Reproductive Performance of a Spider Mite *Tetranychus ludeni* Zacher (Acari: Tetranychidae) in Response to Social Environments

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Abstract

Animals are sensitive to changes in social environments and may plastically adjust their life history traits in response to multiple socio-environmental factors. Population size and density are two key socio-environmental factors affecting female reproductive output and offspring sex ratio. However, previous studies have mostly focused on only one factor at a time, ignoring the other and their interactions. Moreover, the reproductive plasticity of females in response to social environments has been investigated under constant population size and density, but whether they could adjust their reproductive strategies in response to the fluctuations or shifts of population size and density during the breeding period is largely unknown. Using a haplodiploid spider mite, *Tetranychus ludeni* Zacher, I carried out a series of experiments to examine how ovipositing females alter their reproductive investment under different population sizes and densities. Results demonstrate that both population size and density and their interaction significantly affected reproduction and sex allocation. I reveal that when the population was small, females tended to reduce local mate competition among sons by producing more daughters. When the population was large and dense, females were more likely to reduce local resource competition by producing more daughters early in life that would disperse from the natal patch to found new colonies. My findings show that the extremely high female-biased offspring sex ratios in large populations could not be explained by local mate competition models alone, and local resource competition and local resource enhancement might also be involved in sex allocation of *T. ludeni*. However social environments had weaker effects on *T. ludeni* sex allocation compared to the life history traits, and social environments influencing offspring sex ratio was implemented or mediated through other life history traits. *T. ludeni* females adjusted their reproductive strategies in response to the shifts of population density and size during their breeding period. Females constrained fecundity in response to the fluctuating environments. Females traded off egg number for size in response to dynamics of population size and increasing egg size significantly raised the proportion of daughters. They fertilized more larger eggs laid in a small population but fertilized more smaller eggs laid in a large population by lowering the egg size dependent fertilization threshold. This study provides evidence that spider mites can manipulate their reproductive output and adjust offspring sex ratio in response to various static and dynamic social environments.

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Chapter 1

General Introduction

1.1 Introduction

Mites are the largest group of arthropods in Class Arachnida with over 55,000 described species (Dhooria, 2016). About 10% of these species are of economic importance (Walter and Proctor, 1999; Hoy, 2009). Members in the family Tetranychidae are commonly known as spider mites, which spin webs on host plants' leaves, and feed, mate and oviposit under the webs (Dhooria, 2016; Potter and Potter, 2020). Over 1,300 species of spider mites in 95 genera have been recorded (Dhooria, 2016; Migeon and Dorkeld, 2022) with more than 100 species considered as pests and 10 as major plant pests (Dhooria, 2016), including *Tetranychus ludeni* Zacher, *T. urticae* Koch and *T. evansi* Baker and Pritchard (McKinlay et al., 1992; Meyer, 1996; Gerson et al., 2003; Adango et al., 2006), which are recorded now in New Zealand (Fan et al., 2021).

Spider mites injure plant leaves by piercing cells and sucking cell contents, inducing white or yellow spots and reducing photosynthetic surface (Dhooria, 2016). They attack a wide variety of field-grown crops including ornamentals, fruit crops and some vegetable crops in all climate zones in Europe, Asia, Australasia, and North and South America (Gutierrez and Schicha, 1983; Duso et al., 2010; James et al., 2010; Domingos et al., 2014). Some species do severe damages to crops grown in glasshouses (Jeppson et al., 1975; Zhang, 2003; Migeon and Dorkeld, 2006), incurring significant yield losses (Stumpf et al., 2001; Van Leeuwen et al., 2010). *T. ludeni* is a serious pest of economically important crops, such as bean, eggplant, pumpkin and other cucurbitaceous plants in warm areas, and a common pest on greenhouse plants in temperate zones (Jeppson et al., 1975; Reddy and Baskaran, 1991; Kumar and Sharma, 1993; McKinlay et al., 1992; Meyer, 1996; Zhang, 2003; Adango et al., 2006).

Tetranychus ludeni is an invasive species, which is native to Europe and has now become cosmopolitan (Reddy and Baskaran, 1991; Bolland et al., 1998; Zhang, 2003; Beard, 2018). In Palmerston North, New Zealand, all life stages of *T. ludeni* can be found on woolly nightshade *Solanum mauritianum* Scopoli (personal observations) and females reproduce throughout the year under greenhouse conditions (Singh and Chauhan, 2016). Like other *Tetranychus* species, *T. ludeni* has a short lifecycle (about 10 days from egg to adult stage at 27 ºC) and high fecundity (e.g., 84.3 and 111.6 eggs on African eggplant *Solanum macrocarpon* L. and purple amaranth *Amaranthus cruentus* L., respectively) (Adango et al., 2006), and thus build up the local populations quickly. When the population becomes large and dense, females tend to disperse to search for new habitats due to food deficiency and habitat deterioration (Azandémè-Hounmalon et al., 2014; Schausberger et al., 2021; Zhou et al., 2021a).

The silk strands of webs can aid in mite dispersal within or between host plants primarily by walking (MacGregor, 1913; Hussey and Parr, 1963; Dhooria, 2016). The other modes of dispersal are aerial displacement by air currents (e.g., Stabler, 1913; Ebeling, 1934; Marle, 1951; Fleschner et al., 1956; Boyle, 1957; Brandenburg and Kennedy, 1982; Boykin and Campbell, 1984; Silberman, 1984; Smitley and Kennedy, 1988; Li and Margolies, 1993; Osakabe et al., 2008) and transport by phoresy (Boykin and Campbell, 1984; Yano, 2004; Dhooria, 2016; Potter and Potter, 2020). The silk threads produced by spider mites further contribute to long-distance, aerial dispersal (Bell et al., 2005). The sudden infestation of previously uninfested crops mostly occurs through aerial dispersal (Brandenburg and Kennedy, 1982; Margolies and Kennedy, 1985). However, whether and how the dispersed individuals adjust their reproductive strategies in response to the changes in social environments such as population density and size is largely unknown. Understanding these strategies helps predict the colonisation patterns and growth of mite populations.

In this chapter, I outline the relevance of my research and the aim and objectives of the thesis. I then conduct a literature review of current knowledge about the biology and ecology of spider mites and the effects of socio-environmental factors on their life history strategies.

1.2 Relevance of research

Social environment refers to an individual’s physical surroundings, community resources and social relationships, including population composition, fission-fusion dynamics, and the social interactions among group members (Webber et al., 2022). In response to various social environments, such as changes in population size and density during the breeding period, animals may adjust their life history traits to maximise fitness gain (Wauters and Lens, 1995; Estevez et al., 2007; Danko et al., 2018; Webber and Wal, 2018; Burant et al., 2020; Weerawansha et al., 2020, 2022a, 2022b, 2022c). However, the mechanisms underlying the effects of social environments and their frequent changes on reproductive plasticity are still poorly understood, making it difficult to predict the population dynamics in novel habitats and develop pest control programs.

1.2.1 Effects of population density and size on individual reproductive performance

In species that live in groups with overlapping generations, local population density and size are two key social environmental factors that regulate reproduction and population growth (Kunin, 1997a; Estevez et al., 2007; West, 2009; Sprenger et al., 2011; Danko et al., 2018; Ausband and Mitchell, 2021). Population density represents a function of spacing between neighbouring individuals in a finite area of a habitat, and population size is the number of individuals residing in the habitat (Kunin, 1997a). Many studies report that population growth is negatively associated with present and/or past population density (negative density dependence) (Harrison and Cappuccino, 1995; Turchin, 1995; Rotem et al., 2003; Sibly et al., 2005) due to the fast resource exploitation and low provisioning rates of individuals in dense environments (Harrison and Cappuccino, 1995; Ferrer and Donazar, 1996; Rodenhouse et al., 1997; Both, 1998a, 1998b). In nature, population size may also influence individual reproduction (Aviles and Tufino, 1998; Bilde et al., 2007; Estevez et al., 2007; Amsalem and Hefetz, 2011; Grove, 2012; Ruel et al., 2012; Wong et al., 2013; Li and Zhang, 2021; Tinsley-Johnson et al., 2021). However, in many studies, population density is usually used as an explicit proxy of population size owing to their close intercorrelations (Kunin, 1997a; Gaston, 1994; Saether et al., 2002; Sibly et al., 2005), making it difficult to differentiate the effect of population size from that of population density on reproduction (But see Krause and Ruxton, 2002; Vander Wal et al., 2013).

Changes in population density and/or size may also induce sex-specific responses in individuals (Dreiss et al., 2010; Minetti et al., 2013; Szentiványi et al., 2017). For instance, in small populations where related males of one or few mothers will compete for mates, mothers are selected to produce a higher proportion of daughters to reduce the local mate competition among sons (Hamilton, 1967; Sato and Saito, 2006; Macke et al., 2011a, 2014). Alternatively, when the breeding resource is limited in dense populations, females will produce more individuals of the dispersing sex to minimise the local resource competition among siblings or close relatives (Clark, 1978; Silk, 1983; Mari et al., 2008; Hjernquist et al., 2009; Visser et al., 2014; Song et al., 2016). However, most empirical studies focus on how ovipositing females regulate offspring sex ratio in response to either group density or size (Charnov, 1982; Shuster and Wade, 2003; West et al., 2005; West, 2009; Klug et al., 2010), with few studies determining the effects of population density, size, and their potential interactions. Distinguishing the different effects of population density and size and their interactions on reproductive output and sex allocation can help better understand the reproductive plasticity of females and population dynamics under dynamic social environments.

1.2.2 Reproductive adjustment in response to changes of population density and size during the breeding period

Population density and size, especially in group-living species, often vary over time due to deaths, births, aggregation (immigration) and dispersal (emigration) (Roeder, 1992; Roff, 1992; Stearns, 1992; Price and Hunter, 1995; Bowman et al., 2002; Schausberger et al., 2021). Individuals tend to aggregate for multiple benefits (Avilés and Tufiño, 1998; Dyer, 2000; Krause and Ruxton, 2002; Brown and Brown, 2004; Wertheim et al., 2004; Costa, 2006; Mori and Saito, 2006; Sato and Saito, 2006; Le Goff et al., 2010; Ranta et al., 2015), for example, the increase in reproductive success (e.g., Snead and Alcock, 1985; Prokopy and Reynolds, 1998; Le Goff et al., 2010; Pérez-González et al., 2010; Bonsignore and Jones, 2014) and reduction of predation risk (e.g., Spieler, 2003; Morrell and James, 2008; Yano, 2012; Saito and Zhang, 2017).

However, aggregation may increase the competition for food, space, and mates, causing deterioration of environmental conditions (Bilde et al., 2007; Estevez et al., 2007; Grove, 2012; Wong et al., 2013; Li and Zhang, 2021; Tinsley Johnson et al., 2021). As a result, they may disperse to search for new habitats (Ims and Hjermann, 2001; Tien et al., 2011). In response to the changing social environments, individuals are expected to adjust their resource partitioning strategies to optimize their offspring and own fitness (Mueller et al., 1991; McNamara and Simmons, 2017). Nevertheless, in many empirical studies, reproductive fitness has usually been compared between populations (e.g., large and small, or dense and sparse) where the population size and/or density of each population is kept consistent. To date it is not clear how females adjust their reproductive strategies in response to the population density and size shifts during their breeding period.

1.3 Aim and objectives of this study

The present study aims to explore the adjustment of life history strategies of *T. ludeni* in response to varying social environments. The specific objectives are:

(1) to investigate how population density and size affect sex allocation and reproductive fitness in females, and

(2) to determine how females adjust their sex allocation and reproductive fitness in response to the shifts in population size and density during the breeding period.

1.4 Literature review

This section reviews the general biology of *T. ludeni* and literature on various aspects of life history strategies in response to social environments relevant to the current study.

1.4.1 Taxonomy and identification of *Tetranychus ludeni*

The classification for *T. ludeni* is:

Class: Arachnida
  Subclass: Acari
    Order: Trombidiformes
      Family: Tetranychidae
        Genus: *Tetranychus*
          Species: *ludeni* Zacher 1913

Since it was first reported on *Cucurbita* sp., *Salvia splendens* Sellow and Schultes, and *Solanum melongena* L. from France and Germany and named by Zacher (1913), *T. ludeni* has been known by the following other names (Beard, 2018; Migeon and Dorkeld 2023):

Tetranychus desertorum Banks 1900, misidentification by Ehara 1956
Tetranychus desertorum Banks 1900, corrected identification by Ehara & Masaki 1989
Tetranychus (*Epitetranychus*) *ludeni* Zacher 1913, new combination by Zacher 1921
Tetranychus ludeni Zacher 1913, new combination by Pritchard & Baker 1955

Epitetranychus ludeni (Zacher) Zacher 1921

Tetranychus salviae Oudemans 1931, synonym description by Oudemans 1931

Tetranychus salviae Oudemans 1931, synonymy by Pritchard & Baker 1955

Tetranychus (*Tetranychus*) *ludeni* Oudemans, 1931, new combination by Tuttle, Baker & Abbatiello 1976

Septanychus deviatarsus McGregor 1950, synonym description by McGregor 1950

Septanychus deviatarsus McGregor 1950, synonymy by Pritchard & Baker 1955

Tetranychus deviatarsus McGregor 1950, synonymy by Pritchard & Baker 1955

Tetranychus ludeni Zacher, Pritchard & Baker 1955

Tetranychu ludeni is similar to *T. desertorum* Banks and often misidentified as *T. urticae* in the field (Toroitich et al., 2009). Many specimens previously identified as *T. cinnabarinus* (Boisduval) (now *T. urticae*; Auger et al., 2013) is in fact *T. ludeni* (Zhang, 2002). The morphological characteristics of male aedeagus are the most important for identification of *Tetranychus* species (Toroitich et al., 2009; Z-Q Zhang's personal comment). Male aedeagus of *T. ludeni* (Figure 1.1a) differ from that of other *Tetranychus* species (Figure 1.1b–e) in (1) aedeagus bends dorsally, (2) aedeagal knob without a posterior projection, anterior projection small and acuminate, and (3) knob and shaft axis parallel (Figure 1.1a). My study species was identified as *T. ludeni* by Prof Zhi-Qiang Zhang, Landcare Research, Auckland, New Zealand.

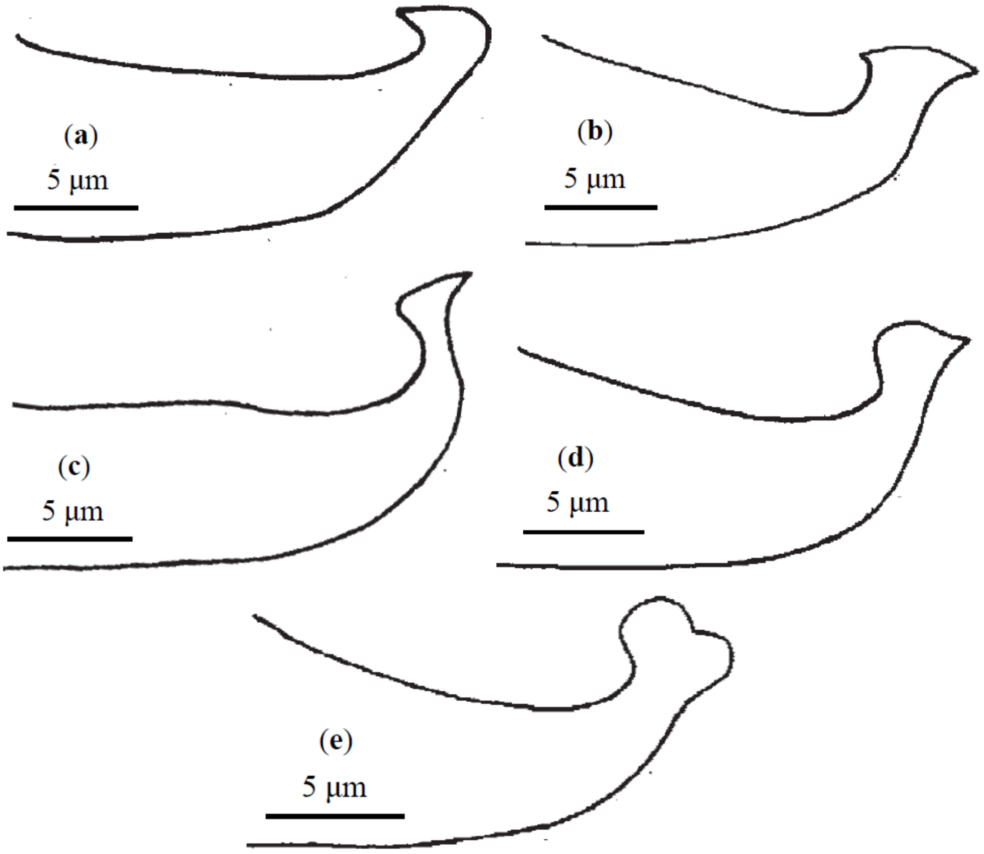


Figure 1.1 Morphology of male aedeagus in *Tetranychus* sp.: (**a**) *T. ludeni,* (**b**) *T. urticae,* (**c**) *T. evansi*, (**d**) *T. lombardiini*, and (**e**) *T. neocaledonicus* [Modified after Meyer (1974) and Toroitich et al. (2009)].

1.4.2 Distribution and host plants of *Tetranychus ludeni*

Tetranychus ludeni is native to Europe (Bolland et al., 1998) and was first reported in Germany (Zacher, 1913). It appears to be better adapted to hot weather and has a more subtropical distribution than the cosmopolitan congeneric *T. urticae* (Martin, 2000; Gotoh et al., 2015). *T. ludeni* is now globally distributed and can be found in many countries and districts (Beard, 2018) including Australia, Algeria, Argentina, Brazil, Canary Islands, Caribbean islands, Chile, China, Colombia, Cook Islands, Costa Rica, Egypt, El Salvador, Eswatini, Fiji, France, French Polynesia, Germany, Greece, Hawaii, Honduras, India, Iraq, Japan, Kazakhstan, Kenya, Madagascar, Madeira Island, Malawi, Mauritius, Mexico, Morocco, Mozambique, New Caledonia, New Zealand, Nicaragua, Paraguay, Portugal, Republic of Benin, South Africa, Spain, Swaziland, Taiwan, Thailand, USA, Venezuela, Zambia, and Zimbabwe. More than 300 species of plants from 60 families are recorded to be hosts for *T. ludeni* across the world (Reddy and Baskaran, 1991; Zhang, 2003; Beard, 2018). This species is a serious pest in greenhouse production and field crops, destroying annual and perennial crops belonging to many economically important families (Zhang, 2003).

1.4.3 General biology of *Tetranychus ludeni*

Tetranychus ludeni pass through several developmental stages: egg, larva, quiescent larva, protonymph, quiescent protonymph, deutonymph, quiescent deutonymph, and adult (Figure 1.2). Eggs are usually laid on the underside of leaves and sphere-shaped. Eggs laid by virgin females are brown and those laid by mated females are pale yellow in colour (Figure 1.2a–b). The larvae (Figure 1.2c–d) have six legs, and nymphs (Figure 1.2e–i) and adults (Figure 1.2j–k) have eight legs. Female adults (Figure 1.2j) are dark red without the two black spots on dorsolateral idiosoma apparently found in those of two-spotted spider mite, *T. urticae* (Zhang, 2003; Adango et al., 2006). Male adults (Figure 1.2k) are spindle-shaped and pale yellow in colour (Kaimal and Ramani, 2011) and smaller than female adults (Figure 1.2j).

Like other spider mites, *T. ludeni* is haplodiploid, where virgin females give birth to haploid sons and mated females produce both haploid sons and diploid daughters. Development occurs above 13 ºC (Ristyadi et al., 2021). At about 26 ºC and 70% RH on common bean *Phaseolus vulgaris* L., egg, larva, protonymph and deutonymph take 4.2, 2.0, 1.8 and 2.1 days, respectively, to develop to next stage, and males and females take about 9.3 and 10 days, respectively, to complete a life cycle (Moros and Aponte, 1994). Under the same environmental conditions, females live for an average of 25 days and produce 84.3 eggs in their lifespan (Adango et al., 2006). Virgin females lay fewer but larger eggs than mated females, giving rise to larger male adults that produce more daughters during their lifespan (Zhou et al., 2018).

