Sex-specific aging in two-spotted spider mite *Tetranychus urticae*:

Effects of diet, social environment and predator-induced stress

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Abstract

Aging is associated with progressive decline in physiological performance and increasing vulnerability to disease with age and is almost universal and inevitable for multicellular organisms. Although our knowledge about aging has advanced greatly during the last few decades, our current understanding of how and why we age remains incomplete. My research examines how abiotic and biotic factors including food regime, social environment and predator-induced stress affect the fitness traits (i.e., survival and reproduction) of the organism. Additionally, I attempted to explore how these factors influenced the fitness of both sexes. Meanwhile, I tested two evolutionary hypotheses, antagonistic pleiotropy hypothesis of George Williams and disposable soma theory of Kirkwood and Holliday, which postulated that the survival and reproduction trade off with each other.

To address these questions, I employed spider mite *Tetranychus urticae* as my model organism, along with its specialist predator *Phytoseiulus persimilis* to generate predator-induced stress. The life-history traits (e.g. survival rate, adult lifespan and reproductive parameters) were compared between treatments to determine the responses of males and females to various environmental factors. Furthermore, the relationship between longevity and lifetime fecundity were assayed with linear regression and path analysis in testing the two evolutionary theories of aging.

To determine the starvation tolerance of spider mites, the lifespan of mites was assayed with mites fed *ad libitum* as control. It showed the females and males have a median lifespan of 6 and 3 days, respectively (Chapter 2). Therefore, in further studies, the spider mites under food regimen were subjected to fasting at most two days. When exposed to four levels of intermittent fasting (IF): 33%, 50%, 67% with control fed *ad libitum*, I found a sex-specific response to IF in lifespan, whereby females showed a significant increase in lifespan at the intermediate level of IF, while males showed a shorter lifespan when subjected to each level of IF. The females under IF decreased or even ceased reproduction in comparison with *ad libitum* fed mites during the reproductive stage. The lifetime fecundity of females fed at 50% IF was significantly lower than that of control (Chapter 3). For both sexes isolated individuals

outlived their counterparts when paired with conspecifics regardless of sex, indicating that the cost of interaction with the same sex and the opposite sex is significant (Chapter 4). Additionally, the time and frequency of sexual interaction can have significant fitness consequence on spider mites. Females and males showed sex-specific plasticity of longevity (Chapter 5). With a comprehensive investigation on the relationship between survival and reproduction traits through path analysis including traits of longevity, female lifetime reproduction, age at first reproduction, early reproductive efforts and late reproductive efforts, I discovered no evidence for trade-offs between these life-history traits (Chapter 6).

I showed that predator-derived odour prolonged the immature duration of both sexes, shortened female adult lifespan (18.8%) but not those of males, and reduced lifetime reproductive outputs of the females (29.8%). Using a full factorial design, I exposed offspring from both risk-experienced mothers and control mothers randomly to leaf discs with predator odour and control. I found that parental effects were significant in the early developing stage, but not in later life stages. There was no significant inter-generational stress influence on adult lifespan and reproduction, and those of the offspring were strongly negatively affected by the stress they directly exposed. Additionally, parental effects in the earlier life stage were sex-specific, with daughters exhibiting delayed hatching when parents were exposed to predation risk, but not sons. These results suggested that predator-induced stress is strongly detrimental and decreases the fitness of prey by accelerating the onset of aging, but the effects are sex-specific. The influence of predator-stress spanned to the next generation and constrained early developmental stages by influencing the hatching age of offspring from risked-experienced parents. (Chapter 7).

My thesis highlights the pronounced influence of food regimen, social interaction with conspecifics and predator-induced stress on the pace of aging and reproductive output of an organism. It also demonstrates clear sex differences in longevity and responses to environmental variation.

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Chapter 1

General introduction

1.1 Introduction to aging

Aging is an inevitable and universal process that occurs to almost all living creatures. It is reflected in an age-dependent decline in physiological function, decrease in reproductive potential, increasing vulnerability to diseases, leading to increased mortality rates that ultimately lead to the end of life (Aunan *et al.* 2016; Fedarko 2011; López-Otín *et al.* 2013). Almost no organism can avoid aging because natural selection on genes expressed in later life is generally weak, allowing the accumulation of late-acting deleterious mutations (Medawar 1952). Additionally, genes that enhance reproduction in early life can lead to somatic deterioration by preferentially allocating the limited resource to reproduction (Bonduriansky *et al.* 2008).

The rates of aging vary across species, with the longevity of organisms ranging from thousands of years to a few days, and a few exceptions which appear to be immortal (Table 1.1). The ocean quahog *Arctica islandica* is well known for its extremely long lifespan (up to 507 years) while budding yeast, a widely used model organism for aging research, only survives for about 14 days (Mei & Brenner 2015). Hydra even seems to be non-aging because mortality rates remain extremely low and constant, and fertility does not decrease with advanced age (Schaible *et al.* 2015).

Although the longevity of species is genetically determined to some extent, it can be influenced within broad limits by many abiotic and biotic factors. For example, families with exceptional longevity have some genetic characteristics that confer them with advantages in survival over others. This was supported by evidence that siblings of centenarians showed a higher probability of surviving to extreme old age. For instance, a study with Americans in the 1900s showed that female and male siblings of centenarian were at least 8 and 17 times as likely to attain age 100, respectively (Perls *et al.* 2002).

TABLE 1. 1 Records of Maximum lifespan for organisms in Fungi, Animalia and Plantae kingdom, with classical model species in bold. All other species belong to Animals except that *Saccharomyces cerevisiae* (Fungi) and *Pinus longaeva* (Plantae). Data were obtained from AnAge database.

Common name	Species	Maximum lifespan(yrs)	References
Yeast	***Saccharomyces cerevisiae***	**0.04**	**Mei and Brenner (2015)**
Worm	***Caenorhabditis elegans***	**0.16**	**Zhao *et al.* (2017)**
Fruit fly	***Drosophila melanogaster***	**0.3**	**Jones *et al.* (2014)**
Norway rat	***Rattus norvegicus***	**3.8**	**Turturro *et al.* (1999)**
Mouse	***Mus musculus***	**4**	**Miller *et al.* (2002)**
Rat	*Rattus rattus*	4.2	Richard Weigl (2005)
Zebrafish	***Danio rerio***	**5.5**	**Gerhard *et al.* (2002)**
Honeybee	*Apis mellifera*	8	Haddad *et al.* (2007)
Gray wolf	*Canis lupus*	20.6	Richard Weigl (2005)
Domestic dog	*Canis familiaris*	24	J. Veronica Kiklevich, pers. comm.
Red junglefowl	*Gallus gallus*	30	Carey and Judge (2000)
Rhesus monkey	*Macaca mulatta*	40	Mattison *et al.* (2012)
Echidna	*Tachyglossus aculeatus*	49.5	Richard Weigl (2005)
Chimpanzee	*Pan troglodytes*	59.4	Richard Weigl (2005)
Gorilla	*Gorilla gorilla*	60.1	Columbus Zoo
Human	*Homo sapiens*	122.5	Allard (1998)
Turtle	*Terrapene carolina*	138	Nigrelli (1954)
Rockfish	*Sebastes aleutianus*	205	Cailliet *et al.* (2001)
Ocean quahog clam	*Arctica islandica*	507	Butler *et al.* (2013)
Pine	*Pinus longaeva*	5062	Schulman and Harlan
Hydra	Hydra	immortal?	Schaible *et al.* (2015)

Nonetheless, even if the heritability component of longevity was about 15% to 30%, a recent study indicated that when accounting for effects of assortative mating, longevity heritability amongst humans is well below 10% (Ruby *et al.* 2018). Therefore, understanding how the pattern of aging is modulated by environmental variations and the underline mechanism are the longstanding interests of researchers in the fields of biology and gerontology. Although

substantial progress has been made in the past few decades, we are just beginning to understand the science of aging.

1.2 The development of evolutionary theories of aging

During the last two centuries, with increasing interests in aging research (Cunnington 2015) and the rapid development of experimental techniques, more than three hundred theories of aging have been proposed to elucidate the exact mechanism of aging, and the number is still growing (Medvedev 1990; Kirkwood 2010). The majority of these theories focus on a particular aspect of aging such as programmed and error theories and some of them interact with one another in a very complicated way (Jin 2010). However, none of them provides an entirely comprehensive explanation to why and how animals age (Kirkwood 2005). From the evolutionary point of view, the differences in aging rates and longevity are driven by the interplay between natural selection and accumulated mutation. To date, four evolutionary theories of aging have been proposed, namely, i) programmed death theory of August Weismann, ii) mutation accumulation theory of Peter Medawar, iii) antagonistic pleiotropy hypothesis of George Williams, and iv) disposable soma theory of Kirkwood and Holliday. Each of these is discussed further below.

1.2.1 Programmed death theory of August Weismann

August Weismann, a great experimental biologist in the 19th century, was the first who attempted to explain the mechanism of aging from an evolutionary perspective (Weismann 1891). He suggested that organisms are programmed to death after a certain period and aging evolves as a consequence of natural selection. This theory proposed that aging is a way to eliminate the old and worn out individuals of populations, for the sake of freeing up resources and making space for younger individuals. The younger generation was assumed to be more evolved than the old organisms. Within this scenario, aging is seen to be an adaptive trait for the whole population, but not necessarily for each individual. The idea was probably

influenced by Alfred Russel Wallace, a contemporary of Darwin, who claimed that natural selection favours races that die immediately after they have successors (Travis 2004).

This theory was incompatible with "Darwin's survival of the fittest concept", which predicted that all organisms strived to live longer in order to reproduce more. Traits were therefore selected to assist survival while maintaining breeding. In general biologists at that time discounted the programmed death theory because of this obvious flaw. Animals in the wild rarely survived to the age when the symptom of aging is apparent because of high levels of extrinsic mortality resulted from disasters, natural enemies, starvation, diseases and extreme temperatures. For example, 90% of the free-living mice die within ten months, despite having an average lifespan of 24 months in the laboratory. Consequently, scarcely any organism living in the wild survived long enough to allow any evolutionary force to pose a longevity limit for them (Ljubuncic & Reznick 2009). Therefore, in natural population, there is no necessity to evolve an aging mechanism to wipe out the old individuals.

Weismann (1891) later proposed a biological mechanism for his theory of programmed death. He speculated there is a limited number of potential cell divisions that are determined in the embryonic period. The lifespan of different species is therefore dependent on the specific limitation of soma cell division of each species. This assumption was first challenged by Alexis Carrel using a tissue culture experiment with embryonic chicken heart in the early 20th century. He demonstrated that when supplied regularly with nutrient these cells can continue growth for over 20 years, which is much longer than the average lifespan of a chicken (Carrel 1912). However, later it was found that Carrel's experiment was non-replicable. In the middle of the 20th century, Hayflick proposed that differentiated cells can undergo only a limited number of divisions before dying, known as the Hayflick limit (Michael & Fossel 2004).

Weismann was also the first person to distinguish soma cells and germ cells (Weismann 1892). The germ cells can develop into new offspring through cell division, while the soma cells can divide and differentiate to specialized cells which contribute to the organism's functionality but cannot produce an offspring themselves (Weismann 1892). He suggested

that the germ cell is immortal and produces mortal soma cell that can sustain the growth and reproduction of organisms. For the unicellular organisms, with no germ/soma cell separation, they have negligible senescence and mortality. This idea has had a formative role in the development of the successive evolutionary theories of aging.

1.2.2 Mutation Accumulation Theory of Medawar

The mutation accumulation theory of aging proposed that aging is caused by random deleterious mutations inducing adverse aging characteristics (Medawar 1946; 1952). According to this theory, the force of natural selection decreases when animals begin to reproduce. In the earlier life stage (pre-reproduction), a deleterious gene can be removed by natural selection. In late life, with the power of natural selection fades with increasing of age, it allows the hazardous late-acting genes to persist and pass down to the next generation. This theory was supported by evidence from age-associated human diseases that occurred as a consequence of errors of the genetic code and increased with age. For instance, progeria, a progressive genetic disorder occurs in children, and cannot be transmitted to the next generation because progeria patients failed to survive to age of reproduction (Turker 1996). In contrast, Alzheimer's disease, usually onsets in over 65-years-old, couldn’t be removed as readily as the disease occurs in early life (Le Bourg 2001). Therefore, this theory predicts that the frequency of and susceptibility to genetic diseases should increase at older ages.

This theory serves as an alternative to the earlier programmed death theory, reflecting contrasting perspectives (Goldsmith 2011). If one would not accept the notion that aging is an evolved trait because few animals can survive to old age and got exposure to natural selection, he should not reject the idea that aging is caused by the accumulation of deleterious gene in late-life since natural selection could not eliminate these genes. It turned the disadvantages of the programmed death theory into its advantage and wasn’t in conflict with Darwin’s survival of the fittest concept.

This theory assumed that equilibrium gene frequency of deleterious mutations should increase with age owing to weaker selection strength against later-acting mutations. In light

of this, we would expect that the relationship between progeny lifespan and parental lifespan should not be linear. Instead, there would be an increase of slope for the dependency of progeny longevity on their parental lifespan. This prediction may also apply to other quantitative traits such as body height. The genealogical data on life expectancy in European royal and noble families verified this assumption (Gavrilova *et al.* 1998). The mathematical model for this population showed that for people survived to age thirty the regression slope between parents and daughters, and between parents and sons both had a steeper increase with age.

Although this theory offers an explanation for the heritability of human longevity, it does not seem to be a plausible hypothesis for aging because of empirical evidence that some species do not demonstrate age-related mortality increase (Jones *et al.* 2014). In contrast, as observed in the classical model organism fruit fly *Drosophila melanogaster* and other organisms, the age-specific mortality in early life did increase approximately exponentially. However, surprisingly, it generally leveled off and reached a plateau at an advanced age. This goes against Medawar's assumption that mortality inexorably climbs with age (Pletcher & Curtsinger 1998).

1.2.3 Antagonistic pleiotropy theory of George Williams

Similar to Medawar, Williams (1957) also considered that the strength of natural selection declines with age. He made two major assumptions, which are fundamental for his antagonistic pleiotropy theory of aging. First, he hypotheses the existence of pleiotropic genes that control not only one fitness trait, but multiple traits of an individual. Second, these genes show completely different influence on the fitness traits of an individual in different life stages. Natural selection favors genes that are beneficial in early life but detrimental in late life. Cellular senescence is a striking example supporting this assumption. For multicellular organisms such as humans and mice, senescent cells show a significant change in gene expression, which arrests growth in early life and fibroblast function in later life. It may be beneficial for young organisms in suppressing the development of cancer. However,

in aged organisms' fibroblasts can induce progression and hyperproliferation of preneoplastic epithelial cells, and accelerate tumorigenesis (Krtolica *et al.* 2001).

Antagonistic pleiotropy theory predicted that gene regulating developmental rate would also have a role in modulating the rate of aging and longevity. Specifically, organisms with rapid development would generally age faster, known as "live fast, die young." It was tested in different species, but the evidence was mixed. Some researchers argued that the developmental time, measured as age maturity, might coevolve with adult lifespan based on a significant positive relationship in empirical studies with Bivalve Molluscs *Arctica islandica* (Ridgway 2010). However, in some organisms such as the fruit fly *Drosophila melanogaster*, no relationship between longevity and rate of development was observed (Economos & Lints 1986).

Meanwhile, this theory also explained why there may be a substantial trade-off between reproduction and lifespan. The genes promoting early reproductive investment should increase the aging rate and reduce longevity or late-life survival. Thus, aging is a critical life-history strategy of the organism to optimize their overall fitness.

1.2.4 Disposable soma theory of Kirkwood and Holliday

The disposable soma theory proposed by Kirkwood and Holliday (1979), which is considered to be a derivative of Williams's theory of aging, also suggested a trade-off between early and late-life fitness (Hughes & Reynolds 2005). The trade-off is thought to have evolved because the pool of resources in most natural environments is restricted and has to be partitioned and allocated differentially to various life-history components (Van Noordwijk & Dejong 1986; Houle 1991; Partridge & Barton 1993; Kokko 1998). A cost of reproduction is accordingly inherent in both life-history theory and evolutionary theory of aging (Roff 1992; Stearns 1992; Rose 1994) and has been shown in a number of studies (reviewed in Reznick *et al.* 2000).

Each living organism has to acquire resources such as nutrients from the environment, processes them into the subunits for its own body, and then allocate this nutrient for different

kinds of functions such as survival, development, somatic maintenance, reproduction, and acquisition of more resources. How to allocate these resources for different kinds of functions is a primary question in life-history theory (Zera & Harsh-man 2001; Roff 2002; Boggs 2009). The two life-history parameters, longevity and reproduction are the most essential fitness components and are suggested to be associated with each other mechanistically and genetically. Theoretically, both these two fitness traits can be maximized when abundant resources are accessible to the organism. However, the life-history theory indicates that these two fitness parameters are negatively related to each other under most circumstances (Clutton-Brock *et al.* 1982; Reznick 1985; Steams 1989; 1992; Roff 1992; Rose *et al.* 1996). That is to say, there is a trade-off between lifespan and reproduction because of the competition between different fitness components for the insufficient resources available.

The underlying principle of trade-off can be interpreted by a two trait Y model (Houle 1991). The stem of Y represents the pool of resources organisms can acquire from the external environment. Generally, the resource pool is insufficient because of the amount present, impeded access or competition from other organisms. Functionally, the organism has to make a decision on the use of resource allocation to optimize its own fitness. When more resources are allocated for somatic maintenance, then fewer are available for reproduction. The trade-off not only occurs between different life-history traits, but also between life-history traits expressed at different periods in the life cycle. In lizards, for example, a common effect of current reproduction may be the reduction of internal reserves, which affect future reproduction either by reducing survivorship or by reducing the nutrient requirement for the future reproduction (Landwer 1994; Schwarzkopf 1994; Doughty & Shine 1997, 1998; Wilson & Booth 1998). Although the trade-off is a decision made by the organism and was regulated by the gene, external factors such as the environment and mating behavior are the trigger and primary cause.

In accordance with the prediction of antagonistic pleiotropy theory and disposable soma theory, the trade-off between early and late-life traits has been observed in artificial selection experiments. Fruit fly *Drosophila melanogaster* that postponed the onset of reproduction showed an increase in lifespan (Partridge *et al.* 1999). Moreover, the population selected for

longevity, showed a decline in early fecundity associated with lifespan extension (Partridge *et al.* 1999), confirming the cost of reproduction to longevity. Although life-history trade-offs were apparent in a number of studies with classic model organisms, it still reminds controversial when it comes to non-model organisms.

After the first report showing that limiting the food intake of rats can delay aging and prolong their lifespan (McCay *et al.* 1935), there has been an ever-increasing interest in exploring environmental factors influencing aging and anti-aging interventions extending lifespan (Walker 2005; Lee 2015). It has been subsequently shown that dietary restriction, irradiation, physical activity, hypergravity, and temperature stress (cold and heat shock) can also increase longevity and delay the onset of aging and avoid the aging-related diseases (Minois 2000; Rattan 2004). However, the social environment, interaction with the conspecifics (mating) and predators (stress), has thus far been neglected. In further research, it is worthwhile to investigate how interaction with conspecifics such as mating, and the predator-induced stress affect the aging and lifespan.

1.3 Food, sex, stress and aging

1.3.1 Dietary restriction and lifespan extension

Dietary restriction (DR) was initially defined as reducing 20-60% of the total calorie intake without severe malnutrition. It was first reported by McCay and Crowell (1935) that limiting calorie intake doubled the lifespan of rats confirming how the aging process can be delayed. During the past few decades, extensive research has confirmed the effect of dietary restriction on model organisms from unicellular yeasts, to worms, flies, rats and mice (Holloszy & Fontana 2007). Conceptually, nutrients now include micronutrients and feeding time restriction impacting on lifespan. Evidence showed that dietary restriction enhanced the fitness of organisms not only by life extension but also through decreasing aging rates, preventing age-related diseases and reducing the incidence of cancer (Varady & Hellerstein 2007; Singh *et al.* 2012; Colman *et al.* 2014; Caramoci *et al.* 2016). Increasing attention has been paid to modification of aging via diet across disciplines from, medical, molecular,

evolutionary, to ecological. Apart from genetic and pharmacological interventions, dietary restriction is extensively studied as a dietary intervention which can prolong median and maximum lifespan.

Many studies demonstrate that females tend to extend lifespan rather than reducing or eliminating reproductive output, which was interpreted as the trade-off between longevity and reproduction. Therefore, the evolutionary hypothesis was proposed to explain the effect of DR. According to the resource allocation theory, the effect of DR on life histories is highly conserved and is an evolved strategy to maintain survival when limited resources are available (Holliday 1989; Kirkwood & Shanley 2005; Speakman & Mitchell 2011). To increase the chance of survival during the famine, re-allocating limited accessible resource from reproduction to somatic maintenance is favored by natural selection (de Jong & van Noordwijk 1992; Kirkwood & Rose 1991; Grandison *et al.* 2009; Partridge *et al.* 2005; O'Brien *et al.* 2008). Once plentiful nutrients are acquired, the organism would resume reproduction by reversing nutrient priorities.

In most previous studies, only the fitness of female was determined in the DR research because both the reproductive output and longevity of females is much easier to measure. On the contrary, few protocols are available and reliable to estimate the reproductive success of the male (Zajitschek *et al.* 2013). In addition, males are frequently discarded after mating to simplify experiments and reducing labour work (Zajitschek *et al.* 2013). However, there is great importance of determining the lifespan extension effect of DR on both sexes because the investment in reproduction is different between them regarding mating efforts and nutrients required for producing gametes. Specifically, males always expend energy in courtship (see Simmons 2001), spermatogenesis and ejaculate production (Dewsbury 1982; Wedell *et al.* 2002). They also produce small but large numbers of gametes, while the females pay the cost of experiencing sexual harassment, producing a large but small number of gametes and parental care (e.g., Roff 1992; Kotiaho & Simmons 2003). This emphasizes the importance of taking both sexes and their differences in reproductive investment into account when assaying the resource re-allocation hypothesis.

Recent research on micronutrient restriction calls into question whether trade-offs between longevity and reproduction are necessary for lifespan extension (Zajitschek *et al.* 2013; Adler *et al.* 2013). Methionine is one kind of essential amino acid which cannot be synthesized by humans and animals but is needed in a variety of biochemical pathways. Previous studies on methionine restriction (Troen *et al.* 2007; Grandison *et al.* 2009; Dick *et al.* 2011; Kabil *et al.* 2011) have found that when adding methionine back to the restricted diet of female *Drosophila melanogaster*, the fruit fly can maintain lifespan as long as its DR counterparts without a reduction in fecundity. This suggests the possibility of experiencing long lifespan without low reproductive output. Other lifespan extension interventions such as single-gene alteration can also prolong lifespan without negatively affecting reproduction (Fontana *et al.* 2010; Magwire *et al.* 2010). However, the fact that high fecundity and long lifespan can co-occur is inconsistent with the idea that any aspect of reproduction directly inflicts damage on the soma to shorten lifespan (O'Brien *et al.* 2008).

Although the effects of DR are extensively studied in several species, the majority of the research failed to investigate sex differences in response to DR. However, existing evidence suggested that females and males respond differently to dietary restriction. For example, when *Drosophila melanogaster* was fed at different levels of dietary restriction, the lifespan of females was prolonged. Surprisingly, the female lifespan was extended 40% at the food concentration of 60% standard laboratory diet while males experienced only 30% longevity extension at the food level of 30% (Magwere *et al.* 2004). This suggested that males are more sensitive to starvation and females experienced a much more pronounced life-extension effect from DR.

1.3.2 Social environment and adult lifespan

Besides dietary restriction, reproduction is another critical factor influencing the resource allocation strategy and altering the longevity of organisms. Reproduction is a cost for both males and females (e.g., Fowler & Partridge 1989; Tatar *et al.* 1993; Westendorp & Kirkwood 1998; Messina & Slade 1999). Overall, the cost can be attributed to the energy

expenditure of courtship and copulation, nutrients required for producing eggs and ejaculate, and increasing the risk of predation and vulnerability to diseases.

Transferring sperms to females by copulation is the primary function of mating, to fertilize eggs. Accompanied by the sperms, many seminal fluid proteins are also transferred to females. These seminal fluid proteins always trigger females to produce eggs, can enhance egg production, reduce receptivity to remating, alter immune responses and feeding behavior, facilitate sperm storage and use, and affect longevity (Avila *et al.* 2011). For females, egg-producing is one of the most important energies and nutrient costs. A large number of studies reported a decrease in lifespan associated with investment in reproduction. The simple explanation is that a large amount of lipids, proteins and carbohydrates with abundant energy from the female were consumed to form oocytes. This is supported by some empirical studies that virgin females without producing offspring lived much longer than the mated females which invest a lot of energy and nutrients in egg production in many insects (Chen *et al.* 2013).

In most insect species single insemination can supply enough sperm to fertilize all the eggs of one female throughout their life (Walker 1980; Thornhill & Alcock 1983; Drummond 1984; Halliday & Arnold 1987). However, multiple mating occurs frequently (Thornhill & Alcock 1983; Eberhard 1996). This possibly results from the fact that the reproductive interests of males are positively correlated with the lifetime mating rates. In polyandrous species, males maximize their fitness by copulation with many females in order to father more offspring and pass their genes to the next generation while reducing the chances of male rivals to mating. Females generally have many opportunities to mate but have limited resources for producing eggs. Theoretically, they can benefit from multiple-matings by increasing gene diversity of offspring and gaining from cryptic sperm selection. However, males appear naturally selected to trigger the immediate investment in the reproduction of the females, which always comes at the cost of few resources for somatic maintenance and increased mortality, resulting in a decreased lifespan of female. There is a conflict between the two sexes in mating rates.

Although multiple mating is beneficial in terms of increasing offspring genetic diversity, some studies showed that these benefits are costly for both males and females (Thornhill & Alcock 1983; Rowe 1994; Chapman *et al.* 1995; Crudgington & Siva-Jothy 2000), as well as the offspring. For instance, in males, both the longevity and the reproductive investment decreased with the increasing of mating frequency in stoneflies *Megarcys signata* (Plecoptera: Perlodidae), Mediterranean fruit flies, *Ceratitis capitata* and almond moth *Cadra cautella* (e.g., Taylor *et al.* 1998; Papadopoulos *et al.* 2010; McNamara *et al.* 2012). Similarly, the lifespan of a multi-mated female may be negatively affected by the mating rates (Wigby & Chapman 2005; Ji *et al.* 2007; Avila *et al.* 2011). It is obvious that the reproductive output of females increases with the frequency of mating, leading to a reduction in lifespan. Increased mating frequency can also impact the next generation, for example lowering hatching rates in *Leptinotarsa decemlineata* beetles (Orsetti & Rutowski 2003).

In summary, it is well documented that the mating status and mating frequency can have a profound effect on the lifespan of the organism. Generally, un-inseminated females not producing eggs outlive inseminated individuals, and the lifespan of females is negatively correlated to the number of matings. However, most of these findings are shown in monandrous and polyandrous insects which have sexual reproduction. Little is known how the mating status and mating frequency influence the reproduction cost in more complex groups such as parthenogenetic and polygamous insects, for instance, spider mites, which can produce unfertilized eggs without mating and give birth to both unfertilized eggs and fertilized eggs when mated.

1.3.3 Predator-induced stress

Every organism is exposed to various kinds of stress during its life. Although the source of stress varies from one individual to another, an organism's reaction to it, behaviourally or physiologically, is always associated with life history changes. Predator-induced stress has been most studied (Clinchy *et al.* 2013). For the predator-prey system, *Tetranychus urticae* and *Phytoseiulus persimilis*, being used in this research, it has been reported that spider mites can perceive predation risk through the chemical trace, metabolic waste and footprints left by

predators on the leaves which leads to behavioural changes to reduce risk. The spider mites actively avoid plants with its specialist predator, *P. persimilis* but showed no discrimination against plants with the generalist predator *Neoseiulus californicus* (Pallini *et al.*1999). In addition, in short-term predation risk lasting for only one or a few days, higher dispersal rate, lower daily fecundity, reduced growth in adult body size and delayed deposition of first egg were observed in a previous study (Škaloudová *et al.* 2007). More interestingly, it is documented that predator-experienced spider mites behaved much bolder than their naïve counterparts and produced a similar number of eggs on both control and predator-visited leaf discs. This indicates that the prey may alter their strategies in response to both temporary, short-term risk and long-term risk. Thus, it is important to examine the impact of chronic predation risk on life-history traits.

Previous research indicated that predation stress led to a decrease in food intake mainly through reduced foraging rates (Lima 1998; Brown & Kotler 2004; Caro 2005). However, unlike simple diet restriction, the predator-induced stress is also accompanied by physiologically responses and changes in the immune system (e.g., Hawlena & Schmitz 2010; Archard *et al.* 2012; Stoks *et al.* 2006). There is evidence that predation stress alters development and decreases reproduction (e.g., Orizaola *et al.* 2013; Zanette *et al.* 2011). Additionally, biomedical studies suggested that predation pressure experienced by animals was comparable to the psychological stress encountered by the human, because predators or predator cues have quantifiable effects on the 'neural circuitry of fear' (Rosen & Schulkin 1998, 2004) and can induce sustained psychological stress in less cognitively sophisticated species. Although post-traumatic stress disorder showed that predator-induced stress resulted in changes in behavior and neurochemistry, gene expression, and glucocorticoid level in mammals (Nanda *et al.* 2008; Creel *et al.* 2009), little attention has been paid to how the lifespan of organisms are impacted by physiological change induced by chronic predation stress.

1.4 *Tetranychus urticae*: A potential model in aging research?

1.4.1 Why we still need to develop a new model for studying aging

Vertebrates such as primates, mice and rats are biologically closer to human and therefore have a pivotal role in aging studies. These species, however, shared several disadvantages which limited their utilization in aging research. They generally have a relatively large size and longer lifespan, requiring large space and much longer experimental periods to test different treatments. The high expenditure of maintaining populations pose a limitation of sample size and statistical power. The escalating ethical restriction also limits treatment options in these organisms (Lyn *et al.* 2011). Despite the difference in morphology with a vertebrate, invertebrates share similar metabolic pathways and cell physiology (Pitt & Kaeberlein 2015). Furthermore, the mechanism of aging is conserved because the sensory and signaling pathways that associated with survival, reproduction, and stress resistance in reaction to environmental cues are assumed to be highly conserved (Pitt & Kaeberlein 2015).

Given the weakness of vertebrates and the conserved aging pathway, invertebrates may be better models for aging research. The classic invertebrate models are the fruit fly *Drosophila melanogaster* and nematode *Caenorhabditis elegans* (Austad 2009). They have the advantage in aging research in that they are easy to mass reared in the laboratory and have a short life cycle (10-12 days for fruit fly and 3-4 days for nematode). Both of them have rapidly expanded our knowledge about the mechanisms and pathways involved in aging. Recently, however, some concerns have been raised because of the awareness of taxonomically biased research with some organisms and the potential value of non-model species in understanding aging (Jones *et al.* 2014).

Expanding aging-related research across a range of organisms would broaden our views of the diversity of aging across the tree of life and highlight generalisations in the effects of aging on lifetime reproduction. For example, social insects have been found to have a special life history compared with classic models (Keller & Jemielity 2006). One of the fundamental evolutionary theory of aging assumed that there is a trade-off between longevity and

reproductive efforts, and this theory has been verified in many vertebrates and invertebrates. Surprisingly, the mated ant showed a 50% longer lifespan than the virgins regardless of whether the male is fertile or sterilized (Schrempf *et al.* 2005). Also, mating increased the lifetime reproduction of the mated queen, indicating the absence of trade-off in this species. Additionally, species with indeterminate growth such as fish, reptiles and some insects may exhibit patterns of senescence that are distinctly different from those of species with determinant growth (Vaupel *et al.* 2004; Finch 1994; Baudisch 2008; Charnov 2001). Therefore, a full understanding of aging will require works on a diversity of organisms.

1.4.2 Common characters of *Tetranychus urticae* as the classical model organism

Tetranychus urticae is a major pest in agriculture and feeds on a large variety of host plants. There are several reasons why we attempt to develop this species as a novel model organism for aging studies. First and foremost, this species belongs to subphylum Chelicerata, one of the four major lineages of the phylum Arthropoda (Table 1.2). Up to now, the most popular invertebrates employed in aging and longevity research is fruit fly *Drosophila melanogaster*, in the subphylum Hexapoda. A number of insects in the same subphylum such as silkworm moth *Bombyx mor*, grasshopper *Romalea microptera*, honeybee *Apis mellifera*, ants *Cardiocondyla obscurior* has drawn the attention of gerontologists and contributed greatly to our knowledge on aging (Lee *et al.* 2015). However, little attention has been paid to organisms in other subphylum, which have tremendous differences in genetics and life history traits with fruit fly and other species belonging to Hexapoda. Consequently, new model organisms in other taxonomic groups, for example phylum might be prospective candidate expanding our knowledge about aging.

TABLE 1. 2 The taxonomic information about *Tetranychus urticae* and its generalist predator *Phytoseiulus persimilis*.

Tetranychus urticae		***Phytoseiulus persimilis***	
Kingdom:	Animalia	Kingdom:	Animalia
Phylum:	Arthropoda	Phylum:	Arthropoda
Subphylum:	Chelicerata	Subphylum:	Chelicerata
Class:	Arachnida	Class:	Arachnida
Subclass:	Acari	Subclass:	Acari
Order:	Trombidiformes	Order:	Mesostigmata
Family:	Tetranychidae	Family:	Phytoseiidae
Genus:	Tetranychus	Genus	Phytoseiulus

So far, lifespan extension effect of DR has been assayed across diverse taxa (see Nakagawa *et al.* 2012) including model organism yeast (*Saccharomyces cerevisiae*), nematode worm (*Caenorhabditis elegans*), fruit fly (*Drosophila melanogaster*). They were selected as model organisms partially because they have the common characteristics of fast development, are easy and cheap to rear in the laboratory, and have a low cost for population maintenance. Considering these merits, *T. urticae* can also become an excellent model organism. At an optimal temperature of 30°C, it develops from egg to adult within nine days. With *r*-selected reproductive strategy, the population always increase rapidly in normal laboratory conditions.

1.4.3 Abundant literature about its biology

In addition to the common characteristics mentioned above, *Tetranychus urticae* also has several advantages over others. Firstly, it is a tiny species (about 0.5 mm long on average) so that rearing them is not space-consuming. One cabinet is roomy for hundreds of spider mites, a considerable sampling size for experiments in ecology and biology. Secondly, as a major worldwide pest in agriculture, *T. urticae* has been extensively studied in areas of ecology, pest control and population biology. There are 10900 entries on spider mite when searching use terms "*T. urticae"* and 1600 entries using the terms "life table" in google scholar (in 2018-12-4). Specifically, we have numerous data about the biology of the spider mite on

various host plants and at different temperatures (e.g. Sabelis 1991). In addition, different from other model systems, spider mite is haplodiploid (Figure 1.1). The female can produce male offspring without fertilization but give birth to both female and male after being fertilised (Macke *et al.* 2010), making it a particularly interesting case for investigating how reproduction and longevity are associated in the process of aging. Furthermore, sexual size dimorphism (SSD) is quite apparent in spider mites: the female and male can be distinguished at the final moulting stage, and females are approximately double the size of the male at the adult stage. Finally, the prey-predator system composed of *Tetranychus urticae* and its generalist predator *Phytoseiulus persimilis* is a well-established system for researching predator-induced risk (Grostal & Dicke 1999), which makes it much easier to study how spider mites change their fitness in response to life-threatening psychological stress.

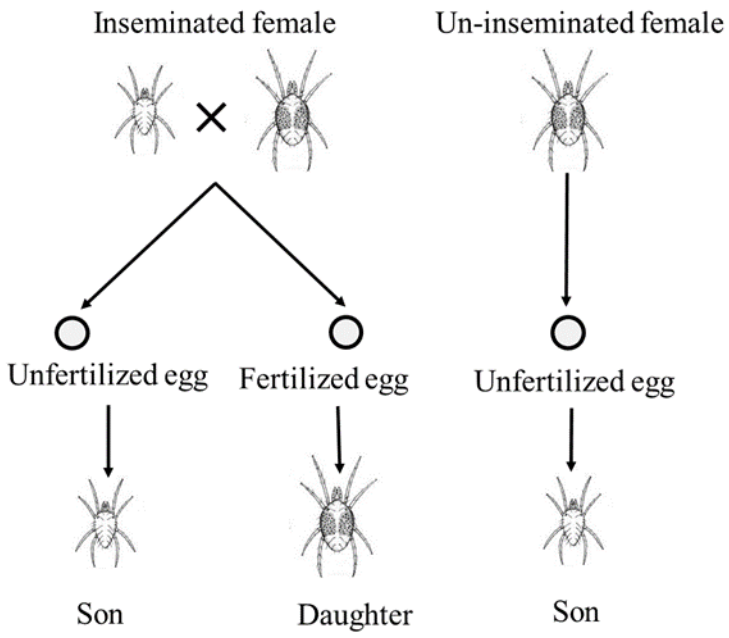

FIGURE 1. 1 The haplodiploid reproductive system of spider mite *Tetranychus urticae.*

Another advantage of spider mite is that its genome has already been sequenced and annotated (Grbić *et al.* 2011), making it a promising model organism for the genetic research on aging. Its small genome distributed on three equal-sized holocentric chromosomes is 75 Mbp (Grbic *et al.* 2007), only 60% of the *Drosophila* genome and 80% of *C. elegans* genome. In addition, the well-established RNAi-mediated gene silencing technology (Khila

& Grbić 2007) by injection of dsRNA, make it possible to generate loss-of-function phenotypes and investigate the function of special genes.

1.5 Biology of spider mites

1.5.1 Life cycle and mating behavior of spider mites

Life history of spider mites

The life cycle of spider mites comprises egg, larva, protonymph, deutonymph, and adult stage (Crooker 1985). Post egg, each of the three immature stages consists of three consecutive phrases including active feeding period, an immobile period with silvery appearance and moulting period. At around 25°C, a whole life cycle (egg to adult) usually takes about ten days (Figure 1.2).

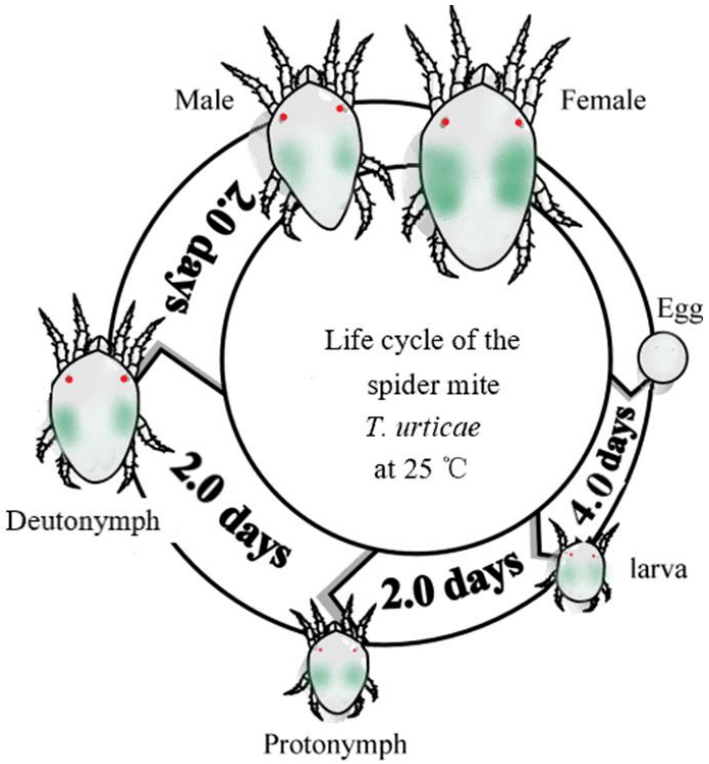

FIGURE 1. 2 The life cycle of two-spotted spider mite *Tetranychus urticae* from egg to adult at 25℃.

Egg stage

The newly produced eggs are translucent, spherical, about 0.13 mm in diameter. It becomes opaque with maturity and finally turns pale yellow. Prior to hatching, the red eyespots of the embryo become visible. At 25°C, egg to hatching usually takes about four days.

Larval stage

The newly incubated larva has three pairs of legs and is just a little bigger (0.15 mm in length) than an egg. It is initially translucent but after feeding turns from pale green to straw color, and the two characteristic spots begin to appear on its dorsum.

Protonymphal stage

After approximately two days the larva develops into protonymph with four pairs of legs, as do all the succeeding stages. The two spots become more pronounced, as the green color becomes darker.

Deutonymphal stage

It usually takes about two days for the protonymph to moult into a deutonymph. In the late deutonymphal stage, the male and female can be distinguished by their body size and the shape of their abdomen. The female is generally much bigger (0.5 mm) than male (0.30 mm) in length with oval rather than pointed abdomens.

Adult stage

The male emerges earlier than the female with a smaller body size and always guards the quiescent deutonymph females to ensure paternity. Different from males, the females show indeterminate growth after sexual maturation and ceases growth at 6-day-old. The female begins to deposit eggs one day after adult emergence.

Spider mites have limited visual capacity. Their eyes can receive the light of wavelength ranging from 350 to 600 nm but are apparently not capable of resolving images (McEnroe & Dronka 1966, 1969; McEnroe 1969). The setae scatted across their whole body serves as sensory organs for touching and smell. Chemical cues are one of the most important ways for them to obtain information from their surroundings. Females attract males mostly through

chemical trails and volatile compounds (Penman & Cone 1974), but sometimes by tactic stimuli such as webs.

Mating behavior of spider mite

Female and male spider mites have different optimal mating regimes to maximize their reproductive potential. For female spider mites, their reproductive success is independent of mating frequency, as sperms received from the earlier adult stage can be stored for fertilization in its later life, and one successful mating is sufficient for their lifelong fertilization. However, the reproductive fitness of the male increases as a function of the number of females they can inseminate during their life. The sexual selection favors males which have characters enabling them to achieve more frequent successful mating (Potter *et al.*1976a). Therefore, male spider mites have evolved with characters and mating strategies to compete against rivals.

Male spider mites employ precopulatory mate guarding strategy to gain an advantage over other competitors. They guard females in the last stage (quiescent deutonymph) before adult emergence in order that they can mate with the female immediately after ecdysis (shedding of the old exoskeleton). This behavior has evolved in many organisms in which females have limited sexual receptivity or show first-male sperm priority (Oku 2014). Females attract males mainly through chemical trails and volatile compounds (Penman & Cone 1974). By releasing sex pheromone, the deutonymph female stimulates the males around to initiate mating-searching. A recent paper by Oku reported that the female deutonymphs change the volatile profile to attract more males, probably for the sake of inducing male competition (Oku *et al.* 2015). In addition, the female deutonymph web also plays a critical role in their reproductive biology (Penman & Cone 1974). It is not only an important component for initial male contact but also a major factor for increasing male attraction to the female. Furthermore, the web also facilitates kin recognition and assists the male to avoid mating with familiar females thereby limiting inbreeding depression (Yoshioka & Yano 2014).

The male also shortens guarding time for each female by evaluating the age of the deutonymph female. They do not show discrimination between quiescent deutonymph

females for up to 12 hours. However, when the time lag is more than 23 hours, males always prefers to guard the females about to become receptive rather than the younger ones in order to minimise guarding time for individual females (Oku & Saito 2014). In addition, deutonymph females are sensitive to the cues from males before moulting. When guarded by a conspecific male, the developmental duration of quiescent deutonymph was shortened by 3-5 % (Oku 2015).

There is also fighting and aggressive behavior between males, particularly when there are a high proportion of males in a population (Potter *et al.* 1976a). When there are intruders, the guarding males raise and spread their first pair of legs in order to strike their rival, extending their stylets to puncture the rival's integument, and attach silk droplet to their rival (Potter *et al.* 1976 b). Sometimes the guarding male (about 30%) is tolerant of the competitor and allows co-guarding (Sato *et al.* 2016). Male size appears decisive for mating success and successful males are always larger than combat losers (Potter *et al.* 1976a). However, male fighting is costly, with fighters having poorer survival than the sneakers that do not involve in mating competition (Sato *et al.* 2016).

Although the females often passively engaged in mating immediately after adult emergence, they choose mates by inducing male competition early in their immobile deutonymph stage. A female guarded by a male is more attractive to other males than solitary females (Oku 2009). Oku *et al.* (2015) also confirmed that female deutonymphs change the volatile profile to attract more males. Once the male competition is triggered, the winner always gets the opportunity of mating with the female.

