banta 2003) and may suffer from high mortality caused by parasites and predators. Therefore, they may represent lower reproductive value as their contribution to the female's overall fitness is reduced and selection may favour reduced reproductive effort. Theoretical models also predict that females should allocate fewer resources to reproductive bouts with lower reproductive value (Begon and Parker 1986; Pianka and Parker 1975).

In our study, the female spiders produced significantly larger eggs for the first egg sac and egg size also correlated positively with the maternal age across all the egg sac order (Figure 2B). The same pattern has been found in various taxa (salamander:

Semlitsch 1985; bird: Christians 2002; fish: Berkeley et al. 2004; Wang et al. 2012; turtles: Wilkinson and Gibbons 2005). However, in arthropods there is not a consistent trend for the effect of maternal age on egg size (reviewed in Fox and Czesak 2000). Assuming the chance of surviving until laying the next clutch is declining because of senescence, Begon et al. (1986) predicted that maternal allocation in eggs decrease with the maternal age. This would be an optimal strategy when clutch size is constrained, however, in their model, age-specific fecundity of females is not considered, a condition that is unlikely to be realistic. Another model proposed by Winkler and Wallin (1987), predicted that maternal allocation should increase with age when the allocation per offspring is low. Egg sacs of *Argiope radon* spiders contains hundreds of eggs, and each egg is around 0.2 percent of the total weight of the egg sac, thus the per capita energetic cost of offspring is very low. That would be an adaptive strategy in the female spider to increase egg size in later egg sacs without a notable reduction in fecundity. Plaistow et al. 2007 showed that soil mite, *Sancassania berlesei* (Acaridae), maternal allocation is dynamic, shifting from maximizing egg number in the early clutches to maximizing egg size later in life. Given that egg sacs laid by the aged female spiders are less likely to develop, increasing the egg size possibly increases the emerging rate and toleration to starvation of the offspring. Unfortunately, we did not count the number eggs in the egg sacs to reveal if fecundity of the female spiders is changed with age.

The protein content of the eggs slightly reduced with maternal age, however, this relationship was not statistically significant. Despite a significant reduction in the mass of the egg sac with age, maternal protein allocation per offspring remained relatively constant, possibly to maximize offspring toleration to starvation emerged from the aged females. Similarly, Pöykkö and Mänttari (2012) showed that the proportion of protein allocated to the eggs in a moth, *Cleorodes lichenaria* (Geometridae), does not vary with maternal age. However, in some parasitic wasps, maternal protein allocation decreases with age possibly because of diminishing maternal reserves (Giron and Casas 2003; Muller et al. 2017). Quantifying other

component of the eggs such as lipids and total energy devoted to the eggs may reveal a clear picture of the temporal variation in egg provisioning and possible consequences for population dynamics such as impacts on sex ratio (Macke et al. 2011).

Emergence time of spiderlings positively correlated with the egg sac order. In insects generally, offspring born from older mothers have longer developmental time due to constrained available resources as a result of low maternal allocation (Fox and Dingle 1994; Fox 1993; Harvey 1977; Leonard 1970; Wasserman and Asami 1985) with few exceptions (Richards and Myers 1980; Vargas et al. 2013). The spiderlings emerged earlier from younger mothers in the first and the third egg sacs in contrast with the second egg sac where spiderlings emerged earlier from older mothers (Figure 4A). The trend we found in the third egg sac could be generated by chance due to the low sample size ($N=7$), however, patterns for the emerging time in the first and the second egg sac with a reliable sample size are opposite which shows a significant interaction between egg sac order and maternal age. Given the difference in emerging time is around 3 days measured in a laboratory test may be unimportant in the field (Heimpel et al. 1998). Moreover, the spiderlings emerging time may not necessarily represent the developmental time of the offspring. When the spiderlings passed through embryonic development, they stay within the egg sac until they moult then the second stage juveniles emerge from the egg sac. Therefore, caution must be exercised for relating these patterns to the field populations dynamics.

Interestingly, spiderlings emerged from the second and the third egg sacs survived around two weeks longer than the first egg sac (Figure 3B), despite the fact that they developed from smaller eggs. This would give them a competitive advantage over the spiderlings emerged from earlier egg sacs (Averill and Prokopy 1987; Kivelä and Välimäki 2008; Messina 1991). Moreover, higher tolerance to starvation would be beneficial if the spiderlings emerged later experience low prey availability. Although, total maternal reproductive allocation reduces over the time, the egg size is

increasing with female age (Figure 2A, B) possibly because of the trade-off between egg size and number (Smith and Fretwell 1974). This can explain higher tolerance to starvation of spiderlings emerged from second and third egg sacs.

There was no significant correlation between neither egg size nor protein content with the offspring emerging time and toleration to starvation. The relationship between maternal egg size and progeny performance has been subjected to a great scrutiny in invertebrates, revealing that offspring from larger eggs may hatch earlier (Rossiter 1991), have higher emergence rates (Richards and Myers 1980), develop faster or develop into larger adults (Rossiter 1991; Steinwascher 1984). The composition of the eggs has also been reported as an influencing factor on offspring performance. However, there are studies that failed to find an observable advantage of larger eggs for offspring performance (Ameri et al. 2014; Vargas et al. 2013). Other factors like lipids content and total energetic content of the egg may play roles in offspring performance (Deventer et al. 2017) and quantifying these factors can illustrate a clearer picture of the maternal reproductive strategy.

This study provided evidences regarding temporal of maternal effects in *A. radon* spider. The results reveal that total resource allocation reduces with maternal age, which results in lower egg sac mass and higher emerging time in the egg sacs laid later in life. However, females increase spiderlings toleration to starvation by increasing the egg size, which suggests there is a trade-off between offspring toleration to starvation and emerging time. These findings showed a strategic maternal allocation in female *A. radon* spiders, and its consequences on offspring performance. The variation between siblings due to the maternal allocation can also have long lasting effects on life history of individuals. This could differentiate the life history traits of the individuals which may adaptively reduce the inter-individual competition and maximize the parent's fitness.

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Tables

Table 1. Generalized linear mixed model analysis of egg sac order and age on reproductive traits in female *Argiope radon* spiders. Asterisks indicate statistically significant p value ($\alpha = 0.05$).

Traits	F	df	p
Egg sac mass			
Egg sac order	10.56	59	0.014*
Age	10.55	45	0.032*
Egg sac order × age	2.13	48	0.344
Random effect (ID)	Variance = 0.001, SD = 0.04		
Residual	Variance = 0.004, SD = 0.06		
Egg size			
Egg sac order	7.85	52	0.049*
Age	10.79	48	0.029*
Egg sac order × age	0.83	52	0.661
Random effect (ID)	Variance = 0.001, SD = 0.02		
Residual	Variance = 0.002, SD = 0.05		
Protein content			
Egg sac order	3.96	50	0.411
Age	1.26	50	0.739
Egg sac order × age	1.21	50	0.544
Random effect (ID)	Variance = 0.001, SD = 0.001		
Residual	Variance = 104703, SD = 323		

Table 2. Generalized linear mixed model analysis of egg sac order and age on progeny performance in female *Argiope radon* spider. Asterisks indicate statistically significant p value ($\alpha = 0.05$).