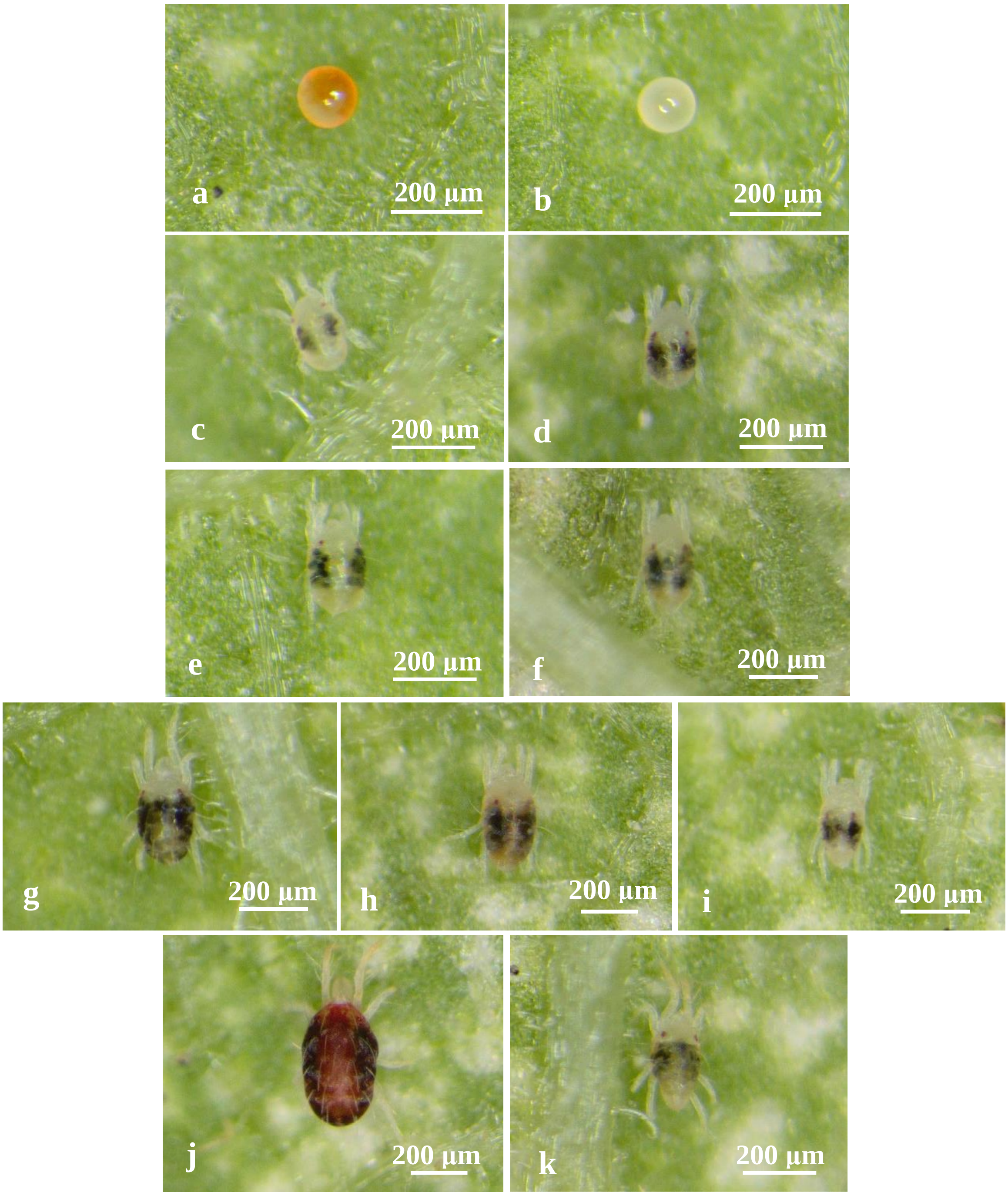


Figure 1.2 Life stages of *T. ludeni*: (**a**) egg laid by a virgin female, (**b**) egg laid by a mated female, (**c**) active larva, (**d**) quiescent larva, (**e**) active protonymph, (**f**) quiescent protonymph, (**g**) active female deutonymph, (**h**) quiescent female deutonymph, (**i**) quiescent male deutonymph, (**j**) active adult female, and (**k**) active adult male.

1.4.4 Life history strategies

Life history strategies are the evolutionary decisions made by organisms on the life history traits such as growth pattern, age and size at maturity, timing of reproduction, the number, size and sex ratio of offspring, adult longevity and so on (Brown and Choe, 2019) in response to the ecological challenges posed by the environment (Roff, 1992; Stearns, 1992, 2000). Life histories are shaped by natural selection and reflect how members of a species allocate their limited resources to survival, growth, and reproduction (Brown and Choe, 2019). The best possible allocation of resources is to allow an organism to obtain the maximum level of fitness through optimal life history strategies (Preston et al., 2014). However, the optimal life history strategy may be different for each species, depending on its traits, environment, and other constraints (Emlen and Oring, 1977).

Mated spider mite females usually allocate more resource to large eggs (Macke et al., 2012a) which are more likely to be fertilised and develop to diploid daughters (Macke et al., 2011a; Weerawansha et al., 2022d) and less resource to small eggs which are less likely to be fertilised and develop to haploid sons (Macke et al., 2011a). Mites often invade their host plants and make full use of local resources (Sances et al., 1979; Park and Lee, 2002), and mated adult females then disperse within the host plant by walking (Brandenburg and Kennedy, 1982; Kondo and Takafuji, 1985; Margolies and Kennedy, 1985) or to new habitats by wind currents, birds, insects or humans (Dhooria, 2016; Potter and Potter, 2020). Over the course of an invasion-exploitation-dispersal cycle, they need to adopt different life history strategies in terms of oviposition and sex allocation in response to the various environments.

1.4.5 Trade-offs between life history traits

The evolution of life history traits by natural selection is the process through which the living organisms are capable of adapting to the changes of environment (Jillson, 1980; Mueller et al., 1991; Muller et al., 2016; Gutiérrez et al., 2020). In nature the pool of resources in most environments is limited, thus to optimise their fitness animals need to adjust their physiology and partition the limited resource for various life-history traits (Van Noordwijk and de Jong, 1986; Houle, 1991; Roff, 1992; Stearns, 1992; Kokko, 1998; Garland, 2014, Cohen et al., 2020; Garland et al., 2022). As a result, allocation of resources to one trait may reduce that to others, leading to trade-offs between traits (Houle, 2001; Lancaster et al., 2017).

The frequently reported trade-offs are clutch size with egg size (Smith and Fretwell, 1974; Parker and Begon, 1986; Stearns, 1989, 1992; Fox and Czesak, 2000; Fischer et al., 2011; Macke et al., 2012b; Walzer and Schausberger, 2015; Maenoa et al., 2020) and fecundity with longevity (Stearns, 1989; De Loof, 2011; Flatt and Heyland, 2011; Blacher et al., 2017; Cingolani et al., 2020). However, some studies have demonstrated a lack of trade-offs between life history traits (Zera and Harshman, 2001; Jordan and Snell, 2002; Bowden et al., 2004; Uller and Olsson, 2005; Weerawansha et al., 2022c) probably due to species-specific effect (Brown and Shine, 2009; Crean and Marshall, 2009).

1.4.6 Sex allocation

Sex allocation is a vital life history trait with substantial effects on population dynamics, because it directly affects the fitness of individuals (Fox and Czesak, 2000; Lebreton et al., 2009; Bondy and Hunter, 2019). For species with sexual reproduction, the allocation of resources to male and female offspring is an important reproductive decision for the parents as it lays the foundation to the way in which natural selection acts (Hamilton, 1967; Charnov, 1982; Hardy, 2002; West, 2009). The Fisher's principle predicts that the sex ratio (female:male) in many animals is 1:1 (Fisher, 1930), assuming that male and female offspring are equally costly for parents to produce, the relatives do not interact with one another, and the environment has no effect (Fisher, 1930, 1958).

However, under some circumstances, females may manipulate brood sex ratios by differential investment in offspring of a particular sex depending on which sex has higher fitness value (Hamilton, 1967; Clutton and Brock, 1984; Hardy, 1992, 2002; Reece et al., 2008; West, 2009; Khidr et al., 2013; Wilkinson et al., 2016). Examples include birds (Komdeur et al., 1997; Veiga et al., 2008; Song et al., 2016; Reviewed in Payevsky, 2021; Rubalcaba and Polo, 2022), protozoan parasites (Read et al., 1992, 1995; West et al., 2001; Pickering et al., 2000; Reece et al., 2008; Lopez et al., 2011), parasitoid wasps (Werren, 1980, 1983; Godfray, 1994; Shuker et al., 2004; Burton-Chellew et al., 2008; Abe et al., 2009; Li et al., 2018; Iritani et al., 2021) and mites (Macke et al., 2011b; Duncan et al., 2018; Rodrigues et al., 2021; Weerawansha et al., 2022a, 2022b, 2022c). Spider mite populations are usually female-biased, with the sex ratio ranging from 2:1 to 9:1 (Carey and Bradley, 1982; Roeder, 1992; Macke et al., 2011a; Shuker et al., 2009; Weerawansha et al., 2022a, 2022b) depending on social environments (Sabelis, 1991; Hales, 1994; Sato and Saito, 2006; Duncan et al., 2018; Weerawansha et al., 2022a, 2022b, 2022c).

1.4.7 Local resource competition and local mate competition

Local resource competition (LRC) (Clark, 1978) and local mate competition (LMC) (Hamilton, 1967) have been frequently used to explain how mothers may adjust their sex allocations to offspring in response to social environments. LRC predicts that when competition occurs among relatives for a restrictive breeding resource in a dense population, mothers should bias offspring sex ratio toward the dispersing sex to avoid local competition with their offspring (Silk, 1983; Mari et al., 2008; Hjernquist et al., 2009; Visser et al., 2014; Song et al., 2016). LMC expects that when mating takes place locally between siblings, females are selected to produce a daughter-biased sex ratio. Under LMC, mothers will only produce a few sons necessary to fertilise all daughters (Herre, 1985).

In haplodiploid arthropods such as spider mites, sex ratio adjustments are likely mediated by differential investments in egg size, with small eggs possibly experiencing lower chances of fertilisation and giving rise to haploid males (Macke et al., 2012a). However, in many cases haplodiploid females produce extremely female-biased offspring sex ratios, which cannot be explained by LMC theory alone (West, 2009; Iritani et al., 2021). Moreover, given that LRC and LMC are likely to take place simultaneously in a patch of the same species (West, 2013), whether LRC mediates the LMC to produce extreme female-biased sex ratios has been poorly investigated.

1.4.8 Population size and density

The local abundance of a species can be represented by either the population size or population density (Kunin, 1997a), and changes in local population density and size may alter the resources available for local individuals (Wauters and Lens, 1995; Estevez et al., 2007; Webber and Wal, 2018; Burant et al., 2020; Weerawansha et al., 2020). However, the local population density and size may affect individuals differently and thus the population dynamics (reviewed in Kareiva, 1983; Kunin, 1997b). Therefore, animals must adjust their life history strategies in order to adapt to such changes in social environments.

In spider mites, individuals inhabit in discrete patches with varying population sizes and densities (Le Goff et al., 2010; Schausberger et al., 2021). They tend to live in clusters or groups on leaves (Iftner and Hall, 1983; Gerson, 1985; Jones, 1990) by which they gain a variety of advantages (Perring et al., 1984) due to the Allee effects (Allee, 1931; Stephens et al., 1999), for example, web sharing and consequently elevating egg production (Oku et al., 2009; Le Goff et al., 2010), and prolonging survival (Le Goff et al., 2010; Yano, 2012) through better protection against abiotic factors (e.g., wind and rain) and predators (Gerson, 1985; Lemos et al., 2010; Yano, 2012). Living in a group may also increase mite interaction and thus reduce time for searching potential mates (Potter, 1979a, 1979b).

However, group living in habitats of high population size and density would lead to overexploitation, competition and deterioration of local resources (Le Goff et al., 2010; Weerawansha et al., 2020, 2022a), and lower reproductive performance due to increasing behavioural interference among individuals (Weerawansha et al., 2020; Li and Zhang, 2021). Furthermore, variations of population size and density may affect the sex allocation behaviour (Hamilton, 1967; Trivers and Willard, 1973; Bulmer and Taylor, 1980; Charnov, 1982; West et al., 2005; West, 2009; Iritani et al., 2021; Weerawansha et al., 2022a). However, previous studies on reproductive performance and sex allocation have usually focussed on the effects of either the population size or density at a time (Charnov, 1982; West et al., 2005; West, 2009), and few studies have disentangled the effects of population density from that of population size due to their high-level interrelationship (e.g., Amsalem and Hefetz, 2011; Li and Zhang, 2021). So far, whether population density and size interact and have different impacts on animal reproductive strategies is largely unknown.

1.4.9 Shift of population size and density

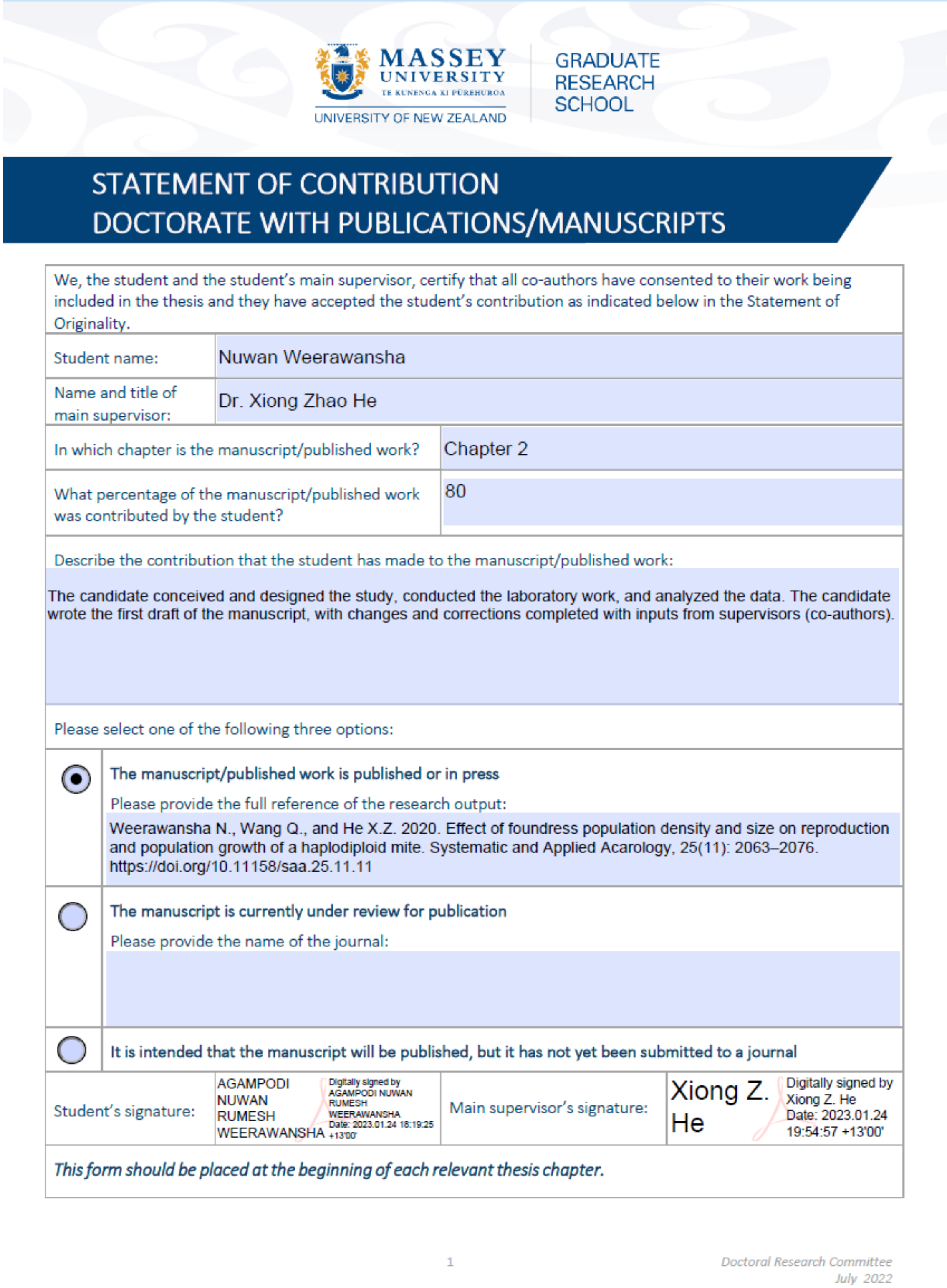
Population fluctuations in local habitats may occur due to primary ecological events such as births, deaths, immigration (aggregation), and emigration (dispersal) (Andrewartha and Birch, 1954; Roeder, 1992; Roff, 1992; Stearns, 1992; Bowman et al., 2002; Pedigo and Rice, 2006; Schausberger et al., 2021). Such changes in social environments would affect the inhabitants beneficially or detrimentally depending on the level of fluctuations (Nicholson, 1957; Whitlock, 1992). Therefore, animals must alter their behaviours and reproductive strategies to adapt to such changes (Lihoreau and Rivault, 2008; Ruan and Wu, 2008; Krams et al., 2009; Schausberger et al., 2017; Li and Zhang, 2021). For example, animals may do trade-offs between their life history traits by making the best use of the limited resources to optimize the fitness of offspring and their own (Roff, 1992).

In spider mites, populations can expand rapidly with an increasing rate up to 40% per day owing to high reproduction rate, short developmental time (Shih et al., 1976) and high female-biased offspring sex ratio (Carey and Bradley, 1982; Macke et al., 2011a; Weerawansha et al., 2022b). To benefit from the cooperative construction of protective webs and increase of egg production, reproducing females tend to aggregate or join small groups (Clotuche, 2011; Clotuche et al., 2011; Le Goff et al., 2010; Yano, 2012). However, when the population becomes larger and denser, females tend to disperse in search of favourable habitats for the next generation (Azandémè-Hounmalon et al., 2014; Schausberger et al., 2021; Zhou et al., 2021a). Therefore, females may need to adjust their reproductive strategies when such shifts occur. However, the mechanisms underlying the adjustment of the reproductive strategies have received less attention.

Chapter 2

Effect of foundress population density and size on reproduction and population growth of a haplodiploid mite

This chapter was published in *Systematic and Applied Acarology* 25(11): 2063–2076 (2020).



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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Nuwan Weerawansha
Name and title of main supervisor:	Dr. Xiong Zhao He
In which chapter is the manuscript/published work?	Chapter 2
What percentage of the manuscript/published work was contributed by the student?	80

Describe the contribution that the student has made to the manuscript/published work:

The candidate conceived and designed the study, conducted the laboratory work, and analyzed the data. The candidate wrote the first draft of the manuscript, with changes and corrections completed with inputs from supervisors (co-authors).

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Doctoral Research Committee
July 2022

Abstract

Tetranychus ludeni Zacher (Acari: Tetranychidae) is an invasive polyphagous haplodiploid pest mite of many vegetable crops in many parts of the world. This study investigated the effects of simultaneous variations in the local foundress population density and size on the reproduction and population growth of *T. ludeni*. I show that negative population-density and -size dependency limited the reproduction of foundresses, where the effects of population size on population growth overweighed that of population density. I further demonstrate that ovipositing females could accelerate population growth rate (intrinsic rate of increase, r_m) at higher population sizes by producing more daughters early during their lifespan, which can be an adaptive strategy to allow more daughters to complete development and disperse to reduce future food competition intensity. This study provided knowledge on population dynamics in response to foundress population density and size in general and *T. ludeni* population forecast and precise timing of pest management in particular.

Key words: *Tetranychus ludeni*, daughter production, disperse, resource competition, life table parameter

2.1 Introduction

The ability of organisms to increase their populations is crucial to their establishment in a new habitat especially for the invasive species. Local foundress population density and size are two important socio-environmental factors that regulate reproduction and population growth (Kunin, 1997a; Danko et al., 2018). The former represents a function of spacing between neighbouring individuals in a finite area and the latter is the number of individuals in a local population (Kunin, 1997a). In nature, resources are unevenly partitioned among individuals, and this inequality increases when resources become scarce with increasing population density and size (Krebs, 1971; Łomnicki, 1978; Rodenhouse et al., 1997; Leips and Travis, 1999; Creighton, 2005; Goubault et al., 2007). Therefore, it is fundamentally important to understand the nature and extent of population regulation by density- and size-dependent processes in population ecology (Levins, 1979; Newton, 1994, 1998; Ferrer and Donazar, 1996; Rodenhouse et al., 1997; Penteriani et al., 2003).