The male spider mite also engages in post-copulatory mate guarding to prevent other males from accessing their partner. Experimental studies indicate that females copulating for 40 seconds produced the maximum number of daughters (Satoh *et al.* 2001), even though the average copulation duration for spider mite is around 190 seconds (Ozawa & Takafuji 1987), far more than the minimum duration for success insemination. Extended copulation duration may act as a tactic to assure paternity because the effectiveness of second mating decreased linearly with the duration of first mating (Potter & Wrensch 1978).

Generally, the sex ratio of spider mite populations is female-biased and round 3:1 (Laing 1969; Overmeer 1972). Once females have mated they tend to disperse to a new colony, whereas males invariably stay in the old colony, resulting in a decline of the sex ratio over time in most colonies. The remained males thus compete with young males for mating opportunities, making mating competition more and more intense. Males characteristically favour virgin females over previously mated females (Oku 2010). The assumption is that mating with a mated female could not increase male reproductive success. In fact, when the virgin female was absent, 60% of males attempted to copulate with mated females in one hour (Oku 2010), which was considered sexual harassment. Possibly adult females disperse to avoid sexual harassment and to find new food resources (Kondo & Takafuji 1985; Oku *et al.* 2002; Oku 2010).

1.5.2 Sex dimorphism of life-history traits and their response to environmental factors

Introduction

Spider mites belong to the order of mites (Acari), the suborder of Prostigmata, the family of Tetranychidae. They are among the smallest arthropods ranging from 200µm to 900µm. Currently, there are 1275 phytophagous species reported, among them about one hundred being considered as a pest (Brandenburg & Kennedy 1987; Xie *et al.* 2006; Migeon & Dorkeld 2015). Ten are major pests, posing a significant threat to agriculture and forestry. The most well-known and widespread is the ubiquitous two-spotted spider mite, *Tetranychus urticae* Koch (Migeon & Dorkeld 2015). It can infest as many as 1100 plant species across 140 families including species known to produce toxic compounds (Grbic *et al.* 2011; Bensoussan *et al.* 2016).

Spider mites prefer to live on the underside the leaves. As a sucking pest, they damage the plants by penetrating epidermal cells and ingesting cell contents, resulting in pale chlorotic spots (Bensoussan *et al.* 2016). The photosynthetic ability of the leaves thereby is affected owing to reduced functional leaf area. Infested leaves present a burnt appearance, and finally

wither and dry off. As a result, the yield and quality of crops are severely affected (Meck *et al.* 2013; Van Leeuwen *et al.* 2015). In addition, the mites transmit pathogens and viruses between plants (Beavers & Reed 1972).

The biological characteristics of spider mite such as short developmental period, relatively high fecundity as well as haplodiploid sex determination, facilitate rapid evolution of pesticide resistance (Van Leeuwen *et al.* 2010; Ay & Yorulmaz 2010; Attia *et al.* 2013). To minimize crop damage from mites, chemical and biological control have been developed (Attia *et al.* 2013). Although releasing predators has proved successful in some agricultural systems, pesticides and acaricides remain widely used around the world. The spider mite is well known for its ability to develop resistance rapidly (Rauch & Nauen 2002) and *T. urticae* is reported to have the highest incidence of pesticide resistance among arthropods (Van Leeuwen *et al.* 2010). In order to establish an effective pest management strategy, numerous experimental studies have been conducted to understand the life-history characteristics of spider mites under different environmental factors, especially temperature and host plants. To date, a large number of research papers have been published about the effect of these factors on the development and fecundity of spider mites, with a few books and literature reviews on these topics (eg., Boudreaux 1963; McMurtry *et al.* 1970; Van de Vrie *et al.* 1972; Sabelis 1985; Sabelis 1991; Zhang 2003; Navajas *et al.* 2013).

In the past decade, some researchers proposed that spider mites, particularly the two-spotted spider mite *Tetranychus urticae* is a promising candidate for research including experimental evolution and plant-pest interaction (Belliure *et al.* 2010; Cazaux *et al.* 2014). Additionally, it is also a potential reference organism for Chelicerates, which is the second largest group of arthropods and of immense importance for fundamental and applied science (Grbic *et al.* 2007). These studies all highlight the critical utility of spider mites in scientific research, and their value in advancing our knowledge of how life history factors interact with environmental and social factors.

Although numerous studies have been devoted to exploring the effect of external factors on the spider mites, few researchers have attempted to provide a systematic review on the life

history traits of spider mites. The exception is Sabelis (1991) who derived life-history traits under standardized conditions to show interspecific variation. He also demonstrated that the intrinsic rate of population increases covaried with other life-history parameters such as faster development, a higher rate of reproduction and higher lifetime fecundity. His work has greatly advanced our understanding of the life history evolution of spider mites. However, Sabelis (1991) is primarily concerned with female reproductive parameters, including oviposition, fecundity and population increase, with life-history traits of males being ignored.

The fact that only one successful mating, unless interrupted, is sufficient for fertilization of all eggs produced by a female has resulted in the role of males being overlooked or down-played in many studies. Nonetheless, with the growing awareness of the contribution of males to the population and the promotion of age-stage, two-sex life table techniques in analyzing life-history parameter of insects and mites (Chi 1988), has prompted more investigations of both males and females. In this review, I focus on both males and females, specifically the sex dimorphism in development and longevity. Since Sabelis (1991) has already presented a comprehensive review of the reproduction parameters of spider mites, I have not undertaken a systematic review here.

The main purpose of this review is to elucidate the effect of temperature and host plant type on the life history traits of different species of spider mites. In this study, I selected research papers from the extensive literature on life-history traits of spider mites with data for both male and female being reported. First, I determined the sex difference in the developmental period and longevity between females and males across species. Second, I determined how two important factors, temperature and host plants influencing the magnitude of sexual dimorphism. With this review, I aimed to provide general information about some life-history traits of spider mites, facilitating their future studies when spider mites are utilized as candidate species. Additionally, I seek to enhance the understanding of how sex differences vary with the external environment.

Data selection and analysis

I compiled literature about spider mites by searching two Google Scholar and ISI Web of Science, with keywords that included spider mite, Tetranychidae, and species name paired with development, longevity, life table, two-sex life table. From the year 1965 to 2018, there are 160 papers published investigating the performance of spider mites, the majority exploring the impact of temperature and host plants on the development and longevity of spider mites.

Papers were assessed according to the following criteria: First, the study should have developmental or longevity data for both males and females reared under the same condition. Second, the study should have detailed information about the species name, host plant and rearing temperature. Third, all these data should be available in the paper or in the supplementary file: mean of development or mean of longevity, sample size, standard dividend or standard error for each parameter. To clarify, throughout this text, the parameter development refers to the whole developmental period from egg to adult. I only included longevity data for mated spider mites. Overall I found 42 papers on 26 spider mite species, while 33 papers included longevity measurements for 16 species.

Based on my primary assumptions, I predicted that three moderators, species, temperature and hostplant might influence the direction and magnitude of sex difference in development and longevity. Therefore, I firstly categorized our data according to species to clarify whether there is species-specific sexual dimorphism within this family.

Second, the temperature has an important influence on the development of insects and spider mites. Temperature required for development and their tolerance to extreme temperature differs significantly between species (Bowler and Terblanche, 2008). In ectotherms, the developmental rate varies non-linearly with temperature with both low- and high-temperature thresholds (Drost *et al.*, 1998; Liu and Tsai, 2000). In the papers used the temperature ranged from 12.5°C to 37.5°C for development, and from 15°C to 37 °C for longevity. To simplify our meta-analysis, I classified them into four temperature groups, I below or equal to 22.5°C, II 23°C to 27.5°C, III higher than 27.5°C. There were eight studies with developmental data and four studies with longevity data not being conducted at a

constant temperature, but under prevailing laboratory condition with fluctuating temperature. These studies were put into the group of fluctuating temperatures (IV).

Thirdly, I distinguished studies according to the host plants spider mites fed on, grouping them into two categories, trees and herbs. These major plant groups have different leaf characteristics and Sabelis (1985) reported that the instinct rate of increase of spider mite feeding on herbs was significantly higher than that on trees. So, I predicted that the type of host plants might also affect the development and longevity, and therefore potentially the level of sex difference.

The meta-analysis was conducted using R (version 3.4.4) with packages "meta" (Schwarzer & Schwarzer, 2018) and "metafor" (Viechtbauer, 2010). The effect size was calculated with the sample size weighted. I ran an initial analysis with all data to check whether the overall effect sizes differ from zero for the two parameters I examined, development and longevity, respectively. The effect size was calculated by weighting the mean difference between male and female in development and longevity against sample size. Then I assayed how the effect size varies with three moderators, i. species, ii. host plant and iii. temperature. The difference between the effect size of host plant was checked using meta-regression with host plant as the main factor and study ID as a random factor. To further explore the differences of the four temperature levels on development and longevity, I first compared each temperature level with the other three levels to obtain *P*-values. Since there are more than two subgroups for this factor, I corrected the obtained *P*-values with the Bonferroni method. I tested for publication bias by inspecting funnel plots.

Results

Sex difference in development across species

There are two subfamilies Bryobinae Berlese and Tetranychinae Berlese in the family Tetranychidae. Although initially, I attempted to include both subfamilies in our meta-analysis to assay how sex difference varies within this family, very few publications included the family Bryobine and it was therefore excluded. Among the 42 studies reporting male and

female development, the majority of the data belongs to the tribe Tetranychini. There are only two studies, one by Imani and Shishehbor (2009) using *Eutetranychus orientalis* and another by Saito and Ueno (1979) reported the development of *Aponychus corpuzae,* belonging to Eurytetranychini. Among the 120 comparisons on development, 71.67% (86 out of 120) are about the major pest *Tetranychus urticae* and 12% of the data is on *Tetranychus turkestani.* Data on other species is relatively few.

Overall, the sex difference in development was significant when all species were combined, with females showing much longer developmental period than the males (SMD=0.6631 [0.5487 to 0.7775], Z=11.36, P< 0.0001; Figure 1.3). But there is great variation in sexual dimorphism across species (Q=325.27, df=25, P< 0.0001; Figure 1.3). One species *Schizotetranychus brevisetosus* showed a significant negative effect size of -0.8132 with a quite wide 95% CI [-2.0442 to 0.4178] (Figure 1.3). Three species *Eutetranychus orientalis*, *Oligonychus litchii* and *Oligonychus mangiferus* showed standard mean difference of 0.0387-0.0712 and -0.0103, respectively, or close to zero (Figure 1.3). In contrast, for species in genera *Tetranychus*, the sex difference in development was consistent and robust, with standard mean difference and confidence interval always greater than zero. Specifically, for *Tetranychus urticae* with the most data, the effects size was 0.6345 with a narrow 95%CI [0.4435 to 0.8255].

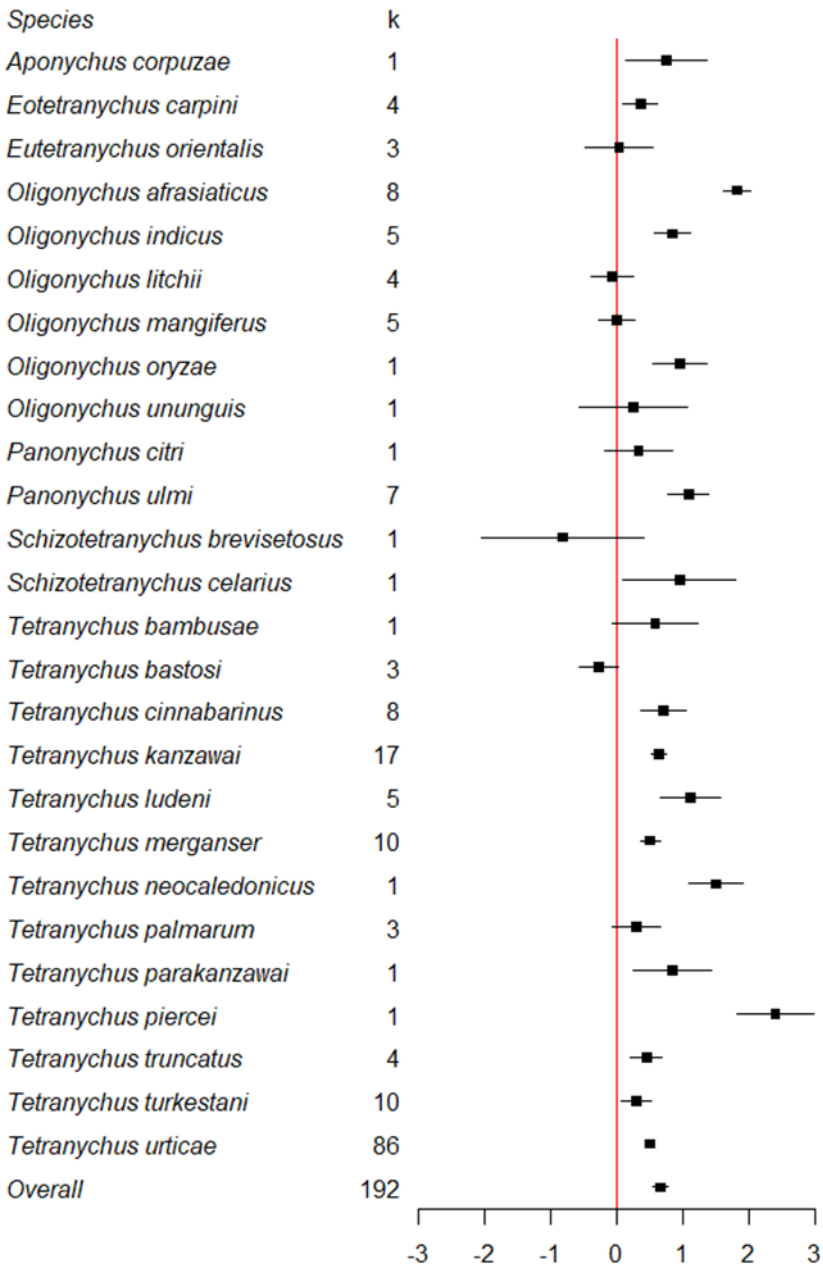

FIGURE 1. 3 The effect size standard mean difference and its 95% CI between female and male development of each species.

Sex difference in longevity across species

There are relatively fewer (39%) papers on longevity in this meta-analysis. All are in the Tetranychini tribe, except one species *Eutetranychus orientalis* belonging to Eurytetranychini tribe. Although the overall effect size showed that the female had a higher longevity than the

male (SMD=0.6043, 95%CI= [0.4054; 0.8031], *Z*= 5.96, *P*< 0.0001, Figure 1.4), but the sex difference in longevity was not consistent across species (*Q*=375.39, *df*=15, *P*< 0.0001). For seven species, their 95% CI covered the value zero, indicating the sexual differences in longevity were not significant. There are two species *Oligonychus mangiferus* and *Tetranychus palmarum* indicating that males tend to live longer than the females (i.e. negative 95% CI. However, for species in the genus *Tetranychus*, with larger sample size, the females showed significantly greater longevity the males, for example, *Tetranychus ludeni* (SMD= 1.4691, N=8), *Tetranychus turkestani* (SMD= 0.4373, N=10) and *Tetranychus urticae* (SMD= 0.8658, N=58).

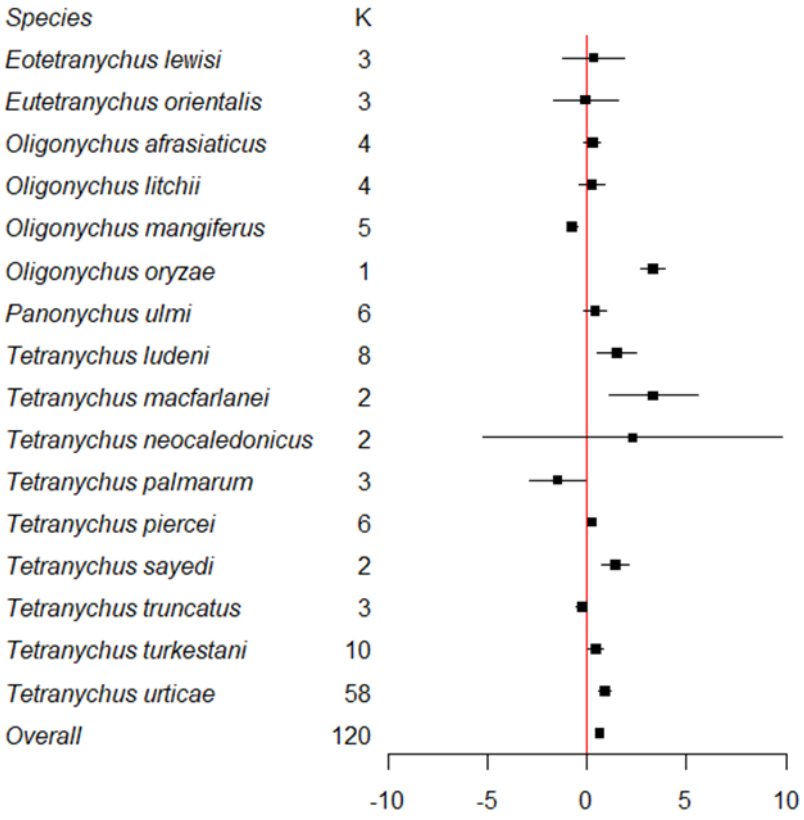

FIGURE 1. 4 The effect size, standard mean difference and its 95% CI between female and male longevity of each species.

Effects of temperature and host plant on sex differences in development rate

Among the four levels of temperatures, below 22.5°C, 22.5°C ~ 27.5°C, higher than 27.5°C and fluctuating temperature, the sample size K was the largest for temperature ranged from 22.5°C to 27.5°C (96 out of 192), but even for the other three treatments (Figure 1.5). When the spider mites were reared at a constant temperature, the difference between female and male in development increased significantly from 0.2803 to 0.6496 when temperature increases from below 22.5 to 22.5~27.5 (Z=2.8951, P=0.0038, 95%CI [0.1408 to 0.7309]). However, these rates showed a slight decrease when the temperature was higher at 27.5°C (Z=-0.8335, P= 0.4046, 95%CI [-0.5068 to 0.2044]). The effect size on fluctuating temperature was the highest among these four levels of temperature, being significantly different from other three levels (fluctuating vs <22.5°C: Z=-4.6346, P<0.006, 95%CI [-1.4505 to 0.5883]; fluctuating vs 22.5°C ~ 27.5°C: Z=-2.7291, P=0.0378, 95%CI [-1.0027 to -0.1645]; fluctuating vs >27.5°C: Z= -3.0345, P=0.0144, 95%CI [-1.2094 to -0.2602]).

For both trees and herbs, the mean effect size weighted by sample size in development between females and males were consistently above zero, with no apparent difference between these two types of host plants (Z=-1.3008, P=0.1933, 95%CI [-0.7816 to 0.1580], Figure 1.5).

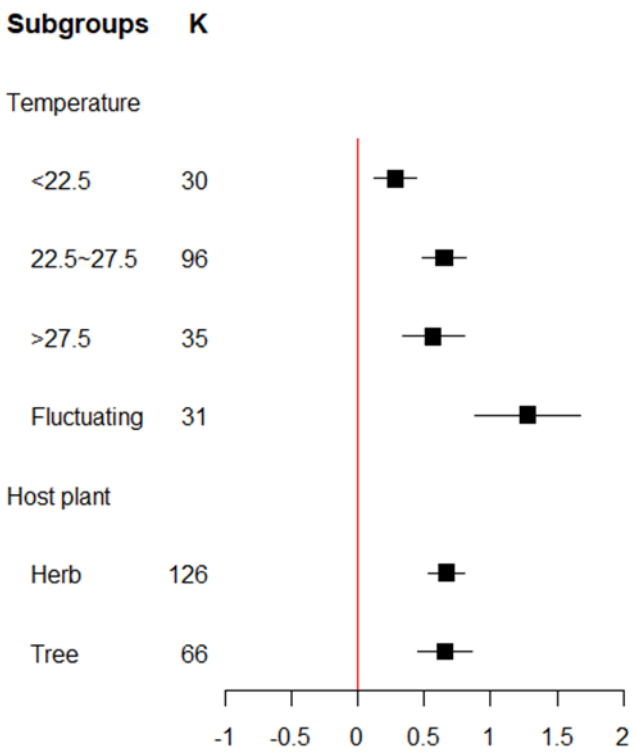

FIGURE 1. 5 Forest plot for mean effect size (SMD with 95% CI) of temperature with four levels and host plant with two levels on the sexual difference in development.

Effects of temperature and host plant on the sex differences in longevity

The sample size was biased for temperature level of 22.5°C ~ 27.5°C (50 out of 120). The sexual difference of male and female spider mites in longevity tends to increase with higher temperatures. When the temperature is blow 22.5°C, the mean effect size included zero (SMD=-0.1535, 95%CI [-0.4516 to 0.1446]; Figure 1.6), significantly lower than that at temperatures above 27.5°C (Z=2.6788, P=0.0444, 95%CI[0.2582 to 1.6660]) and fluctuating temperature (Z=3.0365, P=0.0144, 95%CI[0.6178 to 2.8674]). However, there was no significant difference in longevity for males and females between the other three levels of temperature. Similar to patterns in development, the highest effect size was also shown at fluctuating temperature. For spider mites fed on herbs, the longevity of females was longer than males (SMD=0.824, 95%CI [0.5639 to 1.0841]), but no obvious difference was shown on spider mites fed on trees (SMD=0.1933, 95%CI [-0.0803 to 0.4669]) (Figure 1.6).

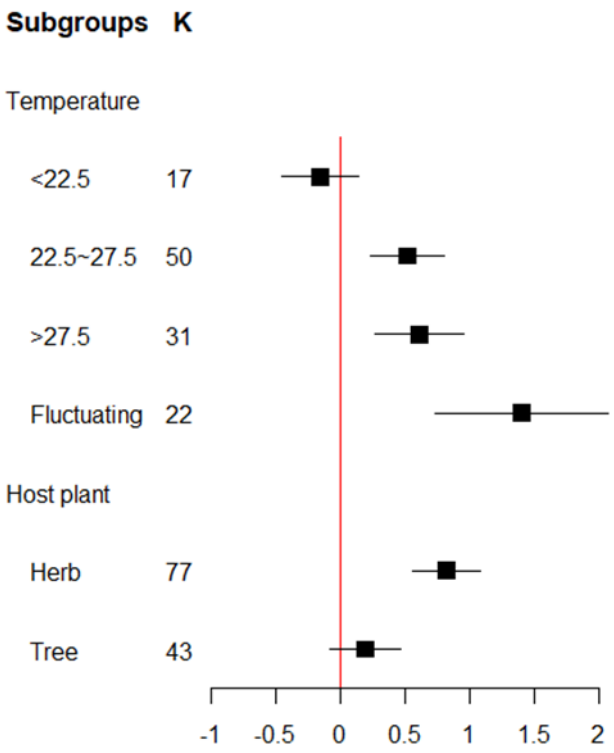

FIGURE 1. 6 Forest plot for mean effect size (SMD with 95% CI) of temperature with four levels and host plant with two levels on the sexual difference in longevity.

Publication bias

The funnel plots for two investigated parameters, development rate (Figure 1.7A) and longevity (Figure 1.7B), both indicate slight publication bias. This can result from studies with relatively small sample size reporting no sex difference or male-biased dimorphism that were under reported in the review. The limitations of this study are discussed in the following part.

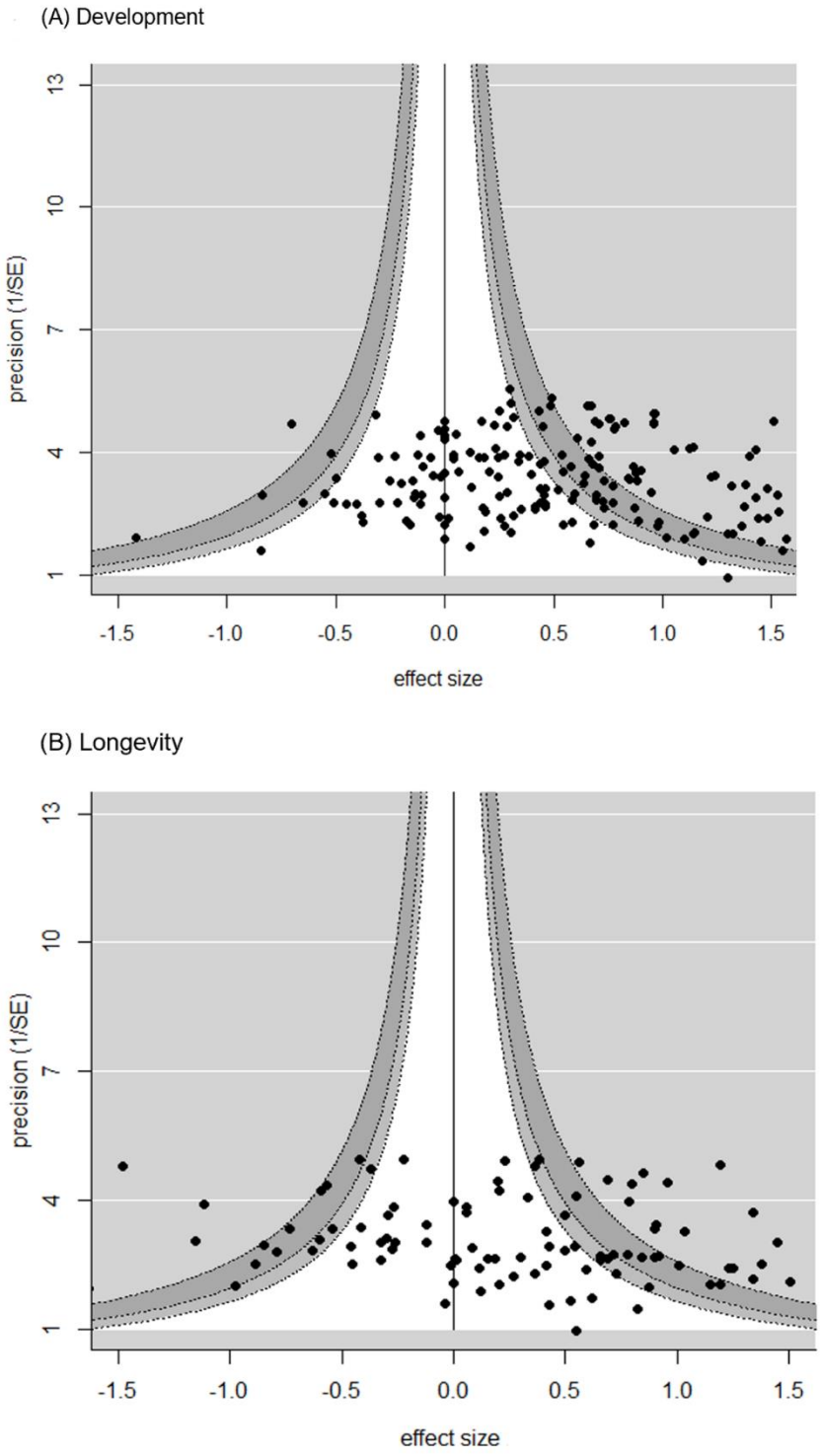

FIGURE 1. 7 Funnel plots of precision (inverse of sampling standard error) as a function of residuals from meta-analytic models for (A) development and (B) longevity.

Discussion

Numerous previous experimental studies using both males and females spider mites have explored the sex-specific response to environmental factors, but very few have attempted to determine the sex difference along gradients of temperature. In this review, I have therefore attempted a meta-analysis to determine sex difference in response to temperature and host plant across species. My results revealed obvious changes in sex difference in spider mite life-history traits to temperature and host plants.

Sexual dimorphism in development and longevity

In this review, I provided a quantitative overview of the sexual dimorphism in spider mites in two main life-history traits, development duration and longevity. The review is possible because of the abundant literature on spider mite life history, specifically in the family Tetranychidae. I showed significant sex differences occur with females having longer developmental period and lifespan than males under certain conditions. This pattern of sexual dimorphism in life-history traits is common for invertebrates. The females always take longer to develop from egg to adult and emerged with larger body size (Jarošík & Honek 2007). The prevailing view is that this pattern evolved as a consequence of sexual selection and reflects the different roles of males and females in reproduction. For females, reproductive success is positively associated with body size. Thus, the bigger female is advantaged over a small one under the pressure of fecundity selection (Honek 1993; Preziosi *et al.* 1996; Monroe *et al.* 2015). Although larger males tend to be more competitive in gaining mating opportunities, males of many species emerged earlier with a smaller body size compared with the female, in order to sire more offspring (Andersson 1994; Jagadeeshan *et al.* 2015).

The sexual size dimorphism and the related sexual dimorphism in development appear universal with a variation in direction and magnitude across taxa. For some species, the females develop through a supplementary instar when they were subjected to relatively high temperature (30℃ to 35℃) or fed with high-quality food to achieve larger body size (Hassall & Grayson 1987; Willott 1998; Esperk *et al.* 2010). However, this is not universal with spider mites or some other animals with sexual dimorphism. Most animals have a fixed number of instars and the same number for both sexes. The developmental difference for these species

can be created by a sex difference in feeding rates, efficiency in converting food into body mass, and metabolic rates. For example, some recent reports showed that females had higher food consumption and assimilation rates than males (Karl & Fischer 2008). For spider mites, in the early immature stages (larva and protonymph), males and females appeared to be similar in body size. Thus, it is unlikely they differed in physiological traits which contributed to their phenotypic differences at later stages. It is more likely in the last deutonymph stage, when the males shortened their developmental period and moulted with smaller body size in comparison with the female.

In this study, although the overall effect size indicated that female spider mites have a survival advantage over males, the sex difference in longevity was not consistently distinct across species and different treatments, just as development. For some species with a large sample size (N>10), for example, *T. urticae*, there is a very clear sex difference. However, for species with small sample size (N< 5) there were few clear differences in lifespan between males and females. Therefore, the results should be interpreted with caution because of the limited sample size for some species. More empirical studies on sex difference in lifespan with other species are needed to further clarify the direction and significance of sexual dimorphism in spider mites.

Sexual dimorphism in response to temperature and host plant

The lack of constant difference between males and females across different temperatures and host plant species in longevity suggests that adult lifespan is labile and susceptible to diverse environmental conditions, such as temperature and host plant/ diet, which are the two most influential factors of life-history traits of animals. I found that there is a significant variation in sex difference in development and longevity across temperature regimes. At constant temperature, there is a substantial increase in sex difference when the rearing temperature increased from lower than 22.5°C to 22.5~27.5°C. However, with the increase in temperature, the difference between male and female appeared to leveled off and displayed no remarkable increase when the temperature was more than 27.5°C. Spider mites showed the most significant sex difference at fluctuating temperature. This may result from the sex-

specific plasticity in response to temperature, when one sex is more plastic to temperature than the other sex, which enlarges the sex difference. However, at around 27.5°C, which is the optimal temperature for many mite species, both males and females reached their maximum growth rate, as well the maximum sexual difference.

When fed on herbs, sex differences between mites are evident for both development and longevity. In contrast, the spider mites reared on trees only show an apparent sex difference in development, but not longevity. Consistent with this study, the extensive literature on plant-feeding insects also document the effects of host plants on the performance traits such as dispersal, consumption rate, growth, survival, fecundity, sex ratio and body size (Reviewed in Awmack & Leather, 2001). The evidence suggests that the quality of plants is a crucial factor influencing the performance and sex-specific plasticity of herbivores. The reason why sex difference in longevity disappeared when mites fed on leaf disc of woody plants may be partially explained by the nutrients demands between males and females, and the different chemical characteristics of trees and herbs. Females have a much higher nutrient requirement for reproduction and survival than males. The woody plants have lower C: N ratio (Chen 2017) than the herbaceous plants, which may limit female performance and decreased the sex difference in longevity.

Conclusion

In this meta-analytic review, I showed sexual dimorphism in development and longevity in spider mites. I also reported for the first time that sexual dimorphism is variable across temperature and type of host plants. However, there is substantial heterogeneity among the literature included in this review, and the females of some species did not differ significantly from males in development and longevity. This may reflect other factors not examined in this study may also have an important role in the sex-specific plasticity, such as previous feeding history. In some empirical studies, mites were exposed to similar host plants before involved in the experiment, whereas for other studies, the experimental mites experienced a host shift. Moreover, I could not completely exclude the possibility that the lack of sex difference arises as a result of the experimental protocol. For example, the development of mites was checked

at varying time periods in different studies (from 8 hours to 1 day) and these differences may have impacted on assessments of developmental rates. Further empirical studies taking these factors into consideration may reinforce and enrich our understanding of the sex dimorphism on development and longevity.

1.6 Outline of this research

In this thesis, the life history performance of the spider mite was determined under three environmental factors: dietary restriction, mating status and predator-induced stress. First, I determined the starvation resistance of spider mites by manipulating food and mating status, and then assayed the survival and reproductive performance of mites to different levels of intermittent fasting. Second, the influences of social context on the fitness of spider mite were explored. Finally, I also examined the impacts of predator-induced stress on the development, survival and reproduction of mites in two consecutive generations.

Existing research mainly focused on females, whereas the male has long been neglected. Given that males responded to lifespan-extension interventions differently in some previous studies, more research is in critical need to investigate the sex-specific response to different conditions. During this study, the survival of and adult lifespan of spider mites were investigated in both sexes. However, the reproduction of males was not scored because no proper method for measuring the reproductive efforts of this species is available to date.

In the first part, from **chapter 2 to chapter 3**, the survival and body size of spider mites were examined by crossing two factors with two levels, food availability (non-fed/ fed *ad libitum*) and mating status (mated/ virgin), to assay the starvation tolerance of both males and females. Meanwhile, I explored whether mating status and body size show any influence on the fitness of mites (**Chapter 2**). This gives accurate information about the starvation tolerance of mites, which is used in the following study (C**hapter 3**), which investigated the response of mites to different levels of intermittent fasting. In this part, I explored the relationship between longevity and lifetime reproduction to test the validity of the trade-off hypothesis.

Secondly, I investigated the influence of social interaction on the performance of mites in chapter 4 and chapter 5. In the first experiment, I examined how male and female longevity varied when they were involved in intersexual and intrasexual interactions (**chapter 4**). I further explored how delayed mating and repeated mating shaped the rate of aging in both sexes and modulated the reproductive schedule of the female by arranging the timing and frequency of mating differently across treatments (**chapter 5**).

Following on the exploration of how different levels of intermittent fasting and mating regimes influence the longevity and reproduction of mites, **Chapter 6** uses the optimal food regime and mating regime for maximizing longevity derived from the preceding chapters to investigate whether these factors have an additive impact on longevity. Instead of checking the relationship among an array of survival and reproductive parameters in pairs, I employed path analysis to explore the interrelation between five fitness parameters under two factors all at once.

Lastly, in **chapter 7**, I determined whether predator-stress is detrimental, for example, perceived predation risk can reduce the forage rates and decrease the daily fecundity in the short term. Thus, it is interesting to explore how chronic stress shapes the lifespan of an organism by changing its feeding rates and investment in reproduction.

At the end of my thesis, the general discussion summarises the results in the preceding research chapters, expanding how life-history traits of spider mites were modulated by biotic and abiotic factors tested in my research. The significance and limitations of this research are discussed in the context of aging research. Directions for future investigation and the potential challenges are considered.

Chapter 2

Does size matter? Fecundity and longevity of spider mites (*Tetranychus urticae*) in relation to mating and food availability

Abstract

Larger animals tend to live longer than the smaller ones across many species, but whether body size also has a robust relationship with survival within species remains uncertain. I investigate the association between body size and fitness traits (fecundity and longevity) under different feeding (starvation and fed *ad libitum*) and sexual history (virgin and mated females) using the spider mite, *Tetranychus urticae*. The longevity of spider mites differed significantly across treatments, with feeding *ad libitum* increasing the survival of both males and females. Mating decreasing male survival when starved and female survival when fed *ad libitum*. The body size of females but not of males increased with food. However, for each treatment, no clear correlations between body size and longevity were found, with the exception of female fecundity which increased with body size. These results suggested that within species there is no strong association between body size and longevity.

Keywords: food availability, starvation tolerance, mating status, body size, fecundity, longevity

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2.1 Introduction

The body size of organisms varies greatly across taxa and considerably within species (Brown *et al.* 2004). It evolves as a consequence of opposing forces of natural selection and sexual selection acting on two main fitness components—survival and reproduction (Blanckenhorn *et al.* 2006). The direction and degree of these selection forces shape the degree of sexual size dimorphism, with female-biased dimorphism, male-biased dimorphism, and monomorphism (Andersson 1994; Dale *et al.* 2007). Body size is an important morphological trait that covaries with many physiological, behavioral, life history (Brown *et al.* 2004), ecological (Cohen *et al.* 2005), and evolutionary (Blanckenhorn 2005) organism traits.

The possible influences of body size on interrelated life-history traits of animals have been extensively explored for vertebrates across a wide range of taxa. For example, small animals can have a relatively lower energy requirement (West *et al.* 1999; Ackerman *et al.* 2004), generally developed faster (Depczynski & Bellwood 2006), and reproduced more quickly (Munday & Jones 1998; Ménard *et al.* 2012; Nash *et al.* 2013). Larger animals, on the other hand, tend to show an advantage over smaller ones because of the dramatic decline of mortality rates with increasing body size under predation pressure (Goatley & Bellwood 2016). Additionally, larger animals live longer than smaller ones, as indicated by the obvious fact that human beings live many times longer than small mice, which in turn greatly outlive insects such as fruit flies. The correlation between body size and lifespan has also been further examined within vertebrate classes such as birds and mammals, suggesting that body size can be a predictor of lifespan (Wilkinson & South 2002; Speakman 2005; Ricklefs 2010; Valcu *et al.* 2014; Scharf *et al.* 2015).

In empirical studies on birds and mammal males are typically bigger than females (Blanckenhorn 2005; Isaac 2005; Székely *et al.* 2007). Generally larger animals live longer than small ones for vertebrates with male-biased sexual size dimorphism. However, invertebrates differed from vertebrates with female-biased dimorphism, male-biased dimorphism, and monomorphism (Fairbairn 1997), and have received relatively little

attention. Moreover, despite clear evidence of the positive relationship between body size and life expectancy in across-taxa research, increasing studies within species have resulted in controversy. In numerous studies of human populations from different countries and people of different occupations, height was observed to be negatively correlated with longevity for both genders due to the increased risk of chronic diseases (Samaras 2007; Samaras 2014).

Within species level, the difference in body size is also substantial. Again, the most striking example is amongst humans, with women being generally shorter in height and lower in weight than men. Some researchers have proposed that variation in body size may be attributable to the sex difference in longevity—a notion supported by evidence that men from America, Poland and Sweden who were 8% taller than females had a 7.9% shorter life expectancy (e.g., Samaras *et al.* 2003). However, the relationship between body size and lifespan between genders for small invertebrates such as arthropods is not clear, although there are extensive reports on how body size covaries with lifespan and reproductive success. Further studies on how body size is related to longevity within species and between genders are therefore important to understand the interrelationship of life-history traits.

Here, I examined the potential influence of body size on the fitness of two-spotted spider mites, *Tetranychus urticae*, a species with female-biased sexual dimorphism. Males and females do not differ in size until the deutonymphal stage, with the larger females having an oval-shaped body, while small males have a tapered abdomen (Delgado *et al.* 1994; see also Fig. 1). Usually, the body mass of female spider mites continues to increase during the early adult stage, by about 100% - 150% and ceases growth at around 6-day post-adult emergence, while the male mite rarely grows after the protonymphal stage. Evidently, food availability can alter the body size and sexual size dimorphism to a large extent (Carabio *et al.* 2017). Another factor that influences resource allocation in adults is the mating status (Branco *et al.* 2017). Being mated can induce the female mites to shift energy and resources from growth and somatic maintenance to reproduction. Although spider mites may re-mate (Oku 2010, Macke *et al.* 2012), only the first mating can effectively inseminate the female, unless it is interrupted (Satoh & Takafuji 2001). The male emerged a few hours earlier than the female and guards the quiescent deutonymphs after emerging to assure paternity (Satoh *et al.* 2001).

To clarify to what extent body size is related to the fitness (i.e., longevity and fecundity), I scored the fitness components of spider mites in different mating status (virgin/mated) and food availability (*ad libitum* fed/starvation). In this study, I aimed to (1) determine the two main fitness traits: survival and reproduction of spider mites and the impact of starvation and feeding *ad libitum*; (2) explore the association between body size with longevity and female fecundity; (3) check whether mating status would be an influential factor on the lifespan of spider mites and the association of body size with fitness traits.

2.2 Material and Methods

2.2.1 Mite species

To study the effects of mating status on starvation tolerance and longevity, I used the two-spotted spider mites because the mating behavior and reproductive system were well known. The spider mites used in the study were from a laboratory-reared population established on common bean (*Phaseolus vulgaris* L.) for nearly 3 years in a greenhouse of Manaaki Whenua – Landcare Research, Auckland, New Zealand.

Experiment preparation and rearing unit

At the commencement of this experiment, I randomly picked about 240 quiescent males and 240 quiescent females in their deutonymphal stages from the bean leaves using a fine brush and transferred them individually onto a bean leaf disc. The freshly emerged adults were used to establish the experiment. The rearing unit for spider mite fed *ad libitum* is a round leaf disc (80 mm in diameter) floating on water in each well of a cell culture plate (24 well plate). The leaf disc was a little smaller than the well in size (well volume 3.4 ml, diameter 15.6 mm), and water around the leaf disc effectively functioned as a barrier to prevent mites escaping. Spider mites in the starvation treatment were raised in similar units, except that the leaf discs were replaced by a black plastic sheet so that the mites had access to water but no food.

2.2.2 Experiment manipulation

I fully crossed two factors, both with two levels: the mating status (mated=a male paired with a female and virgin, that is an isolated male or female without mating) with food availability (starvation and with food *ad libitum*), thus generating four treatments. Two days after the adults emerged, females and males were randomly assigned to each treatment. The survival of males and females was checked daily until animals died. The number of eggs produced by females was also recorded daily. Mites were identified as not dead when they were active or could move when disturbed on their legs by a soft hairy brush. The experiment was conducted in a laboratory under 25 ± 1 °C, 12:12 light cycle, with a relative humidity of 65 ± 5%.

The body width of the mites was also measured as an index of body size for each individual after death to examine to assess whether body size was associated with the starvation tolerance and lifespan of the mites. The dead body of each mite was collected and mounted in Hoyer's medium on microslides, pressed flat, and heated in an oven at 50 °C for 2 weeks. The specimens were then measured in μm at 20-fold magnification using an optical microscope connected to a video camera with NIHimage software on a computer. The body width was measured as the distance between the bases of paired *sce* setae on the propodosoma (shown in Figure 2.1), which is a reliable index reflecting the body size of Tetranychidae (Sato *et al.* 1999). Initially, forty-eight replicates were set up for each treatment; owing to accidental death and escapees, the final sample size ranged from 26 to 44.

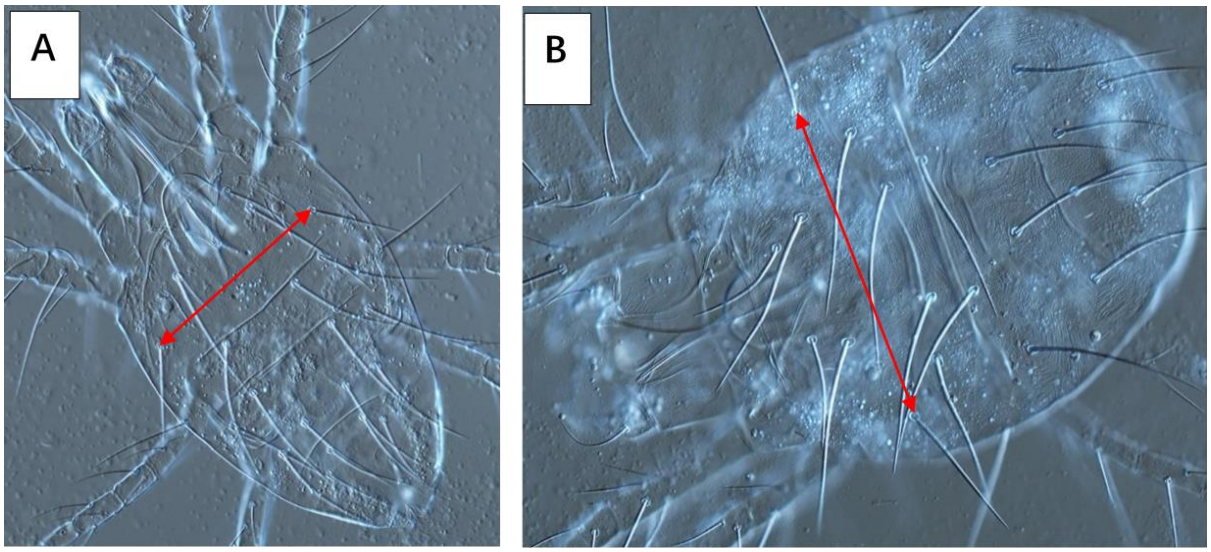

FIGURE 2. 1 Dorsal side of *Tetranychus urticae* showing the measured distance between paired setae *sce* marked with red double-headed arrows for a male (A) and female (B). The females are much larger than males.