Traits	F	df	p
Emerging time			
Egg sac order	9.18	45	0.027*
Age	9.78	31	0.044*
Egg sac order × age	8.80	33	0.012*
Random effect (ID)	Variance = 2.01, SD = 1.42		
Residual	Variance = 3.46, SD = 1.86		
Toleration to starvation			
Egg sac order	3.05	38	0.384
Age	11.93	38	0.018*
Egg sac order × age	2.96	38	0.228
Random effect (ID)	Variance = 0.001, SD = 0.001		
Residual	Variance = 77.72, SD = 8.53		

Figures

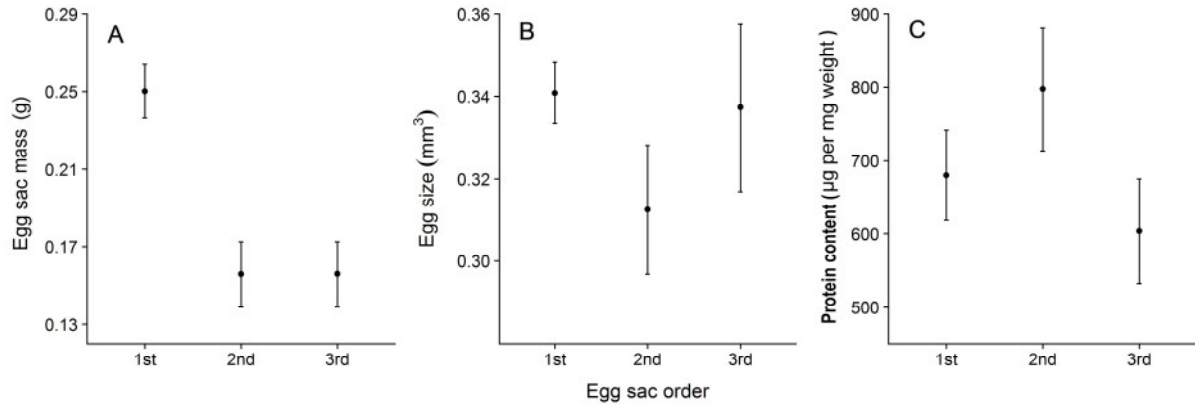


Figure 1. Reproductive strategy (A: egg sac mass; B: egg size; C: egg protein content) of female *Argiope radon* spiders in response to the egg sac order. The solid lines describe the variation in individual egg sacs along egg sac order for 21 adult females. The dashed line describes the average data.

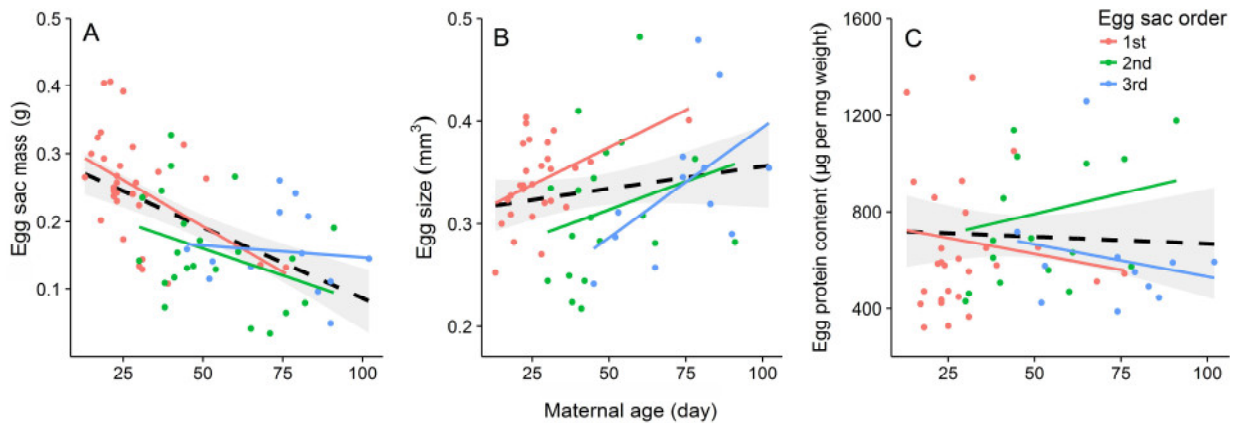


Figure 2. Linear regression of reproductive strategy (A: egg sac mass; B: egg size; C: egg protein content) in female *Argiope radon* spiders relative to maternal age (dots and solid line) and overall average (dashed line) with standard error (shaded area).

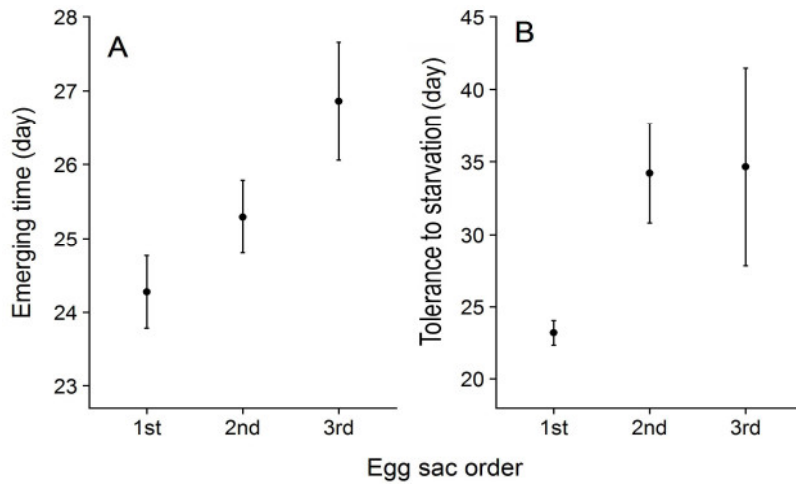


Figure 3. Offspring performance (A: emerging time; B: tolerance to starvation) of female *Argiope radon* spiders in response to the egg sac order. The solid lines describe the variation in individual egg along egg sac order for 18 adult females. The dashed line with dots describes the average of the data.

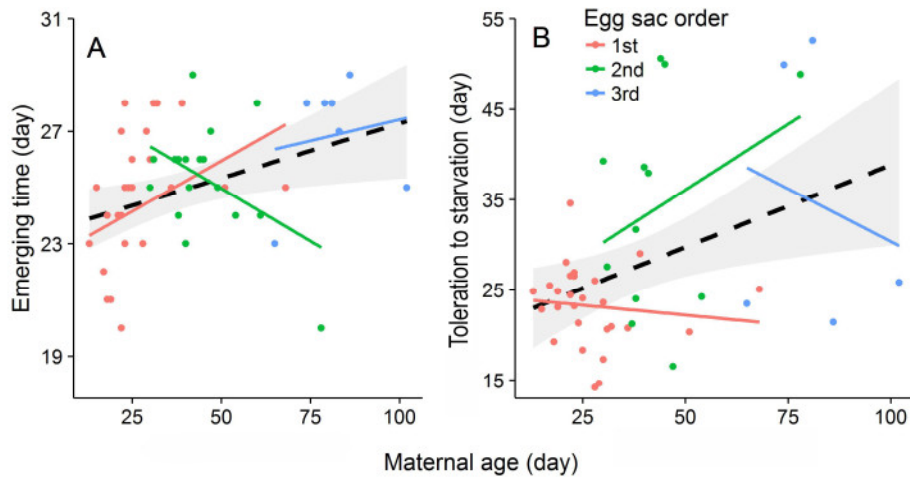


Figure 4. Linear regression of offspring performance (A: emerging time; B: tolerance to starvation) in female *Argiope radon* spiders relative to maternal age (dots and solid line) and overall average (dashed line) with standard error (shaded area).