It is widely reported that population growth is a decreasing function of population density (Harrison and Cappuccino, 1995; Turchin, 1995; Sibly et al., 2005), inducing a negative density-dependent reproduction associated with present and/or past population densities (Adler and Levins, 1994; Sinervo et al., 2000; Rotem and Agrawal, 2003; Meylan et al., 2007; Allen et al., 2008; Creighton, 2005; Leips et al., 2009). The negative density-dependent reproduction could arise either through decreasing territory quality and resource scarcity which limit the individuals to low provisioning rates at high population densities (Andrewartha and Birch, 1954; Pulliam and Danielson, 1991; Dhondt et al., 1992; Ferrer and Donazar, 1996; Rodenhouse et al., 1997; Both, 1998a; Kruger and Lindstrom, 2001; Danko et al., 2018), or by adjusting the reproductive behaviours of individuals as a response to the varying density within the same habitat (Lack, 1954; Both, 1998a; Fernandez et al., 1998). In a similar fashion the size of a population may also affect its dynamics, especially when conspecifics compete for resources (Krebs, 1971; Rodenhouse et al., 1997; Leips and Travis, 1999; Creighton, 2005; Goubault et al., 2007) and when there is direct interference among individuals (Post et al., 1997). However, most studies on density-dependent reproduction are based on the relationship between the mean reproduction and population density (see Sinclair, 1989), without knowing whether density is the only cause of such a relationship or the extent to which individuals respond to the varying density (Kempenaers and Dhondt, 1992; Both, 1998a). It is necessary to understand the role of population size in reproduction (Harris, 1984; Saunders et al., 1991) or whether and how population size could mediate density-dependent reproduction.

Nevertheless, the effect of varying population size on population growth has received less attention. This may be because the different notions of population density and size are often highly intercorrelated in most natural populations (Gaston, 1994; Saether et al., 2002; Sibly et al., 2005) and are hard to be differentiated (Kunin, 1997a). In practice, population density is sometimes used in place of population size when population size is low (Saether et al., 2002; Sibly et al., 2005) and the negative effects of population size are often considered as the results of the density-dependent regulatory process mediated by negative reactions between population density and growth (Murdoch, 1994; Turchin, 1999). However, local population density and size may have different effects on population dynamics (reviewed in Kareiva, 1983; Kunin, 1997b). Therefore, ignoring the role of local population size in population regulation may magnify the impact of local population density. Few studies have quantitatively examined how reproduction or population growth is related to the variations of animal population density and size (Saether et al., 2002, 2016), probably because the spacing between individuals constantly shifts as individuals are mobile (Kunin, 1997a). To date, little is known about whether population density and size interact and regulate population dynamics in the same way and whether individuals will adjust their reproductive strategies in response to variations of these two factors.

In this study, I used *Tetranychus ludeni* Zacher (Acari: Tetranychidae) as the model species to investigate how foundress population density and size affected the reproductive performance and population growth by altering the population size of a given population density and *vice versa*. *T. ludeni* is an invasive haplodiploid spider mite native to Europe and now globally distributed (Bolland et al., 1998). It attacks many vegetable crops in the world (Bolland et al., 1998; Zhang, 2003; Adango et al., 2006; Gotoh et al., 2015). The mite has a short life cycle (10 days at 27–28°C) (Moros and Aponte, 1994; da Silva, 2002; Adango et al., 2006), long longevity (up to 28 days) and high reproductive potential (up to 112 eggs) (Adango et al., 2006). Tetranychid females usually mate at emergence and then disperse to establish new colonies with a female-biased sex ratio (Carey and Bradley, 1982; Macke et al., 2011a). These life history characteristics may encourage the outbreaks of mite populations in the fields or greenhouses.

Based on the knowledge outlined above, I designed a factorial experiment by altering the population density for each test population size and *vice versa*, and tested two hypotheses: (1) ovipositing females make earlier reproductive effort at high population densities and sizes, and (2) ovipositing females have higher reproductive output and population growth at low population densities and sizes. I recorded daily oviposition patterns and lifetime fecundity and daughter production at various population densities and sizes and computed life table parameters. Information generated in this study will contribute to knowledge on population dynamics in response to foundress population density and size in general and *T. ludeni* population forecast and management in particular.

2.2 Materials and methods

2.2.1 Mite colony

A breeding colony of *T. ludeni* was raised from adults collected on *Passiflora mollissima* (Kunth) in Palmerston North, New Zealand in September 2017, and reared on 3- to 5-week-old kidney bean plants (*Phaseolus vulgaris* L.). I maintained the colony on the bean plants in an aluminium-frame cage (30 cm length × 30 cm width × 65 cm height) covered with 200-mesh nylon gauze with a zip door (20 cm width × 55 cm height) at 25 ± 1 °C and 40–50% RH with a photoperiod of 16:8 h (light:dark). I carried out the experiment in a walk-in climate control room under the above environmental conditions and used the first expanded leaves of 1- to 2-week-old bean plants for experiments. The mite colony maintenance and environmental conditions described here were used throughout the thesis unless otherwise stated.

2.2.2 Experimental design and data collection

I set up nine treatments of three population sizes (1, 5 and 10 ovipositing females per leaf square) and three foundress population densities (0.7, 1 and 2 ovipositing females/cm^2), with 15–25 replicates for each treatment. To achieve the required population densities for the experiment, I altered the leaf area while keeping one of the three population size levels constant (Figure 2.1). To obtain mated females, I randomly collected the quiescent female deutonymphs from the colony and individually introduced them onto a 1-cm^2 leaf square placed on wet cotton in a Petri dish (9.5 cm diameter and 1.0 cm height) with a mesh-sealed hole (1 cm diameter) in the middle of the lid. Before the quiescent female deutonymph emerged (silvery in colour), I introduced a newly emerged male produced by a virgin female onto the arena and I removed the male immediately after mating terminated. For each replicate, I transferred mated female(s) of a desired density onto a test leaf square in a Petri dish mentioned above, allowed them to lay eggs for 24 hours. I then transferred them to a new leaf square of the same size once every 24 hours until their death. The number of eggs laid on each leaf square was recorded under a stereomicroscope (Leica MZ12, Germany). The larvae and nymphs were allowed to feed on the same leaf square for five days, after which time they were transferred onto another clean fresh leaf square of the same size where they developed to adults. The number of emerged daughters was also recorded.

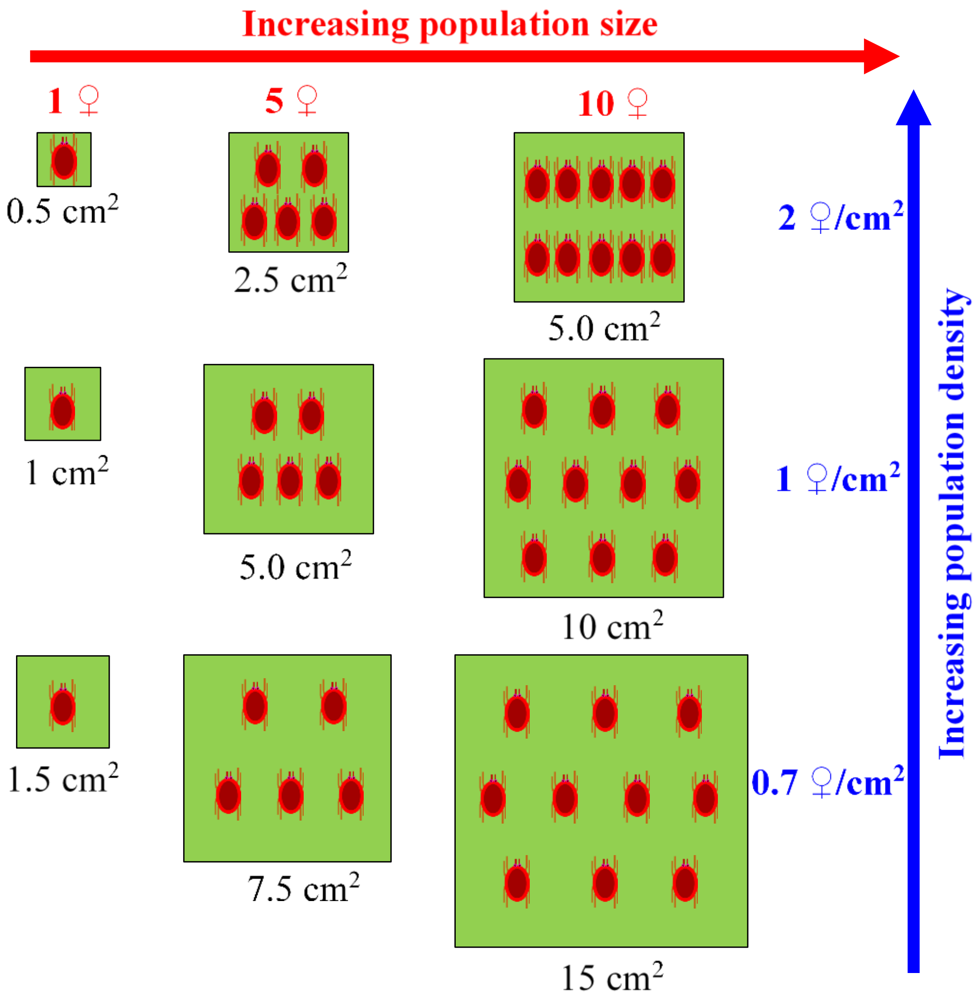


Figure 2.1 A diagram of factorial experimental design showing the population densities and sizes.

The life table parameters **(**Jervis et al., 2005**)** were calculated for each treatment by using the above data on daily survival and reproduction of foundresses. The intrinsic rate of increase (r_m, daughters/foundress/day) was estimated by solving the Lotka-Euler equation, $\sum e^{-r_m x} l_x m_x = 1$, where x is the pivotal age, l_x is the proportion of the foundresses surviving to age x, and m_x is the number of daughters produced per foundress at age x. The pivotal age $x = x_0 + x_i$, where x_i is the age of ovipositing adults and x_0 the developmental time from egg to adulthood ranging from 10.3 to 11.5 days. Other life table parameters included the net reproductive rate ($R_0 = \sum l_x m_x$, daughters/foundress/generation), mean generation time [$T = \log_e(R_0)/r_m$, days], and doubling time [$Dt = \log_e(2)/r_m$, days]. For each treatment, a jackknife method (Caswell, 2001) was used to estimate the life table parameters for each foundress and their means (± SE) for each treatment were calculated (Table S2.1).

2.2.3 Statistical analysis

All data were analysed using SAS software (SAS 9.4, SAS Institute Inc., Cary, NC). Rejection level of H_0 was set at $P < 0.05$. For each treatment, an asymptotic exponential model (Archontoulis and Miguez, 2015) was applied to fit the daily cumulative number of eggs and daughters produced (y) (Figures 2.2 and 2.3):

$y = a/\{1+\exp[-b(x-x_0)]\}$, eq. 1

where a is the maximum y value, x_0 is the inflection point at which the instantaneous growth rate is maximized, b is the constant growth rate of the curve. I developed a nonlinear regression model to fit data of daily number of eggs and daughters produced (y) (Figures 2.2 and 2.3):

$y = a/\{1+[(x-x_0)/b]^2\}$, eq. 2

where a is the maximum y at age x_0, b is a constant rate controlling the width of the peak. For each model, the estimated parameters were compared between treatments according to the 95% confidence limits (CL), i.e., if the 95% CL overlapped, then there was no significant difference between treatments (Tables S2.2 and S2.3). NLIN Procedure was used for modelling.

The life table parameters (y) were analysed by a generalized linear mixed model (GLIMMIX Procedure), with foundress population density and size and their interaction as fixed factors and replicate as a random factor (Figure S2.4):

$y = \exp(a + b\,\mathrm{PD} + c\,\mathrm{PS} + d\,\mathrm{PD}\times\mathrm{PS})$, eq. 3

where a is the intercept, and b, c and d are the estimated constants of population density (PD) and size (PS) and their interaction (PD×PS), respectively. To minimise the potential effect of decreasing population density and size with the progress of experiment, data recorded within 15 days of foundress lifespan were used for analyses in this study because > 85% of lifetime eggs were laid during this period.

2.3 Results

I detected a negative density-dependent production of eggs and daughters (a in eq. 1) at higher foundress population sizes of 5 and 10 but not at the low population size of one foundress (Figures 2.2 and 2.3, Table S2.2). However, the number of eggs and daughters accumulated significantly faster at high population sizes of 5 and 10 than at the population size of only one foundress (b in eq. 1), except at the highest population density and size (Figures 2.2 and 2.3, Table S2.2). The daily number of eggs and daughters produced peaked significantly earlier (< 6 days) at high population sizes of 5 and 10 than that (7.4–8.5 days) at the population size of only one foundress regardless of local population densities (x_0 in eq. 2), with a greater (*a* in eq. 2) and sharper peak (*b* in eq. 2) except at the highest population density and size (Figures 2.2 and 2.3, Table S2.3).

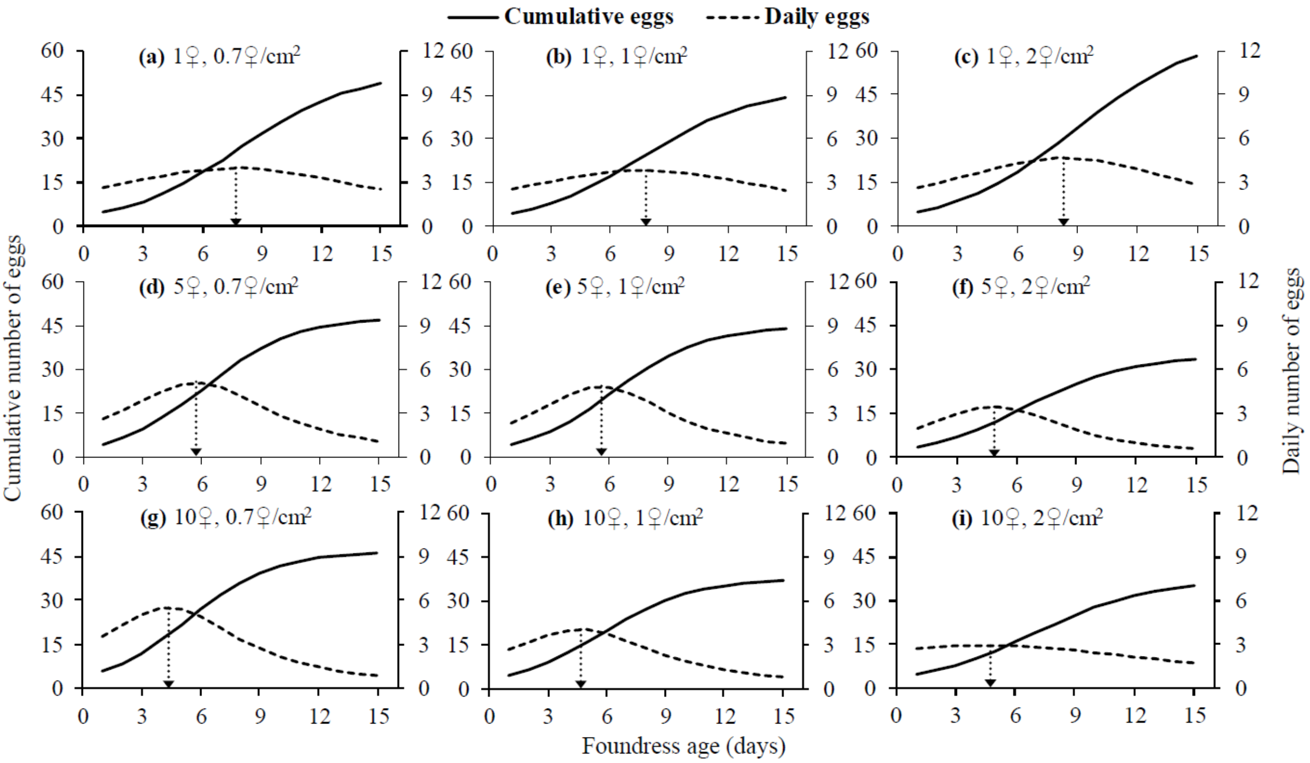


Figure 2.2 Cumulative and daily number of eggs laid by a foundress at different population densities (0.7, 1 and 2 ♀/cm^2) and sizes (1, 5 and 10 ♀). Cumulative number of eggs: $y = a/\{1+\exp[-b(x-x_0)]\}$; daily number of eggs: $y = a/\{1+[(x-x_0)/b]^2\}$. The arrows indicate the timing of peaks of eggs laid. The estimated parameters of models are listed in Tables S2.2 and S2.3.

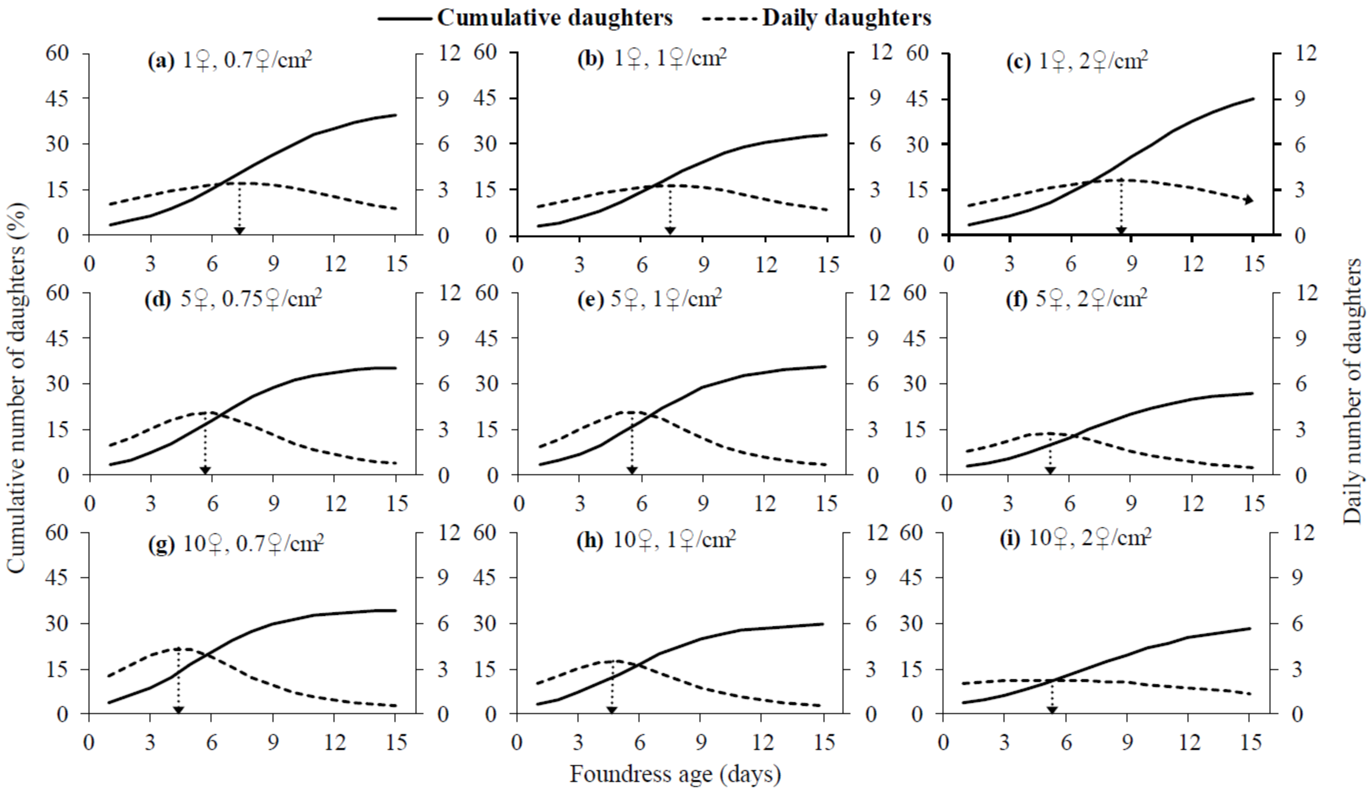


Figure 2.3 Cumulative and daily number of daughters produced by a foundress at different population densities (0.7, 1 and 2 ♀/cm^2) and sizes (1, 5 and 10 ♀). Cumulative number of daughters: $y = a/\{1+\exp[-b(x-x_0)]\}$; daily number of daughters: $y = a/\{1+[(x-x_0)/b]^2\}$. The arrows indicate the timing of peaks of daughters produced. The estimated parameters of models are listed in Tables S2.2 and S2.3.

Population density and size had no significant independent effect on the net reproductive rate (R_0), but their significant negative interaction resulted in a decrease of R_0 when both population density and size increased (Figure 2.4a, Table S2.4). Increase in population density caused a significant decrease of intrinsic rate of increase (r_m) whereas increasing population size incurred a significant increase of r_m (Figure 2.4b, Table S2.4). In contrast, increasing population density significantly prolonged the mean generation time (T) and doubling time (Dt) whereas increasing population size shortened these two parameters (Figure 2.4c–d, Table S2.4).