2.2.3 Data analysis

The survival difference of the spider mites across treatments was analysed with Cox's proportional hazard model with mating treatment, food availability, and sex as fixed factors and body width as a covariate. The possible three- and two-way interactions (mating treatment × food availability × sex, mating treatment × food availability, mating treatment × sex, food availability × sex) were also included in this model. The Kaplan-Meier survival analysis was used for comparisons of survival times across treatments, with Log Rank (Mantel-Cox) tests. The lifetime fecundity of females was analysed using General Linear models, with the mating treatment and food availability as two main factors with the interaction included. The dorsal width of spider mites was fitted in the general linear models with the mating treatment, food availability, sex, and all the possible interactions as explanatory variables. Correlations between longevity and body size were tested using Pearson's product moment correlation.

The analyses were performed in R studio (R version 3.4.3). The Cox's proportional hazard model was performed using function "coxph" in R package survival (Therneau & Thomas,

2015), and general linear models were performed using the function “aov”. Data are given as Mean± SE throughout the text, unless stated otherwise.

2.3 Results

2.3.1 Effects of mating status and sex on survival time of starved mites

The longevity of mites varied significantly, with main factors including their mating status, sex, and food availability. The spider mites showed a sex difference in response to food deprivation, with the females more resilient to starvation—showing a much higher survival rate than the males (χ^2= 71.5, *df*=1, *P* <0.000; Figure 2.2A) regardless of their mating status. When adults were deprived of food for 2 days, the survival rates for females and males were 100% and 86.96%, respectively. The median survival time of food-deprived males was 3 days on average, much shorter than females (6 days; Figure 2.2A). The maximum survival time for males and females were 7 days and 11 days, respectively. There is also a significant interaction between sex and mating status on the survival of starved mites (*z* = 3.288, *P* =0.001): the female lifespan under different mating status did not differ (*P* >0.05), whereas the mated males showed a lower survival than the virgins males (χ^2= 15.4, *df*=1, *P* <0.000).

2.3.2 Effects of mating status and sex on the survival of mites fed *ad libitum*

Spider mites fed *ad libitum* lived longer than the starved ones (*z* = 13.1, *P* <0.000). When fed, the males showed a longer mean lifespan than the females (25 days for males and 12 days for females; $F_{1,145}$= 59.06, *P* <0.001; Figure 2.2B). The mating status had a profound influence on the survival of mites fed *ad libitum*: mated females showed a significant decrease in longevity, while the male longevity was not significantly influenced (*P* > 0.05) —consistent with the results obtained from survival analysis for mites fed *ad libitum* (Figure 2.2B).

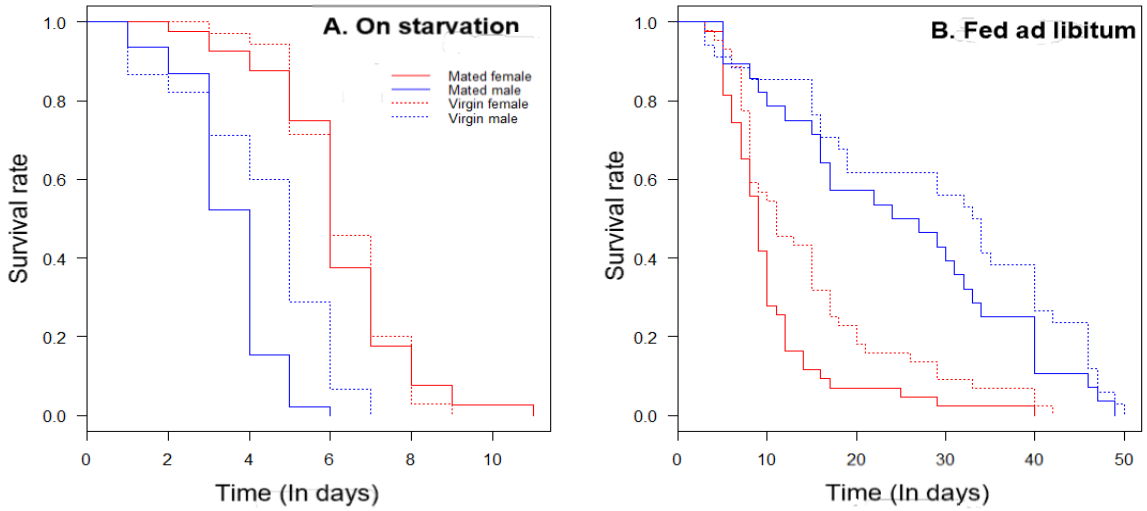

FIGURE 2. 2 The proportion of surviving adult mites under starvation (A) and fed *ad libitum* (B), with the solid red lines for the mated females, dash red line for virgin females, the solid blue line for mated males, dash blue line for the virgin males.

2.3.3 Body size and its relationship with the survival time of starved mites

The female spider mites were significantly larger than males, with the female being on average 480 μm in dorsal length and the male 325 μm (Figure 2.3A). For both sexes, survival time in the absence of food was unrelated to their body size indicated by the linear regressions ($F_{1,38}$= 0.5918, P=0.4465 for mated female; $F_{1,44}$= 2.411, P=0.1276 for mated male; $F_{1,33}$=3.993, P= 0.05399 for virgin female; $F_{1,44}$= 0.4127, P = 0.524 for virgin male, Table 2.1). This is also consistent with the results of Cox proportional hazards model, which indicates that body size has no effect on the survival time of mites under food deprivation (z = –0.04, P =0.96).

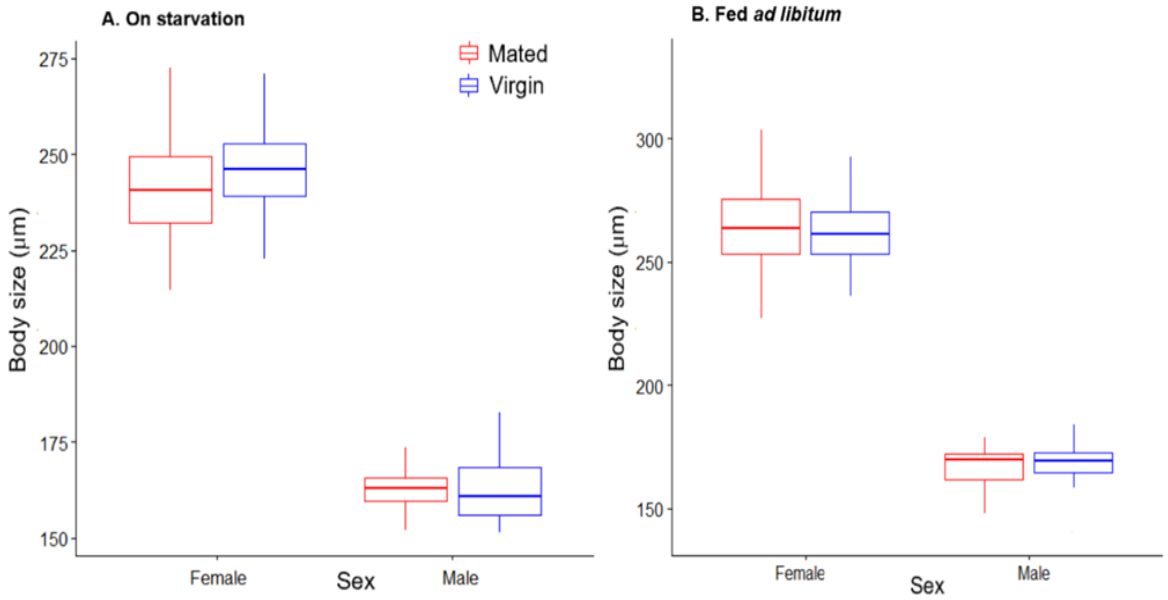

FIGURE 2. 3 Body size (Means±95% percentile) of the mated and virgin mites *Tetranychus urticae* under different conditions (A: on starvation; B: fed *ad libitum*). In both figures, the red boxes denote the mated mites, and the blue boxes represent the virgin mites.

TABLE 2. 1 The correlation between body size and adult lifespan of mated and virgin mites *Tetranychus urticae* with different food availability (on starvation and fed *ad libitum*).

Food availability	Sex	Mated			Virgin		
		slope	R^2	N	slope	R^2	N
On starvation	Female	-0.007	0.01	38	-0.016	0.0809	33
	Male	0.017	0.03	44	0.017	0.009	43
Fed *ad libitum*	Female	0.014	0.018	41	0.055	0.029	42
	Male	0.035	0.029	26	-0.08	0.03	32

2.3.4 Body size and its relationship with the lifespan of mites fed *ad libitum*

As with mites in the starvation treatment, the females were larger than the males in dorsal width ($F_{1,158}$= 740.98, P <0.001 Figure 2.3B). In comparison with starved mites, the body size of females significantly increased when they were fed at the adult stage ($F_{1,158}$= 40.18, P <0.001). However, remarkably, food availability did not alter male body size (P>0.05). The lifespan of mites showed no significant relationship with body size within each treatment (All P >0.05, Table 2.1), suggesting that body size did not contribute to the longevity difference of mites. Across treatments, the influence of body size on the survival of mites was also insignificant (z = –1.258, P =0.21).

2.3.5 Effects of food and mating status and body size on female fecundity

The starved females mostly ceased reproduction on the second day of starvation. Thus, their lifetime fecundity averaged 1.8 eggs per female, significantly lower than that of females fed *ad libitum* (76 eggs per female for fed female; $F_{1,154}$=117.51, P <0.001, Figure 2.4). While mating status also influenced female fecundity, with mated females produced fewer eggs than virgins ($F_{1,154}$=10.33, P =0.002), its interaction with food availability is non-significant ($F_{1,154}$=2.971, P =0.086). Regression analysis showed that for mated starved females, the relationship between lifetime fecundity and body size was not significant ($F_{1,38}$=0.60, P= 0.44, Table 2.2). However, the body size of mated females fed *ad libitum* positively correlated with their fecundity; albeit with lower significant levels ($F_{1,41}$=3.573, P= 0.067). For the virgin females, there is a significant positive relationship between these two parameters, regardless of food availability ($F_{1,33}$=10.44, P= 0.002794 for starved virgin females; $F_{1,42}$=4.818, P= 0.033 for fed *ad libitum* virgin females.

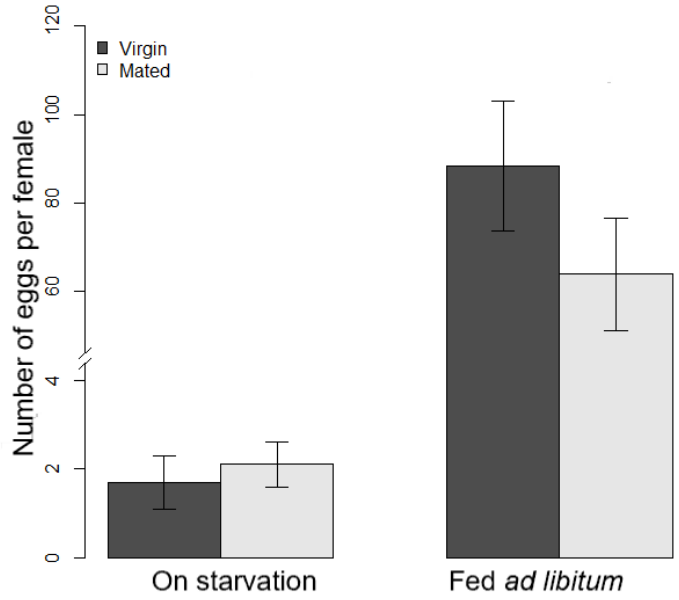

FIGURE 2. 4 Fecundity (Mean±SE) of mated and virgin females *Tetranychus urticae* as a function of food availability (feed *ad libitum* and starvation). Note there is a break on the y-axis, with the lower half for the fecundity of mites on starvation, and upper for the fecundity of females feed *ad libitum*.

TABLE 2. 2 The correlation between body size and female fecundity of mated and virgin mites *Tetranychus urticae* with different food availability (on starvation and fed *ad libitum*).

Food availability	Mated			Virgin		
	slope	R^2	N	slope	R^2	N
On starvation	0.007	0.01	38	0.033	0.217	33
Fed *ad libitum*	0.321	0.056	41	0.379	0.081	42

2.4 Discussion

In this study, I investigate the fitness traits of spider mites in relation to their body size as controlled by food supply. The aim was to determine the impact of body size on survival rates and lifetime fecundity. There was no clear evidence that the variation in body size was

related to starvation tolerance for either sex. When provided with food, small males significantly outlived the large females. Within each sex, no correlation was found between body size and lifespan. The lifetime fecundity appeared to increase with body size, but the positive relationship was not significant for food-limited mated females. The results also showed sex-specific tolerance to starvation, with large females surviving much longer than small males, regardless of their mating status. For males, the mated males that cohabited with their partners were more responsive to starvation and lived for shorter periods than the virgin males.

2.4.1 Sex-specific tolerance to starvation

In the short-term test without any food replenishment, I demonstrated that there was a remarkable sex difference in starvation tolerance: female spider mites could withstand much longer starvation than males, demonstrated by a much higher survival rate. Similar results were also shown in a copepod (*Acartia tonsa*), whose males had a lower survival than females in the absence of food (Finiguerra *et al.* 2013). These sex-specific starvation responses can be attributed to differences in energy reservation. In a study with fruit fly *D. melanogaster*, it was shown that the content of body lipid and starvation tolerance are positively associated with each other, and female fruit flies had a higher amount of body lipid than the males did (Chippindale *et al.* 1996), which is consistent with the size-efficient hypothesis (Threlkeld 1976; Tessier *et al.* 1983). This sex difference in energy storage may also occur in mites, as well as copepods, and could thus contribute to the sex-specific starvation tolerance.

The females under starvation might be able to shift energy between soma maintenance and reproduction is indicated by two main pieces of evidence from these experiments. First, females significantly reduced their reproductive rates on the first day of starvation producing only 1.3 eggs compared to the normal 6~7 eggs on average (Li & Zhang unpublished data). It is possible that starved females retain or absorb the egg when they encounter tight energy budget so that they can re-allocate the resource from reproduction to survival, as seen in other arthropods (Montserrat & Magalhães 2007; Boivin & Ellers 2016). Second, the females eased

reproduction on the second day of starvation, suggesting that they were able to shift energy from egg production to ensure their own survival under adverse conditions.

Another possible factor for sex differences in response to starvation is behavioural as adult male mites are generally far more active than female mites who spend most of the time resting. This was further supported by the observation that many more male than female mites left the floating discs and were trapped in water and that mated males showed lower survival rates than virgins. The higher energy expenditure by males results in an early onset of energy exhaustion and earlier death.

2.4.2 Body size and longevity

The body size of *T. urticae* varied substantially with food availability, and those females that were fed were much larger than starved females (Figure 3). However, the difference in body size has no clear association with their longevity and starvation tolerance, suggesting that body size is not a critical contributor to starvation resistance and longevity of spider mite under our laboratory conditions. This result agrees with other studies that used invertebrates as models to explore size–longevity relationship at the within-species level and found body size or mass is not a reliable/accurate predictor of survival. For example, in a recent study with female *Drosophila melanogaster*, longevity is not significantly affected by body size (Travers *et al.* 2015). The non-significant relationship between longevity and body size was also frequently reported in other model organisms such as *C. elegans* and non-model organisms, including the beetle *Callosobruchus maculatus* (for female only Fox *et al.* 2004), parasitoid *Cephalonomia stephanoderis* Betrem (Lauzière 2014), stinkbug predator *Podisus rostralis* (Zanuncio *et al.* 2002), mosquito *Aedos triseriatus* (Landry *et al.* 1988), and goldspotted oak borer *Agrilus auroguttatus* (Lopez & Hoddle 2014). These reports may suggest that within species the morphology variation in body size does not impact lifespan changes.

The interspecific positive relationship between body size and longevity was based on the oxidative stress theory of aging. It states that small animals have a relatively higher mass-

specific metabolic rate than large species, giving rise to severer oxidative damage and shorter longevity. However, because metabolic rate is not in proportion to body size within species, there is no reason to expect that oxidative damage should relate to body size (Khazaeli *et al.* 2004). It is therefore likely that within species, the range of body size and the metabolic rate variation are not the decisive factors affecting longevity.

2.4.3 Body size and female fecundity

In this study, the body size of the female was found to be positively associated with their fecundity, particularly for virgin females. However, the mated food-deprived females were an exception as they produced very few eggs even when they lived for a few days. This perhaps is not surprising, given that natural selection favours large females that have more energy and nutrient reserve for egg production, producing a fecundity advantage over smaller females. The difference in the relationship across treatments may suggest that other factors such as food and mating status also affect fecundity. In this study, when mated females were deprived of food they produced very few eggs because starvation made them save energy for survival. It seems that the association between body size and fecundity decoupled because of starvation or food level. This was also supported by research with *Agrilus auroguttatus,* in which the females, with unknown feeding history, collected from the field showed that lifetime oviposition was not correlated with any body size indicators, including elytron length, elytron width, or tibia length (Lopez & Hoddle 2014); earlier field research by Branson (2008) and Gotthard *et al.* (2007) also failed to find a positive association between body size and reproductive traits.

In insects and many other animals, high stress resistance to environmental factors can enhance survival probability. Consequently, in some studies stress resistance was deemed an indicator of organisms' fitness (Johnson *et al.* 2002; Lithgow & Walker 2002; Rea *et al.* 2005). This notion was supported by some researchers, who claimed that selected lines of long-lived animals always have better performance when experiencing stress, including starvation and high temperature or *vice versa* (Zwaan *et al.* 1995; Norry & Loeschcke 2003; Stazione *et al.* 2017), although it is not always the case. Our study finds no evidence that the

females highly resistant to starvation lived longer than males. Surprisingly, the females were shown to have a relatively shorter lifespan than the starvation-sensitive males, irrespective of their mating status. The inversed association between starvation tolerance and longevity between male and female was principally due to their difference in reproductive investment across food conditions. The female showed a dramatic increase in reproductive output when food was available, while the reproductive investment of the male varied little between food treatments. A further factor is that this assumption, however, has not yet been demonstrated in this study owing to the lack of an appropriate method for measuring the reproductive efforts in male spider mites. To test this assumption, I suggest employing other model organisms, such as cricket (*Gryllus spp*), whose reproductive investment of the male can be easily measured using calling efforts assessed by electronic monitoring (e.g.: Zajitschek *et al.* 2009, Archer *et al.* 2012; Rapkin *et al.* 2018).

2.5 Conclusion

Although the longevity difference between many mammals and birds can be well explained by their body size, in accordance with the rate of living theory, the relationship between body size and life history fitness within-species for small invertebrates should be interpreted with caution. This study, supported by previous reports, has provided insights that many other factors, including mating status (Himuro & Fujisaki 2010), genetic background (Khazaeli *et al.* 2005), and diet and temperature (Chen *et al.* 2005; Norry & Loeschcke 2002), can more dramatically affect the association between life-history traits. This is particularly important when these environmental factors may be a major source of variation of body size and lifespan or both at the same time. Therefore, the within-species association between body size and longevity will not be fully understood until further research is conducted into the influence of various environmental conditions.

Chapter 3

The sex- and level-dependent effects of intermittent fasting on lifespan and reproduction of spider mite *Tetranychus urticae*

Abstract

Intermittent fasting (IF) is receiving increasing attention as an alternative to continuous restriction of calories because of its benefits in aging-related disease prevention and lifespan extension. However, whether sexually dimorphic species have a similar response to IF have rarely been assayed. In this study, I determined how different levels of IF influence lifespan and whether males and females differed in their responses to IF. I also tested whether there is a trade-off between life span and lifetime reproduction in females under IF. I used spider mite *Tetranychus urticae*, with female-biased sexual size dimorphism (SSD), as our model species. Females showed a curvilinear trend, increasing lifespan under low IF before reaching an asymptote and then finally decreased under diminished food supply, but males showed a decrease in lifespan when subjected to IF. Within each treatment, fecundity was positively associated with female longevity. However, the females fed *ad libitum* had a higher lifetime fecundity with a shorter lifespan, whereas mites fed 50% IF (feeding 2 days out of every 4 days -feed-feed-starved-starved) outlived *ad libitum* fed ones with lower fecundity because of the later onset of reproduction and lower daily fecundity, showing apparent survival and reproduction trade-off when variation of resource availability enhanced across treatments. I showed sex-specific responses to IF for lifespan, indicating that organisms with SSD have different optimal level of IF. These findings showed a trade-off between survival and reproduction between treatments but not within treatments, suggesting that variation in resource availability is the necessary precondition for life-history trade-offs, and IF extends the lifespan of females at the cost of reproductive success.

Keywords: Fasting, sex-specific, longevity, reproductive efforts, trade-off

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3.1 Introduction

Aging is a biological process modulated by both genetic architectures passed down from parents and environmental factors animals experience throughout their lives (Kenyon 2005; Kirkwood 2005; Masoro 2005). To date, a wide array of anti-aging interventions has been proposed to extend healthy lifespan and enhance individual the health status, e.g., dietary restriction (DR), exercise, pharmacological interventions, and hormesis (De Cabo *et al.* 2014; Le Bourg 2009). Among these, dietary restriction, defined as reducing food intake without malnutrition, is the first and most reproducible intervention supported by a large body of empirical studies in the last few decades (McCay *et al.* 1935; Lee & Longo 2016, Liang *et al.* 2018). The concept of DR has been expanded from initial calorie restriction to encompass a wide range of diet-related interventions including short-term starvation, intermittent fasting, and macronutrient (i.e., necessary amino acid, proteins, carbohydrates) restriction (Lee & Longo 2016).

Intermittent fasting (IF) was proposed as an alternative to traditional dietary restriction (Varady & Hellerstein 2007; Teng *et al.* 2011; Lee & Longo 2016). During intermittent fasting, animals are periodically exposed to fasting during which food is withdrawn. IF differs from DR in that it does not limit the total amount of food available. Instead, the animals are offered food *ad libitum* at scheduled feeding periods (Varady & Hellerstein 2007; Mattson & Wan 2005) with IF in-between. DR and IF are both reported to be beneficial for model organisms and humans—the most direct and obvious advantage being the promotion of weight loss (Hsieh *et al.* 2005; Ahmet *et al.* 2005). Besides, they may also generate many physiological benefits, including enhancing glucose tolerance (Weiss *et al.* 2006), decreasing heart rate and blood pressure (Heilbronn *et al.* 2005; Mager *et al.* 2006), and reducing oxidative damage (Varady & Hellerstein, 2007). Cumulatively these result in the extension of lifespan and a lower incidence of aging-associated diseases such as cardiovascular disease and kidney disease (Varady & Hellerstein 2007; Singh *et al.* 2012; Colman *et al.* 2014;

Caramoci *et al.* 2016). DR seems intuitively more challenging than IF to implement for experimental animals; the latter is thus gaining increasing popularity and requires further investigation as a potential strategy for delaying aging and enhancing lifespan.

While dietary interventions have several health benefits for animals, their effects on lifespan demonstrated in previous studies are not always consistent (Longo & Mattson 2014). In some longer-lived animals such as mice, IF (alternative day fasting) beginning at the age of 1–2 months increased adult lifespan (Goodrick *et al.* 1990). Similar results were also observed for short-lived rotifers with an average lifespan of 2 weeks (Kaneko *et al.* 2011). However, some previous studies on IF showed contradicting results. Take the model organism fruit fly *Drosophila melanogaster* for example. In a well-controlled investigation with fruit fly indicated that no positive effects of IF were observed (Grandison *et al.* 2009). However, most recent research showed that IF can benefit fruit fly with longevity extension (Catterson *et al.* 2018). Additionally, in one study with short-lived fish, IF significantly shortened the longevity in comparison with *ad libitum* fed controls (Inness & Metcalfe 2008). Similar results were also shown in phytoseiid mite *Neoseiulus californicus,* with their total lifespan decreased by 40% when subjected to repeated cycles of fasting (Gotoh & Tsuchiya 2009). This disparity in the effects of IF on lifespan for different taxa might be due to their differences in reproductive modes, which have rarely been addressed in most studies.

Although the effects of these dietary interventions on aging and lifespan have been explored extensively across a diverse range of animal taxa, ranging from invertebrates (e.g., *Drosophila melanogaster* and *Caenorhabditis elegans*) to vertebrates (e.g., *Rattus rattus, Mus musculus*, and *Macaca mulatta*) (Speakman & Mitchell 2011), the underlying mechanism by which dietary restriction modulates the aging process remains poorly understood (Kirkwood 2005). In laboratory studies, increased longevity in response to dietary restriction is linked with a decrease in fecundity which indicates that a trade-off between reproduction and survival is the driving force of longer lifespan. This concept has played a crucial role in theory and interpretation of the life history studies (Zera & Harshman 2001). The most prominent theory is the disposable soma theory, which suggests that these two fitness components share a finite resource pool. Thus an increased investment in reproduction would

withdraw resources that might otherwise be available for somatic maintenance (Kirkwood 1977, 1981, 1990). Previous research on aging mainly focused on longevity, with few experimental studies investigating the influence of dietary restriction on reproduction. As a result, more empirical support for the trade-off theory is required before it can be accepted as a general explanation. Moreover, the limited existing evidence is mainly demonstrated in model organisms including fruit fly, worm, and mice, which show more remarkable response to dietary restriction than non-model organisms (Nakagawa *et al.* 2012). Therefore, further investigations that explore the impact of the dietary restriction on both longevity and reproduction are needed, particularly in other non-model species.

It has recently been observed that males and females are not only different in aging rates and lifespan within species, but also display distinct responses to a variety of anti-aging interventions (Austad & Bartke 2016; Austad & Fischer 2016). In many studies, DR has weaker effects on males than on females, both in the model and non-model species (Nakagawa *et al.* 2012). Moreover, there is a sex-specific optimal food regime for lifespan extension. For example, in the *D. melanogaster,* females have longer lifespans on average than the males, particularly up to 60% of the standard laboratory diet, compared with a concentration of 40% for males (Magwere *et al.* 2004). Males generally invest less in reproduction and consequently need comparatively less resource for somatic maintenance and reproduction. However, there is relatively little information about how dietary regimes influence the lifespan of males differently from females.

In this study, I investigated the influence of IF on lifespan and reproduction in a non-model species: the spider mite (*Tetranychus urticae*) ,which belongs to the Acari (mite) family Tetranychidae It is cosmopolitanism on a wide range of host species, including beans, peppers, tomatoes, potatoes, corn, cannabis, and strawberries, with beans being one of its preferred host plants. At 25°C, the spider mites usually develop from egg to adult in about ten days, and the adults live for around two weeks with lifetime fecundity ranging from 50 eggs to 178 eggs (Sabelis 1991). Although there are many studies on the life history traits of female spider mites, the male has been largely overlooked. I included both males and females as subjects, aiming to examine the sex difference in aging by exposing them to three levels of

IF with controls fed *ad libitum*. Our previous study found that bigger females have higher starvation resistance than small males (Li & Zhang 2018). Therefore, I expected that the effects of IF would be level and sex-dependent. I predicted that the long-lived females would show a lower lifetime fecundity as predicted by the life history trade-off model under IF. Specifically, there would be a negative correlation between female longevity and lifetime reproduction.

3.2 Material and methods

3.2.1 Stock culture

The mites used in this study are two-spotted spider mites *T. urticae,* a plant-feeding animal. The stock culture was derived from a small population obtained from Bioforce Ltd, New Zealand in February 2015. It was maintained under controlled conditions in a greenhouse room at Landcare Research, Auckland, New Zealand. This population was reared on potted common bean plants (*Phaseolus vulgaris L.*), a favourable host plant. Newly potted bean plants (about 4 weeks old) were regularly added to the stock culture.

The potted bean plants were sowed every week and cultured in a separate insect-free room in the greenhouse to make sure there were enough fresh bean plants for the mite population. Both the mite population and plants were kept at 20±5 °C, 60±10% RH and the ambient photoperiod in all seasons except for winters, with supplementary light cycle of 16 h light:8 h dark.

3.2.2 Experiment protocol

In our previous starvation tolerance test, *T. urticae* males and females experienced a higher risk of mortality after 2 and 4 days of food deprivation, respectively (Li & Zhang 2018). From this study, I deduced that 2 days fasting is the maximum both females and males can tolerate without extremely deleterious effects. Therefore, the spider mites were exposed to three levels of IF, i.e. 33% IF (feeding them 2 days out of every 3 consecutive days -feed-

feed-starved); 50% IF (feeding 2 days out of every 4 days -feed-feed-starved-starved); and 67% IF (feeding 1 day every 3 days -feed-starved-starved), with spider mites feed at *ad libitum* as control. The mites were transferred to a rearing arena with a black plastic sheet as a substrate during the starvation periods, while placed on fresh leaf discs as a substrate during the feeding period.

The experiment started with males and females on the first day of the final molt. To prepare a large population of mites at this age, the unmated females and mated females from the laboratory population were transferred to leaf discs to lay eggs for 24 hours. These eggs of the same age were then collected and allowed to hatch and develop into adults of similar ages. In all treatments, males and females were paired and fed during the first 2 days of adulthood. They underwent the first starvation period on the third day after the adult emergence when most females started to produce eggs. The mites were then exposed to the four dietary regimes: *ad libitum*, 33% IF, 50% IF, 67% IF, as mentioned above. For all treatments, the leaf discs were changed every 4 days to make sure there was fresh food for mites. The experiment was conducted with 24 well cell culture plates with one pair of mites (male and female) in each cell. The eventual sample size ranged from 23 to 44 for each treatment. During the experiment, survival and female reproduction were measured. The survival of each mite was checked every 24 hours until all mites were dead. The number of eggs produced by each female was recorded each day. The experiment was conducted at 25±2 °C, 65±10% RH and a photoperiod cycle of 16 h light:8 h dark.

3.2.3 Statistics

All statistical analyses were conducted in R software version 3.4.2. For the IF experiment, the survival data were first fitted in the Cox proportional-hazards model with treatment (4 levels) and sex (2 levels) as independent variates. Since the interaction between sex and treatments was significant ($P<0.05$), the survivorship was compared between treatments, for females and males respectively. Pairwise comparison was conducted using Kaplan-Meier method with

log-rank test, taking the censored into consideration. R packages ‘survival’ and ‘survminer’ were employed for computing survival analyses and visualizing the results, respectively. The adult longevity was normally distributed, so two-way ANOVA test (R function ‘aov’) was applied to check the main effects of sex, treatment, and the interaction between them.

The female reproductive parameters, including pre-oviposition period (time period from adult emergence of a female to its first egg being laid), oviposition period (the time period from the first egg of a female being laid to the last egg being laid), post-oviposition period (the time period from the last egg of a female being laid to its death), daily reproductive rates (the number of eggs produced by female per day), lifetime fecundity (total number of eggs produced by a female), maximum daily reproductive rate (the largest number of eggs produced by a female during its life), and the female age at maximum daily reproductive rate (age of the female when a female showed maximum daily reproductive rate), were compared with dietary regimes as the main factor using R function ‘aov’. Post-hoc comparison was performed with TukeyHSD. To clarify the relationship between adult longevity and lifetime fecundity, analysis of covariance (ANCOVA) was conducted with longevity as dependent variable, fecundity as independent variable, and IF levels as covariates (R function ‘aov’). As there is significant interaction between longevity and IF levels, linear regression (R function ‘lm’)was performed for each treatment, respectively.

3.3 Results

3.3.1 Effects of IF on the survival of males and females

The spider mite demonstrated sex-specific response to IF, with females having a higher survival rate at modest fasting, while males having a reduced survival rate under all levels of fasting (Figure 3.1). The survival rate of female spider mites fed at these four dietary regimes showed significant differences, with the females fed at 50% IF showed highest survival, followed by mites fed *ad libitum*, and mites on fasting for 1 day or 2 days every 3 days (χ^2=

9.1, *df*=3, *P*=0.03, Figure 3.1A). Specifically, the *ad libitum* fed females had a similar survival to those fed at 33% IF and 67% IF (χ^2=0.3, *df*=1, *P*=0.6 and χ^2=0.1, *df*=1, *P*=0. 8, respectively; Figure 3.1A), but a marginal difference compared with females fed at 50% IF (χ^2=4.3, *df* =1, *P*=0.04; Figure 3.1A). These results suggested that the optimal regime for lifespan extension of female spider mites was around 50% IF, and neither a lower nor a higher level of fasting was effective in extending lifespan.

In contrast, for the males, the survivorship decreased with the increasing levels of fasting. Males exposed to 33% IF had a similar survival compared with those fed at *ad libitum* (χ^2=3.3, *df*=1, *P*=0.07; Figure 3.1B), but males fed at 50% IF and 67% IF had significantly lower survival rates than the control males (χ^2=20.5, *df*=1, *P* < 0.000 and χ^2=33.6, *df*=1, *P* < 0.000; Figure 3.1B), indicating that males were susceptible to fasting and showed a negative response to the severe fasting.

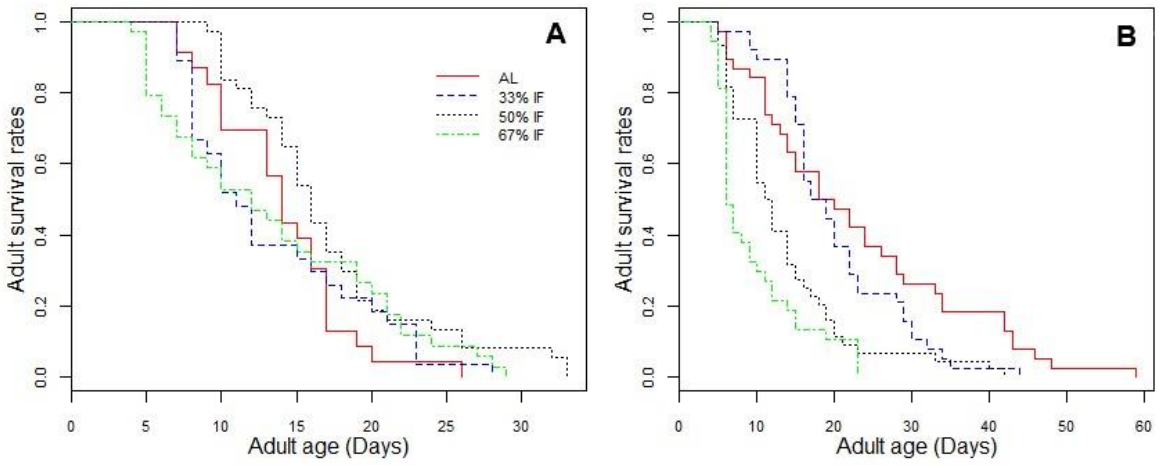

FIGURE 3. 1 Survival curves of spider mites *Tetranychus urticae* under different levels of intermittent fasting (IF). **(A)** Survivorship of female *T. urticae* subjected to three levels of IF in comparison with that of mites fed *ad libitum*. **(B)** Survivorship of male *T. urticae* subjected to three levels of IF in comparison with that of mites fed *ad libitum*. Panel A and B share the same legend.

3.3.2 Effects of IF on the life span of males and females

Both IF and sex showed significant influence on the lifespan of spider mites (Figure 3.2A). With the increase of IF levels, the adult longevity of spider mites (with male and female

pooled together) decreased from 18.41±1.10 days to 11.42±0.99 days ($F_{3, 270}$=8.352, P<0.000, Fig 3.2A). The males showed a reduction in longevity with higher IF levels, while female longevity peaked at 50% and then decreased (Fig 3.2A). Moreover, dietary regimes had a significant interaction with sex on adult lifespan ($F_{3, 270}$=10.612, P<0.000). The males fed at *ad libitum* and at 33% IF lived significant longer than the corresponding females (*AL:* t=3.58, df=48.68, P= 0.001 and 33%IF: t=3.82, df=62.74, P= 0.000), whereas at higher level of IF, 50% IF and 67% IF, the females significantly outlived the males (50%IF: t=2.14, df=78.18, P= 0.03 and 67% IF: t=2.33, df=61.76, P= 0.02; Figure 3.2A).

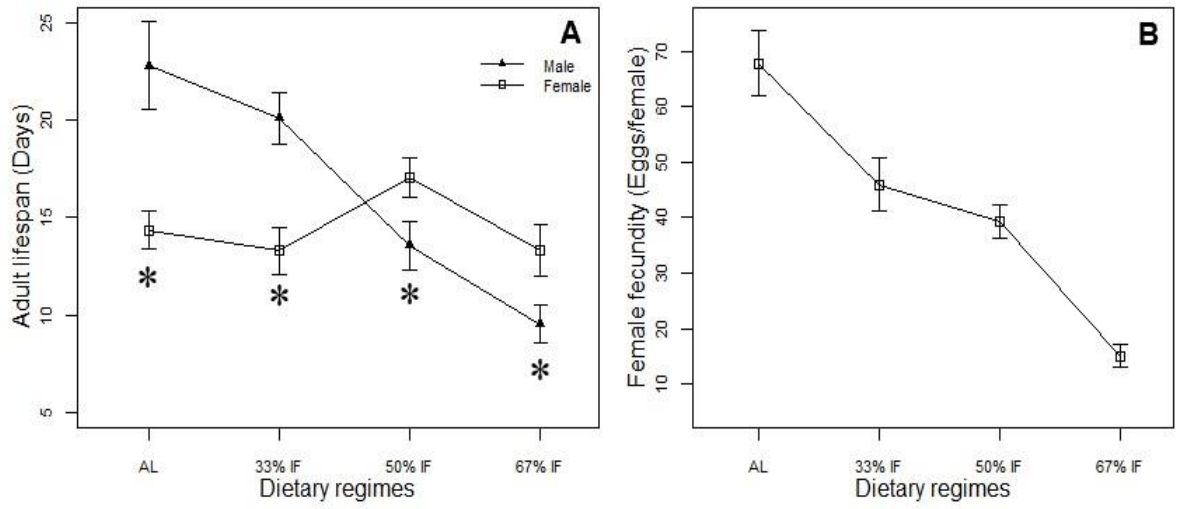

FIGURE 3. 2 Mean lifespan and lifetime fecundity of spider mites *Tetranychus urticae* under different levels of intermittent fasting (IF). (**A**) Effects of feeding regimes: *ad libitum*, 33% IF, 50% IF, and 67% IF on the adult life span of female and male spider mites. Data were shown in means±se (days). The asterisk denotes there was a significant difference in lifespan between male and female at each level of food regime. **(B)** Effects of feeding regimes: *ad libitum*, 33% IF, 50% IF, and 67% IF on female reproductive rates of female spider mites. Data were shown in Means ± SE (eggs/female). Panel A and B share the same legend.

3.3.3 Effects of IF on reproductive parameters

There were no statistical differences in the durations of the pre-oviposition period and oviposition period, even though the mites on IF seemed to delay the onset of the reproduction

(Table 3.1). In addition, no significant variation was shown between female mites fed on these four different food regimes on post-oviposition periods (Table 3.1).

TABLE 3. 1 Effects of dietary regimes on reproductive lifespan and investment of female spider mites.

Parameters	*Ad libitum*	33% IF[1]	50% IF	67% IF	*P*
Pre-oviposition period (days)	1.13±0.07a[2]	1.41±0.14a	1.65±0.14a	1.63±0.19a	*P=0.113*
Ovi-position period (days)	12.21±0.86a	11.80±1.19a	14.57±0.97a	11.28±1.29a	*P=0.067*
Post-oviposition period (days)	2.13±0.44a	1.00±0.00a	2.06±0.28a	1.52±0.16a	*P=0.081*
Daily reproductive rate (Eggs/day)	5.60±0.31a	3.75±0.13 b	2.77±0.10c	1.33±0.55d	*P<0.000*
Maximum reproductive rate (Eggs/day)	9.52±0.46a	8.53±0.32b	7.62±0.28c	2.94±0.12d	*P<0.000*
Adult age at MRR[3] (days)	5.41±0.66b	7.47±0.61ab	8.85±0.68a	5.90±0.60b	*P<0.000*

Note: 1. The abbreviations 33% IF, 50% IF, 67% IF denote feed for 2 consecutive days at the 3-day interval, 2 consecutive days at the 4-day interval, 1 day at 3-day intervals, respectively.
2. Means (±SE) within the same row followed by the different letters are significantly different ($P < 0.05$).
3. MRR is the abbreviation for the maximum reproductive rate.

Although food regimes did not have a significant influence on female reproductive lifespan, fecundity decreasing monotonically with the increasing level of IF (Fig 3.2B). The daily reproductive rate, estimated as the mean number of eggs produced by a female per day, decreased significantly with increasing levels of food deprivation, impacting on lifetime fecundity (Table 3.1). On average, the females fed *ad libitum* produced four times as many eggs as the mites exposed to 67% IF. The maximum daily reproductive rate was the highest for females fed *ad libitum* (9.52 eggs/day), followed by 33% IF (8.53 eggs/day), 50% IF (7.62eggs/day); it was the lowest when mites experienced 67% IF (eggs/day). The females' maximum daily reproductive rate occurred at 8.85 days and 7.47 days old when fed at 33% IF and 50% IF, respectively, much older than those fed *ad libitum* (5.41 days old) and 67% IF (5.90 days old). The daily reproductive rate distribution curve (Fig 3) further indicates that when females were deprived of foods for 2 consecutive days, their eggs were mostly

produced during the feeding period. During the fasting period, egg production decreased on the first day and even ceased on the second day of fasting (Figure 3.3).

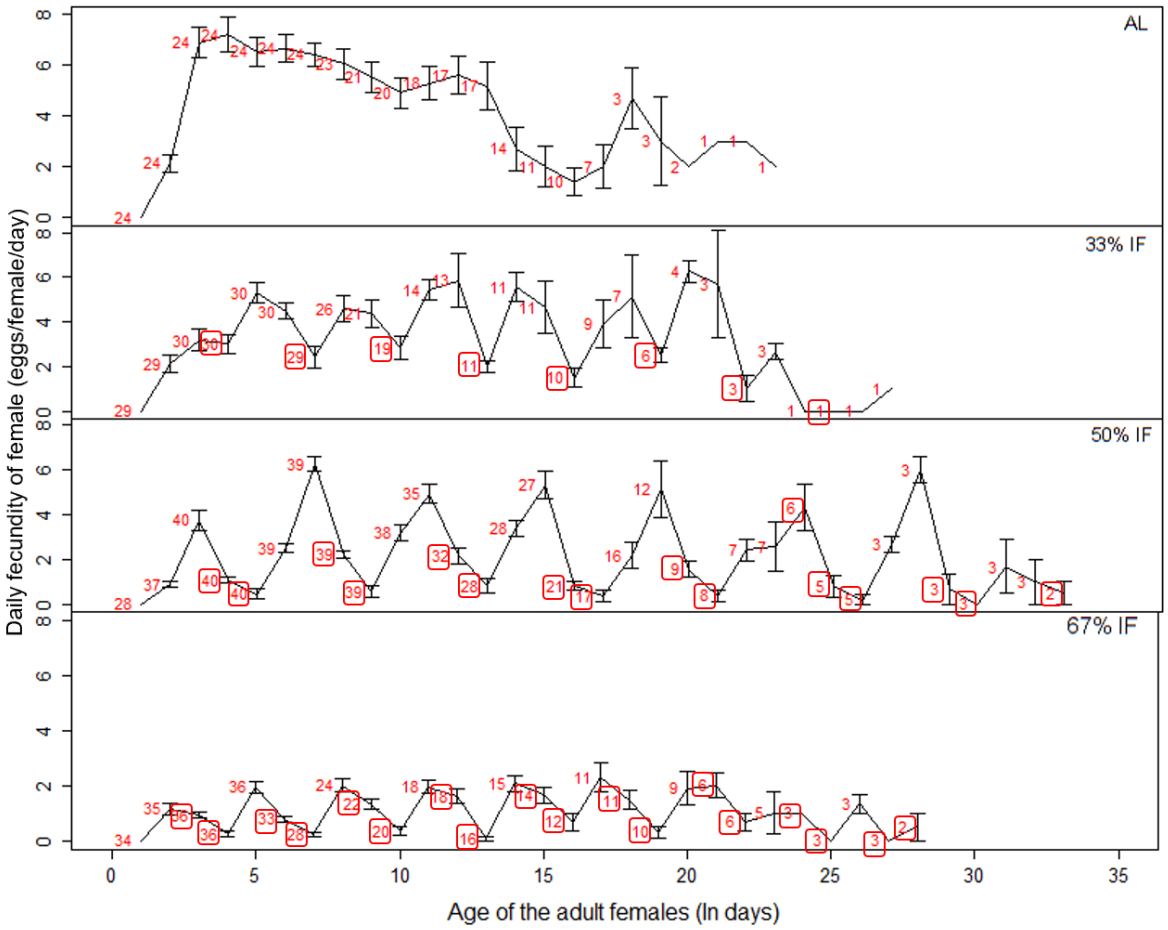

FIGURE 3. 3 The average daily fecundity (Mean± SE) of adult females feeding on the four different dietary regimes: *ad libitum*, 33% IF, 50% IF, and 67% IF. The red numbers beside the line denote the sample size of each data point, and these numbers within boxes indicated the number of mites on starvation. The sample size increased in the earlier stage with more and more females starting to lay eggs, but in the later stage it decreased with the death of some females.