Chapter 4

Genetic and non-genetic parental effects in an orb-web spider, *Argiope radon*

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Abstract

Offspring phenotype is shaped by alleles and non-genetic factors inherited from parents. The magnitude of each source of variation and the interaction between them determines the susceptibility and direction of selection on different life-history traits. In this study we explored the relative importance of non-genetic parental effects (condition) and genetic additive effects using half-sib families in the orb-web spider, *Argiope radon*. Each male spider mated with two different females to produce half-sibs, then the reproductive output and early life-history traits of spiderlings were assessed. Maternal condition is positively correlated with total reproductive allocation, i.e. egg sac mass, while paternal condition has negative relationship with egg sac mass. Interestingly, both maternal and paternal condition was inversely correlated with offspring body size. Quantitative analysis showed body size and toleration to starvation are heritable traits, while no genetic additive variance detected for egg size. Assessing offspring web structure revealed web hub size (non-sticky silk) is heritable while no heritability was detected for features relating to the sticky silk, such as web size, number of spiral threads and capture thread length. These findings suggest maternal and paternal condition can induce asymmetrical alteration in total reproductive mass and offspring body size. Body size and toleration to starvation are less plastic traits, suggesting that these traits are closely linked with fitness of spiderling. Spiderling web analyses suggest that features of the web related to sticky silks are highly plastic traits and subjected to environmental and/or parental non-genetic factors compare to non-sticky silks. This may imbue spiders with selective benefits if they modify their foraging strategy and match it with local condition. Our findings provide insights regarding to the source of variation of early life-history traits of spiderlings, which may have long lasting impact on population dynamics.

Keywords: parental condition, maternal effects, paternal effects, heritability

Introduction

Non-genetic parental factors act in parallel with inherited genes by a variety of mechanisms and represents an important source of phenotypic variation in offspring (Bonduriansky and Day 2009). For a long time, non-genetic effects (i.e. parental effects) have been regarded as nuisance effects in quantitative analyses and evolutionary biologists have only recently begun to address parentally induced plasticity under an evolutionary perspective (Laland et al. 2014; Uller 2008). Both genetic and non-genetic effects and the interactions between them can influence the magnitude and the direction of selection on life history traits and hence the evolutionary population dynamics (Bonduriansky and Day 2009).

Fisher's fundamental theorem stated that 'the rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time' (Fisher 1930). Accordingly, alleles directly related to fitness will be fixed rapidly by selection and traits linked to those alleles have low additive genetic variation (V_A) (Houle 1992; Merilä and Sheldon 1999; Stirling et al. 2002). Falconer (1996) argued that the magnitude of heritability ($h^2 = V_A/V_P$) of a trait represents its importance in an individual's fitness: traits that are closely related to fitness (e.g. sexually selected traits) have higher rates of heritability than traits with weak contributions to fitness (e.g. morphological traits). Empirical evidence has been widely consistent with these prediction (Hartl and Clark. 1990; Mousseau and Roff 1987).

Variation in life history traits induced by non-genetic factors (i.e. parental effects) have been regarded as developmental plasticity (Bonduriansky and Day 2013; Uller 2008). A non-plastic trait is regulated exclusively by genes, while a plastic trait can be influenced by environmental effects provided by parents. Therefore, the degree to which a trait is plastic determines its susceptibility to parental and environmental factors. Moreover, in highly plastic traits, additive genetic effects may be masked by parental effects, suggesting they are more important determinants of an individual's fitness than additive genetic effects (Falconer 1996; Houle 1992; Walsh and Blows

2009). It is necessary to distinguish the contribution of additive genetic effects from those of parental effects on trait variation to explore the adaptive value of the observed variation in any particular trait.

Growing evidence has shown that plasticity induced by parental effects are widespread across taxa and can significantly influence the phenotype of offspring (Gotthard and Nylin 1995; Miner et al. 2005). A variety of extrinsic factors experienced by parents, including diet (Vargas et al. 2014), temperature (Huestis and Marshall 2006; Scharf et al. 2010), photoperiod (Giesel 1988), predation (Li 2002) and intra-specific competition (Marshall et al. 2006) can affect the phenotypic traits of offspring. Shea et al. (2011) described these effects as detection-based factors where the immediate environmental cue detected by parents passes down to the subsequent generations to match offspring phenotype with the environment. These effects have less adaptive significance as they may simply represent the condition detected by parents, in contrast with selection-based effects where the parental effects are imparted to offspring by reliable epigenetic mechanisms and entail an adaptive importance (Bonduriansky and Day 2009; Uller 2008). Example of these effects are parental age (Najafpour et al. 2018; Vargas et al. 2014), predatory induced effects (Benard 2004) and parental resting metabolic rate (Burton et al. 2011; Careau et al. 2008). Detection- and selection-based factors interact with each other and are transferred to offspring thereby contributing to phenotypic plasticity of life history traits.

Spiders forage in heterogenic environments where resources fluctuating throughout the season and a favourable reproductive strategy is to produce high number of small offspring (Stearns 1976; Stearns 1977). However, high rates of mortality in juveniles results in only a very small percentage of the individuals to reach maturity (Valerio 1975). In spiders, mortality occurs mostly during aerial dispersal (i.e. ballooning) where spiderlings are passively dispersing by wind to new sites (Bonte et al. 2007; Bonte and Lens 2007). During this stage spiderlings are exposed to the risk

of starvation and desiccation (Bonte et al. 2007, 2012; Vogelei and Greissl 1989). Survival of early juveniles are a crucial bottleneck to parents' fitness and hence population growth rate, therefore, there may be a strong selection pressure at early stages of juvenile spiders. Surprisingly, quantitative analyses aimed at detecting underlying additive genetic structures of the behaviours in spiders have been largely neglected (aerial dispersal: Bonte & Lens 2007; environmental contamination: Hendrickx et al. 2008).