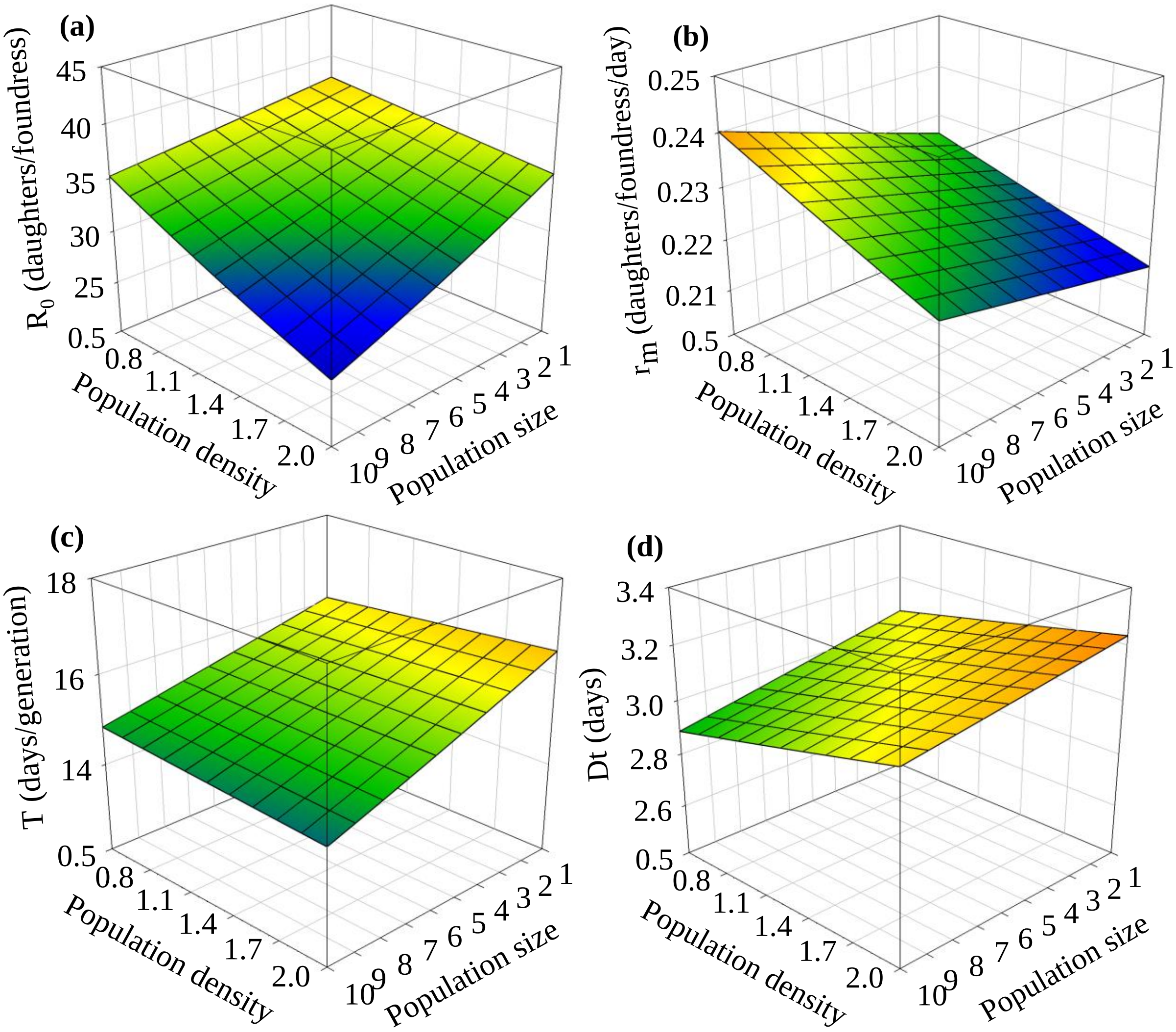


Figure 2.4 Effects of foundress population density (number of foundresses/cm^2) and population size (number of foundresses per leaf square) on life table parameters: (**a**) net reproductive rate (R_0), (**b**) intrinsic rate of increase (r_m), (**c**) generation time (T), and (**d**) doubling time (Dt). The estimated parameters of models are listed in Table S2.4.

2.4 Discussion

In the present study, *T. ludeni* exhibited negative impacts of foundress population density and size on reproduction; however, the negative density-dependent reproduction was detected at higher population sizes of 5 and 10 foundresses but not at the low population size of one foundress, and foundresses at higher population sizes maximised their reproduction significantly faster and earlier during their lifespan, regardless of their local densities (Figures 2.2, 2.3 and 2.4a, Tables S2.2 and S2.3). The results have three implications. First, spider mites feed upon plant leaves by piercing cells and sucking cell contents, which will induce white or yellow spots or ‘stipplings’ and reduce the photosynthetic surface (Dhooria, 2016). The overexploitation associated with quick cumulative excrement on host plants at high population densities may reduce the food availability and quality and thus offspring fitness. Furthermore, spider mites show scramble resource competition with a fast resource depletion when population density or size is high (Krips et al., 1998). Therefore, a decrease of reproduction under the elevated resource competition conditions can be considered as an adaptive strategy of ovipositing foundresses (De Roissart et al., 2016) to reduce the food resource competition of offspring with their mothers or among the offspring (Bonduriansky and Head, 2007).

Second, the life history theory assumes that natural selection on organisms enables them to evolve optimal strategies, including the timing of reproduction and number of offspring produced, to maximize or optimize the reproductive fitness by allocating resources to a range of different demands throughout the lifespan (Stearns, 1992; Roff, 2002). A previous study reports that virgin females adjusted their resource allocations to yield larger sons that could produce more daughters at a higher rate, suggesting that *T. ludeni* has evolved the resource allocation strategy to compensate the loss of producing daughters for females at virgin stage through their sons’ production of more daughters in the next generation (Zhou et al., 2018). My results provide further evidence that foundresses maximising fecundity and producing more daughters during the early lifespan at higher population densities and sizes (Figures 2.2 and 2.3, Tables S2.2 and S2.3) could be a fitness advantage, as production of more daughters early enables more dispersing individuals to complete development, reducing future food competition intensity and elevating population growth (Osakabe et al., 2008; Yano, 2008; Le Goff et al., 2010; Azandémè-Hounmalon et al., 2014).

Third, population density and size are usually intercorrelated in a population (Gaston, 1994; Saether et al., 2002; Sibly et al., 2005) but are hard to be differentiated (Kunin, 1997a). Previous studies have demonstrated the individual adjustment of reproductive behaviours in response to the varying density within a habitat (Lack, 1954; Both, 1998a; Fernandez et al., 1998) rather than to the varying population size. In the present study, I differentiated the functions of population density and size and revealed that foundress population density and size interacted and affected the population dynamics in the same way (Figure 2.4a, Table S2.4); however, the negative density-dependent reproduction and early onset of reproductive peak occurred only when the population size was high. The results suggest that: (1) the individual adjustment of reproductive behaviours results from the increasing intensity of conspecific competition for resources (Krebs, 1971; Rodenhouse et al., 1997; Leips and Travis, 1999; Creighton, 2005; Goubault et al., 2007) and direct interference among individuals (Post et al., 1997) at higher population sizes, and (2) the population size regulates population-density dependency and individual adjustment. Therefore, population size may have overweighed population density as a major socio-environmental factor regulating future population dynamics in *T. ludeni*.

The life table of a species summarises the life history characteristics such as survival, development, time of reproduction, and fecundity or female offspring production of individuals in a population (Jervis et al., 2005), reflecting the potential or capacity of population growth. Previous studies reveal that population density is a decreasing function of per capita growth (Harrison and Cappuccino, 1995; Turchin, 1995; Rotem and Agrawal, 2003). I found that the simultaneous increase of population density and size reduced the net reproductive rate (R_0) (Figure 2.4a). According to the mathematical equations for life table parameter calculation (Jervis et al., 2005), decreasing net reproductive rate (R_0) will result in a smaller intrinsic rate of natural increase (r_m) and longer doubling time (Dt). However, my results only partially agreed with the notion because r_m was higher and Dt was shorter at lower population densities and higher population sizes (Figure 2.4b and 2.4d). These may be attributed to the faster rates of cumulative egg and daughter production and early onset of reproductive peak at higher population sizes (Figures 2 and 3, Tables S2.2 and S2.3). The higher r_m reduced the Dt, and higher r_m with relatively small R_0 shortened the mean generation time (T) (Figure 2.3c; Jervis et al., 2005).

In conclusion, results of this study demonstrate that: (1) both negative population density- and size-dependence may limit *T. ludeni* reproduction, but the effect of population size overweighed that of population density, and (2) ovipositing females are capable to accelerate population growth at higher population sizes through producing more daughters early during their lifespan. My findings that the population grows faster at low population densities and high population sizes could be used to forecast the future population size and decide the timing of pest control.

Table S2.1 The estimated mean (± SE) lifetable parameters at different foundress population densities and sizes.

Parameter	**Population density (♀/cm^2)**	**Population size (♀)**		
		1	**5**	**10**
R_0	0.7	45.16 ± 0.13	37.73 ± 0.08	36.99 ± 0.05
	1	29.36 ± 0.18	35.94 ± 0.10	31.14 ± 0.11
	2	42.12 ± 0.15	24.66 ± 0.09	33.74 ± 0.06
r_m	0.7	0.2274 ± 0.0008	0.2309 ± 0.0000	0.2408 ± 0.0000
	1	0.2091 ± 0.0007	0.2305 ± 0.0000	0.2303 ± 0.0000
	2	0.2208 ± 0.0000	0.2085 ± 0.0007	0.2210 ± 0.0000
T	0.7	16.76 ± 0.05	15.72 ± 0.01	15.00 ± 0.01
	1	16.16 ± 0.04	15.54 ± 0.01	14.93 ± 0.01
	2	16.94 ± 0.01	15.38 ± 0.04	15.92 ± 0.01
D_t	0.7	3.0485 ± 0.0110	3.0014 ± 0.0003	2.8788 ± 0.0002
	1	3.3149 ± 0.0108	3.0067 ± 0.0003	3.0099 ± 0.0005
	2	3.1394 ± 0.0004	3.3260 ± 0.0117	3.1365 ± 0.0003

Table S2.2 Statistical results of modelling of cumulative eggs and daughters produced over foundress' age at different population densities and sizes.

Size (n)	**Density (♀/cm^2)**	***a***	***b***	x_0	**R^2**	**df**	**F**	**P**
Cumulative eggs (Figure 2.2)								
1	0.7	52.74 b	0.35 b	7.81 ab	0.9064	3,347	1120.36	< 0.0001
	1	47.93 bc	0.34 b	7.75 ab	0.8436	3,179	321.73	< 0.0001
	2	65.79 a	0.33 b	8.83 a	0.9557	3,332	238.90	< 0.0001
5	0.7	47.73 bc	0.44 a	6.15 c	0.9513	3,369	2401.88	< 0.0001
	1	44.79 c	0.44 a	6.25 c	0.9477	3,345	2085.73	< 0.0001
	2	34.44 d	0.40 a	6.49 bc	0.8754	3,328	768.20	< 0.0001
10	0.7	46.59 bc	0.46 a	5.34 d	0.9784	3,342	5155.51	< 0.0001
	1	37.65 cd	0.42 a	5.71 cd	0.9303	3,294	1307.84	< 0.0001
	2	37.31 cd	0.34 b	6.93 bc	0.9756	3,311	4143.32	< 0.0001
Cumulative daughters (Figure 2.3)								
1	0.7	42.08 ab	0.38 ab	7.57 ab	0.8897	3,347	933.17	< 0.0001
	1	34.05 cd	0.42 ab	6.79 bc	0.8155	3,179	263.94	< 0.0001
	2	51.02 a	0.33 b	8.90 a	0.9456	3,331	1719.76	< 0.0001
5	0.7	35.93 c	0.47 a	5.99 cd	0.9366	3,369	1817.54	< 0.0001
	1	36.14 bc	0.46 a	6.13 c	0.9355	3,345	1658.99	< 0.0001
	2	27.76 d	0.40 ab	6.61 bc	0.8683	3,328	921.03	< 0.0001
10	0.7	34.61 c	0.48 a	5.24 d	0.9759	3,342	4608.25	< 0.0001
	1	29.98 d	0.45 a	5.55 cd	0.9232	3,294	1178.58	< 0.0001
	2	30.57 d	0.32 b	7.18 bc	0.9690	3,311	3251.40	< 0.0001

For each category, the increase rates of cumulation (a) with the same letter are not significantly difference ($P < 0.05$). Cumulative number: $y = a/\{1+\exp[-b(x-x_0)]\}$, where a is the maximum y value, x_0 is the inflection point at which the instantaneous growth rate is maximum, b is the constant growth rate.

Table S2.3 Statistical results of modelling of daily number of eggs and daughters produced over foundress' age at different population densities and sizes.

Size (n)	**Density (♀/cm^2)**	a	x_0	b	R^2	**df**	**F**	**P**
Daily egg (Figure 2.2)								
1 ♀	0.7	3.96 cd	7.67 a	9.44 a	0.6614	3,356	231.82	< 0.0001
	1	3.80 cd	7.85 a	9.65 a	0.6297	3,187	106.00	< 0.0001
	2	4.64 bc	8.37 a	8.31 a	0.7588	3,314	357.53	< 0.0001
5 ♀	0.7	5.11 ab	5.74 b	4.78 b	0.8699	3,369	822.27	< 0.0001
	1	4.83 b	5.60 b	4.44 b	0.8448	3,352	638.91	< 0.0001
	2	3.44 de	4.91 c	4.48 b	0.7156	3,343	287.62	< 0.0001
10 ♀	0.7	5.50 a	4.38 c	4.51 b	0.8890	3,342	912.72	< 0.0001
	1	4.05 cd	4.64 c	5.03 b	0.8327	3,297	492.83	< 0.0001
	2	2.92 e	4.75 c	12.02 a	0.8117	3,312	448.33	< 0.0001
Daily daughters (Figure 2.3)								
1 ♀	0.7	3.45 c	7.36 a	7.69 a	0.6379	3,356	209.03	< 0.0001
	1	3.27 cd	7.41 a	7.58 a	0.6025	3,187	94.47	< 0.0001
	2	3.67 bc	8.49 a	8.14 a	0.7448	3,314	337.75	< 0.0001
5 ♀	0.7	4.08 ab	5.66 b	4.38 b	0.8422	3,369	656.44	< 0.0001
	1	4.14 ab	5.53 b	4.06 b	0.8229	3,352	545.13	< 0.0001
	2	2.71 d	5.09 bc	4.61 b	0.7144	3,343	286.01	< 0.0001
10 ♀	0.7	4.37 a	4.42 c	4.00 b	0.8796	3,342	833.04	< 0.0001
	1	3.49 c	4.67 c	4.39 b	0.8054	3,297	409.81	< 0.0001
	2	2.27 e	5.36 bc	12.06 a	0.8204	3,312	475.20	< 0.0001

For each category, the estimated timings of oviposition peak (x_0) with the same letter are not significantly difference ($P < 0.05$). Daily number of daughters: $y = a/\{1+[(x–x_0)/b]^2\}$, where a is the maximum y at age x_0, b is a constant rate (slope) controlling the steepness of the peak.

Table S2.4 Statistical results of modelling of life table parameters depending on foundress population density and size.

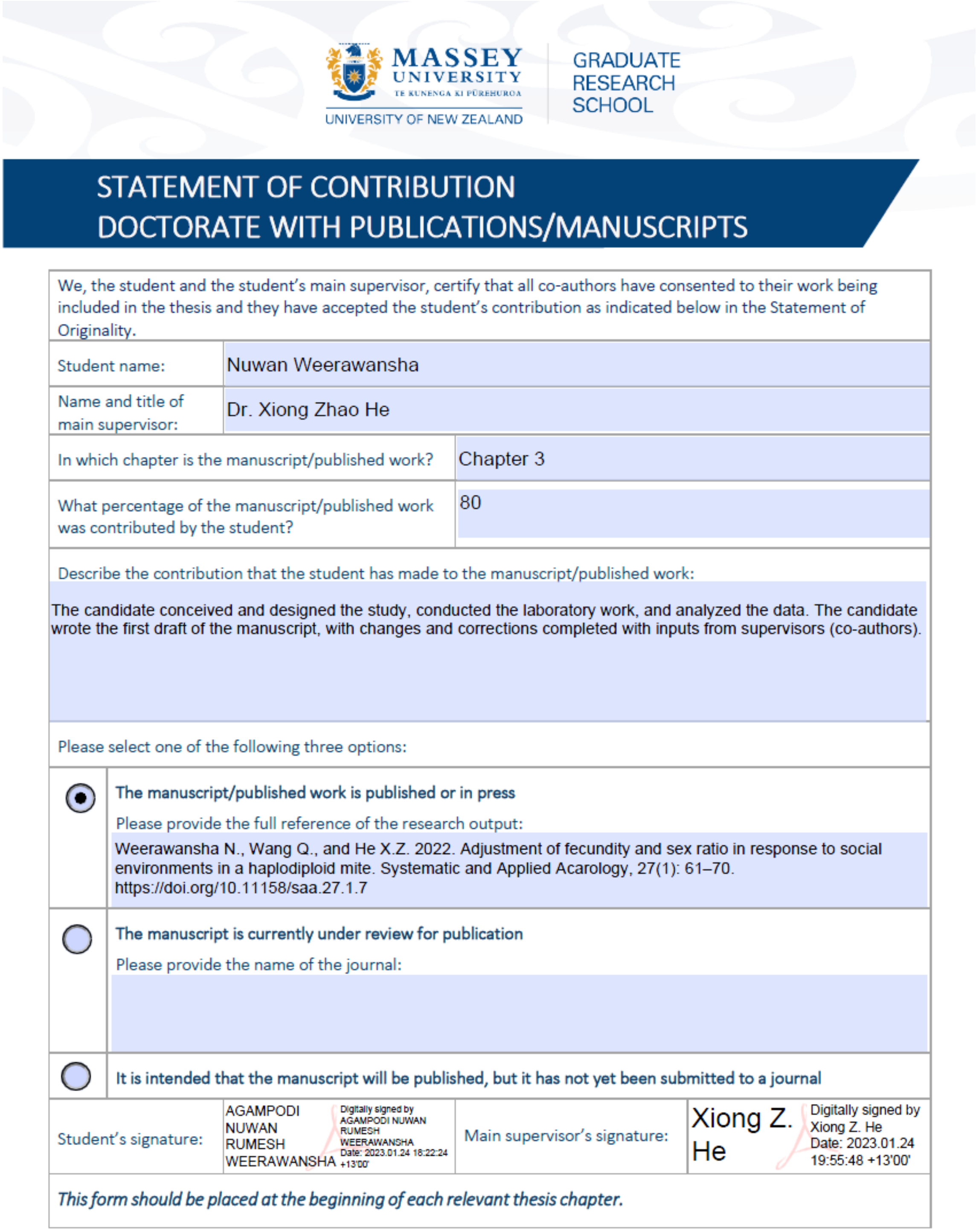
Parameter	Variable	Estimate	SE	$F_{(1,174)}$	P
R_0	Intercept	3.6552	0.0341		
	Density	– 0.0273	0.0249	1.20	0.2743
	Size	0.0005	0.0053	0.01	0.9202
	Density×Size	– 0.0172	0.0039	19.18	< 0.0001
r_m	Intercept	– 1.4763	0.0075		
	Density	– 0.0333	0.0055	37.01	< 0.0001
	Size	0.0074	0.0012	39.47	< 0.0001
	Density×Size	– 0.0014	0.0009	2.70	0.1021
T	Intercept	2.7764	0.0050		
	Density	0.0202	0.0037	30.71	< 0.0001
	Size	– 0.0072	0.0008	84.86	< 0.0001
	Density×Size	– 0.0034	0.0006	34.52	< 0.0001
D_t	Intercept	1.1092	0.0077		
	Density	0.0343	0.0056	37.19	< 0.0001
	Size	– 0.0074	0.0012	37.46	< 0.0001
	Density×Size	0.0014	0.0009	2.59	0.1093

Parameter = exp ($a + b$ PD + c PS + d PD×PS), a is the intercept, and b, c and d are the estimated constants of population density (PD) and size (PS) and their interaction (PD×PS), respectively.

Chapter 3

Adjustment of fecundity and sex ratio in response to social environments in a haplodiploid mite

This chapter was published in *Systematic and Applied Acarology* 27(1): 61–70 (2022).