The ANCOVA analysis indicated that both dietary regimes ($F_{3,106}$=69.91, P<0.000) and longevity ($F_{1,106}$=130.73, P<0.000) have significant effects on the lifetime fecundity, and their interaction is significant ($F_{3,106}$=11.97, P<0.000; Figure 3.4). The lifetime fecundity and adult lifespan of females were positively related when the females are pooled together regardless of food regimes ($F_{1,112}$=45.94, R^2=0.2845, P<0.000). When food regimes were taken into consideration, and the correlation were checked independently across the four

feeding regimes, there was also a significant positive correlation between these two parameters for each treatment (*ad libitum* ($F_{1,21}$=15.75, R^2=0.4014, P=0.001), 33% IF ($F_{1,22}$=175.6, R^2=0.883, P= 0.000), 50% IF ($F_{1,35}$=108.9, R^2=0.749, P= 0.000), and 67% IF ($F_{1,28}$=107.3, R^2=0.785, P=0.000); Figure 3.4).

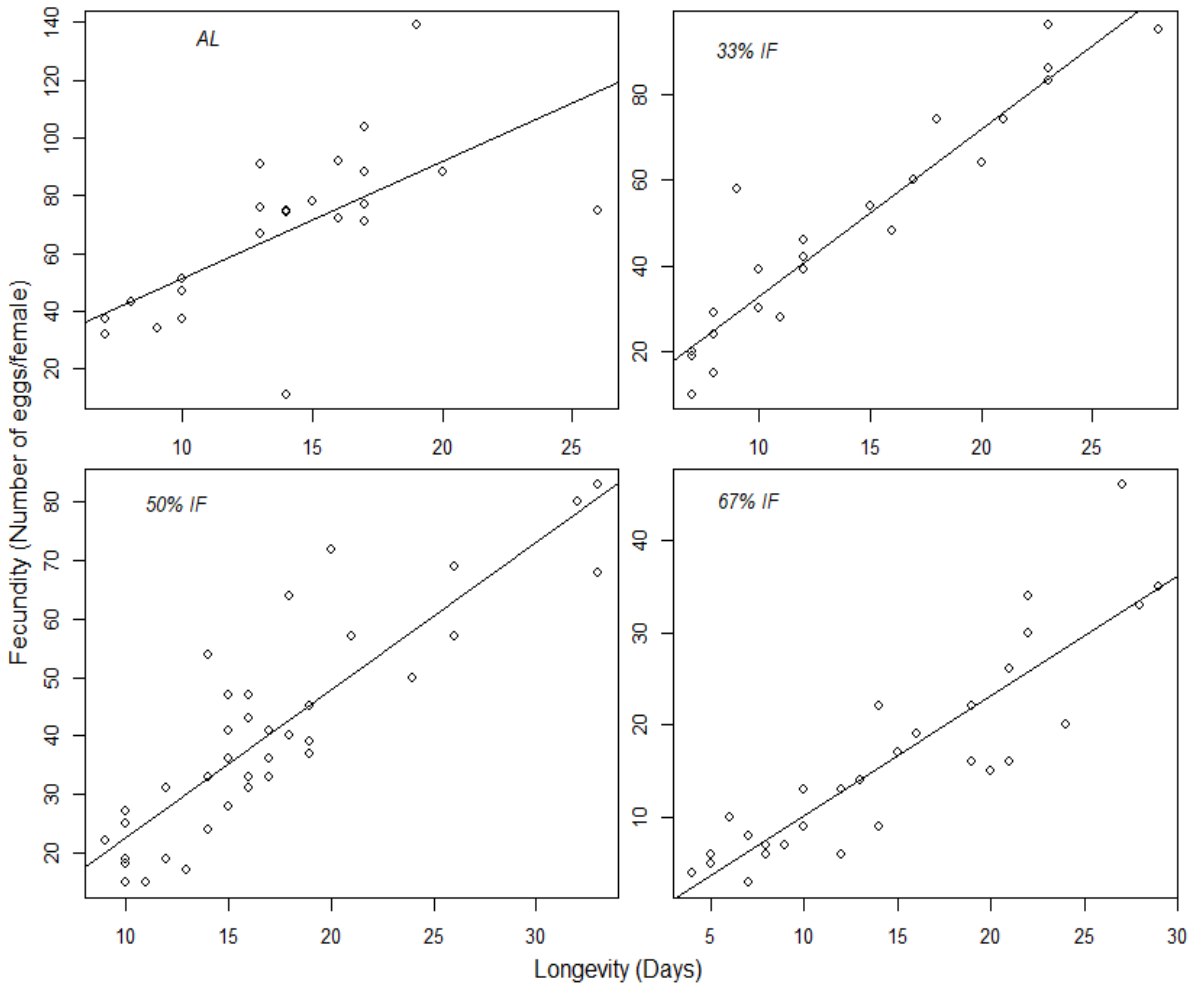

FIGURE 3. 4 Correlations between female adult life span and lifetime fecundity for spider mites fed at four different feeding regimes. Note that axis scales are different for each treatment.

3.4 Discussion

With the emerging evidence indicating that dietary restriction is an efficient protocol extending healthy lifespan, more and more dietary regimens are being explored because the daily food restriction is difficult to implement in practice (Anson *et al.* 2005; Caramoci *et al.*

2016; Wang *et al.* 2017). In this study, I examined the effects of IF, an alternative to DR, on the fitness of spider mites. The male and female spider mites that experienced different levels of IF showed distinct responses. Specifically, the females fed at a moderate level of IF (50% IF) lived longer than the *ad libitum* fed spider mites with a lower lifetime reproduction and older age at the maximum reproductive rate, while males showed a decrease in lifespan with increasing levels of IF. Although between treatments longer lifespan was associated with lower fecundity and delayed onset of maximum reproductive potential, the fecundity showed a positive correlation with the lifespan in female spider mites within each treatment.

3.4.1 Sex difference in lifespan and response to IF

The males and females demonstrated a sex-specific response to different levels of IF. With the increased level of fasting, females showed extended lifespan at 50% IF but these declined at higher levels of IF. However, fasting males exhibited consistently shorter lifespan compared with the control at all levels of IF. This is the first report demonstrating a sex difference in response to IF, mainly because it has not been frequently previously tested in studies. One possible explanation for the sex-specific response to IF is differences in nutrient reserve and resource allocation. Females can store much more nutrients and energy and thus compensate for brief periods of food deprivation. This is supported by the fact that females are more resilient to starvation when food is limited, as shown in our previous study and some other research (Finiguerra *et al.* 2013; Chippindale *et al.* 1996, Li & Zhang 2018). Moreover, from our daily observation of female fecundity, it was clearly shown that when there was no access to food, females could reduce reproduction on the first day and even stop reproduction on the following fasting day. This may indicate that females can divert the limited resource from reproduction to somatic maintenance (Weithoff 2007). Males are more nutrient-limited than females and their reproductive performance is also less likely to be influenced by diet. The key energy source involved is likely to be lipid storage and it would be worth measuring this in these mites.

Another potential factor is sex differences in gene expression when experiencing dietary intervention. Although the females and males have similar genomes, they vary in gene

expression, which can possibly generate sex-specific differences (Tower 2017). This has been tested in *Caenorhabditis elegans*, which is a model organism that displayed lifespan extension in response to DR. Under dietary intervention, the hermaphrodites and males differed in expression levels of gene encoding key enzymes involved in carbohydrate metabolism (Tower 2017). The males have approximately twice as many sex-specific gene expressions than do the hermaphrodites, and 35% of all C-type lectin genes have higher expression levels in males (Miersch & Döring 2012). Understanding the physiological difference associated with gene expression variation between sexes may be crucial for understanding the mechanism of sex-specific aging. Additionally, another study with *C. elegans* indicated that the longevity advantage of hermaphrodites over males was influenced by the terminal transcription factor (TRA-1) of the nematode sex-determination pathway, which can activate the expression of daf-16/ FOXO to regulate development and lifespan (Hotzi *et al.* 2018). However, whether the protein TRA-1 is evolutionary conserved and functions in other species warrants further investigation.

3.4.2 Level-dependent response to IF

In most empirical studies on dietary intervention, the animals are only treated with one level of IF with controls fed *ad libitum*. The protocols of IF applied are usually borrowed from other studies without being adjusted to the species involved. This may explain why there is a disparity in results from different studies. For example, alternative day feeding regimes can be effective in extending the lifespan of some rotifers like *Brachionus plicatilis* (Kaneko *et al.* 2011), but it was reported to be less effective or even detrimental to other species of rotifers such as *Cephalodella spp.* and *E. worallii* (Weithoff 2007). Moreover, the fruit fly *Drosophila melanogaster* experienced starvation for 3 hours and 6 hours per day failed to show longevity extension (Grandison *et al.* 2009). In contrast, female fruit fly benefited with a longer lifespan when starved for 5 days per week in the early adult stage. The males, which was more starvation sensitive, also significantly increased lifespan when fasted for 4 consecutive days per week (Catterson *et al.* 2018). These studies suggested that the effects of intermittent fasting were duration and sex-dependent. Therefore, as discussed above, a well-

designed fasting regime considering factors such as sex and their difference in starvation tolerance is of importance in further exploring the influence of IF on health and lifespan.

3.4.3 Relationship between lifespan and reproduction

Most previous research on intermittent fasting only concerns survival and lifespan, and there have been few attempted to explore its potential influence on reproduction. In this study, I assayed the reproductive success of spider mites on different food-availability regimes and the relationship between longevity and reproduction within and between treatments. A positive linear correlation was found between longevity and lifetime fecundity. The low-quality females died early with the fewer number of offspring, whereas the high-quality females lived longer and produced more eggs. Consequently, the high individual heterogeneity in resource acquisition masked the entailed cost of reproduction. However, across treatments, the females under IF showed a significant decrease in their fecundity with increasing levels of fasting, which is consistent with findings on rotifer *E. worallii* (Weithoff 2007). But among all the investigated levels of food regime, the lifespan of females reached a peak at 50% IF, and decreased at greater levels of IF. Clearly, the females reduced daily fecundity and even ceased reproduction during food deprivation. This indicated that under moderate resource limitation (50% IF) the spider mite diverted the energy and nutrient from reproduction to self-maintenance, in line with the leading hypothesis that longevity extension under fasting is an adaptive plastic response of animals reallocating resource from reproduction to somatic. Furthermore, the long-lived females at 50% IF also demonstrated an older age for achieving maximum reproductive rate, suggesting food restriction retarded the pace of reproduction in comparison with the shorter-lived counterparts fed *ad libitum*. Collectively, despite the individual heterogeneity masked trade-off between survival and reproduction within each population, a clear trade-off was demonstrated among treatments, whereby the spider mites fed *ad libitum* with abundant resource showed highest lifetime reproduction with a shorter lifespan while females at 50% IF with limited resource prolonged lifespan at the cost of reproductive success.

3.5 Conclusions

In this study, I extended previous work in the field of IF by taking the sex of subjects and levels of fasting into consideration, representing the first experimental evidence that the response of lifespan to IF is sex and IF-level dependent. First, the study highlights the fact that females and males differently sensitivity to starvation and fasting; accordingly, they differed in their patterns of aging when exposed to a series of IF regimes. Other animals with sexual dimorphism may also have a sex-specific response to IF. Second, the reproduction of females decreased across all IF treatments, but lifespan extension was demonstrated in females at intermediate fasting level 50% IF. The females at 50% IF with longer lifespan showed decreased lifetime reproductive success and older age at MRR, which suggests there are substantial costs in reproductive fitness for life span extension in females. Additionally, although costs of life span extension are well documented in females, few observations have been reported in males, possibly owing to the protocol challenge of measuring the reproductive efforts of males. Further work revealing the differences in the relationship between lifespan and reproduction between sexes may give insights into the underlying mechanism of sex-specific response to diet intervention.

Chapter 4

The costs of social interaction on survival and reproduction in haploid-diploid spider mite

Abstract:

The social environment (especially interactions with other conspecifics) experienced by animals is a key factor influencing their behavior and even fitness. Traditionally intersexual interaction has been the focus, whereas the costs of intrasexual interaction has been little studied. In this study, I determined the influence of both intersexual and intrasexual interaction on the survival of both sexes in an arrhenotokous spider mite (*Tetranychus urticae*). I also determined the reproductive performance of females in different social environments. Our results show that for both sexes, isolated individuals outlived their counterparts when compared with paired conspecifics, regardless of sex, indicating that the cost of interaction with the same sex and the opposite sex has consequences for fitness. Moreover, the female involved in intrasexual interaction showed reduced lifetime fecundity. These findings underline the cost of social interactions, regardless of sex, influencing the lifespan and reproduction of organisms. Our study highlights that both intrasexual and intersexual interactions can be important determinants in shaping the fitness of animals.

Keywords: spider mite, social environment, longevity, fecundity

4.1 Introduction

The social environment experienced by animals is one of the potential detrimental biotic factors influencing their behaviour and physiology across a diverse range of taxa (West *et al.* 2003; Ruan & Wu 2008; Schausberger *et al.* 2017). This occurs directly by physical contact and indirectly through cues such as chemical cues and auditory stimuli. For example, *Drosophila melanogaster* females exposed to a male-biased population showed a shorter lifespan as consequences of sexual harassment and increased reproduction (Chapman *et al.* 1995). Without physical contact, the males of *Ephestia kuehniella* (Esfandi *et al.* 2015) perceived auditory stimuli from additional females exhibited intense sexually flirtatious behaviour by increasing win fanning duration. The social interaction with conspecifics is now becoming recognized as a key factor modulating life-history traits, particularly in eusocial animals (Boulay *et al.* 1999; Koto *et al.* 2015).

Numerous animals in nature live in groups and have frequent interaction with their conspecifics. Their complex and well-organized social networks are crucial for the survival of these animals. The influences of the social environment have been studied in social animals ranging from ants and honeybee to monkey and human (eg., Holt-Lunstad *et al.* 2010; Gluck & Sackett 1976, Heinze & Walter 2010; Maleszka *et al.*, 2009, Alberts, 2018). Social isolation can be extremely stressful and induce health-related problems (Umberson & Montez 2010; Venna & McCullough 2015), particularly for eusocial insects. For example, social isolation impaired the development of mushroom bodies in ant *C. floridanus*, structures responsible for learning and memory, maintaining the large social networks as well as task allocation (Seid & Junge 2016). Furthermore, the chronic social isolation was associated with increasing vulnerability to diseases such as cardiovascular disorders (Rozanski *et al.* 1999), cerebrovascular disorders (Karelina *et al.* 2009; Venna *et al.* 2014), obesity, type II diabetes (Nonogaki *et al.* 2007) and high risk of mortality, leading to reduction in healthy lifespan of humans.

The profound effects of the social environment documented in social animals may also occur in solitary insects. This assumption has already been proved in insects such as fly *Drosophila* mutants, cricket *Gryllus bimaculatus* and cockroach *Blattella germanica*, mosquito *Aedes aegypti* and others (Ruan & Wu 2008; Kuriwada 2016; Uzsák & Schal 2013; Reiter 2007), although for these species less behavioral interactions were observed between individuals. For some insects, the social experience has been reported to shape developmental rate the reproductive behaviour, time to sexual maturation and animal personalities (Lihoreau & Rivault 2008; Ronnas *et al.* 2010; Holbrook *et al.* 2000; Uzsák & Schal 2012, Schausberger *et al.* 2017). A striking example is the German cockroach *Blattella germanica*. Females started to reproduce much earlier in groups, whereas isolated females showed slower oocyte maturation and later onset of sexual receptivity (Gadot *et al.* 1989a; Lihoreau & Rivault 2008; Uzsák & Schal 2013). Although it is generally accepted that social interaction might have a profound influence on the animals, our understanding of how the long-term fitness of animals is shaped by the presence of conspecifics is still incomplete.

Social experience also can influence the oviposition decision and survival of organisms (Prokopy & Reynolds 1998; Ruan & Wu 2008). The effects of intersexual interaction on male and female fitness have been extensively studied across taxa, whereas little is known about the fitness consequences of intrasexual interaction. Intersexual interaction is a great cost for both males and females, mainly due to the cost of reproduction (Cordts & Partridge 1996). During mating, males demand lots of time and energy for courtship and production of sperm and seminal fluid while females invest energy and nutrients for mating and egg production (Scharf *et al.* 2013; De Loof 2011). In comparison, the intrasexual interaction was less obvious and assumed to entail negligible cost. A few studies have demonstrated that in insects with sexual reproductive systems including carrion fly *Prochyliza xanthostoma*, seed beetle *Callosobruchus maculatus* and wasp *Psyttalia concolor*, the influence of intersexual interaction was more pronounced than that of intrasexual interaction, particularly in females (Maklakov & Bonduriansky 2009; Stojković *et al.* 2010; Benelli *et al.* 2013). Although the possible fitness consequences of intrasexual interaction have been studied in several species of insects, no information is available for spider mites *Tetranychus urticae* Koch. Moreover,

these reported studies only focused on traits such as survival and lifespan, largely ignoring the reproductive traits which are directly coupled with fitness.

Here I investigated how social context affected the longevity and reproduction of arrhenotokous *Tetranychus urticae*. As a group-living arthropod, *Tetranychus urticae* has evolved the capability to detect the presence of conspecifics, as shown by studies that spider mite can discriminate between familiar and unfamiliar females (Yoshioka & Yano 2014), and perceive predation risk from their dead conspecifics (Grostal & Dicke 1999). The female spider mite can reproduce both sexually and asexually (Tehri 2014). The special reproductive mode of the female provides an opportunity to test how the interaction between conspecifics, for example, intrasexual interaction and intersexual interaction, influence the life history traits of individuals in comparison with social isolation when the difference in reproductive cost is taken into consideration. It was hypothesized that the lifetime fecundity of the females involved in intersexual interaction would differ significantly when females are reared together with other females or in isolation. I predicted that the presence of conspecifics could exert a pronounced detrimental influence on the survival of each individual, regardless of their sex, and tested this prediction by comparing the survival and lifespan of mites among those being isolated, reared with the same sex, and reared with the opposite sex.

4.2 Methods and materials

4.2.1 Mite colony

The spider mite originated from Bioforce Ltd, Auckland, New Zealand, and a population was established in the greenhouse of Landcare Research, Auckland, New Zealand in February 2015 (Li & Zhang 2016). The mites were reared on young potted red kidney bean plants (*Phaseolus vulgaris L.*) in a plexiglass box opening on the top. The plexiglass box was put into a much larger box with water to prevent mite dispersal. The climate condition of the greenhouse was maintained at 20 ± 5 °C with a relative humidity of 60 ± 10% and the natural photoperiod in all seasons except for winters with a cycle of 16 h light: 8 h dark. The

population was supplied with fresh young clean beans regularly when the old plants became heavily infested.

4.2.2 Host plant

The host plant provided for the spider mite in the study was the red kidney bean (*Phaseolus vulgaris L.*), one of its favourite host plants (Li and Zhang, in preparation). Another important reason for selecting this plant is that it grows fast and can reach two leaf stages in the greenhouse in about 4 weeks. The bean plants were grown in earth box (120 m L×20 cm W × 30 cm H) with the potting mix at the density of 48 plants in each box. These plants were water regularly, but no fertilization was applied. The earth box was caged to protect the plants from being infested by any insects. These plants were provided with similar climate conditions as the mite population (20±5 °C, 60± 10% RH and the natural photoperiod in all seasons except for winters with a cycle of 16 h light: 8 h dark).

4.2.3 Rearing unit

The mite individuals were reared with detached bean leaf discs. In traditional methods, the leaf discs were placed on cotton pad or foam cub, with their edges surrounded by strips of wet cotton or tissue paper to prevent the escape of the spider mites. There is high mortality as the mites may be trapped on the wet cotton or the tissue paper. In this experiment, I utilized the 24-well cell culture (well volume 3.4 ml, diameter 15.6 mm) plate as the rearing equipment. I first cut the leaf into small discs (8mm diameters) with a puncher, and then placed one leaf disc into each cell of the plate which had been filled with 3.2 ml of tap water. These leaf discs floated on the water and were kept fresh for more than one week and even longer. The isolated leaf disc island in the water served as rearing unit for each mite.

4.2.4 Experimental protocol

The adult males and females just moulting from their last immature stages were used to set up the experiment. To prepare adequate numbers of females and males of the same age, I take

advantage of the fact that the offspring produced by inseminated mothers are mostly females while the offspring of un-inseminated mothers are all males. I collected females and males in their last moulting stages from the stock population. Once moulted, half of females were paired with males and a half left unpaired; both groups of females were allowed to produce eggs on the fifth day for 24 hours. Later the females were discarded and the collected eggs were left to develop into adults. In their deutonymphal stages, the mites were transferred to new leaf discs, one mite per unit to prevent them from mating. These females and males were used in the following experiments.

In this experiment, the spider mites were exposed to three kinds of social environment: i. Isolated, ii. same-sex together, iii. male and female together. In the first treatment, mites were isolated, the male (N=48) and female (N=48) kept individually on the kidney leaf disk all their life in adult stages until death. In the second treatment mites exposed to same sex, either two males (N=24) or two females (N= 24) grouped together on each leaf arena. If one mite in a replicate died, the remaining one was then grouped together with another lone individual (male-male or female-female). In the third treatment, one female was paired with one male (48 replicates) and a similar protocol followed when individuals died. During the experiment, the survival of mites was checked daily, and the leaf disc replaced with a fresh one at every 3 or 4 days. In addition, I recorded the first day each female produced eggs, the number of eggs produced daily during the reproductive stage and the day last egg was produced. By doing this, I can obtain the reproductive parameters for females in all treatments. The experiment was conducted in a humidity-controlled cabinet (60±10% RH) in a room with a constant temperature of 25±2 °C and 12:12 light and dark cycle.

4.2.5 Statistics

To examine the survival difference between female and male mites in the three treatments, I compared the survival curves of mites using Cox proportional hazard regression model with sex and social context as factors. The model is significant as indicated by the overall test (Likelihood ratio test = 45.96, df = 2, P < 0.00). In the multivariate Cox analysis, sex showed a remarkable effect on survival compared with the social environment. To further explore the

effects of social context, I analyzed the survival curve separately for females and males using the Kaplan-Meier method with the log-rank test. The differences between the sexes were also tested across treatments. To clarify the survival differences between the three treatments, a pairwise comparison was conducted for females and males respectively. R packages "survival" Version 2.41-3 and "survmine" Version 0.4.0 were employed to performed survival analysis.

The lifespan of mites was compared using two-way ANOVA with social context and sex as main factors; their interaction was also checked. The reproductive parameters including the pre-oviposition period, oviposition period, post-oviposition, daily fecundity and lifetime fecundity were first checked for their normality. Those not meeting the normality assumption were log-transformed before performing one-way ANOVA. All the statistical analyses were performed in R version 3.4.1 (R Development Core Team 2017).

4.3 Results

4.3.1 Survival rates of males and females in different social contexts

The female spider mite *T. urticae* differed significantly in survival rates (χ^2=18.2, *P*= 0.000) when exposed to different social environments (Figure 4.1A). The intersexual interaction showed higher costs to female longevity with females paired with males having lower survival rates compared with isolated females (χ^2=15.6, *P*< 0.000; Figure 4.1A), and females reared together with the same sex (χ^2=6.8, *P*< 0.009; Figure 4.1A). Females in the intrasexual treatment also had lower survival rates compared with isolated females (χ^2=4.3, *P*< 0.037, Figure 4.1A), suggesting that intrasexual interaction has a similarly negative effect on female's survival.

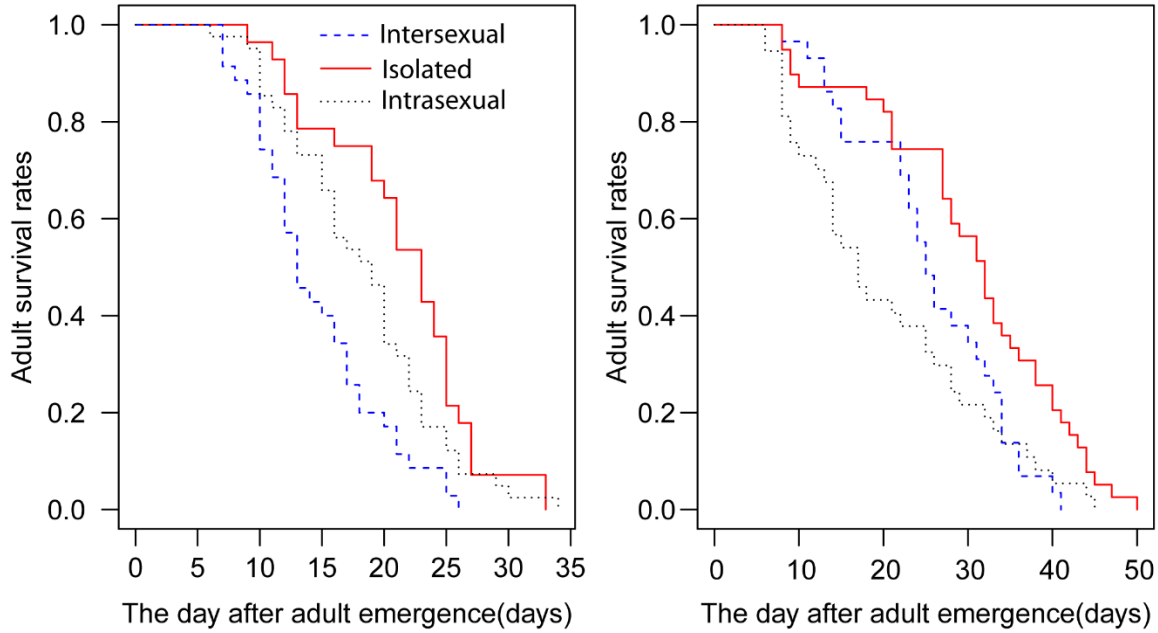

FIGURE 4. 1 Survival plots of female (A) and male (B) spider mites *Tetranychus urticae* in three different social contexts: isolated, reared with same-sex, with different sex. The solid red lines denote female or male being isolated. The black dotted lines denote female or male interaction with the same sex. The blue dash lines denote female and male reared together with the opposite sex.

Males in the three different social environments also exhibited different patterns of survival rates (χ^2=14.4, *df* =2, *P*= 0.001; Figure 4.1B). However, in contrast to females, the survival cost for males paired with females was only marginally significant when compared with isolated males (χ^2=3.7, *d f*=1, *P*< 0.053; Figure 4.1A). However, the effect of intrasexual interaction was less in males. Both the isolated males (χ^2=12.5, *df* =1, *P*< 0.000; Figure 4.1A) and the males paired with females (χ^2=4.1, *df*=1, *P*< 0.044; Figure 4.1A) has had significantly lower survival rates than those in intrasexual treatment.

The results further indicate that there is a strong sex difference in survival between male and female with males outliving the females when they were isolated (χ^2=15, *df*=1, *P*< 0.000) or involved in the intrasexual interaction (χ^2=23, *df*=1, *P*< 0.000). With intrasexual interaction, the survival difference between sexes narrowed (χ^2=0, *P*< 0.936).

4.3.2 Adult lifespan of males and females

There was a significant difference in adult lifespan in response to gender (F=39.097, P<0.00; Figure 4.2) and social contexts (F=11.80, P<0.00 Figure 4.2). Both factors have an interaction effect on the lifespan of mites (F=5.063, P<0.007; Figure 4.2). Specifically, for females, a dramatic decrease in adult lifespan was observed when they were exposed to heterosexual interaction (females: Isolated=21.32days, intrasexual=18.26 days, intersexual=14.51days) while for the males the shortest lifespan was shown when they experienced intrasexual interaction (males: Isolated=30.23 days, intersexual=25.44 days, intersexual=20.32 days; Figure 4.2).

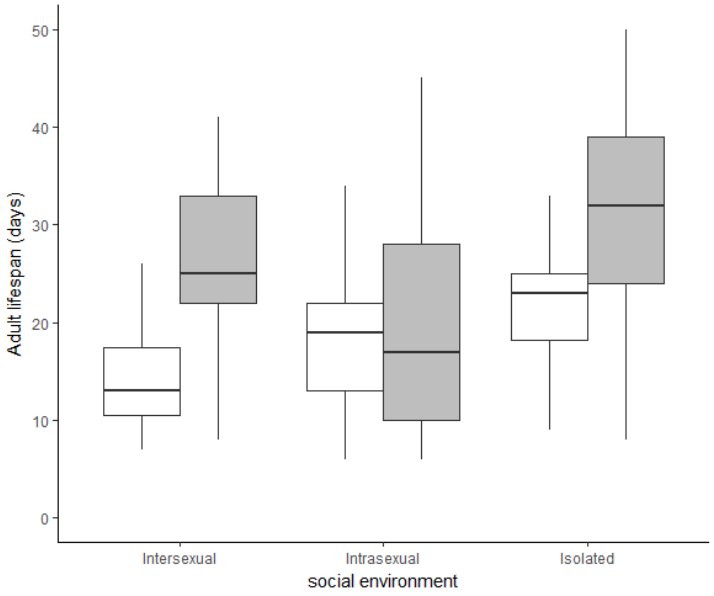

FIGURE 4. 2 Box plot of female and male adult lifespan in three different social contexts: isolated, reared with same-sex, with different sex, with the white box representing the females and the grey box representing the males. The bold lines cross the box indicate the Means.

4.3.3 Reproductive parameters of females

The social environment had a significant influence on the reproductive parameters of females, particularly reproductive lifespan and reproductive rate. When a female co-existed with a male its reproductive lifespan decreased significantly by 5 and 4 days respectively,

compared with isolated females and females housed with the same sex ($F_{2,102}$=6.776, P= 0.002; Figure 4.3). However, the pre-oviposition period ($F_{2,102}$=0.041, P=0.960; ; Figure 4.3) and the post-oviposition period ($F_{2,102}$=0.771, P=0.469; Figure 4.3) were not significantly different between these three treatments (Figure 4.3). The daily fecundity of a female housed with another female was significantly lower by 20% compared with an isolated female and a female paired with a male ($F_{2,102}$=18.331, P<0.000; Figure 4.4). Lifetime fecundity was similar in intersexual and intrasexual treatments—both were significantly lower than those of isolated females (27.6% and 20.5% for females involved in the intersexual and intrasexual interaction, respectively) ($F_{2,102}$=7.141, P<0.001; Figure 4.4).

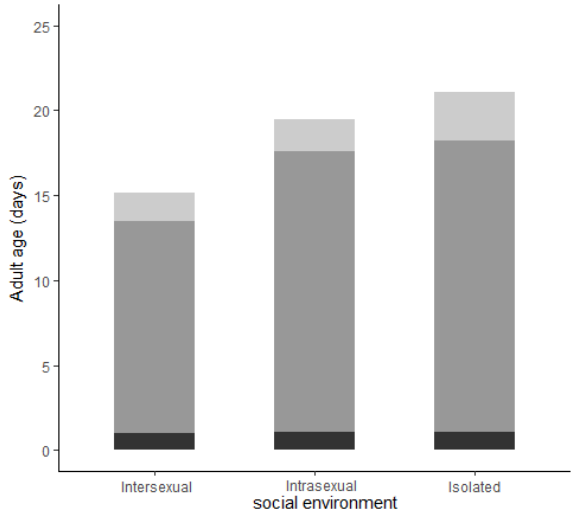

FIGURE 4. 3 The reproductive lifespan of female spider mites *Tetranychus urticae* in three different social environments. The black, dark grey and light grey regions denote pre-reproductive lifespan, reproductive lifespan and post-reproductive lifespan.

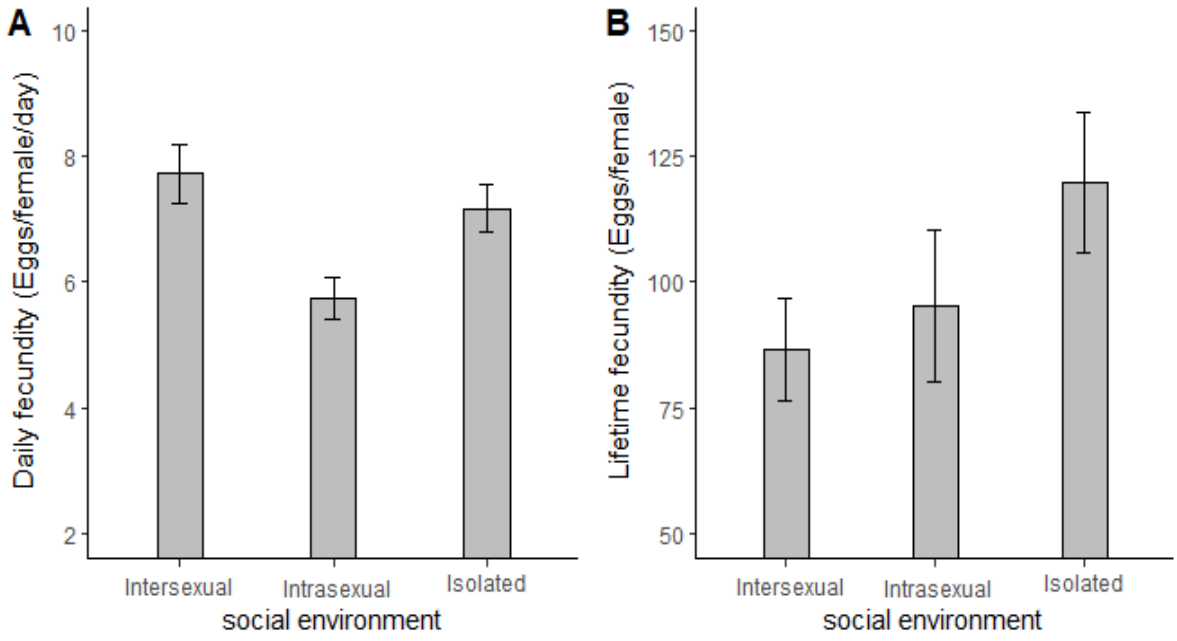

FIGURE 4. 4 Daily reproductive rates and lifetime fecundity of female spider mites *Tetranychus urticae* in three different social environments.

4.4 Discussion

In this study spider mites reproducing asexually and sexually were used to investigate the fitness consequence of intrasexual and intersexual interaction. Our results showed that for both sexes, isolated individuals outlived those paired with conspecifics regardless of sex, indicating that the cost of interaction with the same-sex and opposite-sex is significant. Additionally, there is a sex-specific response in survival in different social environments with females in the intersexual treatment showing a more significant reduction in lifespan, while the males suffered more from the intrasexual interaction.

4.4.1 Influence of intersexual interaction on female and male

In spider mites, the cost of heterosexual interactions incurred a high cost to females, but not males. The females exposed to intersexual interaction showed a lower survival rate, with their lifespan reduced by 32.3% compared with isolated counterparts. These findings are inconsistent with results from sexually reproductive insects such as carrion fly *Prochyliza*

xanthostoma (Maklakov & Bonduriansky 2009). Results from these studies are surprising because it is well known that sexual interaction is energetically costly and time consuming for females. The lifespan of females was reduced possibly because of the high investment in egg production, which can be many times their own body in volume (Mitchell 1973). Additionally, the females experienced frequent sexual harassment from their partners (Li personal observation). It also takes energy and time for females to avoid harmful behavioural interactions with males which can reduce food and resource intake, as has been reported in other insects (Magurran & Seghers 1994; Arrington *et al.* 2009; Ojanguren & Magurran 2007).

For the males, the cost of intersexual interaction is insignificant and not comparable with that of the females. Similar results were also observed in the previous study with this spider mite (Chapter 5), in which both males housed together with a female for periods from only one day to throughout their life showed no significant difference in lifespan with the virgin male. These results suggested that for males the cost of intersexual interaction appears to be negligible for the fitness parameters measured.

4.4.2 Influence of intrasexual interaction on male and female

Intrasexual interaction exerted a profound influence on male survival and decreased the lifespan by 33%; similar results were also shown in previous studies with insect species such as beetles, flies and wasps (Maklakov & Bonduriansky 2009; Stojković *et al.* 2010; Benelli *et al.* 2013). In these studies, males exposed to males demonstrate mimic sexual behaviours such as display, chase and mounting, contributing to the cost of intrasexual interaction. However, I have not observed similar behaviour in spider mites. Fierce combat between males might explain the consequences of intrasexual interaction among mites. Intense aggressive behaviour includes fighting with forelegs and mouthparts or trapping them in defensive webbing, which can lead to injury and may even result in early death (Potter *et al.* 1976; Oku 2014).

The entailed cost of intrasexual interaction is much higher than the intersexual interaction. For example, males housed with a rival had a lifespan on average five days shorter than males housed with females. Similar results were shown in previous studies with fruit fly *Drosophila melanogaster* (Bretman *et al.* 2013), in which both the virgin and mated females exposed to rivals had a lower survival rate compared with mated males with no rival. This suggests that mating and sexual harassment with females is not costly, but the price of interaction with a competitor is high.

Isolated female spider mites lived longer than those paired with another female in our experiment (Figure 2). This is consistent with some previous reports with fruit fly *Drosophila melanogaster ((*Leech *et al.*, 2017) and seed beetle *Callosobruchus maculatus* (Maklakov & Bonduriansky 2009) but not with another insect such as the waltzing fly *Prochyliza xanthostoma* (Maklakov & Bonduriansky 2009). The disparity in these studies reflects methodological differences among them. The animals including mites, fruit flies and seed beetles were kept in groups of two to investigate the cost of intrasexual interaction, but the negative effects disappeared when the group size increased to ten for the waltzing fly. Probably the benefits of larger group size weakened interaction strength and outweighed the cost of intrasexual interaction. This is supported by the evidence that spider *Stegodyphus dumicola* showed increased feeding efficiency and lower mass loss during desiccation with group size increased from one to five and higher (Vanthournout *et al.* 2016). Further research on the effects of the social environment may be required to take the group size into account.

4.4.3 Influence of social environment on the reproductive performance of females

The reproductive performance of females was compared across social contexts. I found that isolated females produced higher numbers of eggs than females companioned with conspecifics, regardless of sex. Our results were consistent with other reports showing that females engaged in intersexual interaction lay fewer eggs than isolated females, owing to the cost of sexual harassment (Campbell 2005; Maklakov *et al.* 2005) This may also result from

the different investment of mothers into fertilized eggs and unfertilized eggs. However, these results conflict with a previous study investigating group-effects on the fecundity of female spider mites, which found no difference between fecundity of isolated virgin females and two virgin females cohabitated in a five-day study (Le Goff *et al.* 2010). However, there was a significant difference in daily egg production between these studies. In this study, the daily egg number per female ranged from 5-7, about 3 to 5 times that reported in Le Goff *et al.* (2010). Possibly females with high fecundity in my study suppressed their reproduction as a strategy to reduce offspring competition, supported by the fact that females reduced the number of eggs laid in response to increasing leaf damage with time (Kant *et al.* 2004).

Although empirical evidence suggesting social environment plays a crucial role in determining the fitness of individuals, and the intrasexual interaction is substantial, the underlying mechanism by which intrasexual interaction shapes the fitness of animals is yet to be fully understood. Many behavioural and physiological responses to intrasexual interaction can cause noticeable life history changes. For example, the social environment has a significant influence on the efficiency of digestion. In a study with ants, the grouped ants were shown to be more efficient in food digestion and lived longer than the isolated ones. When both were starved, the difference in lifespan was reduced (Koto *et al.* 2015). However, the response of solitary animals differed apparently from that of social organisms to similar social context, presumably because of their distinct differences in social structure and life history characteristics. Whether or not they share a similar mechanism by which intrasexual interaction affects the fitness of eusocial animals deserves further exploration.

4.5 Conclusion

In conclusion, this study highlighted that social environment can have major effects on the lifespan of solitary animals and provides evidence that the influence of intrasexual interaction may be comparable to that of intersexual interaction indicating that this comparison needs more attention in the future. Clearly, I only tested the group effect with only two individuals involved in each replicate; however, in natural conditions, the social environment can be much more complicated in terms of group density, the identity of the involved individual, and

resource availability. How these factors interplay and shape the fitness of organisms needs to be further examined.

Chapter 5

Sex differences in lifespan and fecundity of *Tetranychus urticae* (Acari: Tetranychidae) in response to delayed and repeated mating

Abstract

Sexual interaction is an important activity that determines the reproductive schedule of organisms and ultimately influences the fitness traits of both sexes. To understand how mating regimes (timing and frequency) modulate the fitness of males and females, I used spider mite (*Tetranychus urticae*) to investigate the influence of delayed mating and repeated mating on longevity and reproduction. The unmated and the delayed mating females outlived those mated immediately after the adult emergence. The repeated mating shortened lifespan of females mated at 1-day-old, but not those that mated as 7-day-old. However, males showed no detectable changes in lifespan when engaged in different mating regimes. I found although delayed mating significantly decreased the daily reproductive rate of the females, there was no significant difference in lifetime reproduction of females across treatments because of the delayed mating females increased their reproductive lifespan as a compensation. Our study demonstrated that the time and frequency of sexual interaction can have different fitness consequence on male and female spider mites. Sexual interaction incurs a higher cost to females which have a much lower optimal mating frequency than males.

Keywords *T. urticae*; Delayed mating; Repeated mating; Longevity; Reproduction; Sex-specific plasticity

5.1 Introduction

The sexual interaction among organisms plays a crucial role in determining resource allocations and lifetime fecundity (Holt-Lunstad *et al.* 2010; Amdam 2011). An individual can influence its partner directly by sexual interaction or indirectly through sexual pheromones (Shi & Murphy 2014; Gendron *et al.* 2014), especially for sexually matured individuals which may engage in frequent sexual interaction with the opposite sex. For example, males can trigger the earlier onset of female reproduction by transferring seminal fluids and sperm to the female (Gromko *et al.* 1984; Opp & Propopky 1986) and thereby accelerate egg production. This may increase the aging rate and shorten the lifespan of the female.

The cost of reproduction is mainly derived from the different interests of the male and female in the sexual interaction, termed sexual conflict (Arnqvist & Rowe 2005). It arises when the female and male have a different optimal schedule of reproduction and mating frequency. Males and females sometimes employ different strategies to increase their own fitness and realize maximum reproductive success at the expense of the opposite sex (Chapman *et al.* 2001). Often males gain greater reproductive success through mating early. Hence it is stated that selection favours males that "live fast, die young" because they sacrifice longevity for mating opportunities (Vinogradov 1998; Carranza & Pérez-Barbería 2007; Bonduriansky *et al.* 2008). This idea is supported by the evidence that males reduced longevity when invested intensively in seeking partners early in life to gain advantages over the other competitors using secondary sexual trait expression (Prowse & Partridge 1997; Bonduriansky & Brassil 2005). The males also increase early reproductive rates of their partners and reduce their sexual receptivity after mating (Arnqvist & Rowe 2005) to ensure paternity. This is known for animals where one successful mating is enough for female lifetime fertilisation and first fertilisation is advantageous in sperm competition. However, the earlier onset of offspring production can reduce future reproduction and survivorship of females (Chapman *et al.* 2001). Males may also be eager to mate repeatedly to sire as many as offspring as possible to pass their genes to the next generation. In contrast, it is possible for females to maximize their

reproductive success by only one mating or a few matings (Walker 1980; Arnqvist & Nilsson 2000), because there is a limitation on the number of eggs females can produce and a single mating may be sufficient to fertilize them all. The excessive mating attempts from males towards females can therefore be considered sexual harassment (Arnqvist & Rowe 2005).