In this study we tested the relative importance of additive genetic effects and parental effects in early life history traits of an orb-web spider, *Argiope radon* (Araneidae). We conducted a classic full\half sib experiment where each male spider mated with two different females to test additive genetic effects. The effect of phenotypic parental condition was also tested on spiderling traits, including egg size, body size, tolerance to starvation and web structure. Egg size in arthropods is correlated with maternal factors such as condition (Ameri et al. 2014; Fox 1997) and age (Najafpour et al. 2018; Vargas et al. 2014), however, it is only weakly correlated with offspring performance (Fox and Czesak 2000). Similarly, web structure of orb-web spiders is highly flexible and subjected to maternal and environmental factors (Heiling and Herberstein 2000). We hypothesize that additive genetic effects have low influence on egg size and web structure of offspring and these traits are more influenced by phenotypic parental condition. In contrast, we predict that additive genetic effects have high influence on body size as it is closely linked to individual's fitness such as fecundity and longevity (Blueweiss et al. 1978; Peters 1983). Similarly, tolerance to starvation in early spiderlings is a crucial trait as it determines survival and the chances to reach maturity (Valerio 1975). Therefore, we predict tolerance to starvation to be less plastic and due to additive genetic effects rather than parental condition.

Materials and Methods

Study Species

Argiope radon is a common Australian orb-web spider distributed in the Northern Territory and Queensland where they construct their orb webs on trees and shrubs overhanging river banks (Rao et al. 2009). A group of subadult females (N = 40) and males (N = 20) were collected from the banks of the Katherine River in the Northern Territory, Australia, during October 2016. All animals were transferred to the laboratory for further tests (Zschokke and Herberstein 2005). Female spiders were transferred into 3D Perspex frames (50 x 50 x 15 cm), where they could build their typical orb-web over the night. A strip of masking tape was attached to the inner side of the frames to aid the attachment of the silk. Male spiders were kept in 300ml ventilated plastic cups. The females were fed one large cricket (0.368 ± 0.02 g) per week and the males were fed on *Drosophila* flies twice a week, 10-15 flies each time. All animals were housed in a temperature-controlled room ($26 \pm 2^\circ\text{C}$; 70% RH) on a 12:12 hour light:dark cycle and sprayed water two time a week. All experiments were conducted under the same environmental condition.

Experimental procedure

Once individuals moulted to the adult stage, photographs of the patella-tibia length of the right fore leg in females and carapace width in males were taken using a digital camera (CASIO Exilim EX-F1, CASIO Computer Co., Tokyo, Japan) as a linear measurement of body size. Mass of the males and females were measured to the nearest 1 mg using a microbalance (Mettler Toledo PB303-S). Each adult male was crossed randomly with two different unmated females. Females were prevented to cannibalize males by placing a fine paint brush in front of her chelicerae during copulation. This method has no effect on copulation duration or sperm transfer (Herberstein et al. 2011). The age of the females at the time of mating was 8.03 ± 0.91 days since final molt and the age of the males was 25.67 ± 2.56 days since final molt

for the first mating and 32.07 ± 2.94 days since final molt for the second mating, respectively.

The females were kept on standard diet, until they started laying egg sacs. Each egg sac was weighted individually to the nearest 0.01 mg using a microbalance (Mettler Toledo New Classic MS), then opened gently with sterilized forceps and a group of 15 eggs were removed randomly with a fine paint brush. The eggs were photographed under a stereo-microscope (Motic SMZ-171) equipped with a camera (Moticam 3.0MP). The eggs are packed in a very dense batch within egg sac and there is a pressure from the surrounding eggs. Therefore, the eggs might not be perfectly spherical. We ignored these small changes and assumed that eggs are spherical and egg volume, as a proxy of egg size, was estimated, using formula $\frac{4}{3}\pi(R/2)^3$, where R is the egg diameter. This method results in more accurate measurements than weighting the eggs and produced less biased measurements. All photographs in this study were measured using ImageJ 1.49v software (U. S. National Institutes of Health, Bethesda, Maryland, USA).

After egg removal, the egg sacs were kept in 100 ml ventilated plastic cups and sprayed with water twice a week to keep them moisturized. Once the spiderlings emerged from the egg sacs two random groups of full-sib spiderling were selected from each egg sac to measure body size and toleration to starvation. The rest of the spiderlings were kept in a 300 ml ventilated plastic cups. For body size a group of 20 spiderlings were randomly selected from each egg sac. Spiderlings were killed via exposure to -80°C for 5 minutes then air dried for 30 minutes under a fume hood to avoid the effect of condensed moisture left on their body. They were weighted individually to the nearest 0.01 mg using a microbalance (Mettler Toledo New Classic MS). For toleration to starvation, a group of 30 newly hatched spiderlings were randomly selected and kept in a 100 ml ventilated plastic cup. The cups were checked every second day and the mortality of the spiderlings was recorded until all spiderlings were dead.

Full-sib spiderlings in the 300 ml ventilated plastic cups were fed on 20-30 medium size *Drosophila* flies twice a week. After 10 days a group of 10 full-sib spiderlings were randomly selected and transferred individually to a plastic container (15 x 15 x 5 cm) where they can build their web over a night. Web diameter and web hub diameter were measured using a digital calliper to the nearest 0.01 mm. Capture thread length (CTL) was estimated using a modified formula (Heiling et al., 1998): $CTL = [(C_{hub} + C_{web}) / 2] \times [(sp1 + \dots sp4) / 4]$, where C_{hub} and C_{web} are the circumferences of the hub and web respectively and sp1, 4 are the number of spirals in eight sectors of the web, from upper vertical sector moving clockwise every 90 degrees around the entire web.

Statistical analyses

The distribution of the variables was checked before further analysis by calculating quantiles of each variable in the dataset and then plot them against theoretical quantiles of the normal distribution (q-q plot) to check if the empirical quantiles sit within border of normal distribution. The effects of parental condition were analysed with R 3.3 (R Development Core Team). The package 'car' (J. Fox and Weisberg 2016) was used to fit general linear models, testing the significance of the male and female condition, on reproductive output and offspring performance. Then, we estimated the additive and non-additive genetic components of spiderling's performance and web structure using a full\half sib analysis (Falconer 1996). The full\half sib analysis is equivalent to the standard ANOVA approach whereby the sire variance component equals $\frac{1}{4} V_A$ and the dam (nested within sire) component equals $\frac{1}{4} V_D + V_{EC}$ (ignoring epistasis). ASReml software package (Gilmour et al. 2009) was used to estimate genetic variance and narrow-sense heritability. Our design was considerably unbalanced at the family level all genetic (co)variances were estimated using REML rather than least squares techniques, because REML estimates are more robust with respect to variation in group size (Michael Lynch and Walsh 1998).

Results

Of the individuals collected from the field, 18 males mated with 36 females and 30 mated females laid an egg sac. The average number of individuals per clutch measured for egg size was 15.26 ± 0.92 , followed by 19.17 ± 0.92 , 34.21 ± 2.30 and 9.50 ± 0.47 for body size, toleration to starvation and web structure measurements.

Maternal phenotypic condition positively correlated with the mass of the egg sacs while paternal phenotypic condition had a significant negative correlation with the mass of the egg sacs (Table 1, Fig.1a). Egg volume did not vary with parental condition (Table, 1; Fig. 1a). Interestingly, body mass of offspring at the time of emergence significantly decreased with both maternal and paternal condition (Figure 1b). There was a significant interaction between sire condition and dam condition on offspring body size (Table 1). Toleration to starvation of offspring was not influenced by paternal nor maternal condition, similarly, no significant interaction was detected between paternal and maternal condition for offspring toleration to starvation (Table 1; Figure 1c).