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Name and title of main supervisor:	Dr. Xiong Zhao He
In which chapter is the manuscript/published work?	Chapter 3
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July 2022

Abstract

Animals can adjust reproductive strategies in favour of corporation or competition in response to local population size and density, the two key factors of social environments. However, previous studies usually focus on either population size or density but ignore their interactions. Using a haplodiploid spider mite, *Tetranychus ludeni Zacher*, I carried out a factorial experiment in the laboratory to examine how ovipositing females adjust their fecundity and offspring sex ratio during their early reproductive life under various population size and density. I reveal that females laid significantly more eggs with increasing population size and significantly fewer eggs with increasing population density. This suggests that large populations favour cooperation between individuals and dense populations increase competition. I demonstrate a significant negative interaction of population size and density that resulted in significantly fewer eggs laid in the large and dense populations. Furthermore, I show that females significantly skewed the offspring sex ratio towards female-biased in small populations to reduce the local mate competition among their sons. However, population density incurred no significant impact on offspring sex ratio, while the significant positive interaction of population size and density significantly increased the proportion of female offspring in the large and dense populations, which will minimise food or space competition as females usually disperse after mating at crowded conditions. These results also suggest that population density affecting sex allocation in *T. ludeni* is intercorrelated with population size. This study provides evidence that animals can manipulate their reproductive output and adjust offspring sex ratio in response to various social environments, and the interactions of different socio-environmental factors may play significant roles.

Key words: population size, population density, interaction, spider mite, *Tetranychus ludeni*, reproduction, sex allocation

3.1 Introduction

Animals in patchy habitats often face intraspecific competitions (Iritani et al., 2020; Ausband and Mitchell, 2021; Li and Zhang, 2021) and adjust their reproductive strategies in response to social environments (Lihoreau and Rivault, 2008; Ruan and Wu, 2008; Krams et al., 2009; Schausberger et al., 2017; Conroy and Roff, 2018; Crocker and Hunter, 2018; Li and Zhang, 2021). The number of individuals in a local population (population size) and the number of individuals per unit area in a habitat (population density) are two key socio-environmental forces that drive population regulations (Estevez et al., 2007; West, 2009; Sprenger et al., 2011; Weerawansha et al., 2020; Ausband and Mitchell, 2021). Fluctuations in population size and density alter the resources available for local individuals so that they must change their fecundity and offspring sex ratio accordingly to adapt (Wauters and Lens, 1995; Estevez et al., 2007; Webber and Wal, 2018; Burant et al., 2020; Weerawansha et al., 2020). However, in the field populations, the effect of population density is difficult to be disentangled from that of population size and *vice versa* due to their strong inter-relationship (Amsalem and Hefetz, 2011). Few studies have distinguished the distinct effects of animal population density and size on reproduction and determined whether population density and size interact and how their interactions will alter an animal's reproductive strategies.

Larger population size and higher density often result in lower reproductive performance because of the intense competition for resources and increasing behavioural interference (Estevez et al., 2007; Wong et al., 2013; Li and Zhang, 2021). For example, the spider mite *Tetranychus urticae* Koch has lower fecundity in a larger group with a higher density (Li and Zhang, 2021). However, when the population density remains constant, females of the same species in larger groups lay more eggs than those in the smaller ones (Le Goff et al., 2010). Similar findings are also reported in many other group-living species (Avilés and Turino, 1998; (Prokopy and Reynolds, 1998; Estevez et al., 2007; Salomon and Lubin, 2007; Lihoreau and Rivault, 2008; Krams et al., 2009). This phenomenon may be attributed to the fact that group living helps access to food and mates, protect against predators, and increase available time for forage and feeding efficiency (Ranta et al., 1993; Dyer, 2000; Mori and Saito, 2006; Sato and Saito, 2006; Estevez et al., 2007; Vanthournout et al., 2016). To date, how female animals adjust their fecundity in response to variations of both population size and density is still not clear.

Mothers can also alter their offspring sex ratio in response to social environments (Hamilton, 1967; Trivers and Willard, 1973; Taylor and Bulmer, 1980; Charnov, 1982; West et al., 2005; West, 2009; Iritani et al., 2020). For example, when the population density is high where food resources are limited, mothers should skew the offspring sex ratio towards the dispersing sex to reduce resource competition among siblings or close relatives (Clark, 1978; Silk, 1983; Mari et al., 2008; Hjernquist et al., 2009; Visser et al., 2014; Song et al., 2016). However, when food resources are sufficient in a small population, mothers should favor a female-biased offspring sex ratio to reduce the competition for mates between sons (Hamilton, 1967; Sato and Saito, 2006; Macke et al., 2012a, 2014). So far, empirical studies on sex allocation have focused on offspring sex ratio regulation in response to either the population size or density (Charnov, 1982; West et al., 2005; West, 2009), ignoring the impact of potential interactions and relative importance of the population size and density.

Spider mites (Acari: Tetranychidae) are a group of haplodiploid invertebrates where mated females produce both male and female offspring and virgin ones give birth to male offspring only (Oku, 2010; Zhou et al., 2018). They live in groups of patchy habitats and may have developed strategies to maximize their fitness under different population size and density. In the present study, I used the spider mite *Tetranychus ludeni* Zacher as a model species to investigate how ovipositing females adjust their fecundity and sex ratio in response to the variation of social environments. To distinguish the different effects of population density and size and their interaction, I carried out a series of factorial experiments by allowing both population size and density to vary and recorded the number of eggs laid and proportion of female offspring. Information generated from this study provides insights into the mechanisms behind adjustment of fecundity and sex ratio in response to the various social environments.

3.2 Materials and methods

3.2.1 Mite colony

A breeding colony of *T. ludeni* was raised from adults collected on *Passiflora mollissima* (Kunth) in Palmerston North, New Zealand in September 2017, and reared on 3- to 5-week-old kidney bean plants (*Phaseolus vulgaris* L.). I maintained the colony on the bean plants in an aluminium-frame cage (30 cm length × 30 cm width × 65 cm height) covered with 200-mesh nylon gauze with a zip door (20 cm width × 55 cm height) at 25 ± 1°C and 40–50% RH with a photoperiod of 16:8 h (light:dark). I carried out the experiment in a walk-in climate control room under the above environmental conditions and used the first expanded leaves of 1- to 2-week-old bean plants for experiments.

3.2.2 Experimental design and data collection

To test how population size and density affected the reproduction of *T. ludeni*, I conducted a factorial experiment by allowing both population size and density to vary at three levels, with nine treatments: three population sizes (1, 5 and 10 mated females) × three population densities

(0.7, 1 and 2 mated females/cm^2), with 15–25 replicates for each treatment (Table 3.1). To obtain mated females, I randomly collected the quiescent female deutonymphs from the colony and individually introduced them onto a 1-cm^2 leaf square placed upside down on a wet cotton pad in a Petri dish (9.5 cm diameter × 1.0 cm height) with a mesh-sealed hole (1 cm diameter) in the middle of the lid. Before the female emerged (silvery in colour), I introduced a newly emerged male adult produced by a virgin female onto the arena. I removed the male immediately after copulation ended.

Table 3.1 A factorial design showing the leaf area, and population size and density for the experiment.

Leaf area (cm^2)	Population size (♀)	Population density (♀/cm^2)	Replicate (n)
1.5	1	0.7	25
1	1	1	19
0.5	1	2	24
7.5	5	0.7	25
5	5	1	22
2.5	5	2	16
15	10	0.7	18
10	10	1	17
5	10	2	21

For each replicate, I transferred mated female(s) (< 1 hour old) of a desired population size and density onto a leaf square of desired size (Table 3.1) in a Petri dish mentioned above and allowed them to lay eggs for 24 hours. I then transferred the mate females to a new leaf square of the same size once every 24 hours and the process was repeated until they died. I recorded the number of eggs laid on each leaf square. I allowed the larvae hatching from the resulting eggs to live on the same leaf square for five days and subsequently transferred them onto another fresh leaf square of the same size where they developed to adulthood. The sex of the resulting offspring was then recorded once the adults emerged. The mites start laying eggs about a day after mating (unpublished data). I only used data recorded during the first five days of oviposition period for analysis because of the death of many females beyond day 5 of oviposition, which would change the social environment.

3.2.3 Statistical analysis

I analysed the data using SAS software (SAS 9.4, SAS Institute Inc., Cary, NC). Rejection level of H_0 was set at $P < 0.05$. Data on the number of eggs laid and proportion of female offspring were not normally distributed (Shapiro-Wilk test, UNIVARIATE procedure), I thus analysed these data using a generalized linear mixed model (GLIMMIX Procedure), with population size (PS) and density (PD) and their interactions (PS×PD) as the fixed factors and female age as a random factor. I ran a two-factor model to test the effects of PS and PD first, and then a three-factor model to test whether the PS×PD changed the effects of PS and PD.

3.3 Results

3.3.1 Effect of social environments on fecundity

When I tested the effect of population size (PS) and density (PD) simultaneously, I found that the females laid significantly more eggs with increasing PS ($F_{1,843}$= 21.20, $P < 0.0001$) and significantly fewer eggs with increasing PD ($F_{1,843}$= 17.19, $P < 0.0001$) (Figure 3.1a). My analysis of the impact of PS×PD interactions on fecundity shows that when PS and PD were low, mothers laid significantly more eggs with the increase of both PS and PD ($F_{1,842}$= 59.86, $P < 0.0001$ for PS; $F_{1,842}$ = 7.09, $P = 0.0079$ for PD), but when PS and PD were high, females produced significantly fewer eggs with the increase of both PS and PD ($F_{1,842}$ = 40.8, $P < 0.0001$) (Figure 3.1b).

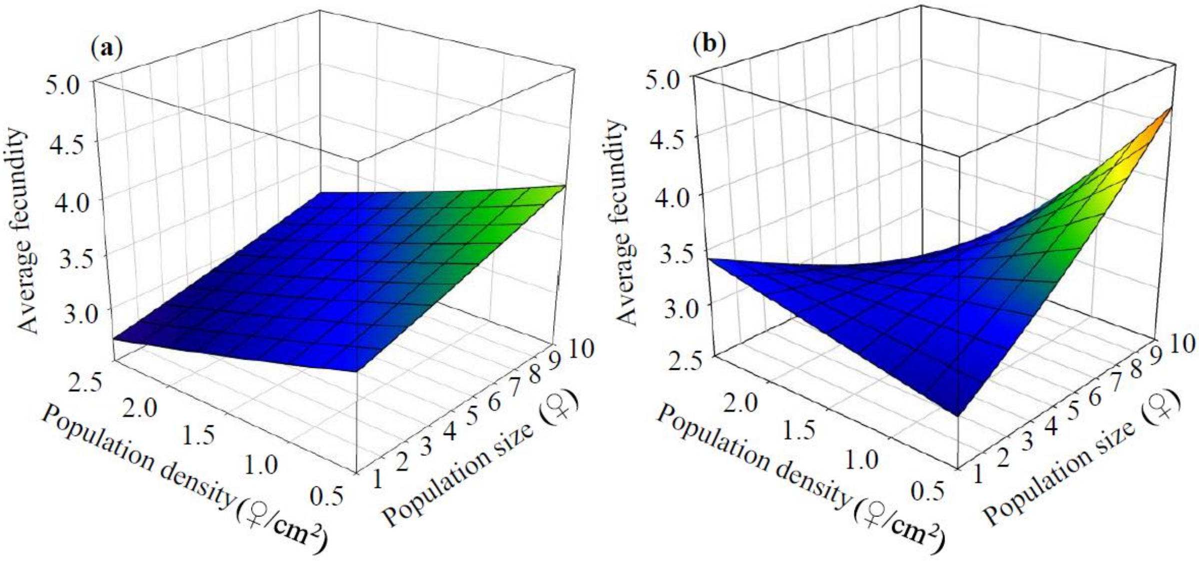


Figure 3.1 Effect of population size (PS, number of females per leaf square), density (PD, number of females/cm^2) and their interactions (PS×PD) on average fecundity of the first five days: (**a**) two-factor model, eggs = exp (1.2514 + 0.0189 PS - 0.1092 PD), and (**b**) three-factor model, eggs = exp (0.9737 + 0.0726 PS + 0.1181 PD - 0.0445 PS×PD).

3.3.2 Effect of social environments on sex allocation

Our results indicate that when only PS and PD were included in the two-factor model, increasing PS significantly reduced the proportion of female offspring produced ($F_{1,804}$= 14.40, P = 0.0002) but PD incurred no significant impact on sex allocation ($F_{1,804}$ = 1.94, P = 0.1637) (Figure 3.2a). In the three-factor model, I found that PS×PD interaction significantly increased the proportion of female offspring at higher PS and PD ($F_{1,803}$ = 10.61, P = 0.0012) but did not significantly alter the effects of PS and PD on sex allocation ($F_{1,803}$ = 20.80, $P < 0.0001$ for PS; $F_{1,803}$ = 3.57, P = 0.0592 for PD) (Figure 3.2b).

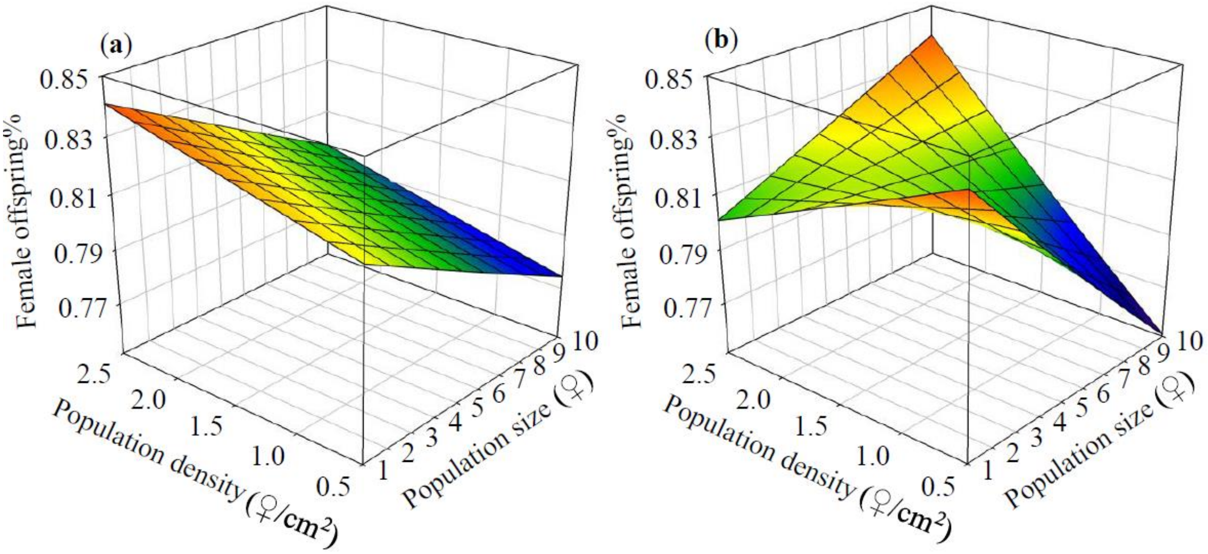


Figure 3.2 Effect of population size (PS, number of females per leaf square), density (PD, number of females/cm^2) and their interactions (PS×PD) on average sex ratio of the first five days: (**a**) two-factor model, female offspring% = exp (– 0.2021 – 0.0061 PS + 0.0140 PD), and (**b**) three-factor model, female offspring% = exp (– 0.1449 – 0.0168 PS – 0.0331 PD + 0.0088 PS×PD).

3.4 Discussion

Animals are believed to have evolved strategies to maximise their reproductive fitness in varied social environments (Estevez et al., 2007; Iritani et al., 2020; Weerawansha et al., 2020; Ausband and Mitchell, 2021; Li and Zhang, 2021). Similar to other species (e.g., Wardhaugh and Didham, 2005; Khan et al., 2018; Li and Zhang, 2021), *T. ludeni* females reduced their fecundity with the increase of population density (Figure 3.1a) because individuals at crowded conditions usually have a higher level of behavioural interferences or aggressions (Estevez et al., 2007; Wong et al., 2013; Li and Zhang, 2021) and intense food competition (Wertheim et al., 2005; Burant et al., 2020). Spider mite populations can grow rapidly due to short lifecycles and high fecundity (Shih et al., 1976; Tuan et al., 2016) but their exponential population growth can end abruptly due to overexploitation of the host plants (Krips et al., 1998). Therefore, fecundity reduction at dense environments may be a flexible strategy of *T. ludeni* females to reduce the intensity of food competition and minimise the possibility of local population extinction.

However, when population size increased, the fecundity significantly increased in *T. ludeni* (Figure 3.1a). This may be attributed to cooperative nature of spider mites, i.e., aggregating and constructing common silk webs under the leaves, which protect them and their offspring from environmental hazards (Davis, 1952; McMurtry et al., 1970; Ashley, 2003; Mori and Saito, 2005; Le Goff et al., 2010). Therefore, group living may increase access to food and time for forage and feeding (Ranta et al., 1993; Dyer, 2000; Mori and Saito, 2006; Sato and Saito, 2006; Estevez et al., 2007; Vanthournout et al., 2016), and in turn promote reproduction. Similarly, many studies also report that the presence of conspecific eggs may stimulate female reproduction, for example, in the tobacco budworm *Helicoverpa virescens* (F.) (Navasero and Ramaswamy, 1993), cactus bug *Chelinidea vittiger aequoris* McAtee (Fletcher and Miller, 2008), and white butterfly *Pieris napi* L. (Raitanen et al., 2014).

In the present study, I found a significant interaction between population density and size, which altered the reproductive behaviour of *T. ludeni* (Figure 3.1b). First, fecundity significantly increased with the increase of population size when the population density was low, or with the increase of population density when the population size was small. Second, fecundity significantly decreased in large and dense populations. These results have two implications: (1) cooperation enhances spider mite reproduction when the population size is small and the population density is low, where the benefits of cooperation exceed the costs of food competition among the ovipositing females; and (2) the costs of food competition outweigh the benefits of cooperation if the populations are large and dense. These findings suggest that group-living females of *T. ludeni* can determine whether they corporate in reproduction or compete for resources under different social environments.

Sex allocation is one of the main life history traits that manipulate resource competition in a local population (Hamilton, 1967; Clark, 1978; West et al., 2005; West, 2009). I demonstrated that *T. ludeni* mothers favoured a female-biased offspring sex ratio in a small population (Figure 3.2a) or in a small population with low density (Figure 3.2b). These results agree to the theorical prediction of local mate competition (Hamilton, 1967) and empirical studies on spider mites (Sato and Saito, 2006; Macke et al., 2012a, 2014), i.e., when mothers oviposit in a small group where their sons compete for mates, they will produce more daughters, reducing the local mate competition.

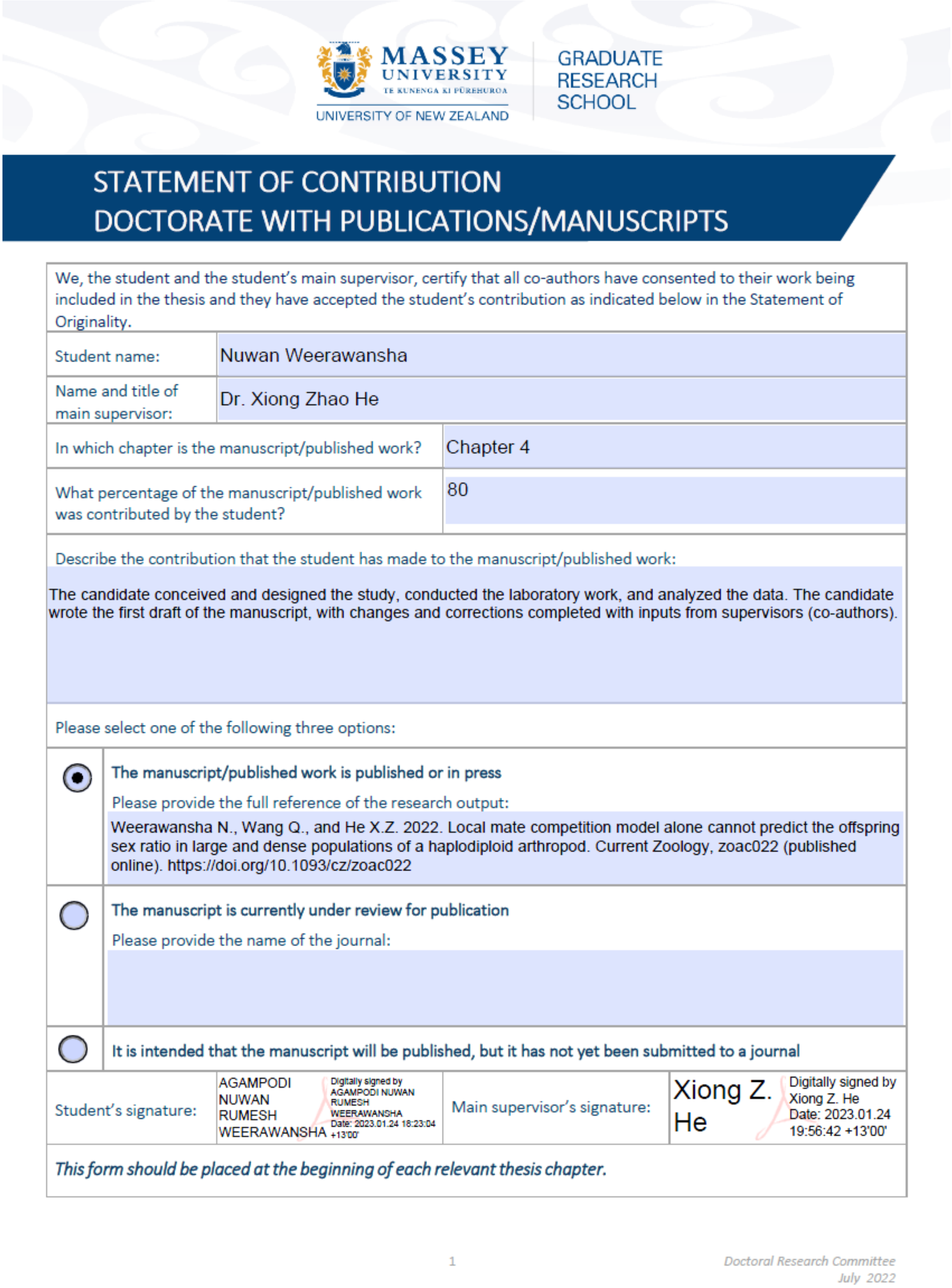
However, the population density did not significantly affect offspring sex ratio regardless of whether its interaction with population size was accounted for analysis or not, while the significant positive effect of interaction resulted in an increase of female-biased sex ratio in large and dense populations (Figure 3.2). I suggest that the effect of population density on sex allocation in *T. ludeni* is intercorrelated with that of population size. In a large and dense population, *T. ludeni* females produce more daughters that will disperse from the dense environments to form new colonies, reducing the competition for food or space (Clark, 1978; Silk, 1983; Mari et al., 2008; Hjernquist et al., 2009; Visser et al., 2014; Song et al., 2016). In addition, the mechanism of sex allocation adjustment in *T. ludeni* may have shifted from reducing mate competition in small and sparse populations to reducing competition for food or space in large and dense populations. Therefore, treating population density and size as independent factors in statistical models may generate misleading conclusions.