The cost of social interactions during reproduction depends on the stage at which they occur: courtship/sexual harassment, copulation, egg production, and parental care (Kotiaho & Simmons 2003; Scharf *et al.* 2013). The cost associated with egg production is the most frequently addressed because of the well-established methodology for measuring the female fecundity. Generally, females experience most of the costs associated with reproduction (Partridge *et al.* 1987; Chapman *et al.* 1998). There are also considerable costs associated with courtship, sexual harassment and copulation owing to the increased risk of physical injury, predation, sexually transmitted diseases and the reduced time for other activities such as feeding and oviposition (Rowe 1992; Cordts & Partridge 1996; Reale *et al.* 1996; Thrall *et al.* 2000; Shine *et al.* 2000; Sakurai & Kasuya 2008). Finally, parental care also requires energy. For example, a study with dung beetles *Onthophagus taurus*, which offers provisions for their offspring growth, found that females with assistance from partners for brood provision lived longer than females that did it alone (Hunt *et al.* 2002).

Numerous studies document the cost of reproduction, but few reports have clarified the entailed cost for males (but Kotiaho & Simmons 2003; Davies *et al.* 2005; Bretman *et al.* 2013). It is traditionally assumed that reproduction incurs no cost for males as they only invest in the offspring by providing genes via the relative cost-efficient sperms (Simmons 2001). However, much evidence has been accumulating that cost can be imposed to males during pre-copulation guarding, fighting with rivals (Bretman *et al.* 2013), gametes production (Van Voorhies 1992), searching and signaling for females (Scharf *et al.* 2013). This emphasizes the need to investigate both sexes and to take the social environment as a contributing factor to fitness components.

Previous studies determining the cost of reproduction for both sexes have usually failed to evaluate separately the costs at different stages, for example, copulation and sexual

harassment. In polyandrous organisms such as fruit fly, a classic model species with the sexual reproductive system, females require several successful mating to achieve their maximum reproductive potential. Consequently, during the experiment, the females are always kept together with males and thus experienced copulation and sexual harassment nearly continuously (e.g. Davies *et al.* 2005; Zajitschek *et al.* 2013). Therefore, it is challenging to decouple the cost of copulation from that associated with sexual harassment.

In this study, the two-spotted spider mites do not need multiple-mating, only one successful mating is enough to fertilize all the eggs. However, the males always coerce females to mate with them to maximize their reproductive output when no virgin females are available (Oku, 2010). Therefore, it provides an ideal opportunity to study the cost of copulation and sexual harassment by only exposing females to males for only 24 hours to allow for mating only and avoiding unnecessary sexual harassment.

In this study, I investigated female and male fitness of arrhenotokous *T. urticae* under different mating timing and frequency to clarify the fitness consequence of sexual interaction. Firstly, the effects of delayed mating were determined by comparing the mites mated at the adult age of day 1 versus day 7. Secondly, I compared the fitness of mites exposed to their partners throughout the life with those were separately reared mites after allowing to mate for 24 h to investigate the influence of repeated mating.

5.2 Materials and methods

5.2.1 Mite colony

T. urticae was obtained from Bioforce Ltd, Auckland, New Zealand, and a population of spider mites were established by releasing hundreds of females on young potted beans (*Phaseolus vulgaris*) in their two-leaf stage. The laboratory colony was maintained by regularly replacing the old bean plants heavily infested with mites with young bean plants. It was kept at the temperature of 20±5 °C, the relative humidity of 60±10%, and the photoperiod of 16 h light: 8 h dark, in the greenhouse, Landcare Research, Auckland, New

Zealand. Before conducting the experiments reported here the population had been kept in our greenhouse for about eight generations.

5.2.2 Host plant

The common beans (*Phaseolus vulgaris*) were used throughout this experiment. They were all planted in the same conditions as the mite population. To make sure there were enough new plants at their two leaf-stages for the experiment, a new batch of beans was planted each week.

5.2.3 Rearing units

The rearing unit consists of a piece of foam cube, detached bean leaf, plexiglass slide and film from the bottom to top. The foam cube 10mm thick was placed on the bottom layer of the leaf disc to keep the leaf fresh. It was kept wet by adding 3-5ml water twice every day. Then a plexiglass slide with a round hole in the center (1.5 cm in diameter) was placed on the upper layer of the detached bean leaf disc. The round hole was covered with a transparent clean film to form a cell for the mite. The unit was fixed together by an elastic band. The film was punctured with a fine insect pin to maintain suitable relative humidity without causing condensation. The experiment units were kept in a room at 25 ±1°C, 12L: 12D photoperiod and 60 ±10 % RH.

5.2.4 Experimental protocol

Before the experiment, newly emerged male and female mites were selected. To obtain virgin males, females from the lab population in their last moulting stages were transferred onto fresh bean leaves and allowed to oviposit after they moulted. All these eggs would develop into males because the females were un-inseminated, whereas the virgin females were produced by the inseminated mothers.

When the virgin males and females emerged, I randomly assigned them to one of the five treatments (two-level full factorial design with unmated mites as control) within 24 h:

(1) Male and female were paired after adult-emergence and allowed to mate frequently throughout their life (referred to M1T hereafter).

(2) Male and female were paired after adult emergence and allowed to mate for only 24 hours, and then were separated to keep them from re-mating throughout their life (referred to M1S).

(3) Male and female were paired at 7-days of age and allowed to mate frequently over the rest of their life (referred to M7T).

(4) Male and female were paired at 7-days of age and allowed to mate for only 24 hours, and then were separated keep them from re-mating throughout their life (referred to M7S).

(5) In the control treatment, one virgin male or female was transferred to each leaf disc and was left un-inseminated throughout their life (referred to Con).

During the experiment, the leaf discs were replaced with new ones regularly to ensure the mites always had access to fresh food. The survival of each female and male was checked daily until their death. The number of eggs produced by each female was also checked daily. Other female reproductive parameters such as their pre-reproductive lifespan, reproductive lifespan, post-reproductive lifespan were calculated. On the 10^{th} day of adult emergence, eggs laid by the females in all treatments were collected and allowed to hatch on different fresh bean leaves. The offspring sex ratio was determined when they developed into adults. I removed from data analysis those females that had cohabited with males but were not successfully mated (i.e. produced only male offspring). Only individuals that made it to the seventh-day post-emergence 'cut-off' were finally included in the data analysis.

5.2.5 Data analysis

The survival of mites under different mating status was firstly analysed in a full model with sex and treatment as main factors (LLR= 7.31, df=2, P =0.02591). The results indicated that there is a significant difference in survival between two sexes (Z= − 2.628, P =0.00858). I

then performed the pairwise comparison for survival curves among different treatments for each sex respectively to further explore the effects of mating status on survival. One-way analysis of variance was conducted to explore the effects of different mating regimes including unmated/mated, delayed mating and repeated mating on the longevity of both sexes, as well as on the reproductive parameters of females, for example, reproductive rate, lifetime fecundity. When the *F* test was significant for a parameter, posthoc comparisons were undertaken with Tukey's Honestly Significant Difference (HSD) post hoc tests to clarify the differences between treatments. To explore whether there is any significant interaction between delayed mating and repeated mating, two-way ANOVA was conducted with the control treatment (unmated males and females) removed in the model. The offspring sex ratio was calculated as the proportion of male offspring in the total number of offspring. For unmated females, no female offspring were observed and they were therefore not included in the final comparison. The male offspring ratios among the other four treatments were analyzed by pairwise comparisons without adjusted p-values using the R function pairwise.prop.test at a significant level of $P = 0.05$. All data analysis were performed with R version 3.4.3 (R Development Core Team 2017).

5.3 Results

5.3.1 Effects of mating regimes on the survival and lifespan of female and male

Females in mating treatments differed significantly in their survival (χ^2=16, *df* =4, *P*= 0.00308; Figure1) but not males (χ^2=1.9, *df* =4, *P*= 0.76; Figure 5.1). For females, both the females mated on day 1 kept ((M1T) with and separated from male partner (M1S) had significant lower survival than the unmated females (M1T vs Unmated, χ^2=13.3, *df* =1, *P*= 0.000264, M1S vs Unmated, χ^2=7.9, *df* =1, *P*= 0.0049; Figure 5.1). The females mated at adult emergence and kept together with male (M1T) also has a lower survival than the delayed mating females (M7S) without repeated mating (M1T vs M7S, χ^2=7.8, *df* =1, *P*= 0.00517; Figure 5.1). The survival rates of unmated females were similar with the females

which had access to males when 7-day-old (Unmated vs M7T and Unmated vs M7S; P >0.05; Figure 5.1).

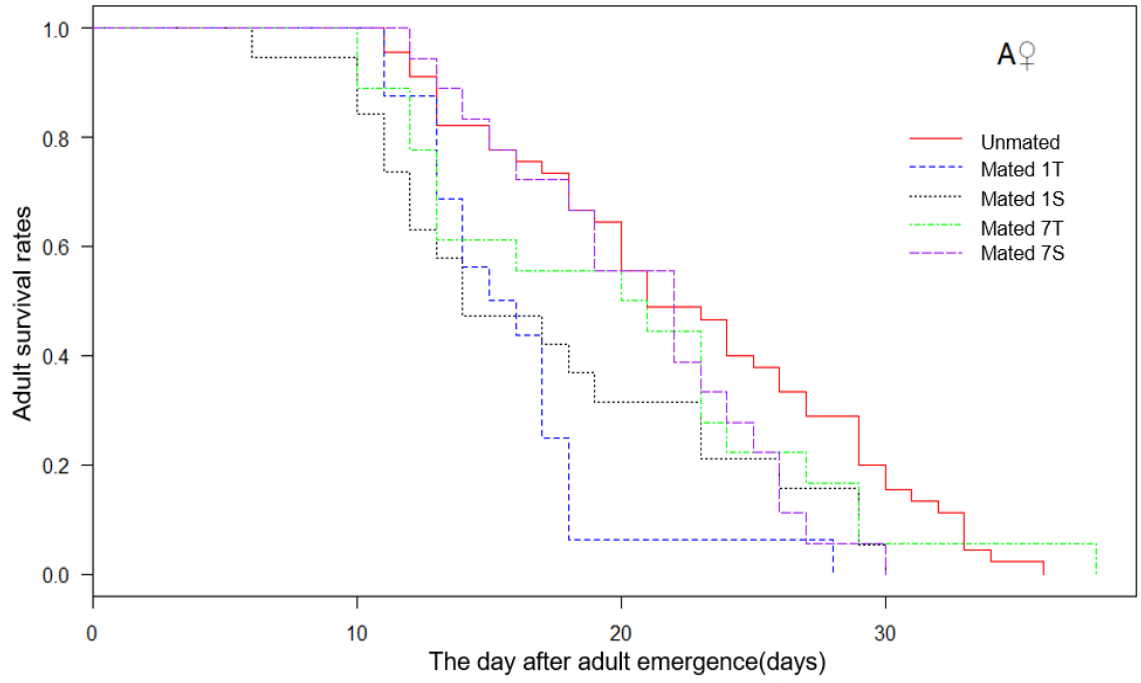

FIGURE 5. 1 Survivorship of female *Tetranychus urticae* in different mating regimes: unmated, mated at one-day-old with repeated mating, mated at one-day-old without repeated mating, mated 7-day-old with repeated mating, mated 7-day-old without repeated mating.

However, males displayed similar survivorship responses to delayed mating and repeated mating (χ^2=1.9, df =4, P= 0.76; Figure 5.2). These results indicated that mating regimes were an influential factor with regard to change in the survival pattern of females.

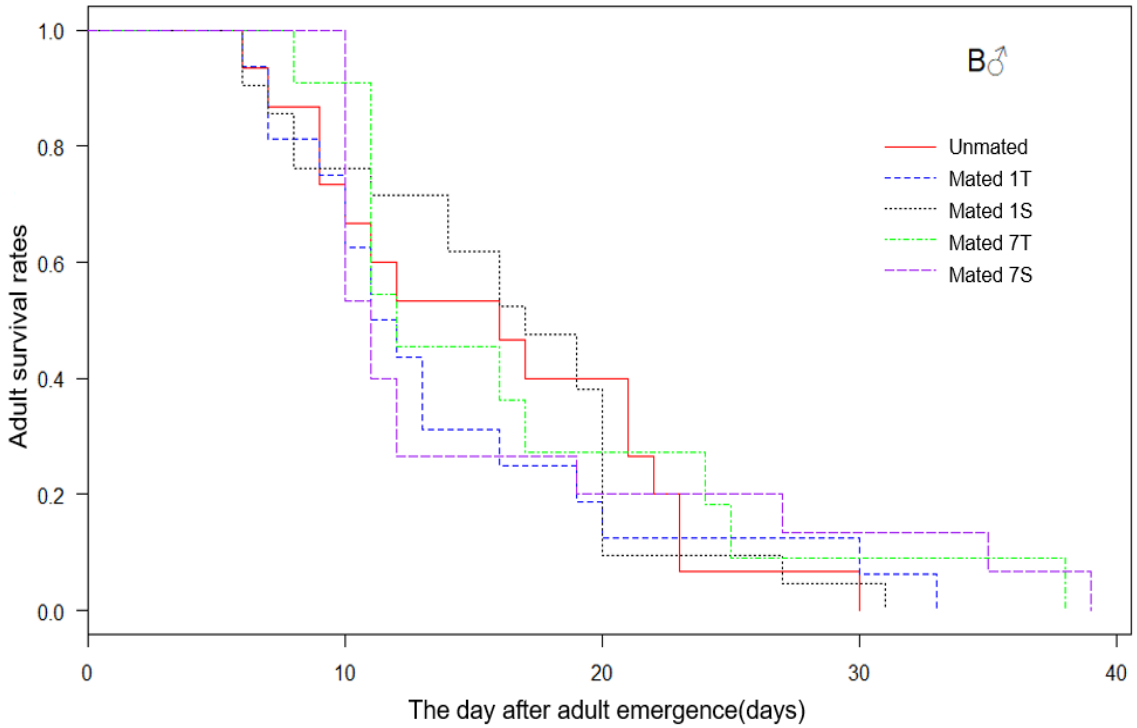

FIGURE 5. 2 Survivorship of male *Tetranychus urticae* in different mating regimes: unmated, mated at one-day-old with repeated mating, mated at one-day-old without repeated mating, mated 7-day-old with repeated mating, mated 7-day-old without repeated mating.

The mean adult lifespan of females was longest in unmated females (Con =22.0 days), followed by the females mated 7-day-old regardless of repeated mating (M7S =20.7 days & M7T =19.8 days; Table 5.1). Within these three treatments, female longevity did not differ significantly. However, they all outlived these mated at 1-day- old (M1S =17.2 days vs M1T =15.8 days; Table 5.1). Moreover, compared with females cohabited with males all along their life and experienced repeated mating, the females separated from males after mating for only one day lived significantly longer when they mated at one-day-old (M1S =17.2 days *vs* M1T =15.8 days; Table 1), but similar effects of repeated mating were not observed between females mated 7-day-old (M7S =20.7 days vs M7T =19.8 days; Table 5.1).

TABLE 5. 1 Effects of mating status: unmated, delayed mating and repeated mating on the female and male lifespan, maximum reproductive rate and post-reproductive lifespan of female *Tetranychus urticae*.

Treatments	Female lifespan (days)	Male lifespan (days)	Maximum reproductive rate (eggs/female/day)	Post-reproductive lifespan (days)
Con	22.0±1.07a	15.80±1.87a	13.81±0.38ab	2.49±0.28a
M1T	15.8±1.00c	14.19±1.96a	14.38±0.67ab	1.75±0.23a
M1S	17.2±1.70b	16.14±1.47a	15.30±0.61a	1.90±0.37a
M7T	19.8±1.86ab	16.73±2.70a	12.79±0.97b	2.78±0.46a
M7S	20.7±1.24ab	15.73±2.54a	14.06±0.66ab	2.67±0.49a

Data (Mean ± SE) presented in the same column with different letters are significantly different at the level of $P = 0.05$.

The two-way ANOVA analysis indicated that delayed mating showed significant influence in female longevity ($F= 5.972$, $P= 0.017$) but not repeated mating ($F= 0.586$, $P = 0.447$; Table 5.2). The males did not differ in their average longevity across treatments, and further analysis also suggested that both delayed mating ($F= 0.251$, $P = 0.618$) and repeated mating ($F= 0.051$, $P = 0.882$; Table 5.2) had no marked effects on male adult lifespan.

TABLE 5. 2 Two-way ANOVA of the effects of the delayed mating (mated at 1-day-old or 7-day-old) and repeated mating (cohabitating with a male for one day or all life-long) on the lifespan of male and female spider mites *Tetranychus urticae.*

Sources	Female lifespan			Reproductive lifespan			Male Lifespan		
	SS	*F*	*P*	*SS*	*F*	*P*	*SS*	*F*	*P*
Delayed mating (D)	247.103	5.972	**0.017**	144.009	4.470	**0.038**	16.952	0.251	0.618
Repeated mating (R)	24.253	0.586	0.447	31.440	0.976	0.327	3.453	0.051	0.822
D × R	0.909	0.022	0.883	0.875	0.027	0.870	32.494	0.481	0.491
Error	41.378			32.218			67.628		

5.3.2 Effects of mating regimes on female reproductive parameters

The differences in the mating status of spider mites also resulted in variation in reproductive parameters in females, especially daily reproductive rates, maximum reproductive rates and reproductive lifespan. The daily reproductive rate was significantly higher with females mated 1-day-old without repeated mating (M1S=10.5 eggs/female/day), following by those mated 1-day-old experiencing repeated mating (M1T=8.8 eggs/female/day), lower for unmated females and delayed mating females (M7S=7.42 eggs/female/day, M7T=7.86 eggs/female/day, Con=7.76 eggs/female/day, Figure 5.3A). Similarly, the highest maximum reproductive rates were also observed in females mated 1-day-old without repeated mating (M1S= 14.38 eggs; Table 5.1). However, females in other treatments did not differ significantly for reproductive parameters. The reproductive lifespan of females was much shorter in females mated 1-day-old experiencing repeated mating with comparison to the unmated (M1T= 13.81 days, Con=18.95 days; F= 5.710, P = 0.010; Figure 5.3B), delayed mating and repeated mating females, of which reproductive life span didn't differ within these treatments (P > 0.05, Figure 5.3B). The post-reproductive lifespan was lower in females mated one day old irrespective of repeated mating but did not differ with that of unmated females and delayed mating females (F= 1.33, P = 0.260; Table 1). In contrast, the total

fecundity of females was not affected by the mating status (F= 0.818, P = 0.516; Figure 5.3C). Additionally, the females did not show differences in male offspring ratio (χ^2 = 2.5406, df = 3, P = 0.468; Figure 5.3D).

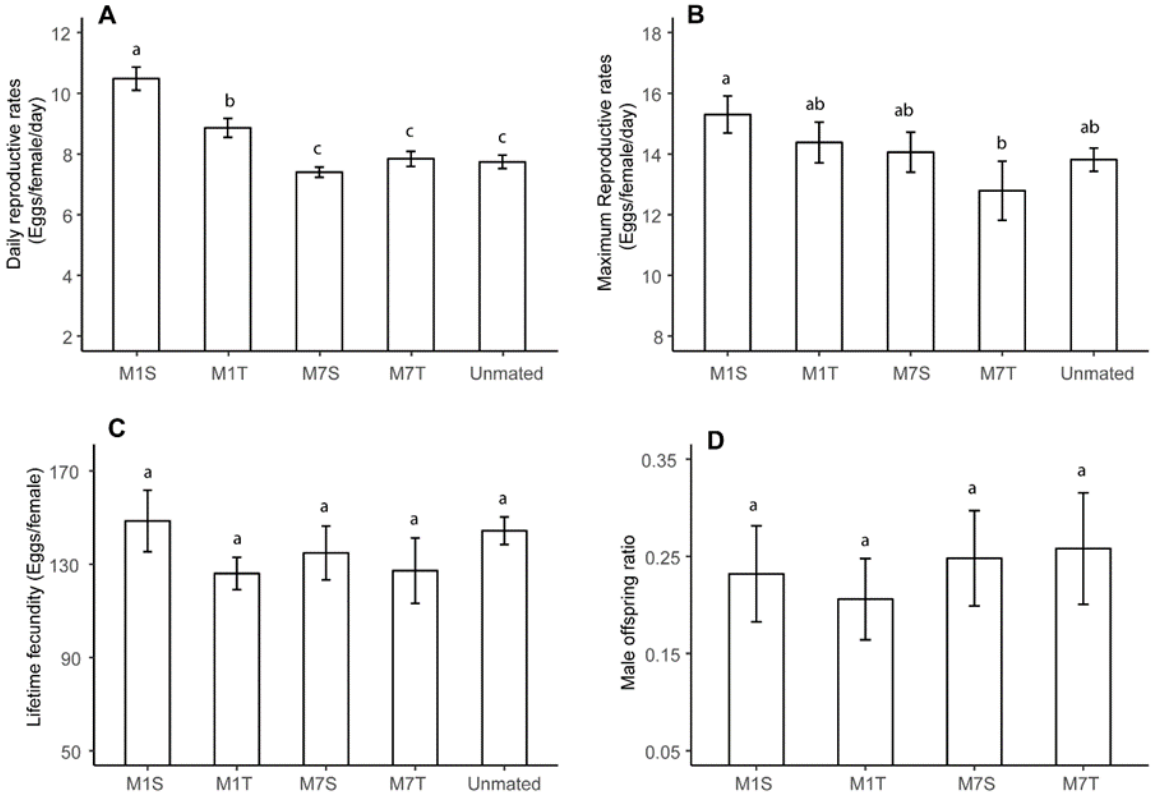

FIGURE 5. 3 Effects of mating status: unmated, delayed mating and repeated mating on the reproductive parameters including mean daily reproductive rates, reproductive lifespan, lifetime fecundity and male offspring ratio of female *Tetranychus urticae*. Data are presented in Mean ± SE. The bars with different letters are significantly different at the level of P = 0.05.

5.4 Discussion

In this study, I determined the effects of mating regimes in a spider mite on longevity and reproductive output. Our results revealed that unmated females outlived the mated individuals. The females with delayed mating for 7 days lived longer than these mated earlier, at 1-day-old. The females mated at 1-day-old were significantly affected by repeated mating, whereby the females coexisting with males after mating had a shorter lifespan than the counterparts that were kept alone after mating for one day. However, for the females mated at 7-day-old, the impact of repeated mating was not significant. In comparison with females, mating status did not show significant influence on the survival and longevity of males. My results suggested that differential optimal mating regime of male and female is a key factor constraining sex-specific plasticity of longevity in this spider mite. Additionally, female reproductive rates were similar across treatments because reductions in daily reproductive rates of delayed mating females were compensated by longer reproductive lifespan.

5.4.1 Effects of mating and delayed mating on female longevity

The virgin females outlived females mated at adult emergence suggested the cost of reproduction for individual survival is substantial to females. Similar results also were reported in life-history studies with other insects such as fruit fly (Flatt 2011). This accords with survival trade-offs against reproduction when constrained by limited resources (Williams 1957; Kirkwood 1977; Atwood & Bowen 2010). However, my study further showed that the trade-off of resource allocation can be shifted by delayed mating. When the female spider mites delayed mating by seven days, their lifespan was prolonged compared with that mated at adult emergence but was not significantly different from those of the virgins. This indicates that mating *per se* may not be costly for females, rather the costs are associated with sexual harassment and subsequent egg production.

Results in my study indicated that females mated at adult emergence cohabiting with males had reduced lifespans compared with females isolated after mating for one day, supporting

that sexual harassment has negative effects on female longevity. Fitness costs of continual exposure of females to males was also reported in other polyandrous insects. The females of *Drosophila simulans* showed a decrease in lifespan when they were continuously exposed to males (Taylor *et al.* 2008). Some studies also documented sexual harassment causing a decrease in female's oviposition (Fowler & Partridge 1989; Gay *et al.* 2009; Li *et al.* 2015). For instance, the female *Thrips tabaci* with a male companion produced fewer eggs even though they have similar longevity to those without a male partner throughout their lifetime (Li *et al.* 2015). The entailed cost of repeated mating on survival and reproduction may also be a consequence of reduced time for feeding (Heubel & Plath 2008; Kohler *et al.* 2011).

The entailed cost on the survival of mated females requires more energy and nutrient investment in egg production compared with virgin females. Although the females across treatments produced a similar number of eggs during their life, the total amount of reproductive investment of unmated females is lower than those of mated females, given that the eggs produced by virgin mothers were much smaller in size than the mated mothers Macke *et al.* (2010; 2012). However, the delayed mated females and the unmated females had a similar lifespan. This suggests that both repeated mating and egg production had a negligible impact of lifespan. The steep decrease in daily reproductive rate after females being 5-day old is a likely explanation. The number of eggs produced in the first seven days of adult life accounts for around 60% of lifetime fecundity (summarized from our raw data). Therefore, the delayed mating females suffered less physiological cost compared with those that mated soon after at adult emergence.

The females mated immediately at adult emergence possibly accelerated aging and lived shorter than females delayed mating because they received seminal fluid much earlier. Although the sperms in the seminal fluid were necessary for fertilization, the sex peptide transferred along with sperms could also influence other traits of females (Ram & Wolfner 2007). It was reported to reduce the female's willingness to remate and increase their reproductive output (Chapman et al. 2003). Moreover, some research indicated the sex peptide increased activity, reduced sleep (Isaac et al. 2009) and enhanced immune responses

(Peng et al. 2005). Considering the high energy costs for these responses, it appeared no surprise that early exposure to male decreased the adult lifespan of the female.

Another important finding of the study was that repeated mating reduced female lifespan when sexual interaction occurred at adult emergence but not in the delayed mating females. This may reflect the decrease in the attractiveness of aged females to males (Rasmy & Hussein 1994) and less frequent copulatory activity. In a previous study, it was demonstrated that 1-day-old virgin males could copulate on average 15.5 times, ranging from 2 to 31 times over the first eight days after adult emergence. By comparison, 8-days-old females copulated for only 3.5 times when virgin females were offered *ad libitum* (Krainacker & Carey 1989). Thus, our results demonstrate that the influence of repeated mating diminished in late age with the decrease in the physical cost of sexual interaction and physiological cost of egg production.

5.4.2 Effects of delayed mating and repeated mating on the reproduction of females

The lifetime number of eggs produced by females across treatments was similar because the females mated at adult emergence were observed to have a higher daily reproductive rates over shorter lifetimes. Repeated mating only decreased mean daily reproductive rates of females mated at adult emergence (10.5 for M1S **vs** 8.88 for M1T), but not that of delayed mating females (7.84 for M7S **vs** 7.42 for M7T). Previous studies also documented that sexual harassment decreased the oviposition rate of females (Oku 2010) in a shorter-term investigation lasting for one day. A possible explanation for the non-significant effects of repeated mating on the delayed mating female is the decrease in sexual harassment with the increasing of age (Krainacker & Carey 1989), as has been found in other organisms (e.g. Li *et al.* 2014).

The male offspring ratio didn't differ between the mated females irrespective of their mating regimes. These results indicated that successful mating on the first day of adulthood can provide the female with the sufficient number of sperms to inseminate eggs. Males can

transfer several hundreds of sperms to female at one time (Pijnacker & Drenth-Diephuis 1972), which is approximately nine times the number of eggs female can produce. Additionally, repeated mating is ineffective and couldn't increase the proportion of daughters produced. Altogether, males seem unlikely to benefit from mating repeatedly with females. Therefore, it is perhaps unexpected that males are willing to engage in repeated mating given they are capable of distinguishing mated females from virgins (Oku 2010; Rodrigues *et al.* 2017).

5.4.3 Effects of mating regimes on male longevity

In contrast to the females, there were no detectable effects of delayed mating and repeated mating on the adult lifespan of males. This result is consistent with our previous study that mated males kept together with a female throughout life have similar lifespans to unmated males (Chapter 4), indicating that there is the negligible cost of copulation, sexual harassment and ejaculation on male longevity. In light of the result of this study and our previous study, where males cohoused with males were reported to show decreased longevity (Chapter 4), it can be concluded that the cost of sexual interaction was less intensive than the inter-sexual interaction. The cost of inter-sexual interaction was mainly in the early adult life of mites when the competition and fighting for newly emerged females are fierce (Oku 2014). It would be important to identify the potential cost in the pre-copulatory mating stage.

It is worth noting that the mating regimes investigated in this study showed distinctly different influences on males and females; similar results were also reported in model species fruit fly *Drosophila melanogaster*, which shows sex difference in response of lifespan to the social environment when reared in separate-sex and mixed-sex groups (Zajitschek *et al.* 2013). This may be attributed to the higher cost of reproduction in females than in males, as has been found with spider mites in this study. Female spider mites suffered great cost from egg production and male sexual harassment during the experiment, whereas there is little cost of ejaculation and sexual attempts for males. Moreover, in our study, the males are free from the cost in pre-copulatory mating stage since they were never exposed to any competitors

throughout their life. Another potential reason for the sex difference in response to social interaction is their difference in energy demand, as generally the female has a higher investment in eggs than the males in sperm.

5.5 Conclusion

In conclusion, I showed that delayed mating and repeated mating have a remarkable influence on the fitness of spider mites. However, these effects were sex-specific, with only females being affected, but not males. The females lived longer when they were unmated and when mating is delayed owing to the effect of reduced sexual harassment. My results provide evidence that for females, superfluous sexual interactions were deleterious. I suggest that the differential optimal mating regime is one of the explanations for sex-specific plasticity of longevity.

Chapter 6

Age-specific mortality and fecundity of spider mite under diet restriction and delayed mating

Abstract

Numerous experimental life history studies on aging are mainly biased on two classic models—fruit fly *Drosophila melanogaster* and nematode *Caenorhabditis elegans*—with relatively little attention given to other organisms with different life history characters. Spider mites differ from other arthropods in that the females have indeterminate growth and can reproduce sexually and asexually. In this study, I examined the influences of dietary restriction and delayed mating on the aging patterns of two-spotted spider mite *Tetranychus urticae.* The aim was to test the prevailing survival and reproduction trade-off hypothesis of aging. I found significant sex-specific responses of *T. urticae*: females showed longevity extension on diet restriction (in the cycle of two-day-feeding after 2-day-fasting) compared with being fed *ad libitum*, and after delayed mating for 9 days, while the males displayed a decrease in lifespan when experiencing diet restriction but were not significantly influenced by delayed mating. I investigated the relationship between mite survival and reproduction traits using path analysis, including traits of longevity, female lifetime reproduction, age at first reproduction, early reproductive efforts, and late reproductive efforts. Overall, I failed to find evidence for trade-offs between these life-history traits. I confirmed the lifespan extension effects of dietary restriction and delayed mating on female spider mites, proving that diet restriction is a robust anti-aging intervention, and that later onset of reproduction can prolong adult lifespan in females. However, the trade-off hypothesis is not supported for this species; possibly the indeterminate growth decoupled survival and reproduction in female spider mites.

Keywords: diet restriction, delayed mating, age-specific mortality, sex difference, early- and late- life trade-off

6.1 Introduction

Aging is associated with a decline of physiological function that gives rise to increasing mortality and decreasing reproductive rate (e.g., Williams 1957; Rose 1994; Partridge & Barton 1996; Promislow & Bronikowski 2006; Fabian & Flatt 2011). Understanding the consequences and mechanism of aging has long been a major research topic in the field of biology and gerontology (Vaupel *et al.* 2004). The evolutionary theory of aging proposed that aging is determined by the resource allocation strategy of organisms. With finite resource available, increased allocation to reproduction can incur a cost to somatic maintenance and repair, resulting in an increased risk of mortality, frequently considered as a trade-off between reproduction and survival (Kirkwood 1977; Kirkwood & Rose 1991; McNamara *et al.* 2009).

The negative correlation between longevity and reproduction is supported by two prominent evolutionary theories of aging: i. the antagonistic pleiotropy and ii. the disposable soma theory (Hughes & Reynolds 2005; Monaghan *et al.* 2008). The former, first advanced by Williams (1957), states that alleles selected for enhancing fitness in early life could have deleterious effects in later life, so-called pleiotropic effects. The disposable soma theory, which originated from physiological ecology, posits that a high risk of extrinsic mortality imposed on the natural population can lead organisms to invest limited resource in early reproductive success at the expense of somatic maintenance and future reproductive success. Although these two theories originated from different perspectives, they both claimed that the high output of reproduction incurs a cost to longevity, and the early investments in fecundity can shorten lifespan and reduce further reproductive success.

There is some empirical evidence for a trade-off between early and late-life fitness in free-living vertebrates including birds, mammals, and reptiles, documented in a review by Lemaître *et al.* (2015). This review showed that 43 out of 74 of the reported relationships (from 26 studies) between early fitness and late fitness indicate that fitness traits such as age at first reproduction or early life reproduction trade-off with late-life fitness including brooding success, age at last reproduction, survival, and onset and rate of actuarial

senescence. However, a general negative association between early and late fitness may reflect publication bias. Furthermore, some empirical studies show the absence of trade-offs or even a positive relationship between lifetime reproduction and survival, questioning the generality of the trade-off hypothesis (e.g., Zajitschek *et al.* 2016, 2018; Panagakis *et al.* 2017). For example, in a study with rotifer *Brachionus plicatilis* in the laboratory, the lifetime fecundity positively correlated with adult lifespan regardless of the intrinsic factors such as mictic status and extrinsic factors such as season (Snell & King 1977; Smith & Snell 2014).

Some researchers suggest that the absence of trait trade-offs can result from methodology problems. First, environmental stressors may be necessary for the expression of trade-offs. In many studies, the animals live in benign conditions with abundant food, and are therefore free from resource constraints, which may be prerequisites for life-history trade-off (Nussey *et al.* 2013). This may explain why trade-offs are less frequently observed in laboratory studies compared with investigations in the wild. Additionally, some argue that measuring multiple traits simultaneously is necessary for testing the trade-off theory because of the complexities associated with allocation strategies in animals. be manifested in different traits. Thus, it is problematic to conclude no trade-off when only a pair of early and late life-stage traits were studied (Nussey *et al.* 2008). Take a longitudinal study on a preindustrial human population, for example, even though the cost of high early-life reproduction to late-life mortality is apparent, the late-life fecundity is positively correlated with early life fecundity under natural selection (Hayward *et al.* 2015). Consequently, to adequately test the trade-off theory, it is important to assess trade-offs across multiple traits.

Patterns of aging and reproduction have been extensively explored under different environmental conditions. However, most previous studies are largely based on well-known model species such as the nematode *Caenorhabditis elega*ns (e.g., McCulloch & Gems 2003; Gruber *et al.* 2007; Casanueva *et al.* 2011) and fruit fly *Drosophila melanogaster* (e.g., Libert *et al.*, 2006; Marshall & Sinclair 2009; Zajitschek *et al.* 2016, 2018). Admittedly, these model species have greatly advanced our knowledge in many fields due to the well-developed tools and techniques for experimental manipulation and the specific databases and infrastructure available (Valenzano *et al.* 2017; Russell *et al.* 2017). However, relatively little is known

about the aging patterns in other taxa, which may have very different evolutionary histories. Furthermore, experiments on these species mostly involve groups rather than individuals in laboratory study. Therefore, it is not possible to examine some life-history traits of each individual, namely age at first reproduction and early/late reproductive efforts.

In this study, I investigate the correlation of multiple life-history traits of a non-model organism the two-spotted spider mite, *Tetranychus urticae,* to better understand the generality of trade-off theory. In light of our previous study with this species, I found that two environmental factors have significant impacts on the longevity and reproductive traits, namely diet and mating regime. As found in other animals, a moderate dietary restriction can extend lifespan and reduce the fecundity of females (Li & Zhang 2019). Delayed mating decreased reproductive rates and delayed onset of reproductive aging (Chapter 5). Therefore, I explored the performance of spider mites in a factorial design with two factors: food level and mating status. This study aims to test the existence of a trade-off between life-history traits under resource constraints. Multiple traits were investigated, including age at first reproduction, maximum reproductive rates, early reproductive efforts, as well as longevity and lifetime reproductive efforts for each individual. To display the direct and indirect influences of diet restriction and mating status on these traits and visualize the relationship between them, path analysis was performed to give an integrated perspective of the data. Additionally, I compared the longevity difference between sexes and further fitted the data in mortality models to explore the underlying mechanism of sex-specific aging patterns.

6.2 Materials and Methods

6.2.1 Maintenance of lab culture

Our model species for the study is the two-spotted spider mites *Tetranychus urticae*, a species with cosmopolitan distribution (Jeppson *et al.* 1975) and broad host range feeding on over 1,100 plant species (Grbić *et al.* 2011). The spider mites used were descended from a population of over 300 mites obtained from Bioforce Ltd in February 2015. They were maintained on fresh bean plants bean (*Phaseolus vulgaris* L.) and allowed to breed

panmictically. The same host plant was used for the experimental mites. The colony was maintained in the greenhouse room with ambient temperature and photoperiod in the spring and summer and 25 ± 1 °C, 16/8 h light/dark cycle in the autumn and winter.

6.2.2 Collection of experimental mites

To prepare a large number of newly emerged females and males, about 100 females and males in their last moulting stage were randomly selected from the stock and transferred to the leaf disc. Emerging females and males were used as parents of the experimental mites. Among these parents, half the number of females were left unmated, and half were mated with males, which gave birth to male offspring and female offspring, respectively. The un-inseminated mothers and inseminated mothers were allowed to lay eggs on new leaf discs for 24 h at the age of 5 (days). Four days later, the newly hatched larvae were transferred to leaf disc (one larva per leaf disc) where they developed until adult emergence.

6.2.3 General experimental protocol

All mites were sexed immediately after adult emerging and randomly assigned to experimental treatments: mated (at first day of the adult) on diet, mated (at first day of the adult) fed *ad libitum*, delay mated for 9 days on diet, delay mated 9 days fed *ad libitum.* The mites on diet went through a diet cycle of being fed *ad libitum* for 2 days after 2-day-fasting. The delayed-mated mites, both male and female, were kept individually before mating on day 9, then the male and female were paired and reared together. At the beginning of the experiment, I had 48 pairs of male and female in each treatment. However, due to accidents, inadvertent injuries, and escapes, the final sample size ranged from 29 to 40 individuals in each group. The experimental arenas and setup were the same as in Li and Zhang (2018) and the mites were kept under standard laboratory conditions at 25 ± 1 °C, 70 ± 10% humidity, 16:8 h light/dark cycle.

6.2.4 Survival and reproduction

During the experiment, the mites were offered new leaf discs every 4 days. The survival of each mite was scored at 24 hour intervals. The reproduction of a female was scored each day by counting the number of eggs produced. The age of first reproduction was determined from the day the egg appeared until the day when it developed to an adult female and produced her first egg. The reproductive lifespan was the period during which females gave birth to eggs. The post-reproductive lifespan started after the last egg produced and ended at the death of the female. To explore the relationship between reproduction and lifespan, I also determined the early reproductive effort (ERE) and late reproductive effort (LRE). The ERE was measured by the number of egg produced by a female until the maximum reproductive rate was achieved. The LRE was measured as the egg production after the day of maximum reproductive rate until the end of oviposition period.

6.2.5 Data analysis

Lifespan and survival analysis

Lifespan is a commonly used parameter in aging studies. It has been noticed that anti-aging interventions such as dietary restriction sometimes increases maximum lifespan without affecting mean and median lifespan, while other interventions, for example, exercise, prolonged mean and median lifespan without increasing maximum lifespan (Wang *et al.*, 2004). Therefore, I first summarised the mean lifespan, median lifespan, and maximum lifespan across treatments to check the influences of diet and mating status on lifespan. The maximum lifespan is based on the upper 90% percentile of lifespan for each sample.

The differences in lifespan across sex, diet regimes and mating regimes were examined by three-factor ANOVA. The full model indicated a significant interaction between sex and the other two factors, diet regime and mating status. Thus each sex was analysed separately in a reduced model with diet regime and mating status as two main factors. The survival difference across treatments was checked using Cox proportional hazards regression, with the

"coxph" function of the "survival" package (Therneau & Lumley, 2011). Sex, diet regime, and mating status were included as explanatory factors in the full model. This part of the analysis was performed in R.

In addition, to fully explore the correlation of the life history traits of the female under two environmental factors—diet and mating—path analysis were conducted with structural equation modelling (SEM) to determine the proportion of observed traits of female that could be attributed to other traits. This analysis was performed in R with the package "lavaan" (Rosseel, 2012).

Mortality model fitting

The mortality data were fitted to Gompertz family of four nested models (Gompertz model, Gompertz–Makeham model, Logistic model, and Logistic–Makeham model) by a maximum-likelihood method in WinModest 1.0. The best model was selected based on the results of likelihood ratio tests (Pletcher, 1999). The Gompertz model was finally employed to estimate the demographic rate of change in mortality with age in this study, which is defined as

$$\mu_x = \alpha e^{\beta x}$$

where μ_x is an estimate of the mortality hazard rate at age *x*, *x* is the age at death, α (Gompertz intercept) is the baseline mortality rate, and β (Gompertz slope) is the rate of increase in mortality with age (Pletcher *et al.*, 2000). I also examined the overall effects of diet regime and mating status on the age-specific aging parameter with aging index $\omega^2 = \alpha * \beta$ (Reznick *et al.* 2004).

Age-dependent reproductive efforts

The difference in lifetime reproductive efforts among treatments was examined using GLM, with diet regimes and mating status as explanatory variables. To understand whether the female spider mites differed in their age-dependent reproductive efforts, I also checked the effects of diet regime and mating status on the early reproductive efforts and late reproductive efforts. Post hoc tests were performed using the Tukey's Honest Significant Differences method to assay diet effects and mating status effects (Tukey HSD).

6.3 Results

6.3.1 Lifespan of spider mites

Lifespan for each group of mites under different treatments are summarised in Table 6.1, and further analyses of how diet and mating influence lifespan for each sex are shown in Table 6S1. Delayed mating for 9 days increased lifespan of spider mites ($F_{1, 287}$ = 10.012, P = 0.0017; Table 6.1). There were no overall effects of diet and sex on lifespan. However, significant interactions between sex with diet and mating were observed (sex and diet: $F_{1, 287}$ = 27.709, P < 0.000; sex and mating status: $F_{1, 287}$ = 5.110, P < 0.024). The females on diet showed an increase in lifespan ($F_{1, 140}$ = 10.418, P = 0.00155), while the males had shortened lifespan ($F_{1, 147}$ = 17.763, $P<0.001$; Table 6.1). Mating status also has sex-specific effects on the lifespan of males and females, whereby delayed mating prolonged the lifespan of females ($F_{1, 140}$ = 19.822, $P<0.001$), but had no significant influence on male lifespan ($F_{1,147}$= 0.422, P = 0.5172). The interaction between diet and mating status was non-significant for females (P >0.05), but significant for males, with the males on diet mated at adult emergence having a shortened lifespan (by about 6 days) compared with delayed mating males ($F_{1, 147}$ =6.508, P = 0.0118). However, the mating status had no significant effects on males when they fed *ad libitum*.

TABLE 6. 1 Summary of lifespan for each sex of spider mite *Tetranychus urticae* (mean ± SE, median, maximum ± SE) under different diet regimes and mating status.

Mating status	Diet	Sex	N	Mean	Median	Maximum
Mated on day1	On diet	♀	39	19.55±1.46bcd	19	36.75±2.50
Mated on day1	On diet	♂	39	13.92±1.22 a	11	30.00±1.78
Mated on day1	Full fed	♀	40	16.28±1.20 ab	14.5	31.50±0.50
Mated on day1	Full fed	♂	41	24.93±2.09e	24	50.25±1.60
Mated on day9	On diet	♀	35	26.14±1.31e	27	40.00±0.71
Mated on day9	On diet	♂	40	18.98±1.05bc	18	32.75±2.59
Mated on day9	Full fed	♀	29	21.45±1.35cde	21	34.67±0.33
Mated on day9	Full fed	♂	31	21.55±2.04cde	18	44.00±7.51

6.3.2 Survival and mortality

The survival analysis with Cox proportional hazards regression model generated qualitatively similar results: females with delayed mating and restricted diet showed a higher survival rates than those mating at adult emergence (χ^2 = 10.02, *df* = 1, *P* = 0.002; Figure 6.1) or fed *ad libitum* (χ^2 = 8.5, df = 1, *P* = 0.004; Figure 6.1); fed on diet significantly lowered the survivorship of males (χ^2 = 18.19, *df* = 1, *P* <0.000, Figure 1; Figure 6.1).