Web structure components of offspring did not vary with parental condition (Table 2, Figure 2a-d), and there was no significant interaction between maternal and paternal condition for web structure components (Table 2).

Half-sib analyses showed that sire (additive genetic) effects were not detectable for egg size, however, 29% of body size variation and 52% of toleration to starvation variation could be attributed to additive genetic effects (Table 3). The sires did not have a detectable effect on offspring web size variance, however, a substantial contribution of 65% of web hub variance was due to sire effects (Table 4).

Discussion

This study attempted to disentangle the influence of additive genetic effects from parental effects in an orb-web spider. We predicted that highly flexible traits such as web structure and egg size are under greater parental effects, while traits strongly

linked to fitness (e.g. body size and toleration to starvation) are influenced by additive genetic effects.

Parental phenotypic effects

Our results showed that maternal phenotypic condition strongly affected the mass of the egg sacs, which is not surprising as presumably females are in full control of allocating eggs to the egg sac (Deventer et al. 2017; Fox and Czesak 2000). However, egg size did not vary with maternal or paternal condition in *A. radon*. Generally, there is no consistent relationship between maternal body size and egg size in arthropods, and when there is a positive relationship, it is weak (Fox and Czesak 2000). Therefore, it has been argued that variation in egg composition (e.g. lipids and proteins) is ecologically more important than egg size *per se* (Begon and Parker 1986; Bernardo 1996). Few data are available to test this hypothesis; however, some studies reported a positive correlation between egg content, specifically protein, and offspring performance (Giron and Casas 2003; Muller et al. 2017)

Contrary to our prediction, both maternal and paternal phenotypic condition negatively correlated with offspring body size. Similar results have been found in some butterfly and moth species, where maternal condition inversely correlated with offspring body size (Harvey 1983; Jones et al. 1982). This can be an adaptive strategy for low condition females in which they increase *per capita* investment in offspring at the cost of reducing total fecundity (Einum and Fleming 2000).

Contrary to maternal phenotypic condition, paternal phenotypic condition inversely correlated with the mass of egg sac and offspring body size, suggesting asymmetrical effect of male and female on offspring performance. This phenomenon may be the result of different epigenetic markers between male and female (Martin and Zhang 2007; Spencer 2002). It has also been shown that paternal condition positively correlated with offspring toleration to starvation possibly as a result of trade-off between body size and toleration to starvation. These findings suggest paternal phenotypic condition can be transferred to offspring as non-genetic source of

phenotypic variation (Crean and Bonduriansky 2014; Curley et al. 2011). The mechanisms mediating paternal effects are not well understood, but growing evidence has shown that paternal effects can alter phenotype of offspring, which may influence fitness of individuals (Bonduriansky and Head 2007).

Additive genetic effects

Consistent with our prediction, egg size is not a heritable trait in *A. radon* spiders (Table 3). Heritability of egg size varies among avian species but generally a very high heritability rate (generally greater than 0.5) was seen in birds (Christians 2002b; Duncan 1987; Moss and Watson 1982; Ojanen et al. 1979). Similarly, egg size has shown to be a heritable trait in different *Daphnia* populations (Warren 1924). Surprisingly, few studies examined heritability rate of egg size in other arthropods. In seed beetles, heritability of egg size varied greatly, ranging from 0.22 to 0.91 depending on rearing condition and population (Fox 1993; Fox 1997). However, in *A. radon* spider no heritability for egg size were detected, suggesting that it is more subjected to parental phenotypic effects, such as maternal age. Egg size varied with maternal age in *A. radon* spiders (unpublished data), suggesting that female spiders temporally adjust egg size to ensure a minimum number of offspring will survive. This is also consistent with theoretical model predictions where plasticity is favoured in heterogenic environments (Uller 2008).

We predicted that body size and toleration to starvation are closely linked with an individuals' fitness hence these traits may have high additive genetic variance. Our results showed that additive genetic effects had a 29% contribution to body size, therefore it is a moderately heritable trait. Body size has been reported as an important trait directly linked with various life-history traits in animals (Lindström 1999). Similarly, in spiders, body size is correlated with egg sac mass (Figure 1a), web building behaviour (Herberstein and Heiling 1999) and courtship behaviour (Wignall et al. 2014), which suggests a large phenotypic effect of body size on various traits. Substantial influence of phenotypic body size may explain low heritability of body

size because with increasing non-genetic effects the total phenotypic variance (V_P) increases in the heritability formula (V_A/V_P) (Houle 1992; Merilä and Sheldon 1999).

Toleration to starvation of the spiderlings showed a high heritability rate of 52%, which is consistent with our prediction. Most of the spiderlings die early in their life when they disperse to new sites, because they will be exposed to the risk of starvation and desiccation. Therefore, mortality at early juvenile stages is a major factor limiting number of individuals that reach to the adult stage (Valerio 1975). This suggests a strong selection pressure on spiderling toleration to starvation, which can result in allele fixation and hence high additive genetic variance.

Web structure of the spiderlings did not vary with parental condition. A high heritability of 65% was detected for web hub size, while no genetic additive variances were detectable for web size, number of spiral threads and capture thread length, suggesting that these traits are mostly affected by ontogenic factors such as age and environmental factors (Heiling and Herberstein 2000). The web of araneid spiders is a multi-function structure, various tasks including capture prey, mating and anti-predator behaviours are directly linked to web structure, hence in heterogenic environments female spiders are face with complex decisions for building their web (Herberstein and Tso 2011). However, variation in web structure is mostly implemented via alteration of sticky spiral threads while the structural part of the web such as radial threads and hub area remain unchanged (unpublished data). This can explain why web hub size is a heritable trait while the other measurements that relying on the spiral threads are more plastic and highly subjected to environmental factors. Moreover, orb-web spiders are passive foragers, which means the quantity and quality of prey in each foraging bout is unpredictable. Therefore, flexibility in sticky silks (overall web size and capture thread architecture) allows them to match their foraging efforts with local condition.

To conclude, maternal phenotypic condition is a strong determinant of total reproductive energy allocation and offspring body size, while paternal condition

seems to trade-off body size of offspring with toleration to starvation. Quantitative analyses showed that body size and toleration to starvation are heritable traits, suggesting they are closely linked to the fitness of spiderlings compare to egg size, which is presumably mediated by maternal phenotypic effects. Similarly, web hub size (non-sticky silks) of spiderlings was a heritable trait, contrary to the traits related to sticky silks (web size and capture thread length). This suggests web size and capture thread length are more subjected to environmental factors and/or non-genetic parental effects, which allows spiders to match their foraging strategy to local environment. These findings provide insights regarding the early life-history traits plasticity of *A. radon* spiderlings, which can have long lasting potential impacts on population dynamics.

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Tables

Table 1. General linear model analyses of paternal and maternal condition on reproductive output and offspring quality of the spider *Argiope radon*.