In conclusion, the present study provides evidence that *T. ludeni* females can immediately manipulate their reproductive output and adjust offspring sex ratio in response to social environments during their early reproductive period. I reveal that ovipositing mothers may have cooperated in a large population with low density or in a small population with high density to increase their reproduction. However, mothers may reduce their reproduction in a large and dense population due to the intense food or space competition. Furthermore, mothers can increase production of daughters in small populations with low density and large populations with high density to minimise mate and food/space competition, respectively. I propose that including interaction of population size and density in the statistical model is essential to generate accurate conclusions of the impacts of social environments on animal reproductive adjustments.

Chapter 4

Local mate competition model alone cannot predict the offspring sex ratio in large and dense populations of a haplodiploid arthropod

This chapter was published in *Current Zoology* zoac022 (2022).



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Doctoral Research Committee
July 2022

Abstract

Many theoretical models have been developed to predict the offspring sex ratio under local mate competition (LMC) conditions. However, the LMC theoretical models alone cannot explain the extremely female-biased offspring sex ratio in numerous haplodiploid taxa. Here I used a haplodiploid mite *Tetranychus ludeni* Zacher to test how mated females adjusted the sex allocation strategies in response to different levels of LMC and local resource competition (LRC), and whether the LMC models could predict the offspring sex ratio under different levels of LMC and LRC. I demonstrate that (1) mated females produced more daughters in small populations to reduce local mate competition between their sons, supporting the LMC theory, and (2) females produced significantly more dispersing daughters in the dense and larger populations, agreeing to the LRC theory. However, LMC models could explain the sex allocation by *T. ludeni* females when they individually found the colonies in new habitats with intensive inbreeding but failed to predict the sex ratios at higher population densities. I suggest that LMC, LRC and/or local resource enhancement (LRE) could be the main mechanisms leading to the extremely female-biased sex ratio in *T. ludeni*.

Key words: female-biased sex ratio, population size and density, spider mite, resource competition, cooperation

4.1 Introduction

Sex ratio in animals has profound impacts on the fitness of mothers and their offspring. When the local competition intensity is sex-specific, selection favors the sex that may mitigate such negative impacts (West, 2009). This may occur under (1) local mate competition (LMC) – in a discrete population established by one or a few foundresses, they tend to produce female-biased offspring sex ratio to reduce the local mate competition between related males (Hamilton, 1967); (2) local resource competition (LRC) – in a dense population where related individuals compete for resources, mothers are selected to produce more offspring of dispersive sex to decrease resource competition (Clark, 1978; Wild and Taylor, 2004; Iritani et al., 2021), and (3) local resource enhancement (LRE) – when one sex helps one another rather than compete with one another, selection favors an excess production of the more helpful sex (Taylor, 1981). These scenarios may take place simultaneously in the same species (West, 2013). However, theoretical models for prediction of sex ratio only consider LMC (Hamilton, 1967, 1979; Herre, 1985; West, 2009).

Under the assumption that mated females disperse to found new colonies, Hamilton (1967) proposes the first theoretical model to predict the evolutionary outcome of offspring sex ratio (proportion of male offspring, s) for diploid species under LMC: $s = (N–1)/(2N)$, (eq. 1), where N is the number of females in a population whose offspring develop and mate on the patch. The model predicts that an increase of LMC (i.e., decrease of foundress population size) reduces the proportion of sons from 50% for a large population to 0% for a single foundress, and the 0% of sons could be interpreted as the minimum proportion of sons needed for fertility insurance even though this minimum is unknown. In haplodiploid species, however, inbreeding is common, and females can control the sex of their offspring in response to the social environment through arrhenotoky (fertilized eggs give rise to diploid female offspring and unfertilized eggs to haploid male offspring) (West, 2009). The LMC with intensive inbreeding in small local populations will promote a female-biased sex ratio (Herre, 1985). Therefore, the effect of inbreeding should be incorporated into Hamilton's (1967) model when predicting sex ratio in this group of animals. If the probability of inbreeding in a population of constant size (N) is fixed, i.e., $k = 1/N$ $(1 \leq k \leq 0)$ (West, 2009), then $s = [(N–1)\ (2N–1)]/[N\ (4N–1)]$, (eq. 2). If N varies across patches in a population and there is an average level of inbreeding k $(1 \leq k \leq 0)$ (Herre, 1985; West, 2009), then $s = [(N–1)\ (2–k)]/[N\ (4–k)]$, (eq. 3) (when $k = 0$, eq. 3 is the same as eq. 1).

In numerous haplodiploid taxa, including many wasps, beetles, thrips and mites, females may produce extremely female-biased offspring sex ratios (West, 2009) which cannot be explained by LMC theory alone, probably because of impacts from LRC and/or LRE (Iritani et al., 2021). However, it is still not clear how LMC, LRC and LRE affect sex allocations in haplodiploid animals. Here I used a haplodiploid mite *Tetranychus ludeni* Zacher to test (1) how females adjusted the sex allocation strategies in response to different levels of LMC and LRC, and (2) whether the LMC theoretical models could predict the offspring sex ratio under different levels of LMC and LRC. *T. ludeni* is an invasive pest of horticultural crop and inhabits discrete patches of various population sizes and densities with varying inbreeding intensity. I designed a factorial experiment by keeping each of the three test population sizes constant but allowing the population density to alter at three levels, and *vice versa* (Table 4.1) and recorded the sex ratio.

4.2 Materials and methods

4.2.1 Mite colony

A *Tetranychus ludeni* Zacher colony started from adults collected on *Passiflora mollissima* (Kunth) in Palmerston North, New Zealand in September 2017. I reared the colony on kidney bean plants (*Phaseolus vulgaris* L.) in an aluminium-frame cage (30 cm length × 30 cm width × 65 cm height) covered with 200-mesh nylon gauze with a zip door (20 cm width × 55 cm height). The colony was maintained, and the experiment was carried out at 25 ± 1 °C and 40–50% RH with a photoperiod of 16:8 h (light:dark). I used the first expanded leaves of 1- to 2-week-old bean plants for experiment.

4.2.2 Experimental design and data collection

To test sex allocation in *T. ludeni* under LMC and LRC, I designed a factorial experiment by allowing both population size and density to vary, with nine combined treatments: three population sizes (1, 5 and 10 ovipositing females) × three population densities (0.67, 1 and 2 ovipositing females/cm^2) (Table 4.1). To obtain mated females, I randomly collected the quiescent female deutonymphs from the colony and individually placed on a 1-cm^2 leaf square placed upside down on a wet cotton pad in a Petri dish (9.5 cm diameter × 1.0 cm height, with a 1-cm mesh-sealed hole in the middle of the lid). Before the female emerged, I introduced a newly emerged male adult produced by a virgin female onto the arena. Mating occurred at female emergence and usually lasted for < 2 minutes.

For each replicate, I transferred mated female(s) of a desired population size or density onto a leaf square of desired size (see Table 4.1) in a Petri dish mentioned above and allowed them to lay eggs for 24 hours. After which time, I then daily transferred them to a new leaf square of the same size. I allowed the larvae and nymphs to feed on the same leaf square for five days, after which time they were transferred onto another clean fresh leaf square of the same size where they developed to adults. The number and sex of emerged adults were also recorded for each leaf square. I only used data recorded during the first five days of oviposition period for analysis, because the social environment changed beyond day 5 of oviposition due to the death of the experimental females.

4.2.3 Statistical analysis

I analysed the data on female offspring sex ratio (female offspring%) using a generalized linear model (GLIMMIX procedure, SAS 9.4, SAS Institute Inc., Cary, NC) followed by a Binomial distribution and a Logit link function for the model. I used a Tukey-Kramer test for multiple comparisons between population sizes of a given density or between population densities of a given size the combined effects of population size and density (Figure 4.1). I used a one-simple t-test to compare the observed female sex ratios in response to the social environment with the predictions of LMC models (eqs. 1–3) (Table 4.1).

4.3 Results

The sex ratio of *T. ludeni* under all tested social environments was low (0.13–0.21) (Figure 4.1, Table 4.1). Results show that when population size was large (5 or 10 females), offspring produced were significantly more female-biased at higher population densities (1 and 2 females/cm^2) than at lower population density (0.67 female/cm^2) ($F_{2,60} = 13.55$, $P < 0.0001$ for 5 females; $F_{2,52} = 26.95$, $P < 0.0001$ for 10 females). However, when population size was small (1 female), population density had no effect on offspring sex ratio ($F_{2,64} = 0.01$, $P = 0.9879$) (Figure 4.1). Furthermore, I detected a significantly lower sex ratio at the small population size of 1 female than at large population sizes (5 and 10 females) with a low population density (0.67 female/cm^2) ($F_{2,65} = 3.17$, $P = 0.0488$) but population size had no significant effect on sex ratio when population density was high (1 or 2 females/cm^2) ($F_{2,53} = 1.35$, $P = 0.2689$ for 1 female/cm^2; $F_{2,58} = 0.15$, $P = 0.8609$ for 2 females/cm^2) (Figure 4.1). When comparing the observed sex ratios with the predictions of the three LMC models, I found that the predictions of LMC models only fitted the observed sex ratios when the population size was 1 female with intensive inbreeding ($k = 1$, in eq. 3), but the observed sex ratio in the larger populations was significantly lower than that predicted by LMC models (Table 4.1).

Table 4.1 Comparisons of observed sex ratios (proportion of male offspring) at different social environments with predictions of LMC models.

Leaf area (cm^2)	Population size (females)	Population density ($females/cm^2$)	Observation		Prediction (eq. 2)	Prediction (eq. 3)		
			n	Mean (SE)		$k = 0$ (eq. 1)[†]	$k = 0.5$	$k = 1$
					0.2143	0.2500[⸸]	0.2142	0.1667
1.5	1	0.67	25	0.1497 (0.0144)	4.47[‡]***	6.94****	4.46***	1.18^{ns}
1	1	1	18	0.1544 (0.0193)	3.11**	4.96****	3.10**	0.64^{ns}
0.5	1	2	24	0.1534 (0.0067)	9.14****	14.51****	9.13****	1.99^{ns}
					0.3789	0.4000	0.3429	0.2667
7.5	5	0.67	25	0.2065 (0.0080)	21.54****	24.18****	17.05****	7.53****
5	5	1	22	0.1484 (0.0106)	21.83****	23.83****	18.42****	11.21****
2.5	5	2	16	0.1520 (0.0089)	25.36****	27.72****	21.43****	12.82****
					0.4385	0.4500	0.3857	0.3000
15	10	0.67	18	0.1934 (0.0086)	28.36****	29.69****	22.25****	12.33****
10	10	1	16	0.1325 (0.0086)	35.76****	37.11****	29.59****	19.58****
5	10	2	21	0.1482 (0.0126)	23.20****	23.93****	18.83****	12.04****

[†], when $k = 0$ (i.e., no inbreeding) in eq. 3, the predicted sex ratios are the same as that in eq. 1.
[⸸], when $N = 1$ in eqs. 1 and 3, the sex ratio $s = 0$ which is logically incorrect, thus the predicted sex ratio $s = 0.25$ when $N = 2$. [‡], t value for the one-sample t-test. ^{ns}, indicates no significant difference between the observed and predicted sex ratios; **, *** and ****, indicate a significantly lower observed sex ratio than the predicted ones at $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively.

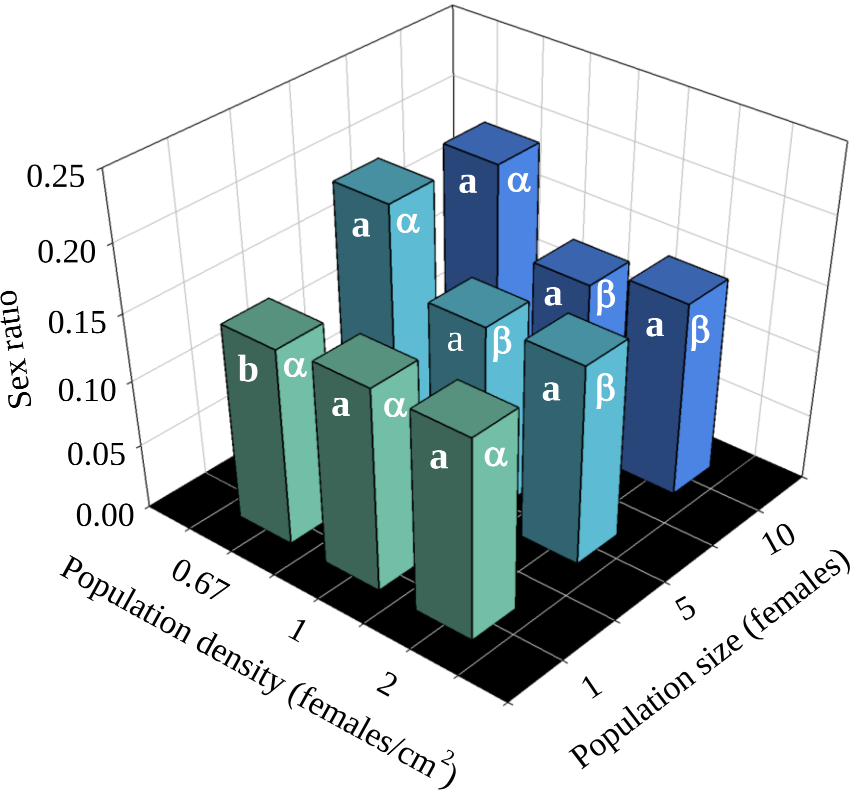


Figure 4.1 Sex ratio (proportion of male offspring) of *T. ludeni* under various social environments. For a given population density, columns with the same English letters are not significantly different between population sizes; for a given population size, columns with the same Greek letters are not significantly different between population densities (Tukey-Kramer test: $P > 0.05$).

4.4 Discussion

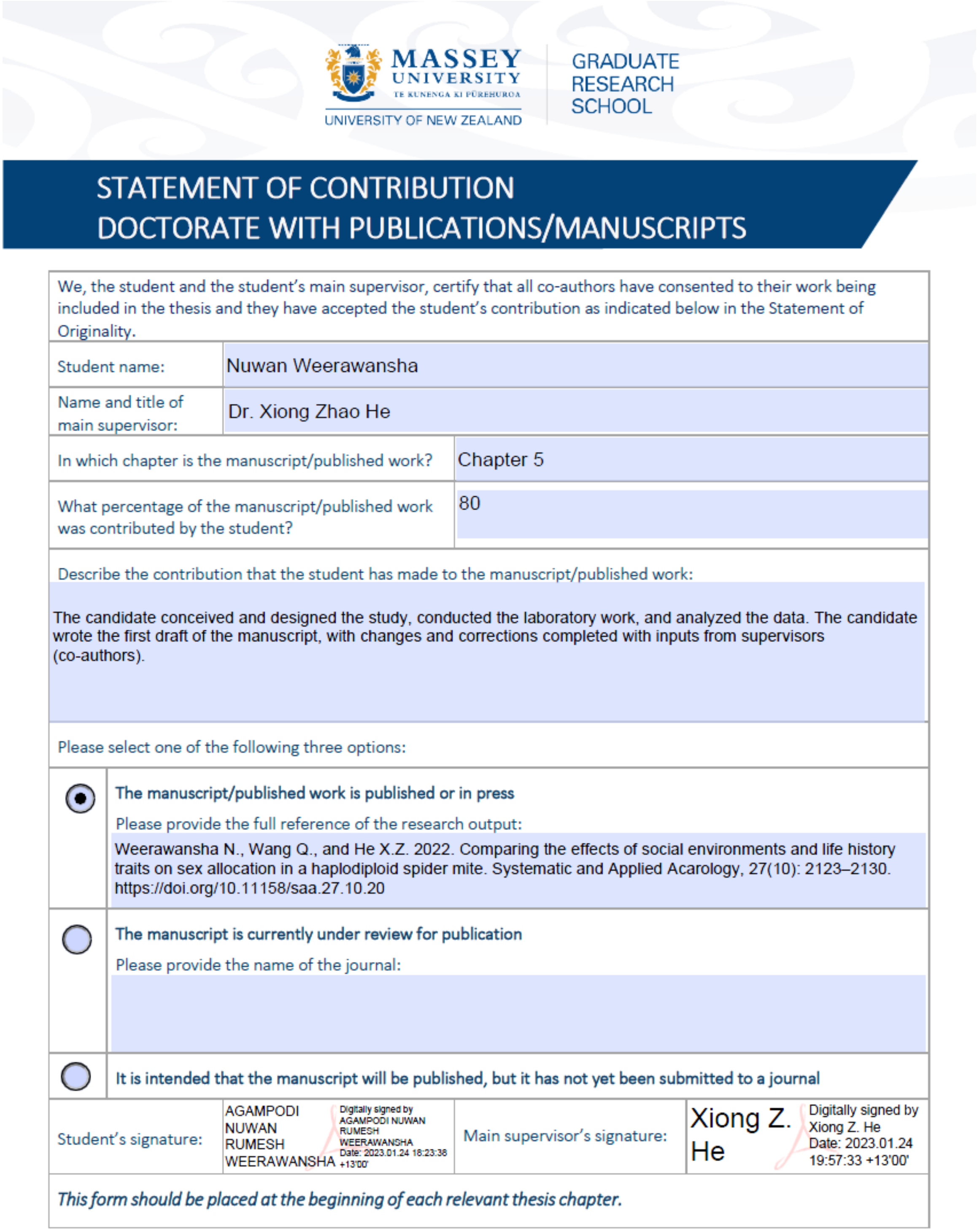
My results only support LMC theory (Hamilton, 1967) when population size is small where mated mothers produce more daughters to reduce local mate competition between their sons (Figure 4.1). Regardless of population size, females may be selected to produce more dispersing daughters in the dense populations (Figure 4.1) to reduce resource competition in the local populations, agreeing to the LRC prediction (Clark, 1978; Wild and Taylor, 2004; Iritani et al., 2021). I suggest that females change their sex allocation strategies in response to social environment. In small populations they produce more daughters to reduce mate competition between sons while in large and dense populations they give birth to more dispersing daughters to reduce resource competition.