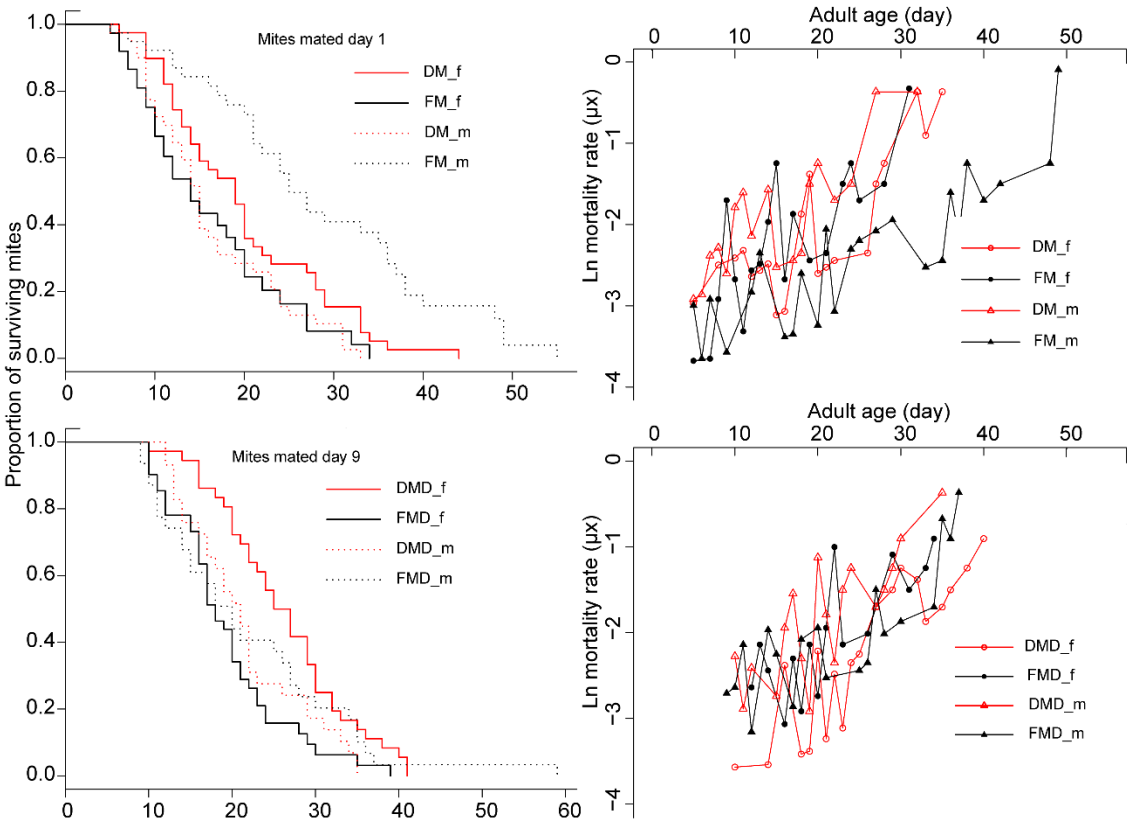

FIGURE 6. 1 Survival plot and age-specific mortality curves for spider mites *Tetranychus urticae* in different treatments. A and B are for mites mated at adult emergence. C and D are for mites mated at 9 days post adult emergence. DM_f: female spider mite mated on day 1 on diet. FM_f: female spider mite mated on day 1 fed *ad libitum*. DM_m: male spider mite mated on day 1 on diet. FM_m: male spider mite mated on day 1 fed *ad libitum*. DMD_f: female spider mite mated on day 9 on diet. FMD_f: female spider mite mated on day 9 fed *ad libitum*. DMD_m: male spider mite mated on day 9 on diet. FMD_m: male spider mite mated on day 9 fed *ad libitum*.

The aging parameters derived from the Gompertz mortality model further explained the difference in mortality parameters (Table 6.2). The Reznick's aging index of delayed mated females was lower than that of those females mated at adult emergence, with much lower baseline mortality (χ^2 = 7.86, P = 0.005) and a higher rate of aging when fed on diet (χ^2 = 4.29, P = 0.038). For mites mated at 1-day-old, neither the initial mortality rate nor the rate of aging showed sex differences. However, sex-specific aging parameters were observed on

delayed-mating mites. When the mites were fed on diet, the initial mortality rate of the male was significantly higher than that of the female (χ^2 = 4.48, P = 0.03), but not the aging rate. In contrast, the females fed *ad libitum* showed a significantly higher rate of aging (χ^2 = 10.54, P = 0.0011) than the males, but had similar initial mortality. Diet shows a remarkable influence on the difference in the rate of aging (ω) between sexes. When fed on diet, males always had a higher rate of aging than females. However, when fed *ad libitum*, those females mated immediately after maturity showed a much higher rate of aging than males, while the difference narrowed when mating was delayed for 9 days (Table 6.2).

TABLE 6. 2 Parameter estimation of mortality model Gompertz for spider mites *Tetranychus urticae* under different mating status, diet treatment and sex.

Mating status	Diet	Sex	Mortality model	α	β	ω
Mated on day1	On diet	♀	Gompertz	0.01453	0.07922	0.0339274
Mated on day1	On diet	♂	Gompertz	0.0197	0.08879	0.041823
Mated on day1	Full fed	♀	Gompertz	0.01765	0.09626	0.0412188
Mated on day1	Full fed	♂	Gompertz	0.01303	0.04624	0.024546
Mated on day9	On diet	♀	Gompertz	0.00309	0.12472	0.0196312
Mated on day9	On diet	♂	Gompertz	0.01019	0.11014	0.0335011
Mated on day9	Full fed	♀	Gompertz	0.00603	0.12345	0.0272838
Mated on day9	Full fed	♂	Gompertz	0.01965	0.04776	0.0306347

Note: α (Gompertz intercept) is the baseline mortality rate, β (Gompertz slope) is the rate of increase in mortality with age, ω is the rate of aging.

6.3.3 Female reproductive efforts

Generally, female spider mites fed *ad libitum* showed a clear sign of reproductive aging after 6 days old, with a steep decline of daily fecundity with age. However, the decrease in

reproductive rates for females on diet was less (Figure 6.2A and Figure 6.2B). Compared with the *ad libitum* fed females, the long-lived females on diet produced significantly fewer eggs (Figure 2; Table 6.S2), and the difference in lifetime reproductive efforts mainly resulted from lower daily reproductive rates during the days when they were deprived of food ($F_{1,142}$ = 541.014, P < 0.000, Figure 6.2A and Figure 6.2B). The delayed mating females showed a higher reproductive effort than those mated at adult emergence ($F_{1,142}$ = 10.76, P < 0.001) due to the longer reproductive lifespan ($F_{1,142}$= 5.81, P < 0.0172).

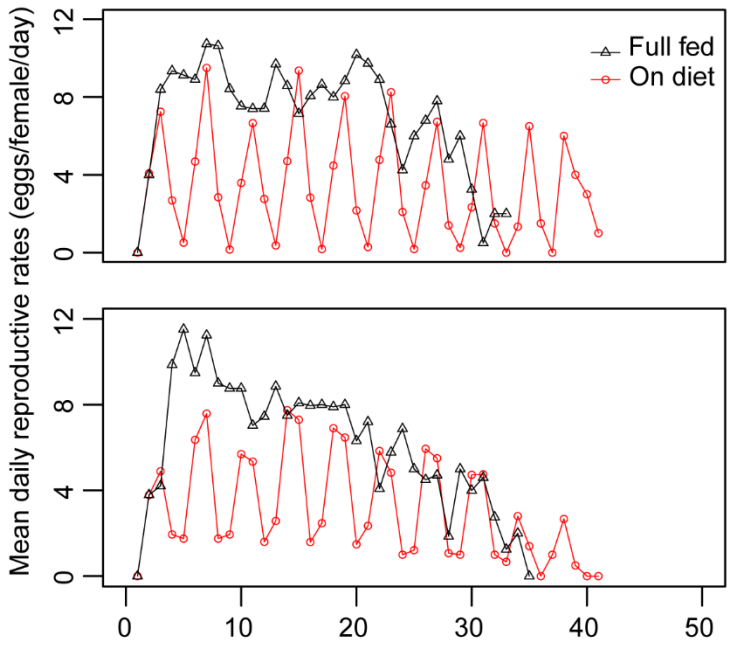

FIGURE 6. 2 (A) The daily reproductive rates of female spider mites *Tetranychus urticae* mated at adult emergence on different diet regimes, on diet (the red dash line) and *ad libitum* (the dark solid line) respectively. **(B)**The daily reproductive rates of female spider mites Tetranychus *urticae* mated at nine days post adult emergence on different diet regimes, on diet (the red dash line) and *ad libitum* (the dark solid line) respectively.

Further exploration of age-dependent reproductive efforts showed that while diet ($F_{1, 142}$ = 82.64, P < 0.000) and mating status ($F_{1, 142}$= 19.02, P < 0.001) showed significant influence on the age-dependent reproductive efforts of females ($F_{1, 142}$= 82.64, P < 0.000), their interaction was non-significant (P > 0.05, Table 6.S2).

Post hoc tests also revealed that feeding mites on a diet led to significantly lower early and late reproductive efforts (All P <0.001). The females with delayed mating showed significantly higher late reproductive efforts than those mated at adult emergence without delay ($F_{1,142}$ = 11.412, df = 1, P < 0.001; Table 6.1).

The path analysis showed the clear influences of diet and mating on lifespan, age-specific reproduction, and total reproduction. It further illustrated the association between the life history traits of female mites. Both early reproductive efforts (Z = 21.992, $P<0.001$; Figure 6.3) and late reproductive efforts (Z = 24.303, $P<0.001$; Figure 6.3) are positively associated with the lifetime reproductive success, while there is no significant correlation between early reproductive efforts and late reproductive efforts (Z = –1.010, P = 0.312; Figure 6.3). Both mites with higher ERE and LRE are likely to have a longer lifespan (Z = 12.23, $P<0.001$ for ERE, Z = 12.99, $P<0.001$ for LRE; Figure 6.3). Mites that lived longer also showed higher lifetime reproductive efforts (Z = 5.981, $P<0.001$; Figure 6.3). The age at first reproduction was not affected by diet or mating status, nor did it affect other fitness traits (All P > 0.05; Figure 6.3).

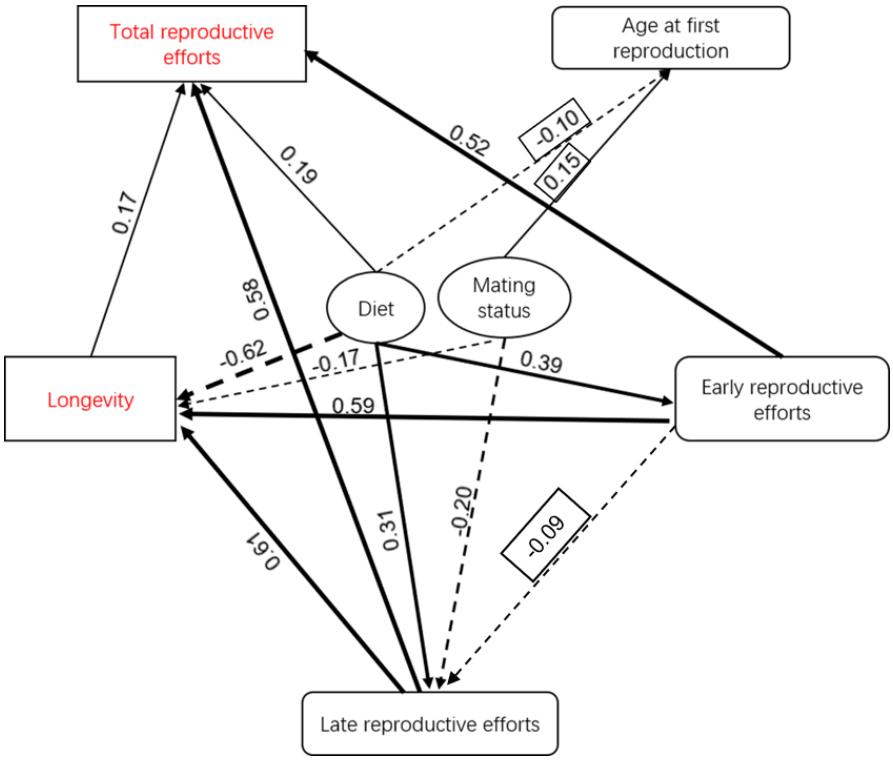

FIGURE 6. 3 Path analytical diagram demonstrating the associations of diet, mating status, age at first reproduction, early reproductive efforts, late reproductive efforts, longevity, and lifetime reproductive efforts of the female spider mite *Tetranychus urticae*. The direction of effects (positive or negative) and path coefficients were given by the arrows and the number on them. The width of the arrow is proportional to the standardised coefficients; the dash arrows indicate a significant negative relationship, while the solid arrows for positive correlation. The circles represent environmental factors (diet and mating status), while the rectangles denote the life history traits of females. Coefficients with boxes indicate they are not significantly different from 0.

6.4 Discussion

My study with spider mites *Tetranychus urticae* determined the effects of dietary restriction and delayed mating on aging patterns and age-dependent reproduction, giving an integrated view of how mites partition their resources to different fitness traits under environmental variation. With path analysis, I considered both direct and indirect effects of diet regimen, mating status, early fitness traits, and late fitness traits on lifetime fitness. Consistent with our early studies, both diet and mating showed a pronounced influence on longevity and fecundity (Li & Zhang 2019, Chapter 5). Although limiting food reduced the lifetime

fecundity of the females and increased their longevity, there was no expected trade-off between longevity and lifetime reproduction because they are positively associated with the long-lived females produced more eggs. Furthermore, there is no evidence for the early and late-life fitness trade-off. The age at first reproduction did not have any adverse effects on longevity and late reproductive efforts. Furthermore, the females were susceptible both to diet restriction and delayed mating, while the males were vulnerable only to diet restriction.

Diet restriction, one of the most explored protocols manipulating resource availability, is known to influence survival and reproductive patterns of many animals, ranging from vertebrates to invertebrate (Swindell 2012; Nakagawa *et al.* 2012; Moatt *et al.* 2016). Many theories on aging propose that abundant resources may allow individuals to achieve their optimal fitness and mask the potential life-history trade-off, while resource limitation intensifies resource competition between survival and reproduction (Reed *et al.* 2008; Nussey *et al.* 2013). As a consequence, one may expect that the females fed *ad libitum* to have higher survival and reproduction, and the females on diet restriction may show survival and reproduction trade-offs. Indeed, between treatments, there is a considerable increase in lifespan of mites on diet accompanied by a reduction in reproduction. However, I found no effects of age at first reproduction on female survival, which is in line with recent studies with other animals (Jankowiak *et al.* 2018; Panagakis *et al.* 2017). Given that this parameter did not show significant relation to either environmental factors or other life-history traits, as indicated by our path analysis, I suspected that for this species, age at first reproduction may be less sensitive and is not a crucial trait for the fitness of an individual.

Despite age at first reproduction not being sensitive to environmental factors in this study and failing to serve as a good indicator of early fitness, I found that early reproductive efforts are significantly influenced by diet and mating. Examining the association between early reproductive efforts and late-life fitness, I again found no evidence for the trade-off. ERF showed negligible association with LRF. Furthermore, females with higher early reproductive efforts survived longer. Some researchers have suggested that confounding effects of environmental variation and individual heterogeneity may make it difficult to detect trade-offs (Hamel *et al.* 2009; Lemaître *et al.* 2015). In this study, which was conducted under

laboratory conditions with controlled food availability, there was little possibility that environmental variation masked potential life-history trade-offs. Another contributing factor could be the quality of males, which may affect the energy and resource allocation of the female. The males of spider mites were documented to have extremely high reproductive potential and one insemination is adequate for a female (Krainacker & Carey 1989). In addition, there is strong heterogeneity of males with a small variation in their reproductive parameters. Consequently, the number of sperms from male partners does not limit female reproduction.

Another possible explanation for the absence of trade-offs is the life history characteristics of spider mites that enables them to continue growth after sexual maturity (Li & Zhang 2018), which may demand the female to withdraw energy and nutrition, and thus break the interdependency between other traits. The traditional "Y" model of resource allocation is the fundamental framework of the life-history trade-off, in which the two branches representing soma and reproduction share a limited common pool of energy and resources (Harshman & Zera 2007). This model is reasonable for animals with determinate growth, including mammals, birds, and most insects because they do not have to allocate resource to growth after maturity. Strikingly, the vast majority of currently available research is highly taxonomically biased, focussing on mammal, bird and some insect species. However, relatively little attention has been paid to fish, reptiles, and plants (Gerhard 2007; Jones *et al.* 2014). Many of these species continued to grow after sexual maturity and reproduction. It is likely that the prevailing model of resource allocation may be less relevant for those species with indeterminate growth, as resource allocation can be more complex than species with determinate growth (Ernsting *et al.* 1993). Further knowledge of the aging and reproductive patterns of animals with diverse life history will be needed before the trade-off hypothesis can be generalized.

This study investigated the trade-offs between life-history traits of spider mites but focused only on the females, although I also recorded the survival of their male partners. With the available data from this study, it is clear that there was a sex-dependent response to diet and mating status. First, the lifespan-extension effects were only in females, which is in line with

most studies that reported on the female-biased effects of anti-aging interventions (Iliadi *et al.* 2009). Further analysis with mortality models showed that the longevity extension effects of diet restriction on the females were achieved by decreasing the baseline mortality rate. Similar effects were also reported in *Drosophila melanogaster* (Magwere, *et al.* 2004), but not in a study of black field crickets, *Teleogryllus commodus,* in which diet showed effects either on the rate of aging or on baseline mortality (Zajitschek *et al.* 2009). Although these studies differed in their subjects, they are consistent in that the effects of diet on aging are sex-dependent, possibly because of sex differences in energy requirement and resource utilisation.

Another interesting finding was that females exposed to males earlier showed a lower survival rate than those females that delayed mating and had higher baseline mortality. On the contrary, the survival of males was not affected by their mating status. Interestingly, mating status showed a significant interaction by diet on male lifespan, with the lifespan of males on diet reduced by almost 6 days when mated early, while when fed *ad libitum* the difference in mean lifespan between different mating regimes was reduced. This study highlights the importance of standardizing social status when exploring the influence of diet and other anti-aging interventions.

6.5 Conclusion

In conclusion, this study demonstrated that environmental factors have a significant influence on the lifespan of male and female spider mites, and that female reproductive schedule and investment were both changed by diet restriction. However, the trade-off between longevity and total reproductive efforts, and early- and later-life history traits, were not detected within treatments when food is constrained. This questions the idea that allocation to reproduction is costly for survival and early investment in reproductive success would come at the cost of late-life performance, which is predicted by the disposable soma theory (Kirkwood 1977) and the antagonistic pleiotropy hypothesis (Williams 1957). It is likely that the cost of sexual harassment from male partners and indeterminate female growth in their adult stage are responsible for the absence of life-history trade-offs. Further research with special attention

to species like fish, reptiles, plants, and some insects with indeterminate growth may be needed to advance our knowledge about how survival and reproductive patterns differed across species and how fitness components like longevity and fecundity are related to each other in taxa with diverse life-history strategies. Furthermore, our study provides insight into how diet and mating status alter sex-specific mortality via baseline mortality and suggests the importance of standardising the social environment to minimise their possible interaction with the targeted factors.

Supplementary Tables

TABLE 6 S 1 GLM analysis for lifespan: complete full factorial model and the effects of diet regimes (a) and mating status on female (b), and male (c). Values given in bold are significant at the level of $P < 0.05$.

Source	d.f.	*F*	*P*
Both sex (a)			
sex	1	0.508	0.42829
diet	1	1.748	0.15994
mating status	1	10.012	**0.00216**
sex × diet	1	28.500	**<0.000**
sex × mating status	1	5.326	**0.02454**
diet × mating status	1	5.223	**0.02033**
sex ×diet × mating status	1	2.826	0.10422
Female (b)			
diet	1	10.418	**0.00232**
mating status	1	19.822	**<0.000**
diet × mating status	1	0.202	0.60116
Male (c)			
diet	1	17.763	**<0.000**
mating status	1	0.422	0.5172
diet × mating status	1	6.508	**0.0118**

TABLE 6 S 2 GLM analysis for reproduction: the effects of diet regimes and mating status on early reproductive efforts (a), late reproductive efforts (b), and lifetime reproductive efforts (c). Values given in bold are significant at the level of $P < 0.05$.

Early reproductive efforts (a)				
	MS	*df*	*F*	*p*
Diet regime	36443	1	49.901	**<0.001**
Mating status	5839	1	7.996	**0.00537**
Diet regime×Mating status	825	1	1.13	0.28967
Residuals	730	142		
Late reproductive efforts (b)				
	MS	*df*	*F*	*p*
Diet regime	19567	1	32.964	**<0.001**
Mating status	6774	1	11.412	**<0.001**
Diet regime×Mating status	88	1	0.148	0.700
Residuals	594	142		
Lifetime reproductive efforts (c)				
	MS	*df*	*F*	*p*
Diet regime	27181	1	23.295	<0.001
Mating status	12555	1	10.76	0.001
Diet regime×Mating status	615	1	0.527	0.469
Residuals	1167	142		

Chapter 7

Predator odour reduced the lifespan of prey mothers and slowed the development of their offspring

Abstract

Non-consumptive effects of predators may have pronounced effects on prey by inducing behavioural, morphological, and physiological changes. Increasing evidence shows that these antipredator responses can also lead to shifts in life-history traits including influencing parental reproduction, particularly for females that experience predation risk in their reproductive stage. However, little is known about how parental experience with predation risk affects the performance of offspring. I raised parental spider mites (*Tetranychus urticae*) either on a leaf disc with or without odour from a natural predator (*Phytoseiulus persimilis*). I showed that predator-derived odour prolonged immature duration of both sexes, shortened female adult lifespan but not that of males, and reduced lifetime reproductive outputs of the females. My studies of offspring from both odour-exposed and control mothers demonstrated that parental effects were significant in the early developmental stage of offspring, but not in later life stages. The offspring were strongly negatively affected by the predation risk when they were directly exposed but not the predation risk experienced by their mothers. Additionally, the parental effects in the earlier life stage were sex-specific, with delayed hatching in daughters (but not sons) when parents were exposed to predation risk. My transgenerational study indicates that there are adverse effects of predator-induced stress on aging and lifespan of prey for both parents and their offspring, although the parental effects appeared to be weak (in the early stage of offspring but diminished in adult stage). My results highlight the sex-difference of prey in response to predation risk and sex-dependent parental effects on the offspring.

Key-words: Predator-induced stress, development, lifespan, reproduction, sex difference, transgenerational effects

7.1 Introduction

Predators can affect the demography of prey by killing them directly. This traditionally well-recognised consumptive effect has received extensive attention in ecology (Trussell *et al.* 2011). However, in the last few decades, growing evidence suggests that non-consumptive effects (i.e., predation risk or the fear of prey to predators) are remarkable, and can even be greater than the consumptive effects (Zanette *et al.* 2011; MacLeod *et al.* 2014). A large body of literature has proved that predation risk can induce prey to alter their behavioural, morphological and physiological responses (e.g. Mondor *et al.* 2005; Brown *et al.* 2013; Culler 2014). For example, to avoid being detected by predators, many organisms reduce their activity, forage less, and spend more time in refuges (McPeek 2004; Donelan & Trussell 2015; Lima & Dill 1990; Verdolin 2006; Lima 1998). Generally, these adaptive behaviours come at the cost of limiting the quantity and quality of food available to the prey (Orrock *et al.* 2013). In response to perceived predation risk with the limited resource available, prey species may change their life history strategies by balancing nutrients and energy for development and fat storage, somatic maintenance and investment in offspring.

Substantial empirical studies have explored how life-history traits of prey were shaped by the predation risk they experienced in a wide range of organisms. In some organisms, growth was slowed because of the reduction in feeding and decreased assimilation efficiency (Stoks *et al.* 2005). However, other studies provided contradictory results. For example, the tobacco hornworm caterpillar (*Manduca sexta*) showed a remarkable increase in assimilation efficiency and quantity of nutrient intake to compensate for the food reduction; they actually gained similar body mass and even developed much faster than controls free of predation risk (Thaler *et al.* 2012). In addition, females exposed to predation risk demonstrated decreased reproductive investment within relatively short experimental periods (Creel *et al.* 2009; Zanette *et al.* 2011; Ferrari & Schausberger 2013). Most studies only focused on the short-term response (e.g., immature development or reproduction) of prey to predation risk. Because the life history components are tightly interrelated with each other, studies are

needed to identify the long-term effects of predation risk on other components of fitness, such as lifetime fecundity and lifespan.

The trans-generational effect has been an area of great interest for the researcher and was assumed to be adaptive when the environment exposed by the mother and offspring are identical or similar according to the predictive adaptive response hypothesis (Mousseau & Fox 1998; Bateson *et al.* 2014). For example, the morphology plasticity of insects varies in response to predation risk. The cotton aphid (Mondor *et al.* 2005) and pea aphid (Weisser *et al.* 1999; Dixon & Agarwala 1999; Kunert & Weisser 2003) exposed to predation risk gave birth to more winged offspring, which ensured their offspring capable of dispersing to low-risk habitat. This adaptive morphological character increased the survival of offspring. However, these advantages may occur at the expense of prolonged developmental time or smaller adult body size. This would consequently impair reproductive fitness in later-life and reduce opportunities for mating, lowering survival rate as well as decreasing fecundity (Magnhagen 1991). Therefore, without rigorous long-term of examination of the parental effects on phenotypic plasticity, the results of short-term tests may fail to detect the potential life-history consequences (Sheriff & Love 2013).

The similarities between the mother's environment and the offspring's future condition appear to benefit offspring by enhancing survival and performance. However, if the environment changed, a mismatch between the offspring and mother's environment may occur and consequently the transgenerational plasticity would be maladaptive (Monaghan 2008; Sheriff & Love 2013). In sheep, the mismatch between prenatal and postnatal nutrient treatments resulted in altered renal function and a higher risk of cardiovascular disease in adulthood (Cleal *et al.* 2007). Even though the adaptive response hypothesis is challenged when the offspring are confronted with different conditions from those experienced by their mother, little is known about the fitness consequences of these environmental shifts.

The response to transgenerational effects is likely to depend on the sex of offspring. A few studies have shown that in the presence of predation risk, parents may allocate resources and hormones differently between sons and daughters. For instance, in a field study with

European starling, mothers exposed to stress transferred higher levels of corticosterone to the eggs which developed into females later. This sex difference between maternal hormone investment led to higher mortality in males (Love &Williams 2008). All these recent research studies suggested that there is value to include the offspring sex as a factor in the future study of parental effects.

Here I addressed this open question by investigating how predation risk affected the lifetime fitness over two generations in a spider mite species. The spider mite (*Tetranychus urticae*), and its specialist predator (*Phytoseiulus persimilis*) are a well-studied predator-prey system for predation risk research (Grostal & Dicke 1999; Škaloudová *et al.* 2007; Choh *et al.* 2010). Spider mites can perceive indirect cues of risk from predators through chemical traces derived from metabolic waste and footprints, as well as from killed conspecifics remaining on the leaf, without direct contact with predators. These detected chemical traces can cause changes in prey behavior, even in the physical absence of the predator (Grostal & Dicke 1999). Spider mites exposed to predation risk showed a range of innate anti-predator behavior including increased dispersal (Grostal & Dicke 1999), more walking activity (Ferrari & Schausberger 2013), altered feeding rates, delayed oviposition of the first egg (Ferrari & Schausberger 2013), and decreased fecundity (Škaloudová *et al.* 2007; Choh *et al.* 2010; Ferrari & Schausberger 2013). In a recent behavioral study, the risk-experienced spider mites also demonstrated bolder behavioral strategies than predator-naïve spider mites (Hackl & Schausberger 2014). Nonetheless, it is not well understood how the life history of spider mite was shaped by anti-predator behaviour when they experience long-term predator-induced stress. Furthermore, the majority of research only focused on the impacts of predator-induced stress either during the immature stage or the reproductive stage within one generation.

In this study, I tested the hypothesis that animals aged faster and had shorter lifespan under predator-induced risk using the spider mite as prey and the odour of its predator as predation risk. The effects of predator-induced risk were investigated for two consecutive generations. I first explored how the development of spider mites was influenced by long-term exposure to predation risk. Second, I investigated how females under chronic predation risk balanced the resource allocation between somatic maintenance (i.e. longevity) and investments in the

offspring (i.e. fecundity). I also examined how males coped with the chronic predation risk to see if the effects of predation risk on aging were sex-specific. Finally, I determined how the parental effects induced by predation risk react with the future environment encountered by the offspring in affecting offspring fitness regarding development, reproduction, and lifespan.

7.2 Material and methods

7.2.1 Mite colony

The spider mite *Tetranychus urticae* (Acari: Tetranychidae) is a herbivorous arthropod feeding on plants from diverse taxa including crops of important agricultural and economic value. A laboratory colony was established on the potted common bean (*Phaseolus vulgaris* L.) in February 2015. The bean plants were sowed and grown in a greenhouse compartment at 20 ± 5 ℃, 60 ± 10% RH, with a photoperiod of 16: 8 (L: D). The spider mite population was offered new bean plants in their two-leaf stages weekly (Li & Zhang 2016).

Phytoseiulus persimilis (Acari: Phytoseiidae) is a specialist predator of *T. urticae*. The predator population was provided by BioForce Ltd, New Zealand, and was maintained on a black plastic sheet placed on a sponge centred in a plastic box (20 cm × 20 cm × 4cm) with water. This box was put into a much larger box with saturated saltwater to maintain high humidity and prevent mites from escaping. The predators were provided with bean leaves infested with spider mites at three-day intervals.

7.2.2 Leaf-discs with and without predation risk

The common bean plants were grown in the greenhouse every week and their leaves used for the experiment at their two-leaf stage. To eliminate the influence of different host plants, the leaves for spider mites with predation risk and without risk treatments were from the same batch of plants. These leaves were hole-punched to create discs (10 mm in diameter) that were placed into each well of a cell culture plate (24-well plate with flat bottom 84 × 126 mm) filled with water. The leaf discs floated on the surface of the water. To generate

predation risk for spider mites, half of the leaf discs were treated with *P. persimilis* by transferring a gravid female onto each leaf disc. The predator odour, metabolic waste and footprints left on the leaf surface (generally termed predator odour) served as predation risk. After 24 hours, the predator and its eggs, if any, were removed. The leaf discs were used for rearing spider mites in the predation risk treatment. But the leaf discs were discarded if the predator escaped or drowned during exposure. The other half of the leaf discs were left untreated without predators for 24 hours (controls) before they were fed to spider mites.

7.2.3 Rearing units

The leaf discs were arranged 12 in each row onto a wet sponge, so they would remain fresh for a few days. The water-soaked sponges (20 cm × 15 cm × 2 cm) were placed in a slightly larger plastic tray with water. Water was added twice a day (in the morning and afternoon) to keep the sponge wet. Each leaf disc was marked and used as the rearing arena for one spider mite. During the experiment, some spider mites drowned in the water and were excluded from data analysis.

7.2.4 Development of spider mites

The experiment was set up by introducing one female from the laboratory colony to each leaf disc and allowing them to lay eggs for 3–4 hours. These females were then removed from leaf discs, and the eggs allowed to hatch. From the third day, the hatched larvae were transferred randomly onto a new leaf disc: half of them onto the leaf discs with predatory cues (risk treatment), the remainder onto the leaf discs without exposure to predator odour (risk-free treatment = control). In both treatments, every spider mite was transferred to new leaf discs in the same manner as the former treatment after the protonymph and deutonymph emerged. Hence, the immature spider mites in the risk treatment were exposed to predatory cues three times over six days. The development of spider mites was observed and recorded at 8- and 16-hour intervals (8:00 am and 4:00 pm every day).

7.2.5 Lifespan and reproduction of females

After the deutonymphs moulted into adults, each female was transferred to a new leaf disc, paired with a male from the same treatment, and then reared together until the end of the experiment, that is when all mites died. The leaf discs were changed at an interval of three days. The pre-oviposition period was recorded from the emergence of the female to the time that the first egg was deposited. During the oviposition period, the number of eggs produced by each female was checked daily. The post-oviposition period began on the day the last egg was deposited and ended with the death of the female. Both females and males were recorded every day until death. To measure body size of mites, the spider mites were mounted in Hoyer's solution on glass slides (Krantz & Walter 2009) after death and dorsal lengths measured under a Nikon E-800 microscope (dorsal shield length being a reliable indicator of mite body size).

7.2.6 Egg size and sex ratio of offspring

To clarify the impact of predation risk on egg size and sex ratio of offspring, the eggs deposited in the first three days were collected. Five eggs from each female were measured in width (a) and length (b) under a Nikon microscope. The egg size was calculated by the formula for a prolate spheroid: $S=\pi ab$. Eggs collected on the 4^{th} day of oviposition were kept on fresh leaves and were transferred to new leaf discs without predation risk after they hatched. The sex was identified and recorded when they developed into adults. The offspring sex ratio was calculated as the proportion of male offspring.

7.2.7 Transgenerational effects

To examine how parental experience affected the life history traits of the next generation, eggs produced by mothers free of risk and under risk were randomly assigned to two treatments: (1) offspring under risk; (2) offspring free of risk, generating four factorial treatments in the offspring generation (as shown in Figure 7.1). To obtain the hatching time

and developmental time, the development of these eggs was observed at 8- and 16-hour intervals (8:00 am and 4:00 pm) until adults emerged. Females were paired with a male from the same treatments and the survival and reproduction of female offspring were checked. The males were dropped out of our study in their adult stages because the results on the first generation indicated that males' longevity was not affected by predator-induced risk. All the experiments were conducted under the conditions of 26 ± 1℃, 60 ± 10% humidity, with a photoperiod of 12: 12 (L: D).

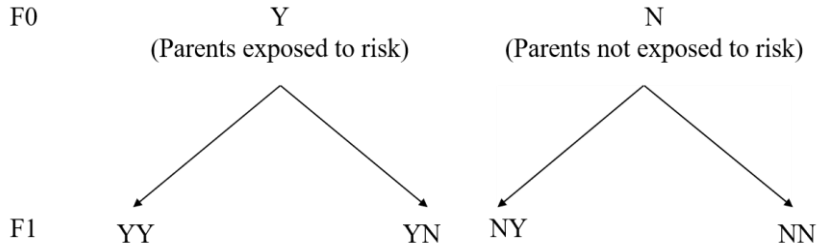

FIGURE 7. 1 Experimental designs for determining the influences of predator odour-induced risk on spider mites across two generations. Y is parents exposed to risk, N is parents not exposed to risk. YY indicates offspring from risk-exposed parents under risk, YN indicates offspring from risk-exposed parents free of risk; NY indicates offspring from risk-free parents experienced risk directly, NN indicates offspring from risk-free parents didn't experience risk.

7.2.8 Statistical analyses

The proportional data, including immature survival rates and the male offspring ratios of spider mites across treatments, were compared with the Chi-square test. The adult survival rates were analysed using the Kaplan-Meier technique by log-rank test respectively for females and males with perceived predation risk as a factor. Prior to analysis, the other parameters were checked by Kolmogorov-Smirnov test to see whether they are normally distributed. When necessary data were log-transformed to improve normality. However, data presented in the results are all untransformed means, unless otherwise indicated. If transformed data still did not show normality, then non-parametric methods were used. In testing for the effects of parental experience with risk and offspring sex on the hatching age, a

two-way analysis of variance (ANOVA) was performed including parental effects and sex as two main factors. In order to check whether the developmental periods vary with indirect environment (parental experiences) and direct environment (offspring environments), I analysed the immature duration (from egg to adult emergence) using MANOVA including parental effects, offspring experience and sex as explanatory variables. The female lifespan, reproduction parameters such as pre-oviposition period, oviposition period, post-oviposition period, total fecundity, daily fecundity were analysed by two-way ANOVA including parental effects and offspring experience as two main factors. All data analyses were performed with R (Version 3.4.3).

7.3 Results

7.3.1 Effects of predation risk on survival and immature development of parents

To determine the effects of predation risk on lifespan and other aspects of prey fitness, I exposed spider mites to predator odour throughout their life. I first examined the survival and development of immature spider mites exposed to predator odour. The survivorship of immature spider mites exposed to predator odour (82.2%) was not significantly lower from those without exposure (88.9%) (χ^2 =1.61, df = 1, P = 0.203) (Figure 7. 2A). However, immature development was prolonged by exposure to predator odour regardless of sex (W = 118.5, P < 0.000 for female; W = 395, P = 0.046 for male) (Figure 7. 2B), suggesting that both sexes were very sensitive to perceived predation risk as juveniles. Furthermore, females that had experienced predator odour were statistically smaller than control females (W = 715, P = 0.013), while males were the same size independent of treatment (W = 88, P = 0.856) (Figure 7. 2C).

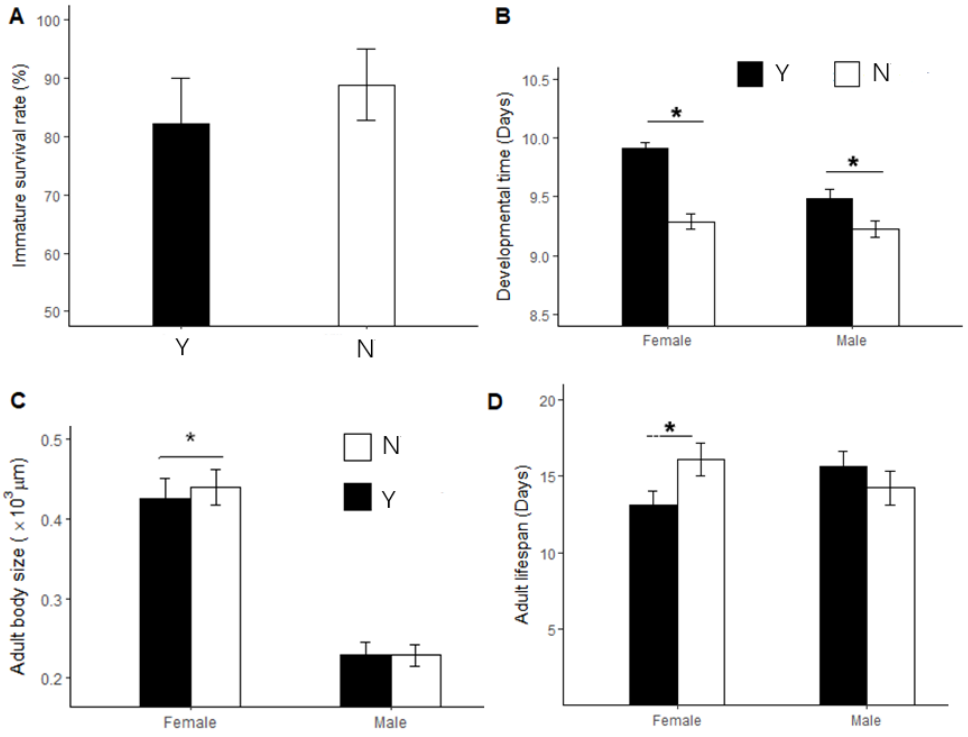

FIGURE 7. 2 Immature survival, developmental time, adult body size and lifespan of spider mites (*Tetranychus urticae*) exposed to odour from predator (*Phytoseiulus persimilis*) compared with mites free of risk. **(A)** The proportions of spider mites that survived to adulthood was not different between mites exposed to predator odour (under predation risk) and controls. **(B)** Both females and males prolonged their development in response to predator odour. **(C)** Adult body size of mites showed no response to predation risk when female and male data were pooled together. But the females that experienced predation risk were statistically smaller, while there is no evident effect of perceived predation risk on males. **(D)** The adult lifespan of females reduced by 3 days (18.8%) when they were exposed to predator odour, whereas the males showed no response to predator odour. The asterisks indicated there were significant differences between treatments. Values are Means ± SE.

7.3.2 Influences of predation risk on lifespan and reproduction of parents

On the first day of adult emergence, female and male spider mites were paired and the lifespans of both sexes monitored. Sex interacted with predator odour to influence the lifespan of spider mites: females exposed to predator odour showed a significant reduction in lifespan by 3 days (t = 2.132, df = 47.621, P = 0.0381; Figure 7. 2D), whereas the lifespan of

males was not significantly influenced (t = –0.988, df = 44.265, P = 0.328; Figure 7. 2D). Further survival plots demonstrate that the females exposed to predator odour had much lower survival rates than the control (χ^2 = 5.1, df = 1, P = 0.025) (Figure 7. 3A), suggesting that predation risk increased the mortality risk of females, but not of males (χ^2 = 1.3, df = 1, P = 0.255) (Figure 7. 3B). Thus, there is a sex difference in response to perceived predation risk: female spider mites are more susceptible to risk in terms of aging and lifespan, whereas the males are resilient to perceived predation risk in their adult stage.

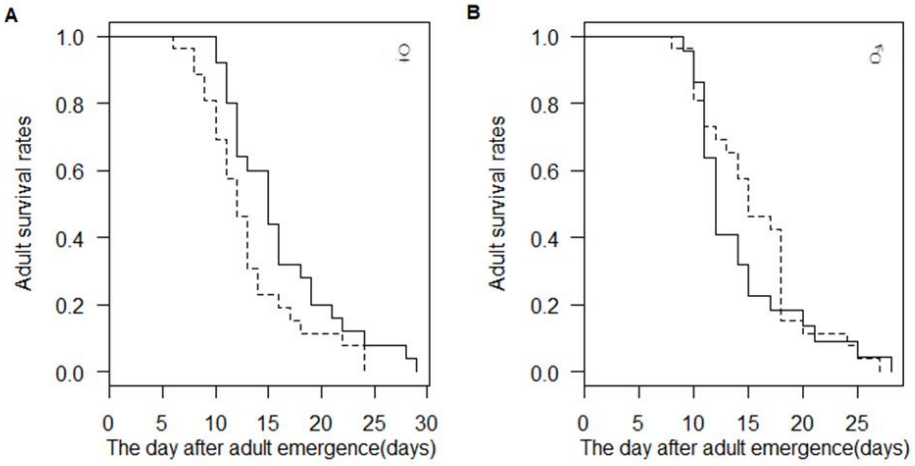

FIGURE 7. 3 Age-specific survival rates of spider mite females and males in response to predator odour. **(A)** For the females, significant influence was observed between females exposed to predator odour (dash line) and the controls (solid line), as much higher survival rates were shown in females free-of risk. **(B)** Predator odour did not exert significant effects on male adults.

Female spider mites also changed their reproduction strategies when they were exposed to predation risk: compared with females without exposure, they reduced both daily reproductive rates (t = 2.742, df = 47.676, P = 0.009) and total fecundity by 29.8% (t = 3.693, df = 46.89, P = 0.001) (Table 7.1 & Figure 7. 4A). In addition, they delayed the onset of reproduction (W = 360.5, P = 0.0020) (Table 7.1), with a remarkably shorter oviposition period (t = 2.077, df = 48.918, P = 0.043) (Table 1); however, there was no significant difference in post-oviposition period (Table 7.1). Perceived predator risk not only decreased the commitment to reproduction but also accelerated the reproductive aging of females. The

offspring sex ratio and egg size were checked to further clarify the reproductive investment of females: both showed no significant response to the perceived predation risk (χ^2 = 0.621, *df* = 1, *P* = 0.431 for offspring ratio; *W* = 948, *P* = 0.320 for egg size) (Fig 7.4B & 7.4C).

TABLE 7. 1 Comparison of reproductive parameters between females exposed to predator odour (under predation risk) and the controls (without predation risk). The females under predator odour had a longer pre-oviposition period, but shorter oviposition period. With a remarkable lower daily reproductive rate, the females reduced their total fecundity. Additionally, there was no significant difference in post-oviposition. Values are means ± SE.