Source of variation		df	F	p value
Egg sac mass				
	paternal condition	1,22	4.45	0.046
	maternal condition	1,22	9.10	0.006
	paternal condition × maternal condition	1,22	4.71	0.041
Egg size				
	paternal condition	1,19	4.14	0.056
	maternal condition	1,19	2.87	0.106
	paternal condition × maternal condition	1,19	3.21	0.088
Body size				
	paternal condition	1,22	5.39	0.004
	maternal condition	1,22	7.84	0.005
	paternal condition × maternal condition	1,22	7.25	0.011
Toleration to starvation				
	paternal condition	1,21	0.28	0.599
	maternal condition	1,21	0.01	0.924
	paternal condition × maternal condition	1,21	0.03	0.858

Table 2. General linear model analyses of paternal and maternal condition on web structure of offspring in *Argiope radon*.

Source of variation	df	F	p value
Capture area			
paternal condition	1,13	0.41	0.534
maternal condition	1,13	0.23	0.635
paternal condition × maternal condition	1,13	3.69	0.786
Web hub size			
paternal condition	1,13	0.87	0.356
maternal condition	1,13	0.01	0.335
paternal condition × maternal condition	1,13	0.71	0.415
No. spiral threads			
paternal condition	1,13	1.32	0.271
maternal condition	1,13	1.93	0.187
paternal condition × maternal condition	1,13	1.75	0.209
Capture thread length			
paternal condition	1,13	1.64	0.221
maternal condition	1,13	1.88	0.193
paternal condition × maternal condition	1,13	1.99	0.181

Table 3. Estimates of narrow-sense heritability (h^2) on egg size and offspring quality for half-sib offspring of *Argiope radon*.

Traits	n	variance (\pm SE)	genetic components (\pm SE)	h^2 (\pm SE)
Egg size				
Sire	18	0.00001 (\pm 0.00001)	$V_A = 0.00005$ (\pm 0.00005)	0.0001 (\pm 0.0001)
Dam	30	14.21 (\pm 9.41)	$V_P = 140.93$ (\pm 15.39)	
Residual	190	126.72 (\pm 13.74)		
Body size				
Sire	18	1.67 (\pm 3.10)	$V_A = 6.69$ (\pm 12.41)	0.29 (\pm 0.53)
Dam	30	8.52 (\pm 3.74)	$V_P = 22.99$ (\pm 3.10)	
Residual	556	12.80 (\pm 0.79)		
Toleration to starvation				
Sire	18	5.34 (\pm 6.94)	$V_A = 21.38$ (\pm 27.59)	0.52 (\pm 0.64)
Dam	30	14.89 (\pm 6.89)	$V_P = 41.28$ (\pm 6.04)	
Residual	958	21.04 (\pm 0.97)		

Table 4. Estimates of narrow-sense heritability (h^2) on web structure for half-sib offspring of *Argiope radon*.

Traits	n	variance (\pm SE)	genetic components (\pm SE)	h^2 (\pm SE)
Web size				
Sire	18	0.00017 (\pm 0.00001)	$V_A = 0.0007$ (\pm 0.00006)	0.001 (\pm 0.0001)
Dam	30	180.04 (\pm 127.69)	$V_P = 1935.01$ (\pm 210.25)	
Residual	190	1754.98 (\pm 190.75)		
Web hub size				
Sire	18	20.52 (\pm 16.16)	$V_A = 82.10$ (\pm 64.58)	0.65 (\pm 0.46)
Dam	30	9.48 (\pm 10.53)	$V_P = 127.18$ (\pm 17.45)	
Residual	190	97.18 (\pm 10.51)		
No. spiral threads				
Sire	18	0.00003 (\pm 0.00001)	$V_A = 0.0002$ (\pm 0.00002)	0.0001 (\pm 0.0001)
Dam	30	9.11 (\pm 5.06)	$V_P = 64.23$ (\pm 0.732)	
Residual	190	55.12 (\pm 5.97)		
CTL				
Sire	18	0.08 (\pm 8.2)	$V_A = 0.33$ (\pm 50.69)	0.004 (\pm 0.66)
Dam	30	36.47 (\pm 16.81)	$V_P = 76.14$ (\pm 11.39)	
Residual	411	39.57 (\pm 2.85)		