The extremely female-biased sex ratio in *T. ludeni* may be attributed to the life history characteristics of spider mites. First, if the increase in fitness return per unit investment is greater in daughters than in sons, an optimal sex allocation is expected to be female-biased (Seger and Stubblefield, 2002). In *T. ludeni*, females are larger in size than males, thus investing more resources to daughters is advantageous for the mothers. Second, although daughters are more likely to compete for local food due to their large body size, they usually disperse from the dense conditions after mating. Thus, production of more dispersing daughters will eventually reduce LRC as well as LMC. Third, spider mite females may cooperate as a form of LRE in spinning silk webs which are crucial for dispersal and protection against environmental hazards, and group-living females produce more silks per mite than single females (Le Goff et al., 2010). In the scenario of LRE, females are the helpers and thus production of more daughters may reduce both local mate and resource competition. Although production of more mates or helping and dispersing daughters may potentially intensify local resource competition, the benefit gained from more daughters produced under LMC, LRC or LRE outweighs the costs of resource competition among females. I suggest that under varying social environments, LMC, LRC and LRE could be the main mechanisms leading to the extremely female-biased sex ratio in *T. ludeni*.

Chapter 5

Comparing the effects of social environments and life history traits on sex allocation in a haplodiploid spider mite

This chapter was published in *Systematic and Applied Acarology* 27(10): 2123–2130 (2022).



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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Nuwan Weerawansha
Name and title of main supervisor:	Dr. Xiong Zhao He
In which chapter is the manuscript/published work?	Chapter 5
What percentage of the manuscript/published work was contributed by the student?	80

Describe the contribution that the student has made to the manuscript/published work:

The candidate conceived and designed the study, conducted the laboratory work, and analyzed the data. The candidate wrote the first draft of the manuscript, with changes and corrections completed with inputs from supervisors (co-authors).

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Doctoral Research Committee
July 2022

Abstract

Many factors including social environments and life history traits may directly and/or indirectly influence sex allocation in animals. In the present study, I estimated and compared the effects of social environments (female population size and density and their interaction) and life history traits (maternal age and egg number and size) on sex allocation in a haplodiploid spider mite *Tetranychus ludeni* Zacher. Results of a path analysis show that maternal age had significantly positive direct and indirect effects on offspring sex ratio (daughters%), and the significantly indirect effect was through its significantly positive influence on egg number and size. I further reveal that (1) population density only imposed significantly positive indirect effects on sex ratio due to its significantly positive effect on egg number; (2) population size had no significant direct or indirect effects on sex ratio, and (3) the interaction of population size and density had significantly positive direct effects on sex ratio while its significantly negative effects on egg number and size might counteract its indirect effects. A stepwise-selection method demonstrated an order of factors significantly affecting *T. ludeni* sex allocation: maternal age > egg number > egg size > interaction of population size and density. Therefore, the effects of life history traits on *T. ludeni* sex allocation outweighed that of social environments.

Key words: sex ratio, population size and density, maternal age, egg number and size

5.1 Introduction

Social environment refers to an individual's physical surroundings, community resources and social relationships, including population size, population composition, fission-fusion dynamics, and the social interactions among group members (Webber et al., 2022). Population size and density are two key socio-environmental factors that affect the offspring sex ratio in group-living animals. For example, in small populations founded by one or a few foundresses, females tend to produce female-biased offspring to reduce the local mate competition between related males (Hamilton, 1967; Krainacker and Carey, 1991; Roeder, 1992; Sato and Saito, 2006; West, 2009; Macke et al., 2012a, 2014), whereas in dense populations mothers incline to produce more offspring of dispersive sex to reduce resource competition (Clark, 1978; Mari et al., 2008; Hjernquist et al., 2009; Iritani et al., 2021; Weerawansha et al., 2022a, 2022b). Social environments may affect offspring sex ratio directly by altering mothers' sex allocation strategies (Clark, 1978; Iritani et al., 2021; Weerawansha et al., 2022a, 2022b) or indirectly through mediating their other life history traits such as clutch size (West, 2009) and egg size (West, 2009; Macke et al., 2011b, 2012a).

Moreover, previous studies reveal that in haplodiploid species (i.e., whose fertilized eggs give rise to diploid female offspring and unfertilized eggs to haploid male offspring), life history traits such as the maternal age can also directly affect sex allocation due to the age-specific fertilization (i.e., more daughters produced at specific ages) (Roy et al., 2003; Ueno, 2014; Weerawansha et al., 2020). Nevertheless, maternal age may also affect offspring sex ratio indirectly through age-specific investment in clutch size (Weerawansha et al., 2020) and egg size (Reviewed in Mousseau and Fox, 1998). Therefore, multiple factors of social environments and life history traits may simultaneously act on offspring sex ratio during the females' reproductive period. However, studies usually test the influence of social environments and life history traits on sex allocation separately and focus on the direct effect only (West, 2009; Macke et al., 2011a, 2012a; Katlav et al., 2021). Due to the lack of appropriate methodology, few studies have investigated (1) whether social environments and maternal age have both direct and indirect effects on sex allocation, and (2) whether social environments impose weaker or stronger effects on offspring sex ratio than the life history traits. Here I used a haplodiploid spider mite *Tetranychus ludeni* Zacher (Acari: Tetranychidae) as a model species to estimate and compare the effects of social environments and life history traits on sex allocation.

5.2 Materials and methods

5.2.1 Experimental design and data collection

I established a colony of *T. ludeni* Zacher from adults collected on *Passiflora mollissima* (Kunth) in Palmerston North, New Zealand. The colony was maintained, and the experiment was carried out at 25 ± 1 °C and 40–50% RH with a photoperiod of 16:8 h (light:dark). I used the first expanded leaves of 1- to 2-week-old bean plants (*Phaseolus vulgaris* L.) for the experiment. To estimate the effects of social environments and life history traits on sex allocation in *T. ludeni*, I designed a factorial experiment by allowing both population size and density to vary, with nine combined treatments: three population sizes (1, 5, and 10 ovipositing females) at three population densities (0.67, 1, and 2 ovipositing females/cm^2). To obtain mated females, I randomly collected the quiescent female deutonymphs from the colony and individually placed them on a 1-cm^2 leaf square placed upside down on a wet cotton pad in a Petri dish (9.5 cm diameter × 1.0 cm height, with a 1-cm mesh-sealed hole in the middle of the lid). Before the female emerged, I introduced a newly emerged male adult into the mating arena. The males were produced by virgin females that developed from individually reared quiescent female deutonymphs randomly collected from the colony. Mating occurred at female emergence and usually lasted for < 2 mins (NW personal observations).

For each replicate, I transferred mated female(s) of a desired population size or density onto a leaf square of the desired size (Table 5.1) in a Petri dish mentioned above and allowed them to lay eggs for 24 hours. After which time, I then transferred them to a new leaf square of the same size daily until they died. I counted the number of eggs laid on each leaf square, and measured egg diameter under a stereomicroscope (Leica MZ12, Germany) connected to a digital camera (Olympus SC30, Japan), using an imaging software (CellSens® GS-ST-V1.7, Olympus, Japan). I calculated the egg size: volume = $4/3\pi r^3$, where r is radius (= diameter/2). As up to 10, 35, and 85 eggs could be laid on a leaf square by 1, 5, and 10 females, respectively, I measured all, 10 and 15 eggs on leaf squares with 1, 5 and 10 females feeding on, respectively. I allowed the larvae and nymphs to feed on the same leaf square for five days, after which time they were transferred onto another clean fresh leaf square of the same size where they developed to adults. The number and sex of emerged adults were also recorded and the offspring sex ratio [daughter% = daughters / (daughters + sons)] was calculated for each leaf square. I only used data recorded during the first five days of the oviposition period for analysis, because the social environment changed beyond day 5 of oviposition due to the death of experimental female mites.

Table 5.1 Experimental design for nine combined treatments of three population sizes at three population densities.

Leaf area (cm^2)	Population size [female(s)]	Population density [female(s)/cm^2]	Replicate (n)
1.5	1	0.67	25
1	1	1	18
0.5	1	2	24
7.5	5	0.67	25
5	5	1	22
2.5	5	2	16
15	10	0.67	18
10	10	1	16
5	10	2	21

5.2.2 Statistical analysis

I analysed the data using SAS 9.4 (SAS Institute Inc., Cary, NC). A path analysis (CALIS Procedure) was applied to estimate the direct effects of population size and density, their interaction, maternal age, and egg number and size on sex ratio, whereas the indirect effects of population size and density, their interaction, or maternal age on sex ratio were assessed through their influences on egg number and size (Figure 5.1). I then used a multivariate linear regression model (REG procedure) to compare the direct effects of social environments and life history traits, according to the partial regression coefficient (R^2, measuring the proportion of variance in sex ratio that can be explained by a given factor, the larger the R^2, the more variance could be explained) of each factor (Table 5.2). The partial R^2 of each factor was calculated as the Type I Sum of Square of a factor divided by the Uncorrected Total Sum of Square of the model. Using the same multivariate linear regression model, I employed the stepwise-selection method to organise the significant factors in order by adding them one by one into the model, with the most significant factor entering the model first and the non-significant ones being excluded from the final model (Table 5.2). To detect the cause of the positive effect of egg number on sex ratio, a linear regression (REG procedure) was used to determine the relationships between the number of eggs and daughters/sons produced, and the significant difference in regression coefficient (i.e., slope) of regression lines was indicated by the non-overlapped 95% CLs of coefficients. As the measuring scales and units of different factors were highly different, I standardised each factor as well as data on offspring sex ratio to have a mean of 0 and a standard deviation of 1 (STANDARD procedure) before path analysis and multivariate regression, so that all factors were comparable.

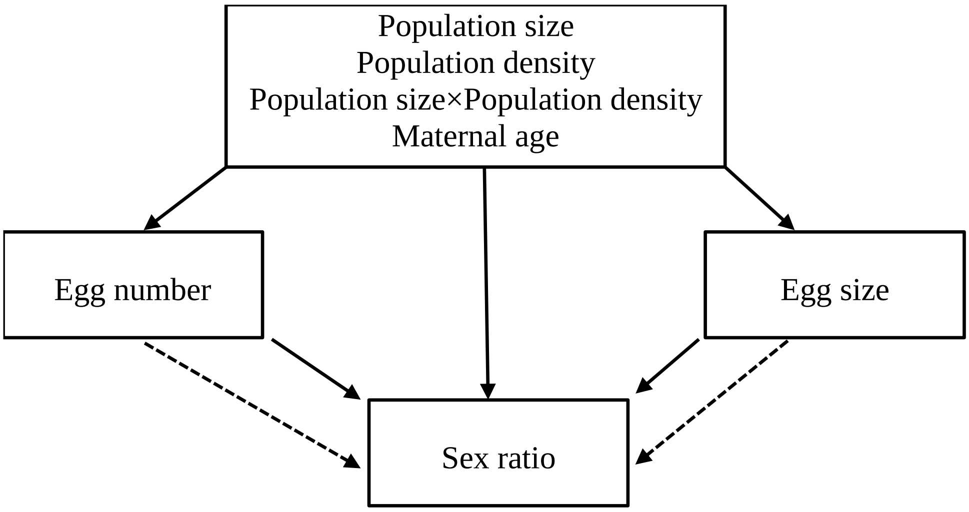


Figure 5.1 A diagram showing the paths of direct (solid line) and indirect (dashed line) effects of social environments [population size and density and their interaction (Population size×Population density)] and life history traits (maternal age, egg number, and egg size) on sex allocation in *Tetranychus ludeni*. Arrows of solid and dash lines indicate the directions of direct and indirect effects, respectively.

Due to the possible correlations of population size and density and maternal age with the egg number and size, multicollinearity might exist, which could result in inaccurate interpretations of the effects of explanatory factors as the change of one factor would inevitably lead to the change of another (Tay, 2017; Frost, 2019). I thus examined the variable multicollinearity through the variance inflation factor (VIF) in the above-mentioned multivariate linear regression model. The VIF measures the inflation in the variances of factors. Results show that the VIF = 1.09 ~ 1.03, indicating a lack of multicollinearity between factors (i.e., VIF < 5) (Tay 2017; Frost 2019), while the VIFs of population size and its interaction with population density were > 5 (Table 5.2) when the interaction was included into the model. However, the large VIF value for population size was caused by the introduction of interaction into the model, and the impact of interaction was a by-product that I specified in the model. Therefore, the large VIF values of population size and interaction would not cause real multicollinearity problems (Frost, 2019).

5.3 Results and discussion

Results of path analysis show that both egg number and size had significant positive direct effects on sex ratio (Figure 5.2, Table 5.2). The significant positive effect of egg number on sex ratio may be because the number of both daughters and sons increased significantly with increasing egg number (coefficient = 0.9518 and 0.4431, and t = 95.16 and 15.06 for daughters and sons, respectively, $P < 0.0001$), but the rate of increase (i.e., coefficient) was significantly greater for daughters than that for sons (non-overlapped 95% CLs of coefficients), entailing a strong female-biased sex ratio at the fecund patches. The significant positive direct effects of egg size on sex ratio suggest that *T. ludeni* females could adjust offspring sex ratio by altering the egg size and fertilizing larger eggs that develop to daughters. Similar findings have also been reported in other haplodiploid species, such as the spider mites *T. urticae* Koch (Macke et al., 2011b, 2012a) and thrips *Pezothrips kellyanus* (Bagnall) (Katlav et al., 2021).

I further reveal that maternal age had both significant positive direct and indirect effects on sex ratio (Figure 5.2). The results suggest that the mated mothers did not experience any sperm depletion as single mating can lead to extremely female-biased sex ratios (Weerawansha et al., 2022b), and the aging mothers tend to increase fecundity as well as daughter production continuously, especially during their early life (Table 5.3; Weerawansha et al., 2020). Moreover, maternal age had significant positive effects on egg number (effect = 0.4530, t = 16.76, $P < 0.0001$) and size (effect = 0.1163, t = 3.57, $P = 0.0004$), leading to a significant positive indirect effect on sex ratio (Figure 5.2).

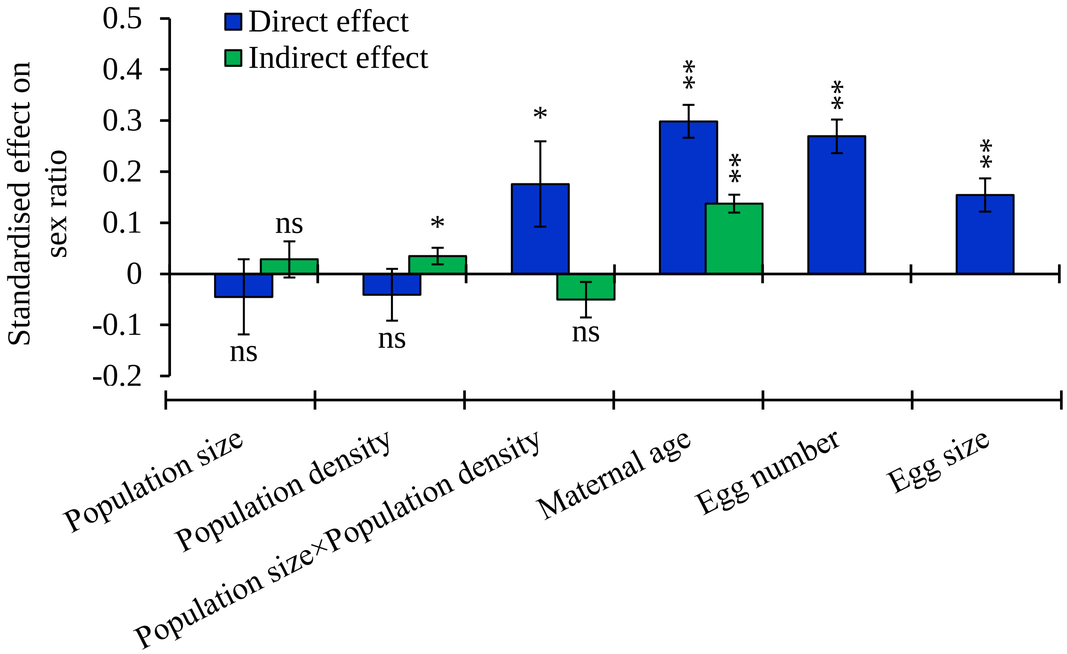


Figure 5.2 Standardized direct and indirect effects of social environments (population size and density and their interaction) and life history traits (maternal age, and egg number and size) on sex ratio in *Tetranychus ludeni*. For egg number and size, the indirect effect was not estimated. Standardised direct effect = – 0.0452 × PopuSize – 0.0411 × PopuDens + 0.1758 × PopuSize×PopuDens + 0.2983 × MaternalAge + 0.2693 × EggNo + 0.1544 × EggSize, R^2 = 0.2729. ^{ns}, not significant; *, $P < 0.05$; **, $P < 0.0001$.

I provide strong evidence that social environments had weaker effects on *T. ludeni* sex allocation compared to the maternal age. First, population density significantly and indirectly affected the sex ratio probably due to its significant direct effect on egg number (effect = 0.1471, t = 2.90, P = 0.0037) (Figure 5.2). Second, population size had no direct effect on sex ratio; in addition, it exerted significant positive direct effect on egg number (effect = 0.4953, t = 8.56, P < 0.0001) but significant negative effect on egg size (effect = –0.6960, t = –12.02, P < 0.0001), which might have decreased the indirect effect of population size on sex ratio (Figure 5.2). Third, the interaction of population size and density had a significant positive direct effect on sex ratio; however, its significant contrary effects on egg number and size (i.e., effect = –0.5233 and 0.5093, and t = –7.34 and 7.13 for egg number and size, respectively, P < 0.0001) might also counteract the indirect effect as did the population size (Figure 5.2). These results suggest that population size affecting *T. ludeni* sex allocation was implemented with population density and *vice versa* (Weerawansha et al., 2022a).

The full model equations of path analysis and multivariate linear regression that predicted the direct effects of different factors on sex ratio were similar (Figure 5.2, Table 5.2). Results of these models indicate that the direct effect of maternal age on sex ratio (effect ≈ 0.30) was about 6.0, 7.0, and 1.6 times higher than that of population size, population density, and their interaction, respectively (Figure 5.2, Table 5.2). Moreover, results of the multivariate linear regression model show that maternal age had a much higher coefficient of determination (R^2 = 0.1848) than the social environments (R^2 = 0.0153) (Table 5.2), suggesting that maternal age alone explained more variance of offspring sex ratio compared to the social environments. The stepwise-selection method in the multivariate linear regression model further demonstrated an order of factors significantly affecting *T. ludeni* sex allocation: maternal age > egg number > egg size > interaction of population size and density (Table 5.2).

Table 5.2 Comparison of standardised direct effects of social environments [population size (PopuSize) and density (PopuDens) and their interaction (PopuSize×PopuDens)] and life history traits [maternal age (MaternalAge), egg number (EggNo), and egg size (EggSize)] on sex ratio in *Tetranychus ludeni*.

Factor	Effect (SE)	95% CLs	t	P	Partial R^2	VIF
Full model						
PopuSize	-0.0485 (0.0751)	-0.1960–0.0990	-0.65	0.5186	0.0030	6.37
PopuDens	-0.0367 (0.0522)	-0.1391–0.0657	-0.70	0.4816	0.0095	3.03
PopuSize×PopuDens	0.1805 (0.0868)	0.0102–0.3507	2.08	0.0378	0.0029	8.17
MaternalAge	0.3071 (0.0351)	0.2382–0.3760	8.75	< 0.0001	0.1848	1.31
EggNo	0.2841 (0.0368)	0.2119–0.3563	7.72	< 0.0001	0.0484	1.35
EggSize	0.1541 (0.0333)	0.0888–0.2195	4.63	< 0.0001	0.0185	1.26
Final model						
MaternalAge	0.3084 (0.0347)	0.2401–0.3766	8.87	< 0.0001	0.1874	1.29
EggNo	0.2800 (0.0357)	0.2099–0.3501	7.84	< 0.0001	0.0434	1.27
EggSize	0.1574 (0.0303)	0.0978–0.2170	5.18	< 0.0001	0.0232	1.05
PopuSize×PopuDens	0.1226 (0.0303)	0.0630–0.1821	4.04	< 0.0001	0.0126	1.00

Full model: standardised direct effect = – 0.0485 × PopuSize – 0.0367 × PopuDens + 0.1805 × PopuSize×PopuDens + 0.3071 × MaternalAge + 0.2841 × EggNo + 0.1541 × EggSize, R^2 = 0.2671.

Final model: standardised direct effect = 0.3084 × MaternalAge + 0.2800 × EggNo + 0.1574 × EggSize + 0.1226 × PopuSize×PopuDens, $R^2 = 0.2666$.

Table 5.3 Variations of egg number, egg size (×10^6 μm^3) and sex ratio (daughters%) of *Tetranychus ludeni* females over five days at different social environments of population size [PopuSize, female(s)] and density [PopuDens, female(s)/cm^2].