Treatments	**Pre-oviposition period (days)**	**Oviposition period (days)**	**Post-oviposition period (days)**	**Reproductive rate (egg/female/day)**
Under risk	1.11±0.03	11.57±0.86	1.05±0.09	6.64±0.29
Without risk	0.99±0.02	14.13±0.88	1.78±0.43	7.71±0.25
t* (W), *df	360.5	2.077, 48.918	112.5	2.7415, 47.676
P	**0.002**	**0.043**	0.0645	**0.009**

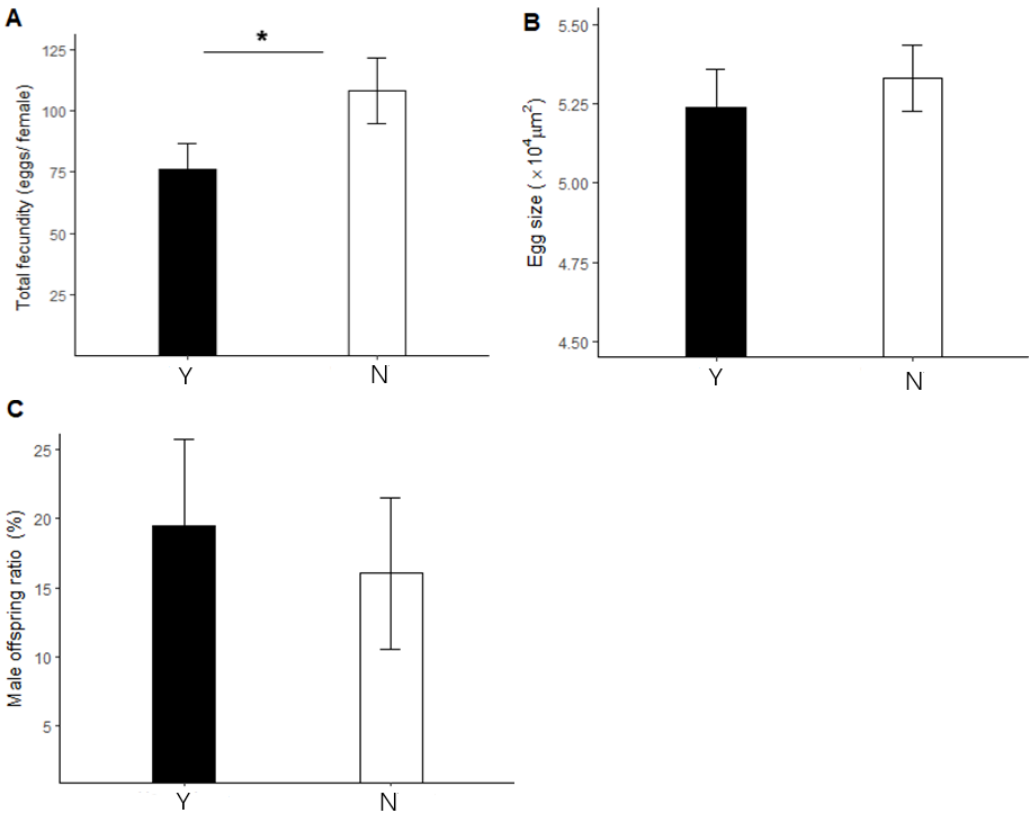

FIGURE 7. 4 Effects of predator-induced risk on the lifetime fecundity of female, egg size and offspring sex ratio. **(A)** The females exposed to predator odour produced significantly fewer eggs compared with controls. **(B)** Egg size was not affected by mothers' experience with predation risk. **(C)** Male offspring ratio did not differ between mothers exposed to predation risk and control mothers. Values are Means ± SE.

7.3.3 Trans-generational effects of predation risk on hatching age of offspring

There is a marginal difference in the hatching times of eggs produced by mothers with predation risk and those without risk ($F_{1, 299}$ = 3.255, P = 0.072, Figure 7.5), and the parental environment interaction with sex influencing the hatching age of the offspring ($F_{1, 299}$ = 12.291, P = 0.001). The daughters of mothers who had experienced risk hatched significantly later than those of mothers without exposure to predation risk ($F_{1, 236}$ = 0.985, P = 0.033), while the sons did not show the same pattern ($F_{1, 59}$ = 0.110, P = 0.912). Additionally, the female offspring hatched much earlier than the male offspring ($F_{1, 299}$ = 55.720, $P<0.001$).

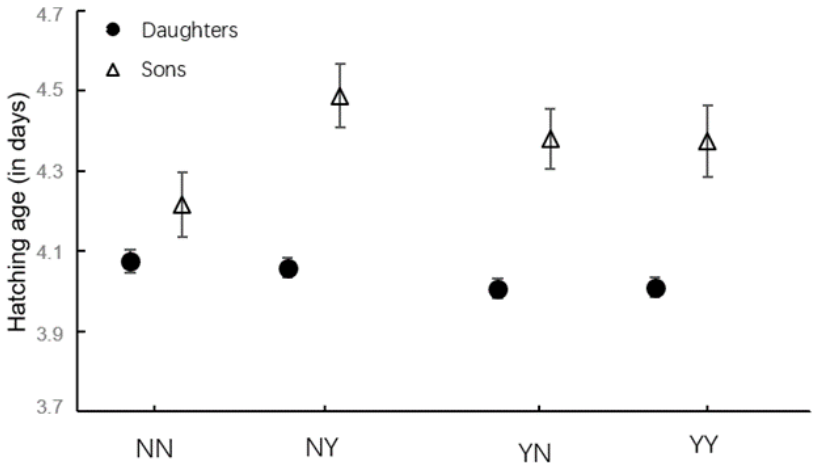

FIGURE 7. 5 Hatching age of daughters and sons from parents exposed to risk or free-of risk. Sons hatched later than daughters across treatments.

7.3.4 Trans-generational effects of predation risk on immature survival of offspring

The immature survival rate of offspring differed significantly across treatments (χ^2 = 11.980, *df* = 3, *P* = 0.003, Fig 7.6A). The lowest survival rate was observed in risk-exposed offspring produced by odour-exposed mothers (77%), which differed significantly from odour-exposed offspring (86.74%, χ^2 = 3.932, *df* = 1, *P* = 0.047) and risk-free offspring produced by mothers without exposure to risk (93.75%, χ^2 = 11.144, *df* = 1, *P* = 0.001), which did not differ from that of risk-free offspring produced by odour-exposed mothers (82.35%, χ^2 = 1.486, *df* = 1, *P* = 0.223). Furthermore, the survivorship of risk-free offspring of non-risked mothers was 11.4% higher than that of risk-free offspring of odour-exposed mothers (93.75% VS 82.35%, χ^2 =5.026, *df* = 1, *P* = 0.025). The offspring's experience did not influence the offspring survival when their mothers were not exposed to predation risk (86.74% VS 93.75%, pair-wise comparison, χ^2 = 2.256, *df* = 1, *P* = 0.133).

7.3.5 Trans-generational effects of predation risk on immature development of offspring

The maternal experience of predator-induced risk did not show significant influence on offspring developmental time ($F_{1,284}$ =1.252, P = 0.264, Fig 7.6B & 7.6C), nor did it interact with the offspring experience to affect the development of offspring ($F_{1, 284}$ = 1.811, P = 0.179). The other two main factors, offspring risk experience ($F_{1,284}$ = 64.271, P <0.000) and sex ($F_{1,284}$ = 82.505, P < 0.000) had pronounced influence on the developmental time of spider mites, with the risk-free offspring developing much faster than those of risk-experienced offspring (Fig 7.6B & 7.6C), and sons showed a shorter immature stage. The interaction between the offspring's risk experience and sex was nonsignificant ($F_{1,284}$ = 0.872, P = 0.351).

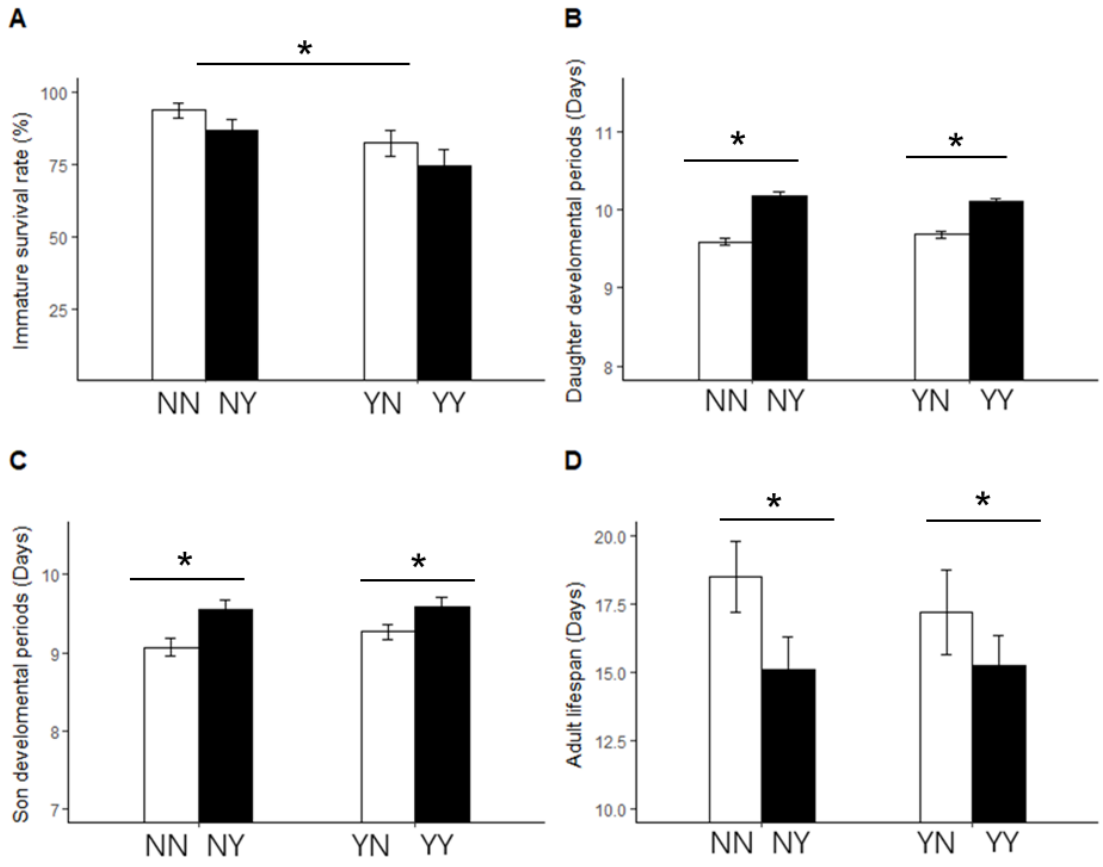

FIGURE 7. 6 Effects of parental effects and offspring exposure to predation risk on the immature survival, female and male offspring developmental period and adult lifespan. **(A)** The immature survival rates of the offspring were significantly influenced by the mother's experience with risk and the offspring's experience. **(B)** No significant influence of parents' experience but the daughters exposed to predation risk prolonged their development. **(C)** The development of sons was not influenced by their parents' experience, but sons experienced risk showed longer immature duration. **(D)** The females experienced predation risk has a shorter adult lifespan regardless of their mothers' experience with risk. Values are Means ± SE.

7.3.6 Trans-generational effects of predation risk on lifespan and survival offspring

There is no significant influence of the mother's predator experience on the lifespan of female offspring ($F_{1, 128} = 0.344$, $P = 0.558$), nor the interaction between mother's risk

experience and offspring's experience ($F_{1,128}$= 1.816, P= 0.180). However, the offspring's exposure to risk had a significant adverse impact on their lifespan ($F_{1, 128}$ = 5.107, P = 0.026, Fig 7.6D), whereby on average the daughters without exposure to risk outlived those daughters that experienced chronic risk by 2.7 days (Fig 7.6D).

The log-rank test showed that there was evidence of a difference in survivorship between the four treatments (χ^2 = 7.867, df = 1, P = 0.049). In pair-wise comparison, it was further showed that the offspring of mothers exposed to risk did not differ in survival whether the offspring environment matched with their mother's or not (χ^2 = 0.224, df = 1, P = 0.636). However, the offspring of mothers without the experience of risk differed depending on the offspring's experience, with the odour-exposed offspring having a lower survival rate than that of risk free (χ^2 = 6.457, df = 1, P = 0.011). In comparison, the odour-exposed mothers enhanced the survival of their offspring in a stressful environment, while the offspring of free-risk mothers were more sensitive to predator odour.

7.3.7 Trans-generational effects of predation risk on reproduction of offspring

The reproductive parameters were resilient to the parental effects but strongly affected by the offspring environment. There is no significant influence on reproductive parameters of parental experience with predator odour. However, the daughters raised under predation risk changed their reproduction investment: they produced their first egg much later ($F_{1, 167}$ = 5.178, P = 0.024, Table 7.2), had a shorter reproductive lifespan ($F_{1, 132}$ = 178.241, P = 0.036) and lower daily fecundity ($F_{1, 131}$ = 15.366, P < 0.000), ultimately reduced their total investment in eggs by 24% ($F_{1, 131}$ = 20.177, P < 0.000). Additionally, no interaction between parental effects and offspring environmental was detected.

TABLE 7. 2 Summary of analysis of variance on reproductive parameters including pre-oviposition period, oviposition period, post-oviposition period and lifespan of the daughters. Generally, the mother's experience showed non-significant effects on the daughters' reproduction while the experience of daughters affected their reproduction. Significant effects are shown in bold.

Pre-oviposition period	SS	df	F	P
Parental treatment	0.039	1	0.428	0.514
Offspring treatment	0.47	1	5.178	**0.024**
Parental × offspring treatment	0.16	1	1.763	0.186
Error	0.091	167		
Oviposition period				
Parental treatment	0.386	1	15.291	0.535
Offspring treatment	4.505	1	178.241	**0.036**
Parental × offspring treatment	0.158	1	6.251	0.692
Error	39.565	132		
Post-oviposition period				
Parental treatment	0.259	1	0.035	0.852
Offspring treatment	4.388	1	0.593	0.444
Parental × offspring treatment	0.097	1	0.013	0.909
Error	7.402	61		
Daily fecundity				
Parental treatment	1.69E-	1	0.000	1.000
Offspring treatment	34.282	1	15.366	**0.000**
Parental × offspring treatment	0.913	1	0.409	0.524
Error	2.231	131		

7.4 Discussion

Our results provide evidence that chronic predation risk (exposure to predator odour) prolonged the immature stage of spider mite as previously reported and also indicate for the first time that the stress accelerated aging processes and shortened the adult lifespan of female spider mites, resulting in reduced reproductive success. Furthermore, I demonstrate that parental effects were significant in the early hatching stage. In later life stages, however, there was no significant inter-generational influence on adult lifespan and reproduction, and offspring were strongly negatively affected by the predation risk to which they had been directly exposed. The interaction between parental and offspring environment was also non-significant. Additionally, the parental effects in the earlier life stage were sex-specific. It was found that daughters delayed hatching when parents were exposed to predation risk. In contrast, sons did not show a similar pattern. To our knowledge, this is the first study in which a full factorial design was used by crossing the parental and offspring environment to measure effects of predator-induced risk on long-term fitness consequences (lifespan).

7.4.1 Effects of predator odour on development

There was evidence that predation risk decreases growth rate with ignorance of sex in a wide variety of species (Altwegg 2002; Griffis-Kyle & Ritchie 2007; McPeek *et al.* 2001). However, several studies have suggested that females and males are different in behaviour when faced with predator-induced risk. Females may be less bold than males (Giles & Huntingford 1984) and show stronger response to predation risk in terms of vigilance, activity, foraging and sensitivity to environmental conditions (Creel *et al.* 2005; Mikolajewski *et al.* 2005, Mikolajewski *et al.* 2008, Christianson & Creel 2008). Even though sex differences in response to predation risk are evident it has been unclear how the life history of the risk-experienced organism is affected (but see Mikolajewski *et al.* 2008). In this study, the development and lifespan of risk-experienced spider mites were analysed with sex as a factor. Overall, sexual difference in developmental and aging plasticity was shown. As has been shown previously males under food limitation or predation risk were less plastic

than females in development (Walzer & Schausberger 2011). The extra behavioural response to predation risk by males was one of the possible explanations. However, males benefit from emerging earlier while females benefit from emerging at a larger body size (De Block & Stoks 2003; Mikolajewski *et al.* 2005). Males were sexually selected to emerge earlier to compete against their kin in guarding the deutonymphal females and gain mating opportunity. The females were supposed to emerge at maturity with large size to enhance reproductive ability.

7.4.2 Effects of predator odour on lifespan and aging

In this study, the spider mites were exposed only to the olfactory cues of predators, without any direct contact with the predator. Sensory perception thus plays a crucial role in modulating aging in spider mites. Previous studies have shown that environmental cues, such as food-derived odour, reduced the lifespan of long-lived flies under dietary restriction (Libert *et al.* 2007), and that female sexual pheromones decreased the lifespan of males through the olfactory regulation (Gendron *et al.* 2014). In addition, olfaction and sensory deficient mutants have extended lifespans in model organisms such as worms and flies (Apfeld & Kenyon 1999; Alcedo & Kenyon 2004, Libert *et al.* 2007). The evolutionarily conserved aging regulation pathway, the insulin signalling pathway, was assumed to be involved in modulating the lifespan when animals are exposed to olfactory cues, but it remains unknown how environmental cues alter gene expression and longevity.

The underlying biomedical mechanism of how predator-induced risk modulates aging processing and longevity is unclear. It is possible that induced stress shortens the lifespan through physiological responses such as elevated concentrations of stress proteins including Hsp60 and Hsp70, which have already been reported both in invertebrates (Pauwels, Stoks & De Meester 2005) and vertebrates (Kagawa, Ryo & Mugiya 1999; Fleshner *et al.* 2004). These stress proteins are closely associated with survival and reproduction as they increase oxidative stress and trigger telomere erosion, although these two processes are not mutually exclusive in regulating aging (Haussmann & Marchetto 2010). The exacerbated generation of free radicals in cells not only causes damage to protein, lipoproteins and lipids but also to

nuclear acids. The accumulation of the damage on molecular and cell structure contributes to the organisms' vulnerability to diseases and senescence.

7.4.3 Effects of predator odour on the reproductive success of female

The lifetime reproductive outputs in spider mites exposed to predator-risk were reduced by 29.8% as a consequence of delayed reproduction and reduced daily fecundity. Perceived predation risk has also been observed to decrease annual reproduction in song sparrows (*Melospiza melodia*) (Zanette *et al.* 2014), and songbirds under nest predation reduced foraging and gained less body fat, which contributed to the smaller clutch size (Zanette *et al.* 2011). Previous studies on the neriid fly *Telostylinus angusticollis* (Adler *et al.* 2013) demonstrated a trade-off between longevity and reproduction following mild stress such as dietary restriction. However, allocation trade-offs were not apparent in this study. When female spider mites experienced chronic predator-induced risk, they showed a decline in both longevity and fecundity. The results suggest that under chronic predator-induced risk the spider mites failed to balance the high energy-demanding somatic maintenance and reproduction investment.

7.4.4 Sex-specific response to predator odour

Adult lifespan was negatively influenced by predator-induced risk only in females. Male spider mites are very aggressive, and sometimes even kill their conspecifics (Potter *et al.* 1976) and deutonymphs of other mite species (Lee *et al.* 1969). Therefore, male spider mites are less sensitive to predation risk than females. Sex-specific response to predation risk could also result from their sex differences in investment in reproduction and somatic maintenance. Males cease growth after maturity and contribute to the offspring only by providing the female with sperms that are less costly than eggs. However, females keep on increasing body biomass in the first week after adult emergence (Mitchell 1973; Bowler *et al.* 2013) and invest heavily in reproduction by producing eggs daily, equivalent to 11% to 48% of the weight of the female (Mitchell 1973). A combination of higher resource requirements and

greater vulnerability to predation likely accounts for this shortened longevity following perceived predation risk. This result is consistent with other research that links sex-specific response in longevity to factors such as dietary restriction, temperature, immune status, inbreeding, and sexual selection (Nakagawa *et al.* 2012, Archer *et al.* 2015, Kelly *et al.* 2014, Bilde *et al.* 2009, Maklakov *et al.* 2007). The underlying mechanisms of sex-specific response to environmental factors deserve further exploration.

7.4.5 Life-stage-dependent parental effects

In this study, the transgenerational effects of predator-induced risk were found in the early developmental stage of offspring. The predator risk experienced by parents had a negative influence on the hatching of daughters. A previous study with *T. urticae* focusing on the developmental plasticity to predator-induced risk further suggested that the parental effects persisted in the next generation until the larval and protonymphal stages (Freinschlag & Schausberger 2016). However, the parental effects on offspring disappeared in later life stages, both in this study and in a previous study (Freinschlag & Schausberger 2016). Furthermore, a mother's experience with risk showed no significant influence on the immature duration. A similar response of offspring to predation risk was also observed in the great tits *Parus major*. It was reported a shorter winging was measured on 8-day-old daughters, but not in 14-day-old nestling daughters (Coslovsky & Richner 2011). Also the body mass at 3 days post-hatching was significantly influenced by yolk carotenoids, while other traits measured including tarsus length, immune response or breast plumage colouration were not affected in later life stages (Marri & Richner 2014). Overall, inter-generational effects appear during early life history phases and dissipate with age of the offspring. The predator-induced risk decreased the mothers' longevity by 3 days and the response of risk-experienced daughters was of similar magnitude (2.7 days) and direction. Thus, the daughters did not show anticipatory parental effects. This is in line with a meta-analysis that found weak empirical support for adaptive transgenerational plasticity from 58 studies of both animals and plants (Uller *et al.* 2013).

The transgenerational effects have been reported in a wide range of taxa in the last few decades, even though the underlying mechanisms have not been fully understood. The transgenerational effects have been attributed to maternal provision or epigenetic inheritance (Giesing *et al.* 2010; Morosinotto *et al.* 2013, Mueller & Bale 2008; Oberlander *et al.* 2008; Rubenstein *et al.* 2016). According to the trade-off theory of reproduction and survival, in adverse environments, the mothers tend to affect their offspring by manipulating sex ratio and the number and size of eggs (Clout *et al.* 2002; Vijendravarma *et al.* 2009). In this study, I found that mothers exposed to risk indeed showed a reduction in lifetime reproductive success, but there was no decrease in egg size. Since the eggs produced by risk-exposed mothers hatched later, it is possible that the composition of eggs, such as deficits of energy and nutrient or elevated content of stress hormone, accounted for the delayed hatching of the daughter produced by risk-experienced mothers. In this scenario, at later developmental stages, the spider mites might become more independent of maternal investment and manage to compensate for negative effects of parents on development by increasing foraging and metabolism efficiency (Donelan & Trussell 2015; Hawlena & Schmitz 2010). However, it is less likely that epigenetic inheritance such as DNA methylation was also involved in shaping the offspring fitness because epigenetic programmed parental effects were likely to have more profound effects and might extend to the late-life stage or even whole life, as indicated by recent studies (Greer *et al.* 2011). Future studies are needed to advance our knowledge of the potential underlying mechanisms involved in the transmission of maternal effects.

7.5 Conclusion

In conclusion, this study reveals that predator-induced risk is deleterious for spider mites in terms of retarding development, accelerating aging and decreasing lifetime fecundity. I also demonstrated that although the stressful environment experienced by parents could indeed influence the subsequent generation, the parental effects were limited to the earlier developmental stage of female offspring. In the adult stage, both the male and female offspring were not affected by the transgenerational effects, suggesting there is little evidence for the anticipatory parental effects when the offspring environment matched the maternal

environment. However, the environment experienced by the offspring directly had a substantially stronger influence than the maternal environment. I assumed the sex difference in response to parental effects was observed because of sex-biased investment or sex-specific response to stress hormone. Further investigations are warranted to determine how the proposed mechanisms shape the difference in responses to parental effects between male and female offspring.

Chapter 8 General discussion

8.1 Background

Aging is a topic which attracts the attention of researchers from multiple disciplines such as biomedical gerontology, biology as well as the specialists in other related fields (Victor 2004). It remains an active area of research not only because of human interest in aging and immortality (Lucke & Hall 2005) but also because of the wide variety of aging patterns displayed across taxa (Jones *et al.* 2014).

From the perspective of a gerontologist, it is of great value to gain a better knowledge of the underlying mechanisms of how we age, which may offer us reasonable approaches to delay aging and decrease the incidence of age-related diseases. During the past few decades, spectacular advances have been achieved in understanding aging and exploring anti-aging interventions. A variety of anti-aging interventions, including diet restriction, genetic manipulation and pharmaceutical administration, have been tested on well-recognized model species (Longo *et al.* 2015; Austad & Bartke 2016; Vaiserman *et al.* 2016). Among these, dietary restriction was proved to be the most reliable and robust approach to prolong lifespan and delay the onset of aging-related diseases (Kapahi *et al.* 2017; Liang 2018). Although a large body of literature on model species demonstrates that animals tend to share conserved pathways regulating aging, there is also emerging research generating surprising and unexpected results. For example, dietary restriction (DR), one of the first and reasonable anti-aging intervention being investigated, failed to prolong lifespan of some non-classical model systems, for example, tephritid flies (Carey *et al.* 2002), housefly (Cooper *et al.* 2004), stick insect (Roark & Bjorndal 2009), and some species of rotifer (Kirk 2001) and fish (Inness & Metcalfe 2008). This challenged the perspective that aging is conserved, and the pathways regulating aging are broadly shared by organisms across the tree of life. This suggested that aging intervention functions may be taxa specific.

Studies of aging patterns of a range of animals, such as yeasts, nematodes, fruit flies, rats and mice, monkey, have led to a series of evolutionary theories for explaining aging. The leading

theories, namely Williams's pleiotropy theory (1957), Kirkwood's disposable soma theory (Kirkwood 1977, 1990; Kirkwood & Holliday 1979; Kirkwood & Rose 1991) and reproductive-cell cycle theory postulated by Bowen and Atwood (2004, 2011), all suggest that there is trade-off between the two life-history components, longevity and fecundity, since they share the limited pool of resources available for individuals. Their relationship has been investigated in both invertebrates including yeast *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and vertebrates such as house mouse *Mus musculus*, including humans, but yielded conflicting results (Le Bourg 2001; Flatt 2011).

8.2 Results summary

In this study, with a newly proposed model species, two-spotted spider mite *Tetranychus urticae* for aging, I investigated the patterns of survival and reproduction under various conditions. First and foremost, I examined how food regimes, social environment and stress modulate the aging pace of animals. Furthermore, I determined how males and females differ in their responses to these biotic and abiotic factors. I also explored the relationship between lifespan and lifetime fecundity to the test the evolutionary theories of aging.

The main body of this research is presented in six chapters (2 to 7), with each one focusing on a different major question. To begin with, the starvation tolerance of both sexes with different mating status was investigated (Chapter 2). In the short-term test in which two-day-old adults were deprived of food, I found significant sex differences in starvation tolerance: female spider mites could withstand much longer starvation than males and showed higher survival rates without any food replenishment. The mating status showed sex-specific influence on starvation resistance of spider mites, whereby the female lifespan under different mating status did not differ, whereas the virgin males significantly outlived the mated males. I also determined whether sex dimorphism in size is associated with the fitness of spider mites. Although the fecundity of females showed a positive relationship with body size regardless of mating status and food regimes, longevity of mites was not associated with their body size, failing to support the "rate of living theory" which assumed that larger animals

with slower metabolisms live longer than the smaller ones with a higher rate of metabolism (Chapter 2).

Building on my data about starvation tolerance of males and females, I exposed the spider mites to three levels of intermittent fasting (IF) with control being fed *ad libitum* and explored the response of males and females to different levels of IF (Chapter 3). I showed the sex-specific response to IF in lifespan, whereby females showed a significant increase in lifespan at intermediate IF, but the adult lifespan of males decreased with fasting before levelling off. The females under IF decreased or even ceased reproduction in comparison with *ad libitum*-fed mites during the reproductive stage. The lifetime fecundity of females at 50% IF was significantly reduced compared with the control, suggesting that females extended lifespan at the cost of reproduction, compared with ad-libitum females. This suggests the great variation in resource across treatments induced a trade-off between survival and lifetime fecundity. However, within each treatment, longevity was positively associated with reproduction, indicating that a substantial difference in resource available is prerequisite for trade-offs between fitness traits (Chapter 3).

My results showed that mating status influenced the fitness of spider mites, and I undertook experiments to explored how social environment influences the life history traits of males and females, respectively. I first determined how inter- and intra-sexual interaction shape the fitness of mites (Chapter 4). My results show that for both sexes, when paired with conspecifics the isolated mites survived longer than their counterparts regardless of sex, indicating that the cost of interaction with the same sex and the opposite sex is significant. Male lifespan was shortest when paired with the same sex, but female lifespan was shortest when paired with the opposite sex. This indicated that different social environments have sex-dependent influences on mites. Moreover, the female involved in male-female interaction showed relatively lower lifetime fecundity. These findings underline the cost of interactions, regardless of sex, influencing both the lifespan and reproduction of the organisms (Chapter 4).

I also investigated the potential influence of delayed mating and repeated mating on longevity and reproduction (Chapter 5). The lifespan of female was longest for virgin female, followed by females delayed mating by seven days, all of which outlived the females exposed to males immediately after the adult emergence. The females mated at 1-day-old were significantly affected by repeated mating, whereby the females kept together with males throughout adult life had a shorter lifespan than the counterparts that were mated for one day only. However, for the females mated at 7-day-old, the impact of continuous exposure to males was not significant. The results suggested that being exposed to males entails costs for the female in terms of reduced lifespan, and the earlier the exposure the higher the cost. Males showed no apparent changes in lifespan across treatments when engaged in various mating regimes (Chapter 5). The sex-specific plasticity in longevity indicated that sexual interaction incurred a higher cost to females which have a much lower optimal mating frequency than males. I also found that delayed mating significantly decreased the daily reproductive rate of the females, but there was no significant difference in lifetime reproduction of females across treatments because of increased reproductive lifespan as compensation. My results demonstrated that the time and frequency of sexual interaction could have a great influence on the fitness of females but not males (Chapter 5).

I examined the influences of dietary restriction and delayed mating on the aging patterns of two-spotted spider mite *T. urticae* and tested the prevailing survival and reproduction trade-off hypothesis of aging. I found sex-specific responses of *T. urticae,* with females having greater longevity on diet and with delayed mating. Males displayed shorter lifespan with food restriction and they were not significantly influenced by delayed mating. I investigated the relationship between survival and reproduction traits through path analysis including longevity, female lifetime reproduction, age at first reproduction, early reproductive efforts and late reproductive efforts. Surprisingly, I failed to get evidence for trade-offs between these life-history traits. To conclude, I confirmed the lifespan extension effects of dietary restriction and delayed mating on female spider mite, confirming that diet restriction is a robust anti-aging intervention. Later onset of reproduction can also prolong adult lifespan.

However, the trade-off hypothesis is not valid for this species, possibly indeterminate growth decouples the trade-off between survival and reproduction (Chapter 6).

I raised parental spider mites either on leaf disc with or without odour from a natural predator (*Phytoseiulus persimilis*). Predator-derived odour prolonged immature duration of both sexes shortened female adult lifespan but not that of males, and reduced lifetime reproductive outputs of females. My studies of offspring from both odour-exposed and control mothers demonstrated that parental effects were significant only in the early developmental stage (hatching stage) of the offspring. Offspring were strongly negatively affected by the direct stress but there was no evidence of transgenerational influences. Additionally, the parental effects in the earlier life stage were sex-specific, with delayed hatching in daughters (but not sons) when parents were exposed to predation risk. With this long-term transgenerational study, I indicated that there were adverse effects of predator-induced stress on aging and lifespan of prey, and the parental effects appeared to be weak and in the early stage of offspring, but diminished in the adult stage. My results highlight the sex-difference in response to risk and sex-dependent parental effects on the offspring (chapter 7).

This research takes a step forward to understand how males and females respond to intermittent fasting and how the social environment (interaction between sexes) affects the life history traits of spider mites, as well as the sexual dimorphism in development duration, size and lifespan under predator-induced risk.

8.3 General discussion

8.3.1 Sex dimorphism in the lifespan of spider mite *T. urticae*

The observation that mated males had a higher survival rate and lived longer than the mated females goes against many previous studies with spider mites *T. urticae*. Among the 23 studies which reported longevity data for both males and females, only *4/23* showed similar results to this study. The average lifespans reported for males and females were 16.9 days and 15.8 days, respectively (Table 8.1). By comparison, the results of our study showed that the

average lifespan of females and males were 16.3 days and 24.9 days, respectively, with male lifespan being significantly higher in our study. Although it is possible the disparity may result from differences in rearing condition and host plant, but it is more likely to be attributed to the methodology improvements in my study, which lowered the escaping and drowning rate of mites, especially for males. Traditionally, rearing units are composed of leaf discs enclosed by a stripe of wet cotton or tissue paper (Figure 8.1A). However, often, the males are easily trapped by cotton and tissue creating high accidental mortality of males, which are unreported. The traditional assumption is that male spider mites generally have little contribution to population increases. However, I used water as a barrier to prevent mites from escaping by making the leaf disc afloat on the surface of the water in every cell (Figure 8.1 B). This dramatically reduced the probability of drowning and escaping of small males and therefore improved the accuracy of data obtained.

TABLE 8. 1 Summary of studies on male and female longevity of two-spotted spider mites *Tetranychus urticae*.

	References	Temperature	Hostplant	Mating status	Female lifespan	Male lifespan
1	Sedaratian *et al.* 2009	28	Soybean	mated	15.86	14.06
2	Sedaratian *et al.* 2009	28	Soybean	mated	10.62	16.29
3	Sedaratian *et al.* 2009	28	Soybean	mated	12.88	10.89
4	Sedaratian *et al.* 2009	28	Soybean	mated	13.51	18.38
5	Sedaratian *et al.* 2009	28	Soybean	mated	14.23	14.71
6	Sedaratian *et al.* 2009	28	Soybean	mated	20.74	15
7	Sedaratian *et al.* 2009	28	Soybean	mated	13.05	14.76
8	Sedaratian *et al.* 2009	28	Soybean	mated	17.43	16.31
9	Sedaratian *et al.* 2009	28	Soybean	mated	13.97	21.24
10	Sedaratian *et al.* 2009	28	Soybean	mated	13.48	14.88
11	Sedaratian *et al.* 2009	28	Soybean	mated	14.93	14.53
12	Sedaratian *et al.* 2009	28	Soybean	mated	22.66	21.41
13	Sedaratian *et al.* 2009	28	Soybean	mated	17.36	15.34
14	Sedaratian *et al.* 2009	28	Soybean	mated	13.19	20.78
15	Riahi *et al.* 2013	25	Peach	mated	12.91	6.8
16	Riahi *et al.* 2013	27	Peach	mated	5.92	3.75

17	Riahi *et al.* 2013	30	Peach	mated	3.56	3.2
18	Riahi *et al.* 2013	33	Peach	mated	6.53	5.23
19	Khanamani *et al.* 2013	25	Eggplant	mated	9.1	10.2
20	Khanamani *et al.* 2013	25	Eggplant	mated	7.84	9.73
21	Khanamani *et al.* 2013	25	Eggplant	mated	7.22	8.16
22	Khanamani *et al.* 2013	25	Eggplant	mated	7.1	7.8
23	Khanamani *et al.* 2013	25	Eggplant	mated	6.93	6.5
24	Khanamani *et al.* 2013	25	Eggplant	mated	7.5	9.09
25	Khanamani *et al.* 2013	25	Eggplant	mated	7.89	7.71
26	Bayu *et al.* 2017	15	Common Bean	mated	67.6	74.6
27	Bayu *et al.* 2017	20	Common Bean	mated	38.1	41.2
28	Bayu *et al.* 2017	25	Common Bean	mated	28.3	23
29	Bayu *et al.* 2017	30	Common Bean	mated	13.8	13
30	Bayu *et al.* 2017	35	Common Bean	mated	9.9	5.3
31	Bayu *et al.* 2017	15/5	Common Bean	mated	79.9	83.2
32	Bayu *et al.* 2017	20/10	Common Bean	mated	67.2	64.8
33	Bayu *et al.*, 2017	25/15	Common Bean	mated	31.3	39.4
34	Bayu *et al.* 2017	30/20	Common Bean	mated	19.3	17.3
35	Bayu *et al.* 2017	35/25	Common Bean	mated	12.9	12.5
36	Bayu *et al.* 2017	40/30	Common Bean	mated	11.2	16.9
37	Esmaeily *et al.* 2017	25	Bean	mated	20.33	14.11
38	Tuan *et al.* 2016	25	Runner Bean	mated	8.86	6.44
39	EI Taj *et al.* 2016	PLC	Brinjal	mated	25.08	24.28
40	EI Taj *et al.* 2016	PLC	Cowpea	mated	24.88	23.86
41	EI Taj *et al.* 2016	PLC	Cabbage	mated	25.88	25.31
42	EI Taj *et al.* 2016	PLC	Bean	mated	26.5	25.94
43	EI Taj *et al.* 2016	PLC	Bean	mated	14.54	11.61
44	EI Taj *et al.* 2016	PLC	Cowpea	mated	16.31	13.25
45	Kavousi *et al.* 2009	25	Bean	mated	3.83	4.41
46	Kavousi *et al.* 2009	25	Bean	mated	4.71	5.09
47	Shih *et al.*1976	27	Bean	mated	19.1	14.6
48	Motahari *et al.* 2014	25	Cucumber	mated	13.75	32.25
49	Motahari *et al.* 2014	25	Cucumber	mated	15.47	29.25
50	Motahari *et al.* 2014	25	Cucumber	mated	13.71	14.16
51	Motahari *et al.* 2014	25	Cucumber	mated	16.47	31.33
52	Shah & Shukla 2014	Summer	Gerbera	mated	10	8.8

53	Shah & Shukla 2014	Summer	Gerbera	unmated	11.46	8.8
54	Shah & Shukla 2014	monsoon	Gerbera	mated	12.36	9.73
55	Shah & Shukla 2014	monsoon	Gerbera	unmated	16.33	9.73
56	Shah & Shukla 2014	Winter	Gerbera	mated	19.84	11.46
57	Shah & Shukla 2014	Winter	Gerbera	unmated	17.46	11.46
58	Veerendra 2013	26	Grape	mated	15.9	8.63
59	Veerendra 2013	26	Mulberry	mated	12.8	7.12
60	Havasi *et al.* 2018	25	Bean	mated	13.01	10.56
61	Kim *et al.* 2008	17	Eggplant	mated	20.7	16.2
62	Kim *et al.* 2008	22	Eggplant	mated	14.3	12.8
63	Kim *et al.* 2008	27	Eggplant	mated	10.6	7.2
64	Kim *et al.* 2008	32	Eggplant	mated	9.2	8
65	Kim *et al.* 2008	37	Eggplant	mated	5.4	5
66	El-Saied Sholla 2016	26	Caster	mated	17.61	14.83
67	El-Saied Sholla 2016	30	Caster	mated	12.71	10.43
68	Chauhan 2016	Summer	Bean	mated	7.3	4.9
69	Chauhan 2016	Monsoon	Bean	mated	7.4	4.55
70	Chauhan 2016	winter	Bean	mated	7.65	6.1
71	Ali *et al.* 2017	25	Cucumber	mated	12.09	10.64
72	Ali *et al.* 2017	30	Cucumber	mated	7	7
73	Bhusal 2011	summer	Chrysanthemum	mated	12.89	8.93
74	Patil *et al.* 2014	PLC	Carnation	mated	12.6	11.57
75	Sarwar 2014	25	Bean	mated	23.57	13.85
76	Sarwar 2014	25	Bean	mated	25	15.28
77	Sarwar 2014	25	Bean	mated	19.28	10.71
78	Abd El-Wahed & El-Halawany2012	Summer	Gerbera	mated	10	8.8
79	Rajakumar *et al.* 2005	PLC	Jasmine	mated	18.7	12.1
80	Shoorooei *et al.* 2018	25	Common Bean	mated	21.42	17.77
81	Shoorooei *et al.* 2018	25	Common Bean	mated	19.75	16.41
82	Shoorooei *et al.* 2018	25	Common Bean	mated	19.2	15.08
83	Shoorooei *et al.* 2018	25	Common Bean	mated	21.22	16.74
84	Shoorooei *et al.* 2018	25	Common Bean	mated	19.41	14.86
85	Shoorooei *et al.* 2018	25	Common Bean	mated	21.55	14.65
86	Shoorooei *et al.* 2018	25	Common Bean	mated	23.43	17.69

Note: 1. PLC denotes Prevailing laboratory condition.

2. The lifespan of female and male spider mites were given in days.

FIGURE 8. 1 Laboratory rearing methods for two-spotted spider mite, with (A) traditional rearing cells and (B) new rearing cells in our research.

8.3.2 Dietary restriction: a healthy lifestyle for aging?

Although there seems to be ample evidence proving that dietary restriction is an effective strategy to delay aging and prolong the lifespan, it is still questionable whether it is suitable for human beings. On the one hand, it is not certain whether the beneficial effects of dietary restriction reported in model organisms can also apply to human beings. While the striking benefits of lifespan-extension have been reported for some species conducted in different laboratories, the data obtained from primates, the genetically closest model organism related to human were inconclusive. One study by Wisconsin National Primate Research Center (WNPRC) reported that monkeys subjected to 30% calorie restriction had a higher and healthier lifespan (Colman *et al.* 2009; Colman *et al.* 2014) than controls. By contrast, National Institute on Ageing (NIA) Intramural Research Program found that monkeys exposed to the same diet intervention only showed a delay in the onset of age-related disease but no improvement in lifespan (Mattison *et al.* 2012). More relevant, the dietary restriction

also was reported to have adverse effects on humans and other organisms. For example, in a study with humans, CR intervention, decreasing energy intake by 16% during the first three months and by 20% during the remaining nine months, reduced bone mineral density, which can increase fracture risk during old age (Villareal *et al.* 2006). A study with fruit fly claimed that the effects of dietary restriction are age-dependent, with DR enhancing starvation resistance early in life, but lowering starvation resistance late in life (Burger *et al.* 2007).

Although DR can extend healthy lifespan, this benefit appeared to be associated with an extremely high cost of reproduction, clearly for females. In our study, the female spider mite prolonged lifespan by approximately 19% (3 days) at the cost of a reduction in lifetime fecundity of 42%. A much higher cost of lifespan extension was recorded on neriid fly *Telostylinus angusticollis* when females deprived of protein failed to produce any offspring (Adler *et al.* 2013). Because the reduction in reproduction was always related to lifespan extension, some researchers proposed trade-offs between these two fitness components as the mechanism whereby DR prolongs lifespan. Le Bourg and Minois (1996) suggest DR could extend the longevity of fertile females with a decrease in egg production but not that of males or virgin females. However, this was refuted by the discovery that DR could in fact prolong the lifespan of both males and virgin females (Magwere *et al.* 2004; Adler *et al.* 2013). It is clear that reproduction cost is not necessary for the prolonged lifespan because it can be modified by manipulating micronutrients. Grandison *et al.* (2009) showed that fruit fly fed on DR food with supplementary methionine alone showed an increase in fecundity, comparable to that of well feed counterparts, without any reduction of lifespan. Consequently, full-feeding may enhance fecundity simultaneously and decrease lifespan through the availability of particular nutrients. It is possible that both these two fitness traits can be maximized without any trade-off between them when nutrients components are well balanced.

8.3.3 Social environment and its fitness consequence

My research highlights the importance of considering social environment, intersexual interaction, and intrasexual interaction when determining lifespan, reproduction and life history trade-offs. In other model organisms for aging research, in particular, small species,

such as worms, fruit flies and rotifers, which are generally reared in groups in empirical studies. This facilitates frequent interactions likely influenced by variation in group size and sex ratio that could impact their reproductive investments and in turn, affect the pace of their aging and lifespan. However, research considered these factors is sparse. One study evaluating the potential influence of social environment on lifespan and reproduction in *Drosophila melanogaster* has demonstrated that females housed in single-sex groups after mating lived much longer and produced more offspring than those reared in mixed-sex groups. The males in single-sex group also outlived their counterparts from the mixed-sex groups (Zajitschek *et al.* 2013). However, another experimental study with neriid fly *Telostylinus angusticollis* showed no significant difference in lifespan between flies reared in mixed-sex and single-sex for both sexes (Adler *et al.* 2013). This disparity may result from the difference in mating system and density of the group, as well as the proportion of each sex involved in the study.

To date, our understanding of how social environment influences aging patterns and lifespan of organisms remains incomplete. Since species across taxa have different mating systems and sex ratios, it appears to be challenging to generalize findings across taxa. To develop our understanding, empirical studies with a wide range of species should be carried out to assay the effects of social environment on reproduction and lifespan. Social environment can interact with other factors, for instance, diet and nutrient, shaping the fitness of the organism. Larval diet significantly increased the lifespan of virgin flies, with males and females on poor diet displayed a 7% increase compared with control. By contrast, it showed no influence on the lifespan of mated flies, regardless of their mating frequencies (May *et al.* 2015). These findings indicate that social environments need to be considered in future aging research and life history study.

The possibility of trans-generational influences on the fitness of animals is rarely considered. For example, Gasparini *et al.* (2012) showed that female guppies experiencing high sexual harassment gave birth to daughters with smaller body size, and sons with shorter gonopodia that were less attractive to females and less successful in coercive mating than offspring produced by mothers under low sexual harassment. Transgenerational effects may also be

expressed in life-history traits. Mold mite offspring derived from mother or father caged with extra-pair (1:3) of mates showed lower fitness than those from parents in a pair, with daughters having a shorter lifespan and lower fecundity and sons with reduced lifespan (unpublished data).

This study illustrated the critical importance of considering social environment when investigating aging, and fitness consequences of the organism under various conditions. Neglecting social environment or using problematic husbandry protocols would introduce confounding factors, masking the influence of the targeted factor and making it hard to interpret the results (Mair 2005).