Figures

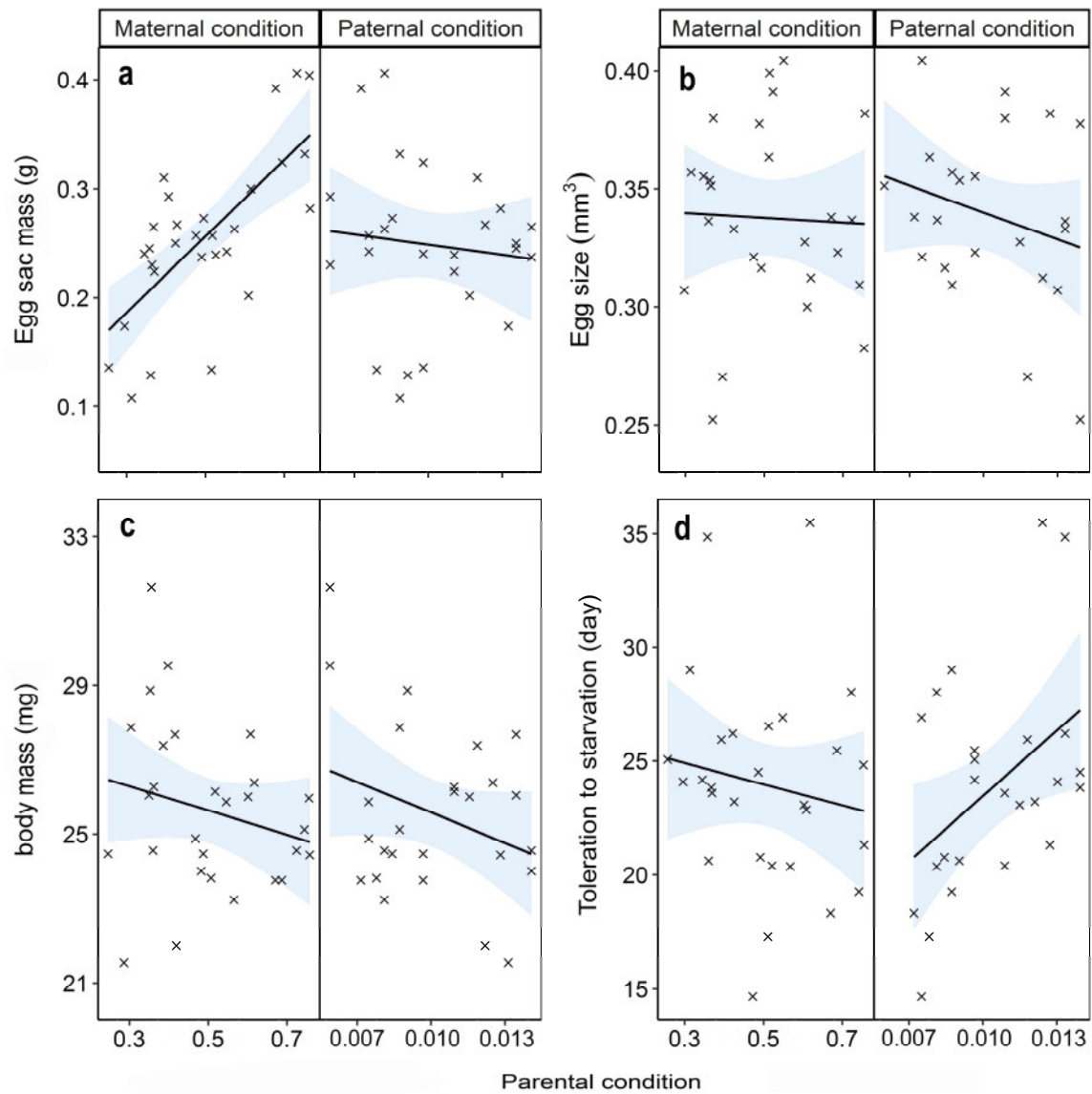


Figure 1. General linear model of parental condition with reproductive allocation and offspring quality in *Argiope radon* (a: egg sac mass; b: egg size; c: body size; d: toleration to starvation).

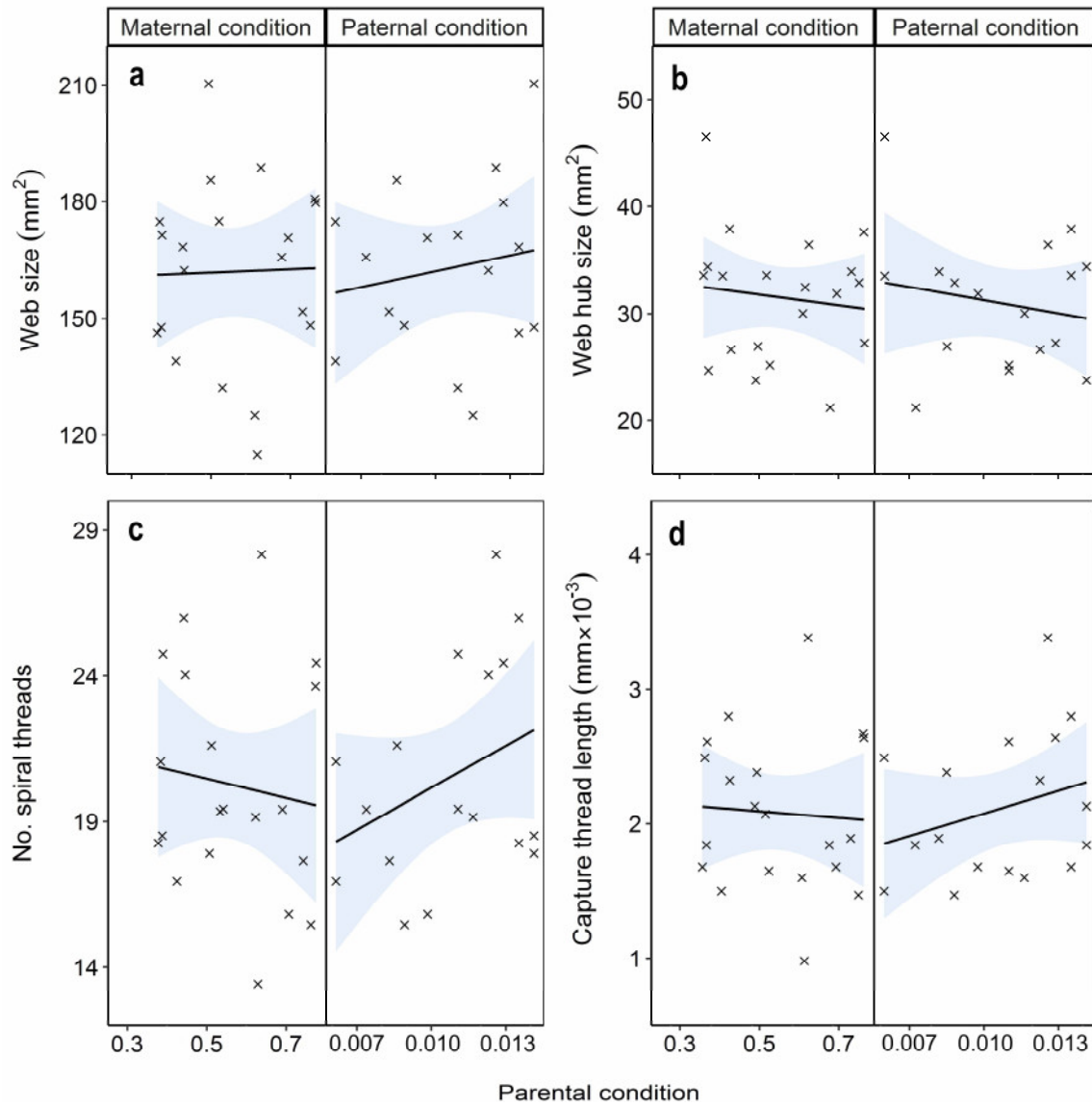


Figure 2. General linear model of parental condition with offspring web structure in *Argiope radon* (a: web size; b: hub size; c: no. spiral threads; d: capture thread length).

Conclusions

This study linked physiology with behaviour and reproductive outputs in the orb-web spider genus *Argiope*. I first assessed the effects of predation risk on foraging behaviour and metabolic rate of spiders. Next, I tested the effects of metabolic rate, age and condition on reproduction and finally, I examined how additive genetic factors impact reproductive output and offspring performance in orb-web spiders.

The presence of predatory cue induced short-term changes in attack behaviour and web rebuilding frequency in the female spiders *A. keyserlingi* (Chapter 1). Overall, the spiders constrained their foraging time budget under predation pressure and were also less likely to rebuild a damage web. Presumably, this behavioural change has a selective benefit by reducing being detected by predators. Quantitative analyses of half-sib spiderlings of *A. radon* in Chapter 4 showed that some web elements are heritable (web hub size) while others are highly plastic (web size and capture thread length) and thus are more affected by maternal and environmental effects (Herberstein and Tso 2011). This is consistent with empirical evidence reporting high flexibility of web building behaviour in orb-web spiders under different environmental conditions (Eberhard 1988; Heiling and Herberstein 2000) including airborne predatory cues (Nakata 2008; Nakata and Mori 2016). However, web size and capture thread length of *A. keyserlingi* females did not vary with predation pressure. Similar results were found in Bruce and Herberstein (2006) where predatory mantid cues did not induce changes in web architecture, suggesting changing web structure is not a favourable response of *A. keyserlingi* under predation pressure.

Resting metabolic rate (hereafter: RMR) is a fundamental trait of animals and represents the minimal energy expenditure of an individual during periods of inactivity (Brown et al. 2004). Despite the consistency of RMR over their life-time (Chapter 2), exposure to the predatory cues significantly elevated RMR of the female spiders. This relatively rapid physiological response may facilitate anti-predatory escape behaviour by redirecting energy to locomotory tissues (Chapter 1). Moreover,

the observed elevation in RMR could influence energy processing and the amount of available resources to be allocated to various functions such as reproduction (Burton et al. 2011; Careau et al. 2008), which was tested in Chapter 2.

My thesis investigated how RMR of *Argiope* spiders is influenced by environmental factor such as predation (Chapter 1) and intrinsic factor such as mating status (Chapter 2). A common pattern is considerable intra-individual variation in RMR of animals (Biro and Stamps 2010; Burton et al. 2011). However, RMR in *A. radon* females appeared to be temporally consistent within individuals, with no overt effect of age. This is possibly due to the cost of long term raising or lowering RMR, which includes modification of tissues and metabolic pathways (Haggerty et al. 2008; Speakman et al. 2004). However, mating status of females did affect RMR, the female spiders reduce their RMR by around 35 percent after mating (Chapter 2). This suggest that the 'compensation model' is a favoured strategy in which female spiders decrease RMR during energy demanding activities such as reproduction (Speakman et al. 2004). A lower RMR allows female spiders to mediate energetic costs of self-maintenance and accumulate more resources. Chapter 4 shows that maternal condition is positively correlated with egg sac mass and offspring body size, therefore an increase in mass gain can increase reproductive resource allocation. The other advantages of this strategy are reduced production of reactive oxygen species (ROS) factors and prevention of exhausting current energetic reserves during food scarcity (Jensen et al. 2010; Slos and Stoks 2008).

Despite relative consistent RMR within individuals, there was considerable variation in RMR between individuals in males and females. In Chapter 2, I investigated what the fitness consequences of this variation might be, by looking at reproductive output and offspring performance. I found no correlation between RMR of the sire or the dam with egg sac laying time, egg sac mass, offspring emergence and body size. Similarly, other studies failed to find a significant correlation between RMR and reproductive output (Burton et al. 2011). One explanation might be the *ad libitum*

access of the spiders to food (Alvarez and Niecieza 2005; Killen et al. 2011). When individuals are faced with food scarcity they have to trade-off different traits, resulting in a detectable reduction in some life-history traits, such as reduced growth rate or fecundity (Roff 2002).

A central question of my PhD was how parental effects (non-genetic effects) impact reproductive outputs. Assessing temporal variation of maternal effects in the female spiders revealed that egg sacs laid early in life were heavier, had larger eggs and offspring emerged sooner than the egg sacs laid later in life (Chapter 3). This is possibly due to a depletion of the accumulated resources over the time (Fox and Czesak 2000) and reduced metabolic efficacy of the females (Beckman and Ames 1998; Harman 1956; Rose 1991). Spiderlings that emerged from early egg sacs were more likely to develop, however, these offspring had reduced toleration to starvation than the egg sacs laid by old females (Chapter 3). This suggests that female spiders allocate resources strategically over their reproductive life-time. This is consistent with the findings in Chapter 4, where no heritability was detected for egg size, suggesting large maternal phenotypic effects on maternal reproductive allocation. Generally, egg sacs laid later in life may be exposed to higher predation rate, and reduced foraging opportunities as less prey are available at the end of the season (Crouch and Lubin 2000; Halaj et al. 1998; Riechert and Tracy 1975). Therefore, the reproductive value of clutches drops with maternal age; theoretical models predict females should allocate less resources to those reproductive bouts that carry lower reproductive value (Begon and Parker 1986; Bernardo 1996a, 1996b).

While I found no relationship between parental condition and offspring toleration to starvation, egg sacs laid later in the reproductive life-time of dams had significantly higher toleration to starvation (Chapter 3). These findings indicate that offspring toleration to starvation is a plastic trait mostly affected by maternal age. Egg sacs laid later in life are more likely to face food scarcity (Crouch and Lubin 2000; Halaj et al. 1998; Riechert and Tracy 1975), therefore maternal effects that increase offspring

toleration to starvation by increasing the egg size. However, this may entail some costs including reduced emerging success rate of the egg sacs (Ameri et al. 2014).

Perhaps not surprisingly, maternal condition positively influenced the egg sac mass. What was not expected was that paternal condition inversely correlated with egg sac mass (Chapter 4), which suggest asymmetrical effects of parents on total reproductive allocation as a result of different epigenetic markers between males and females (Martin and Zhang 2007; Spencer 2002). I found no significant correlation between parental condition and egg size (Chapter 4), however, egg size of the spiders was positively correlated with maternal age (Chapter 3). Half-sib spiderling analyses showed that egg size is not a heritable trait, thus egg size in *Argiope* spiders appears to be a trait that is mostly influenced by maternal non-genetic effects. This suggest that egg size is not under strong selection pressure, which is consistent with empirical evidence in arthropods where generally there is a weak relationship between egg size and offspring performance (Fox and Czesak 2000).

I predicted that offspring size is a highly heritable trait because it is directly linked to various life-history traits such as fecundity and longevity and hence the fitness of individuals (Lindström 1999). However, half-sib analyses revealed a low heritability of 29% for body size (Chapter 4). Low heritability rate of body size may be due to large parental non-genetic effects because with increase in non-genetic effects the total phenotypic variance (V_P) increases in the heritability formula (V_A/V_P) (Houle 1992; Merilä and Sheldon 1999). Toleration to starvation of the spiderlings showed a high heritability rate of 52%, which is consistent to our prediction (Chapter 4).

In summary, predation pressure affects foraging behaviour and RMR of female spiders, therefore, it is very likely that prey capture success rate and hence the physiological state of the spider is influenced by predation risk. RMR does not directly correlate with reproductive output and offspring quality, however, female spiders adaptively reduce RMR after mating thereby reducing self-maintenance

energetic costs and accumulate more energy after mating and gain more weight. This is supported by the positive correlation between maternal condition with egg sac mass. Similarly, maternal and paternal condition were correlated with offspring body size, however in different directions, suggesting asymmetrical parental effects. There is also temporal variation in maternal effects, early egg sacs were larger and emerged quicker, but later egg sacs had higher toleration to starvation, suggesting a trade-off between total resources allocation and emerging time with toleration to starvation. Quantitative analyses of half-sibs showed that body size and toleration to starvation are heritable traits while egg size mostly influenced by parental non-genetic effects. Finally, web hub size (non-sticky silk) is heritable while web size and capture thread length (sticky silks) are more plastic traits which allow female spiders to match their web structure to local condition.

Future questions

This thesis provides a body of evidence on the parental effects on reproductive output and offspring quality in two species of the orb-web spider genus *Argiope* spp. The following questions are suggested to provide a clearer picture of the effects of transgenerational signals and the effects of parents on offspring life-history traits.

- 1) What are the effects of dietary restriction (quantity and quality) on female reproductive output and its consequences on offspring performance?
- 2) How variable are parental effects between different geographical populations of the same species?
- 3) How does the quantity and quality of male seminal fluid affect offspring traits?
- 4) How is the sperm depleted in the reproductive system of females and how does it influence offspring performance?

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