8.3.4 Stress responses and its trans-generational effects

Animals have always been exposed to some kinds of psychological stress. These subtle but chronic stresses may cause the early onset of aging-associated diseases and premature aging (Epel *et al.* 2004). In the last few years, some biomedical research indicates that predator-induced stress resembles chronic psychological stress humans are exposed to. Later animal model systems such as rodent-cat were developed to test post-traumatic stress disorder (PTSD), cellular aging and cognitive aging. Yet little is known about the fitness consequence of chronically or repeated exposure to stress. By employing an acarine system, I managed to shed light on how animals facultatively alter life-history traits throughout life and the trans-generational response to stress.

Although I know a lot about how animals age in response to environmental stress such as nutritional, temperature, radiation, osmotic stress, little is known how psychological stress is experienced by humans, especially daily hassles, time pressure, and social conflict or chronic stressors such as financial strain, job strain, caregiving, marital strain influences healthy lifespan and aging. My study provides a more comprehensive picture of how animals age when subjected to psychological stress at the individual and population levels. My study highlights that stress can have a sex-specific influence on lifespan and aging pattern of animals. While females exposed to predator-induced stress have shortened lifespan, the males

were resilient to risk. Sex-specific response to stress has been documented in Azure damselfly, *Coenagrion puella*: a significant interaction between sex and predation risk was observed (Mikolajewski *et al.* 2013). Without the presence of a predator, the male was larger than females, whereas, the female grew larger than males when they exposed to predation risk. The difference in behavioral and physiological changes to stressors between males and females may be responsible for their sex-difference in phenotypic plasticity. This assumption was supported by increasing evidence showing that males differed from the females in the magnitude of responses to predator-induced risk in vigilance, foraging, immunity and metabolic rates (Childress & Lung 2003; Mikolajewski *et al.* 2008; Lagos & Herberstein 2017). Chronic and repeated exposure to stress is detrimental. It can not only accelerate cognitive aging (Sterlemann *et al.* 2010), but also reduce lifespan and lower the reproductive success, decreasing lifetime fitness of organisms in this study.

8.3.5 Spider mite as potential candidate for aging research

Our studies highlight the suitability of *T. urticae* as a model species in gerontology and anti-aging research. First, it has the common characteristics of the classical model organisms such as the nematode and fruit fly in that it develops fast, can be easily reared in the laboratory and has low cost for population maintenance (Grbic *et al.* 2007). It is also an excellent candidate for genetic investigation because its genome has already been sequenced and annotated, making it a promising model organism for the genetic research on aging. Its small genome distributed on three holocentric chromosomes is 75 Mbp (Grbic *et al.* 2011), only 60% of *Drosophila* genome and 80% of *C. elegans* genome. In addition, the well-established RNAi-mediated gene silencing technology for spider mite by injection of dsRNA (Suzuki *et al.* 2017) makes it possible to generate loss-of-function phenotypes and investigate the function of specific genes.

8.4 Limitations and further direction

It is interesting to note that males and females differ in their response to DR, with female lifespan extended at 50% IF, whereas the male lifespan decreased with the increasing level of

intermittent fasting. Although I failed to find beneficial effects of IF for the male in this study (Chapter 3), it did not indicate that IF is harmful to males. It is possible that the investigated levels of intermittent fasting were too high and beyond the optimal point to prolong the lifespan of the male. To check this assumption, I suggested that further studies with much more gradients of IF are necessary when determining the influence of IF on animals.

To date, there are relatively few experimental studies on how males age, how their reproductive mode changes with the aging process, and how they age differently from the females. Therefore, further research with males being taken into consideration is warranted. Furthermore, in the majority of the well-established aging model organisms, there are few available techniques to measure the investments of males in reproduction. One approach used in crickets was to measure mating song duration and frequency (Zajitschek *et al.* 2009). Therefore, I suggested that a new model organism in which the male reproduction cost can be qualified precisely would be of significant advantage in providing further insights into the cost of reproduction for males.

8.5 Concluding remarks

In my research, I examined multiple factors affecting the pattern of aging in animals, with a newly-developed model organism, two-spotted spider mite. This research takes a step forward to understand how males and females respond to intermittent fasting differently, and how the interaction between sexes affect the life history traits of spider mites, as well as the sexual dimorphism in development and lifespan under predator-induced risk. Although this study further advanced our knowledge of aging of both sexes under various conditions, I only explored the potential explanations from the perspective of resource allocation and the trade-off between fitness traits. The mechanism that mediates the sex-specific response to diet, social environment and stress is far more complicated and remains to be further explored. The future challenges for the successful research with spider mites are to implement the advanced molecular techniques such as RNA inference as well as bioinformatics tools to further explore why and how the organisms age at the molecular level. Furthermore, integrating findings from multiple disciplines and cooperating using both evolutionary and

molecular theories of aging together may give us a much more comprehensive understanding of aging.

Appendix

TABLE A1. Effects of temperature and host plants on the sex difference in the development of mites in the Acari (mite) family Tetranychidae.

Species	Temperature	Host	Nfemale	Nmale	MeanF	SD_F	MeanM	SD_M	References
Aponychus corpuzae	22.5~27.5	tree	24	20	12.54	0.39	12.2	0.49	Saito & Ueno 1979
Eotetranychus carpini	<22.5	tree	47	22	27.9	4.94	27	4.92	Bounfour & Tanigoshi 2001
Eotetranychus carpini	<22.5	tree	38	24	18.4	1.54	17.3	3.38	Bounfour & Tanigoshi 2001
Eotetranychus carpini	22.5~27.5	tree	44	24	14.9	1.99	14.1	2.25	Bounfour & Tanigoshi 2001
Eotetranychus carpini	>27.5	tree	55	19	12	0.67	11.7	0.74	Bounfour & Tanigoshi 2001
Eutetranychus orientalis	<22.5	tree	17	7	22.32	1.65	22.57	1.24	Imani & Shishehbor 2009
Eutetranychus orientalis	22.5~27.5	tree	16	9	17.03	1.68	17	1.32	Imani & Shishehbor 2009
Eutetranychus orientalis	>27.5	tree	15	6	12.43	1.20	12.08	0.98	Imani & Shishehbor 2009
Oligonychus afrasiaticus	22.5~27.5	tree	64	30	11.1	0.90	9.9	0.70	Chaaban *et al*. 2011
Oligonychus afrasiaticus	22.5~27.5	tree	58	28	13	1.00	11	0.70	Chaaban *et al.* 2011
Oligonychus afrasiaticus	22.5~27.5	tree	62	19	11.9	1.20	11.1	1.10	Chaaban *et al.* 2011
Oligonychus afrasiaticus	22.5~27.5	tree	65	18	11.9	0.70	9.7	0.80	Chaaban *et al.* 2011
Oligonychus afrasiaticus	>27.5	tree	67	25	8.2	0.50	7.5	0.50	Samah & chermiti 2010
Oligonychus afrasiaticus	>27.5	tree	64	24	8.3	0.30	7.7	0.20	Samah & chermiti 2010
Oligonychus afrasiaticus	>27.5	tree	58	23	9	0.30	7.9	0.40	Samah & chermiti 2010
Oligonychus afrasiaticus	>27.5	tree	58	17	9.5	0.60	8.4	0.60	Samah & chermiti 2010
Oligonychus indicus	Fluctuating	herb	25	25	9.32	0.76	8.32	0.76	Chundawat 2006
Oligonychus indicus	Fluctuating	herb	25	25	7.28	1.59	6.45	0.90	Chundawat 2006
Oligonychus indicus	Fluctuating	herb	25	25	6.84	1.04	6.64	0.89	Chundawat 2006

Oligonychus indicus	Fluctuating	herb	25	25	6.79	1.02	5.2	1.13	Chundawat 2006
Oligonychus indicus	Fluctuating	herb	25	25	8.556	1.82	7.32	1.01	Chundawat 2006
Oligonychus litchii	<22.5	tree	56	6	39.3	5.50	40.3	6.80	Chen *et al.* 2016
Oligonychus litchii	<22.5	tree	31	19	17.9	1.70	18	2.20	Chen *et al.* 2016
Oligonychus litchii	22.5~27.5	tree	16	20	11.5	0.80	11.6	0.70	Chen *et al.* 2016
Oligonychus litchii	>27.5	tree	31	20	10.6	1.40	10.6	1.00	Chen *et al.* 2016
Oligonychus litchii	>27.5	tree	18	1	9.3	1.00	8	0.00	Chen *et al.* 2016
Oligonychus mangiferus	<22.5	tree	59	11	34.4	1.54	35.8	2.32	Lin 2013
Oligonychus mangiferus	<22.5	tree	60	27	17.4	1.55	17.4	1.56	Lin 2013
Oligonychus mangiferus	22.5~27.5	tree	32	5	14.8	1.13	14.6	0.89	Lin 2013
Oligonychus mangiferus	>27.5	tree	87	21	9	0.93	8.8	0.46	Lin 2013
Oligonychus mangiferus	>27.5	tree	50	8	9.1	0.71	8.8	0.85	Lin 2013
Oligonychus oryzae	22.5~27.5	herb	50	50	10.47	0.51	9.87	0.72	Aswin 2015
oligonychus ununguis	22.5~27.5	tree	14	10	10.39	0.56	10.22	0.79	Saito 1979
Panonychus citri	22.5~27.5	tree	31	27	11.98	1.22	11.61	0.94	Saito 1979
Panonychus ulmi	22.5~27.5	tree	24	10	12.6	0.88	11.4	0.70	Yin *et al.* 2013
Panonychus ulmi	22.5~27.5	tree	24	10	12.54	0.64	11.67	0.44	Yin *et al.* 2013
Panonychus ulmi	22.5~27.5	tree	28	12	13.68	0.48	13.36	0.69	Gotoh 1987
Panonychus ulmi	22.5~27.5	tree	26	11	12.05	1.73	11.25	1.76	Dar *et al.* 2015
Panonychus ulmi	22.5~27.5	tree	27	12	13.4	2.03	10.38	1.84	Dar *et al.* 2015
Panonychus ulmi	22.5~27.5	tree	16	7	16.44	3.00	13	3.02	Dar *et al.* 2015
Panonychus ulmi	22.5~27.5	tree	15	6	21.62	2.52	16.8	2.38	Dar *et al.* 2015
Polyphagotarsonemus latus	22.5~27.5	tree	24	11	3.47	0.39	3.18	0.10	Ho 1991
Polyphagotarsonemus latus	22.5~27.5	tree	20	28	3.6	0.80	3.86	0.10	Ho 1991
Polyphagotarsonemus latus	22.5~27.5	herb	42	16	4.12	0.52	4.13	0.90	Ho 1991

Schizotetranychus brevisetosus	22.5~27.5	tree	22	3	22.6	3.10	25.3	4.20	Tamura & Ito 2017
Schizotetranychus celarius	22.5~27.5	tree	15	10	14.05	0.58	13.42	0.73	Saito & Ueno 1979
Tenuipalpus pacificus	22.5~27.5	herb	14	14	49.6	8.23	47.2	5.24	Wang & Lin 2009
Tenuipalpus pacificus	>27.5	herb	8	9	33.4	2.55	30	3.90	Wang & Lin 2009
Tenuipalpus pacificus	>27.5	herb	4	10	22.8	2.20	22.6	1.58	Wang & Lin 2009
Tenuipalpus pacificus	>27.5	herb	25	11	19	2.50	20.1	2.32	Wang & Lin 2009
Tetranychus bambusae	22.5~27.5	tree	21	17	9.48	0.60	9.12	0.62	Ullah *et al.* 2014
Tetranychus bastosi	22.5~27.5	herb	122	14	10.5	3.20	10.3	3.03	de Lima *et al.* 2017
Tetranychus bastosi	22.5~27.5	tree	93	18	11.2	1.74	11.7	1.06	de Lima *et al.* 2017
Tetranychus bastosi	22.5~27.5	tree	86	20	12.3	2.23	13.4	1.43	de Lima *et al.* 2017
Tetranychus cinnabarinus	22.5~27.5	herb	16	8	10.84	0.80	10.38	0.23	Kazak & kibritci 2008
Tetranychus cinnabarinus	22.5~27.5	herb	16	8	10.81	0.44	9.88	0.51	Kazak & kibritci 2008
Tetranychus cinnabarinus	22.5~27.5	herb	19	5	10.53	0.48	10	0.49	Kazak & kibritci 2008
Tetranychus cinnabarinus	22.5~27.5	herb	16	8	10.28	0.44	10.51	0.88	Kazak & kibritci 2008
Tetranychus cinnabarinus	22.5~27.5	herb	19	5	10.61	0.48	9.9	0.54	Kazak & kibritci 2008
Tetranychus cinnabarinus	22.5~27.5	herb	20	4	10.8	0.67	10.38	0.24	Kazak & kibritci 2008
Tetranychus cinnabarinus	22.5~27.5	herb	16	8	10.72	0.60	10.31	0.34	Kazak & kibritci 2008
Tetranychus cinnabarinus	22.5~27.5	herb	17	7	10.47	0.74	10.29	0.37	Kazak & kibritci 2008
Tetranychus kanzawai	<22.5	herb	43	37	24.5	1.38	24.4	2.43	Ullah *et al.* 2011
Tetranychus kanzawai	<22.5	herb	39	40	21.7	1.37	20.9	1.26	Ullah *et al.* 2011
Tetranychus kanzawai	<22.5	herb	53	39	15.6	0.66	15.1	0.62	Ullah et al 2011
Tetranychus kanzawai	<22.5	herb	61	36	12.1	0.55	12	0.66	Ullah *et al.* 2011
Tetranychus kanzawai	22.5~27.5	tree	29	34	12.2	0.70	11.6	1.05	Gotoh & Gomi 2003
Tetranychus kanzawai	22.5~27.5	tree	35	18	9.7	0.59	9.2	0.51	Gotoh & Gomi 2003

Tetranychus kanzawai	22.5~27.5	tree	36	23	10.8	0.72	9.7	1.10	Gotoh & Gomi 2003
Tetranychus kanzawai	22.5~27.5	tree	39	20	9.9	0.56	9.4	0.58	Gotoh & Gomi 2003
Tetranychus kanzawai	22.5~27.5	tree	36	17	9.3	0.78	8.8	0.41	Gotoh & Gomi 2003
Tetranychus kanzawai	22.5~27.5	tree	32	21	9.4	0.62	8.6	0.50	Gotoh & Gomi 2003
Tetranychus kanzawai	22.5~27.5	tree	33	17	9.6	0.63	8.6	0.70	Gotoh & Gomi 2003
Tetranychus kanzawai	22.5~27.5	herb	55	37	9.8	0.74	9.5	0.55	Ullah *et al.* 2011
Tetranychus kanzawai	22.5~27.5	herb	61	40	8.2	0.47	7.8	0.51	Ullah et al 2011
Tetranychus kanzawai	>27.5	herb	61	34	6.3	0.39	6.2	0.52	Ullah *et al.* 2011
Tetranychus kanzawai	>27.5	herb	57	40	5.6	0.30	5.4	0.25	Ullah *et al.* 2011
Tetranychus kanzawai	>27.5	herb	61	40	5.3	0.31	5.1	0.25	Ullah *et al.* 2011
Tetranychus kanzawai	>27.5	herb	39	19	5.6	0.37	5.4	0.35	Ullah *et al.* 2011
Tetranychus ludeni	<22.5	herb	11	3	20.77	0.90	18.83	0.88	da Silva 2002
Tetranychus ludeni	22.5~27.5	herb	22	10	16.75	1.08	15.45	1.08	da Silva 2002
Tetranychus ludeni	22.5~27.5	herb	28	7	13.29	0.48	12.64	0.48	da Silva 2002
Tetranychus ludeni	>27.5	herb	33	6	9.79	0.52	9.5	0.61	da Silva 2002
Tetranychus ludeni	>27.5	herb	28	2	8.5	0.63	7.75	0.62	da Silva 2002
Tetranychus merganser	<22.5	herb	37	26	38.7	2.19	38.1	2.80	Ullah *et al.* 2011
Tetranychus merganser	<22.5	herb	62	21	28.2	1.50	27.8	1.37	Ullah *et al.* 2011
Tetranychus merganser	<22.5	herb	52	31	18.8	1.37	17.3	1.28	Ullah *et al.* 2011
Tetranychus merganser	<22.5	herb	52	30	12.8	0.65	12.4	0.49	Ullah *et al.* 2011
Tetranychus merganser	22.5~27.5	herb	68	39	10.4	0.82	9.8	0.75	Ullah *et al.* 2011
Tetranychus merganser	22.5~27.5	herb	56	28	8.8	0.37	8.3	0.63	Ullah *et al.* 2011
Tetranychus merganser	>27.5	herb	61	32	6.7	0.39	6.7	0.51	Ullah *et al.* 2011
Tetranychus merganser	>27.5	herb	58	17	6.1	0.30	5.6	0.66	Ullah *et al.* 2011
Tetranychus merganser	>27.5	herb	56	30	5.5	0.30	5.5	0.38	Ullah *et al.* 2011

Tetranychus merganser	>27.5	herb	33	10	6	0.40	6.2	0.73	Ullah *et al.* 2011
Tetranychus neocaledonicus	22.5~27.5	herb	100	39	11.94	0.28	11.48	0.36	Neto *et al.* 2017
Tetranychus neocaledonicus	>27.5	tree	10	10	10.9	0.47	9.5		Kaimal & Ramani 2007
Tetranychus palmarum	<22.5	tree	52	15	18.5	2.16	17.9	3.37	Noronha *et al.* 2018
Tetranychus palmarum	22.5~27.5	tree	54	11	13.3	1.25	12.4	1.53	Noronha *et al.* 2018
Tetranychus palmarum	>27.5	tree	35	11	11.3	1.06	11.3	3.15	Noronha *et al.* 2018
Tetranychus parakanzawai	22.5~27.5	tree	34	19	9.3	0.82	8.7	0.44	Gotoh & Gomi 2003
Tetranychus piercei	22.5~27.5	tree	69	24	10.19	0.50	8.96	0.54	Ullah *et al.* 2014
Tetranychus truncatus	22.5~27.5	tree	50	32	8.82	0.49	8.88	0.57	Ullah *et al.* 2014
Tetranychus truncatus	>27.5	herb	65	17	7	0.32	6.7	0.37	Islam *et al.* 2017
Tetranychus truncatus	>27.5	herb	64	21	7.4	0.56	7.2	0.64	Islam *et al.* 2017
Tetranychus truncatus	>27.5	herb	52	48	6.9	0.29	6.6	0.48	Islam *et al.* 2017
Tetranychus turkestani	<22.5	herb	27	8	50.11	4.10	51.6	3.03	Karami-Jamour & Shishehbor, 2012
Tetranychus turkestani	<22.5	herb	30	30	16.14	1.04	15.91	1.81	Karami-Jamour & Shishehbor, 2012
Tetranychus turkestani	22.5~27.5	herb	31	17	12.17	1.34	11.35	1.20	Sohrabi & Shishehnor 2008
Tetranychus turkestani	22.5~27.5	herb	27	11	11.79	0.88	1.36	1.03	Sohrabi & Shishehnor 2008
Tetranychus turkestani	22.5~27.5	herb	19	14	10.18	0.78	10.78	1.09	Sohrabi & Shishehnor 2008
Tetranychus turkestani	22.5~27.5	herb	23	9	10.27	1.34	10.04	0.87	Karami-Jamour & Shishehbor, 2012
Tetranychus turkestani	>27.5	herb	37	9	7.56	0.73	7	0.90	Sohrabi & Shishehnor 2008
Tetranychus turkestani	>27.5	herb	43	7	7.24	0.85	6.92	1.80	Sohrabi & Shishehnor 2008
Tetranychus turkestani	>27.5	herb	27	10	7.03	0.62	6.7	0.92	Sohrabi & Shishehnor 2008
Tetranychus turkestani	>27.5	herb	38	9	7.734	0.86	7.37	0.96	Karami-Jamour & Shishehbor, 2012
Tetranychus urticae	<22.5	herb	26	11	61.5	3.21	62.2	3.15	Bayu *et al.* 2017
Tetranychus urticae	<22.5	herb	61	29	35.9	1.33	34.3	1.56	Bayu *et al.* 2017
Tetranychus urticae	<22.5	tree	42	11	30.33	21.06	25.57	2.16	Riahi *et al.* 2013

Tetranychus urticae	<22.5	herb	55	48	25	1.19	24.5	1.11	Bayu *et al.* 2017
Tetranychus urticae	<22.5	tree	35	10	28.63	13.02	23.57	1.68	Riahi *et al.* 2013
Tetranychus urticae	<22.5	herb	79	32	16.2	0.44	15.7	0.96	Bayu *et al.* 2017
Tetranychus urticae	<22.5	herb	68	52	12.2	0.58	11.9	0.65	Bayu *et al.* 2017
Tetranychus urticae	22.5~27.5	tree	22	13	13.23	1.17	12.23	1.48	Riahi *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	33	11	9.82	1.09	9.09	0.66	Khanamani *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	40	13	10.28	1.33	9.76	0.56	Khanamani *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	32	14	10.59	1.58	9.86	0.86	Khanamani *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	31	13	10.6	1.06	10.32	0.76	Khanamani *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	23	10	11.16	0.67	11	1.33	Khanamani *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	28	12	10.58	1.01	9.9	0.90	Khanamani *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	28	12	10.36	1.11	10.5	0.59	Khanamani *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	72	44	10.4	0.42	10	0.40	Bayu *et al.* 2017
Tetranychus urticae	22.5~27.5	herb	58	18	9.1	0.61	8.67	0.59	Tuan *et al.* 2016
Tetranychus urticae	22.5~27.5	tree	35	10	9.43	1.06	9.75	1.08	Riahi *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	80	41	8.4	0.27	8.1	0.38	Bayu *et al.* 2017
Tetranychus urticae	>27.5	tree	30	4	11.25	1.31	11.25	0.94	Riahi *et al.* 2013
Tetranychus urticae	>27.5	herb	70	35	6.4	0.25	6.7	0.65	Bayu *et al.* 2017
Tetranychus urticae	>27.5	herb	73	45	6.1	0.34	5.9	0.20	Bayu *et al.* 2017
Tetranychus urticae	>27.5	tree	27	13	11.03	1.56	11.19	1.48	Riahi *et al.* 2013
Tetranychus urticae	>27.5	herb	80	30	5.5	0.36	5.4	0.33	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	67	39	45.9	4.01	47.3	5.00	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	80	35	27.3	1.70	25.9	0.59	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	68	37	37.4	1.57	36.9	1.58	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	58	62	25.9	0.76	19.6	1.34	Bayu *et al.* 2017

Tetranychus urticae	Fluctuating	herb	86	31	15.2	1.02	15.2	1.45	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	64	61	11.9	0.80	11.6	1.17	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	70	46	9	0.08	8.8	0.47	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	76	43	7.3	0.78	7.1	0.33	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	82	37	6.4	0.91	6.2	0.49	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	56	53	6.3	0.45	6.1	0.36	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	71	34	6	0.08	5.5	0.17	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	13	8	39.81	0.76	38.61	1.16	El Taj *et al.* 2016
Tetranychus urticae	Fluctuating	herb	16	7	41.94	0.72	41.07	0.48	El Taj *et al.* 2016
Tetranychus urticae	Fluctuating	herb	12	8	39.33	0.76	37.88	1.13	El Taj *et al.* 2016
Tetranychus urticae	Fluctuating	herb	10	9	39.5	0.85	38.61	0.69	El Taj *et al.* 2016
Tetranychus urticae	Fluctuating	herb	14	9	11.25	1.01	10.39	2.01	El Taj *et al.* 2016
Tetranychus urticae	Fluctuating	herb	16	8	11.94	1.28	10.81	0.85	El Taj *et al.* 2016
Tetranychus urticae	<22.5	tree	42	13	25.3	3.56	24.9	2.31	Bounfour & Tanigoshi 2001
Tetranychus urticae	<22.5	herb	16	16	16.6	0.91	17	0.63	Kim *et al.* 2008
Tetranychus urticae	<22.5	tree	39	24	16	2.75	15.9	2.40	Bounfour & Tanigoshi 2001
Tetranychus urticae	<22.5	herb	15	15	8	0.82	8.1	0.93	Kim *et al.* 20092008
Tetranychus urticae	22.5~27.5	herb	10	9	8.36	1.04	4.67	0.98	Monteiro *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	41	41	13.13	3.14	13.25	4.48	Motahari *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	30	30	12.53	1.97	12.7	3.29	Motahari *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	27	27	10.98	2.29	11.2	2.08	Motahari *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	28	28	10.74	2.01	9.38	2.65	Motahari *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	8	11	15.41667	1.28	21.02917	2.63	Monteiro *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	12	7	13.91667	1.72	10.66667	1.16	Monteiro *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	15	4	11.9125	1.58	14.91667	1.54	Monteiro *et al.* 2014

Tetranychus urticae	22.5~27.5	herb	10	9	13.03333	1.38	20.15	2.03	Monteiro *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	11	8	17.54583	2.13	11.625	1.81	Monteiro *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	9	10	17.25833	1.59	20.1	2.32	Monteiro *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	10	9	19.36667	1.42	13.33333	1.91	Monteiro *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	15	4	14.54167	1.79	11.91667	1.14	Monteiro *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	30	9	9.97	0.33	9.91	0.36	Uddin *et al.* 2015
Tetranychus urticae	22.5~27.5	herb	39	7	9.69	0.44	9.7	0.40	Uddin *et al.* 2015
Tetranychus urticae	22.5~27.5	herb	34	7	9.61	0.35	9.3	0.34	Uddin *et al.* 2015
Tetranychus urticae	22.5~27.5	herb	29	21	9.86	0.86	9.54	0.73	Saito 1979
Tetranychus urticae	22.5~27.5	tree	42	13	13.9	2.40	10.7	1.62	Bounfour & Tanigoshi 2001
Tetranychus urticae	22.5~27.5	herb	77	28	7.6	1.00	5.6	1.40	Shih *et al.* 1976
Tetranychus urticae	22.5~27.5	herb	18	18	5.8	0.67	4.9	0.64	Kim *et al.* 2008
Tetranychus urticae	22.5~27.5	herb	86	19	15.96	1.85	15.89	1.05	Najafabadi *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	82	19	18.44	2.08	18.88	1.05	Najafabadi *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	87	19	12	1.59	12.19	1.09	Najafabadi *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	83	20	20.65	1.46	20.49	0.89	Najafabadi *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	77	20	24.74	2.11	23.34	1.39	Najafabadi *et al.* 2014
Tetranychus urticae	22.5~27.5	herb	80	20	22.74	1.52	21.94	1.39	Najafabadi *et al.* 2014
Tetranychus urticae	22.5~27.5	tree	50	14	10.26	2.12	10.54	0.97	Riahi *et al.* 2011
Tetranychus urticae	22.5~27.5	tree	51	14	9.43	1.29	9.75	1.27	Riahi *et al.* 2011
Tetranychus urticae	22.5~27.5	tree	57	13	9.88	1.28	10.18	2.20	Riahi *et al.* 2011
Tetranychus urticae	>27.5	tree	40	23	7.4	1.71	6	1.44	Bounfour & Tanigoshi 2001
Tetranychus urticae	>27.5	herb	18	18	4.2	0.65	3.9	0.67	Kim *et al.* 2008
Tetranychus urticae	>27.5	herb	10	10	3.5	0.97	3.5	0.58	Kim *et al.* 2008
Tetranychus urticae	Fluctuating	herb	35	14	10.89	1.10	8.44	1.75	Patil *et al.* 2014

Tetranychus urticae	Fluctuating	tree	15	12	12.36	0.68	10.7	0.45	Rajakumar *et al.* 2005
Tetranychus urticae	Fluctuating	herb	25	15	10.92	1.19	11.61	1.34	Shah & Abhishek 2014
Tetranychus urticae	Fluctuating	herb	20	20	13.48	1.25	12.9	1.27	Chauhan 2016
Tetranychus urticae	Fluctuating	herb	25	15	14.81	2.37	9.28	0.73	Shah & Abhishek 2014
Tetranychus urticae	Fluctuating	herb	36	14	10.78	1.13	8.67	3.85	Bhusal 2011
Tetranychus urticae	Fluctuating	herb	20	20	13.92	1.89	11.39	1.00	Chauhan 2016
Tetranychus urticae	Fluctuating	herb	25	15	32.13	4.40	13.89	1.07	Shah & Abhishek 2014
Tetranychus urticae	Fluctuating	herb	20	20	23.3	1.27	17.67	2.19	Chauhan 2016

Nfemale and Nmale represent the sample size for female and male, respectively; MeanF and MeanM represent the mean development of female and male, SD_F and SD_M are the standard deviation of MeanF and MeanM.

TABLE A2. Effects of temperature and host plants on the sex difference in the longevity of mites in the Acari (mite) family Tetranychidae.

Species	Temperature	Host	Nfemale	MeanF	SD_F	Nmale	MeanM	SD_M	Mean difference	References
Eotetranychus lewisi	<22.5	tree	20	12.2	5.86	24	19.4	6.52	-7.20	Lai & Lin 2005
Eotetranychus lewisi	22.5~27.5	tree	20	16	2.73	28	12.3	2.43	3.70	Lai & Lin 2005
Eotetranychus lewisi	>27.5	tree	13	9.6	2.13	16	7.9	2.92	1.70	Lai & Lin 2005
Eutetranychus orientalis	<22.5	tree	17	16.57	3.50	7	21.8	2.22	-5.23	Imani & Shishehbor 2009
Eutetranychus orientalis	22.5~27.5	tree	16	11.75	2.40	9	8.67	2.10	3.08	Imani & Shishehbor 2009
Eutetranychus orientalis	>27.5	tree	15	7.5	1.20	6	7.5	0.42	0.00	Imani & Shishehbor 2009
Oligonychus afrasiaticus	<22.5	tree	23	33.23	12.45	7	29.14	5.28	4.09	Al-Jboory & Al-Suaide 2011
Oligonychus afrasiaticus	22.5~27.5	tree	22	20	12.62	8	18.71	6.38	1.29	Al-Jboory & Al-Suaide 2011
Oligonychus afrasiaticus	>27.5	tree	21	14.3	7.92	9	11.4	4.14	2.90	Al-Jboory & Al-Suaide 2011

Oligonychus afrasiaticus	>27.5	tree	19	11.55	8.77	11	10.14	5.48	1.41	Al-Jboory & Al-Suaide 2011
Oligonychus litchii	<22.5	tree	56	33.2	11.20	6	25	12.10	8.20	Chen *et al.* 2016
Oligonychus litchii	<22.5	tree	31	27.4	5.40	19	28.2	8.00	-0.80	Chen *et al.* 2016
Oligonychus litchii	22.5~27.5	tree	16	13.1	2.80	20	15	4.90	-1.90	Chen *et al.* 2016
Oligonychus litchii	>27.5	tree	31	12.5	4.40	20	8.3	5.10	4.20	Chen *et al.* 2016
Oligonychus litchii	>27.5	tree	18	6.8	3.30	1	5	0.00	1.80	Chen *et al.* 2016
Oligonychus mangiferus	<22.5	tree	58	18.8	6.09	11	24.4	8.95	-5.60	Lin 2013
Oligonychus mangiferus	<22.5	tree	60	13.7	6.20	27	17.7	7.79	-4.00	Lin 2013
Oligonychus mangiferus	22.5~27.5	tree	32	11.9	4.53	5	16.4	5.14	-4.50	Lin 2013
Oligonychus mangiferus	>27.5	tree	87	6.4	3.73	21	11	5.50	-4.60	Lin 2013
Oligonychus mangiferus	>27.5	tree	50	4.8	2.12	8	5.5	2.26	-0.70	Lin 2013
Oligonychus oryzae	22.5~27.5	herb	50	10.34	0.04	50	8	1.00	2.34	Aswin 2015
Panonychus ulmi	22.5~27.5	tree	22	10.88	2.91	10	13.46	2.88	-2.58	Yin *et al.* 2013
Panonychus ulmi	22.5~27.5	tree	22	7.7	2.20	10	7.67	1.30	0.03	Yin *et al.* 2013
Panonychus ulmi	22.5~27.5	tree	15	15.75	2.52	6	13.6	2.28	2.15	Dar *et al.* 2015
Panonychus ulmi	22.5~27.5	tree	16	14.78	1.44	7	13.2	0.98	1.58	Dar *et al.* 2015
Panonychus ulmi	22.5~27.5	tree	26	12	1.58	11	10.75	1.23	1.25	Dar *et al.* 2015
Panonychus ulmi	22.5~27.5	tree	27	10.87	1.82	12	10	1.59	0.87	Dar *et al.* 2015
Tetranychus ludeni	<22.5	herb	11	24.16	9.33	3	16.94	5.52	7.22	da Silva 2002
Tetranychus ludeni	22.5~27.5	herb	22	23.74	10.94	10	14.5	0.58	9.24	da Silva 2002
Tetranychus ludeni	22.5~27.5	herb	14	9	2.81	4	7.25	2.98	1.75	de Andrade Rode *et al.* 2018
Tetranychus ludeni	22.5~27.5	herb	15	9.8	4.38	3	8	2.65	1.80	de Andrade Rode *et al.* 2018
Tetranychus ludeni	22.5~27.5	herb	16	8.81	4.56	3	9	5.56	-0.19	de Andrade Rode *et al.* 2018
Tetranychus ludeni	22.5~27.5	herb	28	17.38	4.84	7	8.83	2.63	8.55	da Silva 2002
Tetranychus ludeni	>27.5	herb	33	13.88	4.29	6	7.75	2.22	6.13	da Silva 2002

Tetranychus ludeni	>27.5	herb	28	11.93	0.53	2	5.73	1.25	6.20	da Silva 2002
Tetranychus macfarlanei	>27.5	herb	5	16.4	3.22	2	7	0.78	9.40	Biswas *et al.* 2013
Tetranychus macfarlanei	>27.5	tree	5	16	0.83	2	11	1.22	5.00	Biswas *et al.* 2013
Tetranychus neocaledonicus	22.5~27.5	herb	100	44.3	3.38	39	48.9	2.21	-4.60	Neto *et al.* 2017
Tetranychus neocaledonicus	>27.5	tree	10	7.325	0.22	10	6.175	0.11	1.15	Kaimal & Ramani 2007
Tetranychus palmarum	<22.5	tree	52	27.4	9.30	15	36.5	19.79	-9.10	Noronha *et al.* 2018
Tetranychus palmarum	22.5~27.5	tree	54	11.2	6.61	11	33.7	9.82	-22.50	Noronha *et al.* 2018
Tetranychus palmarum	>27.5	tree	35	14.7	7.63	11	21.4	10.71	-6.70	Noronha *et al.* 2018
Tetranychus piercei	<22.5	tree	46	33.5	29.84	20	32	6.71	1.50	Fu *et al.* 2002
Tetranychus piercei	<22.5	tree	63	34.5	65.88	23	15.9	2.88	18.60	Fu *et al.* 2002
Tetranychus piercei	22.5~27.5	tree	64	21.8	63.20	29	11.4	1.62	10.40	Fu *et al.* 2002
Tetranychus piercei	>27.5	tree	71	16.1	45.50	24	8	2.45	8.10	Fu *et al.* 2002
Tetranychus piercei	>27.5	tree	26	12.4	16.32	53	7.3	0.73	5.10	Fu *et al.* 2002
Tetranychus piercei	>27.5	tree	50	8.2	25.46	21	7	2.29	1.20	Fu *et al.* 2002
Tetranychus sayedi	22.5~27.5	tree	10	32.5	4.59	10	25.1	3.13	7.40	Mondal & Gupta 2017
Tetranychus sayedi	>27.5	tree	10	12.9	2.34	10	10.5	1.80	2.40	Mondal & Gupta 2017
Tetranychus truncatus	>27.5	herb	52	20.8	2.52	48	22	3.19	-1.20	Islam *et al.* 2008
Tetranychus truncatus	>27.5	herb	64	24.6	4.00	21	24.6	3.16	0.00	Islam *et al.* 2008
Tetranychus truncatus	>27.5	herb	65	23.6	3.63	17	24.6	2.06	-1.00	Islam *et al.* 2008
Tetranychus turkestani	<22.5	herb	27	30.22	14.96	8	30.38	8.94	-0.16	Karami-Jamour & Shishehbor 2012
Tetranychus turkestani	<22.5	herb	30	14.85	6.57	30	18.28	16.82	-3.43	Karami-Jamour & Shishehbor 2012
Tetranychus turkestani	22.5~27.5	herb	19	9.05	5.19	14	5.78	2.21	3.27	Sohrabi & Shishehbor 2008
Tetranychus turkestani	22.5~27.5	herb	27	13.22	3.48	11	9.54	5.34	3.68	Sohrabi & Shishehbor 2008
Tetranychus turkestani	22.5~27.5	herb	31	12.91	2.67	17	11.76	2.97	1.15	Sohrabi & Shishehbor 2008
Tetranychus turkestani	22.5~27.5	herb	23	6.74	3.26	9	8.34	4.14	-1.60	Sohrabi & Shishehbor 2008

Tetranychus turkestani	>27.5	herb	27	6.96	3.90	9	2.72	0.99	4.24	Sohrabi & Shishehbor 2008
Tetranychus turkestani	>27.5	herb	28	11.44	4.34	9	6	4.41	5.44	Sohrabi & Shishehbor 2008
Tetranychus turkestani	>27.5	herb	37	7.79	3.47	7	5.81	2.41	1.98	Sohrabi & Shishehbor 2008
Tetranychus turkestani	>27.5	herb	38	5.78	0.62	9	5.21	4.35	0.57	Sohrabi & Shishehbor 2008
Tetranychus urticae	Fluctuating	tree	15	18.7	2.87	12	12.1	2.56	6.60	Rajakumar *et al.* 2005
Tetranychus urticae	<22.5	herb	61	67.6	12.11	29	74.6	12.60	-7.00	Bayu *et al.* 2017
Tetranychus urticae	<22.5	herb	16	20.7	3.35	16	16.2	3.19	4.50	Kim *et al.* 2008
Tetranychus urticae	<22.5	herb	79	38.1	9.69	32	41.2	3.17	-3.10	Bayu *et al.* 2017
Tetranychus urticae	<22.5	herb	15	14.3	1.61	15	12.8	2.64	1.50	Kim *et al.* 2009
Tetranychus urticae	22.5~27.5	herb	52	19.75	3.75	17	16.41	3.50	3.34	Shoorooei *et al.* 2018
Tetranychus urticae	22.5~27.5	tree	22	12.91	7.74	13	6.8	4.07	6.11	Riahi *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	23	6.93	2.45	10	6.5	3.64	0.43	Khanamani *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	28	7.5	2.86	12	9.09	1.39	-1.59	Khanamani *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	28	7.89	1.96	12	7.71	2.88	0.18	Khanamani *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	31	3.83	1.50	13	4.41	2.31	-0.58	Kavousi *et al.* 2009
Tetranychus urticae	22.5~27.5	herb	31	7.1	3.06	13	7.8	1.01	-0.70	Khanamani *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	31	4.71	2.34	13	5.09	4.65	-0.38	Kavousi *et al.* 2009
Tetranychus urticae	22.5~27.5	herb	32	7.22	3.62	14	8.16	1.53	-0.94	Khanamani *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	33	9.1	4.48	11	10.2	1.59	-1.10	Khanamani *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	40	7.84	3.54	13	9.73	1.30	-1.89	Khanamani *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	47	23.43	5.90	16	17.69	4.44	5.74	Shoorooei *et al.* 2018
Tetranychus urticae	22.5~27.5	herb	49	19.2	5.32	26	15.08	5.10	4.12	Shoorooei *et al.* 2018
Tetranychus urticae	22.5~27.5	herb	58	8.86	4.49	18	6.44	5.90	2.42	Tuan *et al.* 2016
Tetranychus urticae	22.5~27.5	herb	58	21.55	5.64	23	14.65	3.60	6.90	Shoorooei *et al.* 2018
Tetranychus urticae	22.5~27.5	herb	60	21.22	5.65	38	16.74	4.62	4.48	Shoorooei *et al.* 2018

Tetranychus urticae	22.5~27.5	herb	66	21.42	5.61	31	17.77	4.57	3.65	Shoorooei *et al.* 2018
Tetranychus urticae	22.5~27.5	herb	67	20.33	1.88	19	14.11	1.53	6.22	Esmaeily *et al.* 2017
Tetranychus urticae	22.5~27.5	herb	72	28.3	5.18	44	23	2.85	5.30	Bayu *et al.* 2017
Tetranychus urticae	22.5~27.5	herb	78	19.41	5.39	29	14.86	2.32	4.55	Shoorooei *et al.* 2018
Tetranychus urticae	22.5~27.5	herb	100	13.01	0.20	100	10.56	1.80	2.45	Havasi *et al.* 2018
Tetranychus urticae	22.5~27.5	herb	50	17.61	3.46	50	14.83	6.08	2.78	El-Saied Sholla 2016
Tetranychus urticae	22.5~27.5	tree	35	5.92	3.25	10	3.75	1.96	2.17	Riahi *et al.* 2013
Tetranychus urticae	22.5~27.5	herb	18	10.6	1.24	18	7.2	2.59	3.40	Kim *et al.* 2010
Tetranychus urticae	22.5~27.5	herb	77	19.1	6.00	28	14.6	4.60	4.50	Shih *et al.* 1976
Tetranychus urticae	>27.5	tree	30	3.56	2.96	4	3.2	3.32	0.36	Riahi *et al.* 2013
Tetranychus urticae	>27.5	herb	70	13.8	2.34	35	13	1.95	0.80	Bayu *et al.* 2017
Tetranychus urticae	>27.5	herb	50	12.71	7.21	50	10.43	4.24	2.28	El-Saied Sholla 2016
Tetranychus urticae	>27.5	herb	18	9.2	2.07	18	8	2.34	1.20	Kim *et al.* 2011
Tetranychus urticae	>27.5	tree	27	6.53	2.91	13	5.23	3.35	1.30	Riahi *et al.* 2013
Tetranychus urticae	>27.5	herb	80	9.9	1.88	33	5.3	0.80	4.60	Bayu *et al.* 2017
Tetranychus urticae	>27.5	herb	10	5.4	1.56	10	5	1.41	0.40	Kim *et al.* 2012
Tetranychus urticae	Fluctuating	herb	67	79.9	16.53	39	83.2	11.24	-3.30	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	80	67.2	11.54	35	64.8	7.40	2.40	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	86	31.3	6.49	31	39.4	5.46	-8.10	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	70	19.3	4.18	46	17.3	3.46	2.00	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	82	12.9	1.81	37	12.5	1.46	0.40	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	71	11.2	1.69	34	16.9	2.16	-5.70	Bayu *et al.* 2017
Tetranychus urticae	Fluctuating	herb	13	25.08	1.37	8	24.28	0.68	0.80	EI Taj *et al.* 2016
Tetranychus urticae	Fluctuating	herb	16	24.88	1.48	7	23.86	1.11	1.02	EI Taj *et al.* 2016
Tetranychus urticae	Fluctuating	herb	12	25.88	1.07	8	25.31	1.27	0.57	EI Taj *et al.* 2016

Tetranychus urticae	Fluctuating	herb	10	26.5	0.89	9	25.94	0.96	0.56	EI Taj *et al.* 2016
Tetranychus urticae	Fluctuating	herb	14	14.54	1.05	9	11.61	0.84	2.93	EI Taj *et al.* 2016
Tetranychus urticae	Fluctuating	herb	16	16.31	0.96	8	13.25	0.76	3.06	EI Taj *et al.* 2016
Tetranychus urticae	Fluctuating	herb	15	16.33	2.19	15	9.73	1.03	6.60	Shah & Abhishek 2014
Tetranychus urticae	Fluctuating	herb	20	7.4	0.88	20	4.55	0.94	2.85	Chauhan 2017
Tetranychus urticae	Fluctuating	herb	25	12.36	1.38	15	9.73	1.03	2.63	Shah & Abhishek 2014
Tetranychus urticae	Fluctuating	herb	15	11.46	2.16	15	8.8	1.20	2.66	Shah & Abhishek 2014
Tetranychus urticae	Fluctuating	herb	25	10	1.19	15	8.8	1.20	1.20	Shah & Abhishek 2014
Tetranychus urticae	Fluctuating	herb	20	7.3	0.86	20	4.9	0.85	2.40	Chauhan 2016
Tetranychus urticae	Fluctuating	herb	15	17.46	2.03	15	11.46	1.06	6.00	Shah & Abhishek 2014
Tetranychus urticae	Fluctuating	herb	25	19.84	1.46	15	11.46	1.06	8.38	Shah & Abhishek 2014
Tetranychus urticae	Fluctuating	herb	20	7.65	0.93	20	6.1	0.79	1.55	Chauhan 2016

Nfemale and Nmale represent the sample size for female and male, respectively; MeanF and MeanM represent the mean longevity of female and male, SD_F and SD_M are the Standard Deviation of MeanF and MeanM. Mean difference is the difference between MeanF and MeanM.

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