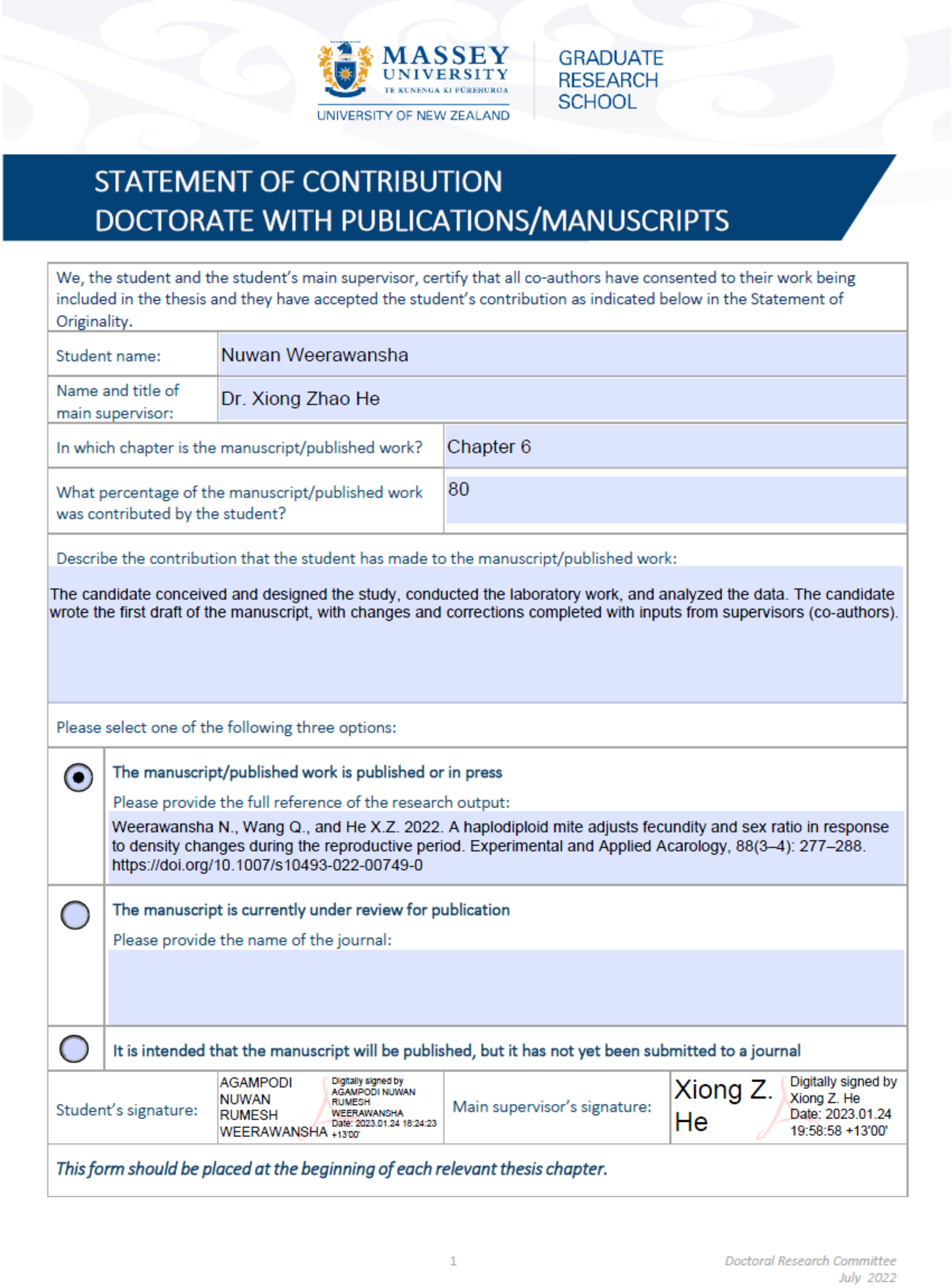
Parameter	PopuSize	PopuDens	Day 1	Day 2	Day 3	Day 4	Day 5
Egg no.	1	0.67	0.48±0.13	2.76±0.30	5.08±0.41	3.72±0.38	3.84±0.55
		1	0.76±0.22	2.33±0.37	4.61±0.48	4.33±0.62	3.11±0.53
		2	0.13±0.07	2.58±0.38	3.88±0.33	5.21±0.43	4.46±0.44
	5	0.67	1.66±0.14	3.50±0.23	4.32±0.33	5.08±0.27	4.90±0.28
		1	1.33±0.15	2.91±0.20	4.07±0.31	4.88±0.41	4.80±0.37
		2	0.96±0.24	2.86±0.42	4.08±0.48	4.22±0.43	4.36±0.48
	10	0.67	1.46±0.08	5.64±0.18	6.41±0.37	6.81±0.34	5.67±0.36
		1	1.41±0.10	4.20±0.28	4.97±0.45	4.88±0.44	4.39±0.45
		2	0.39±0.18	2.64±0.16	4.71±0.24	3.93±0.20	3.07±0.14
Egg size	1	0.67	1.130±0.041	1.160±0.018	1.152±0.017	1.156±0.019	1.174±0.021
		1	0.888±0.027	1.049±0.023	1.100±0.014	1.118±0.024	1.100±0.016
		2	1.145±0.031	1.101±0.018	1.100±0.014	1.106±0.012	1.136±0.018
	5	0.67	1.026±0.010	1.022±0.006	1.024±0.010	1.010±0.008	1.028±0.009
		1	1.097±0.017	1.090±0.011	1.113±0.011	1.097±0.010	1.095±0.011
		2	1.152±0.032	1.131±0.018	1.142±0.015	1.148±0.012	1.146±0.010
	10	0.67	1.017±0.007	1.014±0.008	1.019±0.009	1.015±0.006	1.014±0.006
		1	1.035±0.014	1.060±0.017	1.050±0.014	1.070±0.160	1.070±0.022
		2	1.104±0.041	1.099±0.014	1.092±0.012	1.102±0.015	1.099±0.014
Sex ratio	1	0.67	0.300±0.153	0.759±0.067	0.865±0.044	0.948±0.017	0.767±0.067
		1	0.188±0.132	0.637±0.110	0.918±0.033	0.887±0.034	0.869±0.062
		2	0.000±0.000	0.567±0.072	0.874±0.035	0.872±0.043	0.924±0.023
	5	0.67	0.594±0.031	0.772±0.022	0.819±0.012	0.803±0.011	0.834±0.013
		1	0.530±0.047	0.822±0.028	0.898±0.016	0.859±0.015	0.900±0.014
		2	0.733±0.053	0.782±0.039	0.885±0.024	0.865±0.017	0.865±0.015
	10	0.67	0.513±0.037	0.836±0.009	0.816±0.014	0.814±0.009	0.847±0.012
		1	0.439±0.045	0.939±0.012	0.906±0.011	0.894±0.008	0.926±0.011
		2	0.785±0.138	0.864±0.013	0.809±0.023	0.878±0.011	0.896±0.008

In summary, I deciphered the direct and indirect effects and compared the direct effects of social environments and life history traits on sex allocation in *T. ludeni*. I demonstrate that the effects of life history traits on *T. ludeni* sex allocation outweigh that of social environments, and social environments influencing offspring sex ratios are implemented or mediated through life history traits, e.g., the egg number and size. However, a large amount of variance in sex ratio remains unexplained, indicating sex allocation in *T. ludeni* is more complex than expected. Therefore, further experiments integrating more life history traits and socio-environmental factors may help understand the complexity of sex allocation strategies in haplodiploid organisms.

Chapter 6

A haplodiploid mite adjusts fecundity and sex ratio in response to density changes during reproductive period

This chapter was published in *Experimental and Applied Acarology* 88(3–4): 277–288 (2022).



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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Nuwan Weerawansha
Name and title of main supervisor:	Dr. Xiong Zhao He
In which chapter is the manuscript/published work?	Chapter 6
What percentage of the manuscript/published work was contributed by the student?	80

Describe the contribution that the student has made to the manuscript/published work:

The candidate conceived and designed the study, conducted the laboratory work, and analyzed the data. The candidate wrote the first draft of the manuscript, with changes and corrections completed with inputs from supervisors (co-authors).

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Weerawansha N., Wang Q., and He X.Z. 2022. A haplodiploid mite adjusts fecundity and sex ratio in response to density changes during the reproductive period. Experimental and Applied Acarology, 88(3–4): 277–288. https://doi.org/10.1007/s10493-022-00749-0

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1

Doctoral Research Committee
July 2022

Abstract

Population density is one of the main socio-environmental factors that have critical impacts on reproduction of animals. Consequently, they need to adjust their reproductive strategies in response to changes of local population density. In this study I used a haplodiploid spider mite, *Tetranychus ludeni* Zacher (Acari: Tetranychidae), to test how population density dynamics during the reproductive period altered female reproductive performance. I demonstrate that females produced fewer eggs with a significantly higher female-biased sex ratio in dense populations. Reducing fecundity and increasing daughter production in a dense environment could be an advantageous strategy to minimise the intensity of local food competition. However, females also reduced their fecundity after arrival in new site of larger area from a dense population, which may be associated with higher web production costs because females need to produce more webs to cover the larger area. There was no trade-off between egg number and size, and egg size had little impact on reproductive fitness. Therefore, *T. ludeni* females could adapt to the shift of population density during their reproductive period by manipulating the fecundity and offspring sex ratio but not the egg size.

Key words: spider mite, fecundity, sex ratio, resource competition, social environment

6.1 Introduction

Population density is one of the major components of social environments that can affect population dynamics. A local population density may vary over time due to aggregation (immigration), dispersal (emigration), or mortality (Roeder, 1992; Roff, 1992; Stearns, 1992; Bowman et al., 2002; Schausberger et al., 2021). Animals may aggregate in a habitat to increase reproduction opportunities (e.g., Snead and Alcock, 1985; Bengtsson, 2008; Wheeler and Jr Welsh, 2008; Le Goff, et al., 2010; Pérez-González et al., 2010; Bonsignore and Jones, 2014; DeVries et al., 2017. Dar et al., 2021) or to reduce predation risk (e.g., Spieler, 2003; Morrell and James, 2008; Yano, 2012; Clotuche et al., 2014). However, aggregation may raise population density in the habitat, leading to intensive resource competition and reducing reproductive fitness (Li and Zhang, 2021; Weerawansha et al., 2020, 2022a). In this scenario, the reproductive females would disperse to seek new habitats for the next generation (Schaub and von Hirschheydt, 2009; Azandémè-Hounmalon et al., 2014; Lutz et al., 2015; Kingma et al., 2017; Kusch et al., 2020; Manguette et al., 2020; Vaishali and Krushnamegh, 2020; Schausberger et al., 2021; Zhou et al., 2021b). With a few exceptions (e.g., Roeder, 1992; Fox et al., 1997; Matsuura and Kobayashi, 2010; Maenoa et al., 2020), studies on the effects of population density on reproductive plasticity have been carried out under constant population densities during female breeding time (e.g., Wrensch and Young, 1978; Fischer et al., 2011; Weerawansha et al., 2020, 2022a). To date, it is still unclear how females alter their reproductive strategies in response to the varying population density during their reproductive life.

After settling in new habitats, females are expected to adjust their reproductive strategies to optimize their fitness (Roff, 1992; Stearns, 1992; West et al., 2005; Fischer et al., 2011; Bowers et al., 2017; Maenoa et al., 2020; Weerawansha et al., 2022a). For example, if the population is dense, females may lay fewer (van Noordwijk and de Jong, 1986; Khan et al., 2018; Li and Zhang, 2021) but larger eggs (Parker and Begon, 1986; Sibly et al., 1988; Fischer et al., 2011), trading-offs the number with size of eggs to make best utilization of limited resources and maximise offspring fitness (Smith and Fretwell, 1974; Parker and Begon, 1986; Stearns, 1992; Fox and Czesak, 2000; Fischer et al., 2011; Macke et al., 2012b; Walzer and Schausberger, 2015; Maenoa et al., 2020). In species with sexual size dimorphism, resource-deficient females either reduce the egg size of the larger sex (Fox and Czesak, 2000; Walzer and Schausberger, 2013, 2015) or produce fewer eggs of the larger sex (Trivers and Willard, 1973; Charnov, 1982; Walzer and Schausberger, 2015) to optimize their reproductive fitness returns. Moreover, if one sex is dispersive and the other is philopatric, females often skew investment towards philopatric offspring when local resources are abundant but allocate more resources to the dispersive sex when local resources are deficient (Clark, 1978; Silk, 1983, 1984; West et al., 2005; Hjernquist et al., 2009; West, 2009).

Spider mites (Acari: Tetranychidae) are phytophagous invertebrates, often living as groups (Gutierrez and Helle, 1985; Le Goff et al., 2010; Schausberger et al., 2021) in discrete patches (Mitchell, 1973; Nachappa et al., 2011; Sarwar, 2013). Female adults are larger than male adults (Mitchell, 1973) and thus more likely to compete for food with their mothers or siblings (Young et al., 1986). However, female adults, rather than male adults and immature nymphs, may disperse to found new colonies (Mitchell, 1973; Brandenburg and Kennedy, 1982) especially when the populations are crowded or when food is insufficient or poor in quality (Suski and Naegele, 1968; McEnroe, 1969). As spider mites are haplodiploid, mated females can manipulate offspring sex ratio by fertilizing relatively larger eggs that develop to daughters (Young et al., 1986; Roeder et al., 1996; Macke et al., 2011a). It has been reported that females produce fewer eggs with more dispersing daughters in large and dense populations to reduce local competition for food (Weerawansha et al., 2022a). Moreover, spider mites aggregate and cooperate in spinning silk webs for dispersal and protection against environmental hazards (Le Goff et al., 2010; Yano, 2012), and group-living females produce more silks and lay more eggs per mite than single females (Le Goff et al., 2010). Therefore, spider mites should be able to adjust offspring sex ratio in response to the social environments.

Here I used an invasive spider mite pest, *Tetranychus ludeni* Zacher (Zhang, 2003), to examine how changes in population density during female reproductive life altered egg production and sex allocation. I simulated the aggregation by moving females from low to high population density and the dispersal by shifting females from high to low population density. I recorded the number and size of eggs laid and offspring sex ratio (i.e., proportion of daughters) before and after density changes. Based on the knowledge outlined above, I hypothesise that (1) females lay fewer but larger eggs and produce offspring with a more female-biased sex ratio in response to the aggregation scenario, and (2) the opposite case occurs in response to the dispersal scenario. This study provided insights into the mechanisms behind the adjustment of fecundity and sex ratio in response to the varying social environments.

6.2 Materials and methods

6.2.1 Mite colony

I maintained a colony of *T. ludeni* on the kidney bean plants (*Phaseolus vulgaris* L.) in the laboratory and carried out the experiment at 25 ± 1 ºC, 40 ± 10% RH and a photoperiod of 16:8 h (light:dark). I used the first expanded leaves of 1 to 2-week-old plants for the experiment.

6.2.2 Experimental design and data collection

To determine how females adjusted their fecundity and sex allocation in response to population density dynamics in *T. ludeni*, I set up two treatments, each with 32 leaf squares as replicates. Treatments 1 and 2 tested the effects of density changes from high to low (Figure 6.1a) and from low to high (Figure 6.1b), respectively. Briefly, I randomly selected the quiescent female deutonymphs just before emergence (silvery in colour) from the colony. I individually transferred them onto 1-cm^2 leaf squares placed upside down on a water-saturated cotton pad in a Petri dish (9.5 cm diameter × 1.0 cm height) with a hole (1 cm diameter) in the middle of the lid covered by a fine metal mesh (aperture size = 0.25 mm × 0.25 mm). I then introduced a newly emerged virgin male adult produced by virgin females onto each square. I monitored the pair until the end of copulation, after which time, I removed the male. For each replicate in

Treatment 1, I introduced 16 newly mated females onto a 1-cm^2 clean leaf square and allowed them to stay on the square for one day. I then transferred them to a new square daily for two consecutive days. On the fourth day, I randomly selected 16 of the 32 leaf squares and transferred mites from each leaf square onto a new 16-cm^2 leaf square (from high to low density) and those from each of the remaining 16 leaf squares onto a new 1-cm^2 leaf square (from hight to high density as control) daily for three consecutive days. The same procedure was carried out for Treatment 2 except that I transferred mites from low to high density and from low to low density (as control).

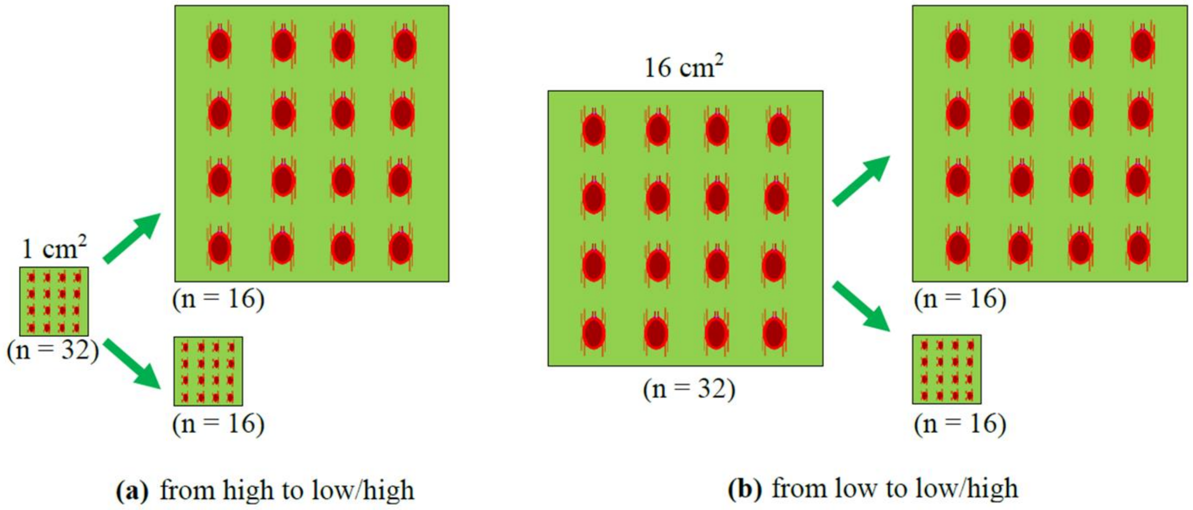


Figure 6.1 Diagram of experimental design to test the effect of population density shift on reproduction in *Tetranychus ludeni*: (**a**) from high to low/high density (Treatment 1) and (**b**) from low to low/high density (Treatment 2). n, number of leaf discs (replicates).

I checked each leaf square twice a day during the six oviposition days and replaced any dead females immediately with females of the same age and social experience. I recorded the number of eggs laid on each leaf square. To determine the egg size, I randomly selected 30 eggs from each leaf square and individually measured their diameter under a stereomicroscope (Leica MZ12, Germany) connected to a digital camera (Olympus SC30, Japan) and an imaging software (CellSens® GS-ST-V1.7, Olympus, Japan). I calculated the egg radius (r = diameter/2) and egg size (volume = $4/3\pi r^3$). After eggs hatched, I transferred all live individuals onto a clean leaf square of the same size once every five days and recorded the sex of newly emerged adults.

6.2.2 Statistical analysis

I analysed all data using SAS 9.4 with a rejection level set at $\alpha < 0.05$. Data on the number of eggs laid and egg size were normally distributed (Shapiro-Wilk test; UNIVARIATE procedure). I analysed the data on egg number and size using a linear mixed model (GLM procedure) with treatment (i.e., density shift) as a main factor and replicate as a random factor, and a Tukey-Kramer test for multiple comparisons (Figure 6.2). The mean egg size and number for each female before and after density shift were calculated and used for analysis. The data on sex ratio (proportion of daughters) were analysed by a generalized linear model (GLIMMIX procedure) with a binomial distribution and a *Logit* link function after the model, and a Tukey-Kramer test was applied for multiple comparisons (Figure 6.4). A general linear regression model (GLM procedure) was applied to determine the relationships between egg size and number, between immature survival rate and egg size (Figure 6.3) as well as between the sex ratio and egg size (Figure 6.5). The mean egg size and number, immature survival rate, and sex ratio for each female were used for regressions.

6.3 Results

6.3.1 Effect of population density shifts on fecundity, egg size and immature survival

Our results show that females at low density laid significantly more eggs than at high density before density shift but laid significantly fewer eggs after the shift regardless of whether it was from high to low or from low to high ($F_{5,107} = 28.79$, $P < 0.0001$) (Figure 6.2a). If the shift occurred at the same density levels, females produced similar number of eggs before and after shift (Figure 6.2a). However, population density and its shift had no significant impact on egg size ($F_{5,107} = 0.37$, $P = 0.8700$) (Figure 6.2b). Moreover, increasing number of eggs laid did not significantly reduce the egg size (Figure 6.3a) and egg size had no significant impact on immature survival rate (Figure 6.3b).

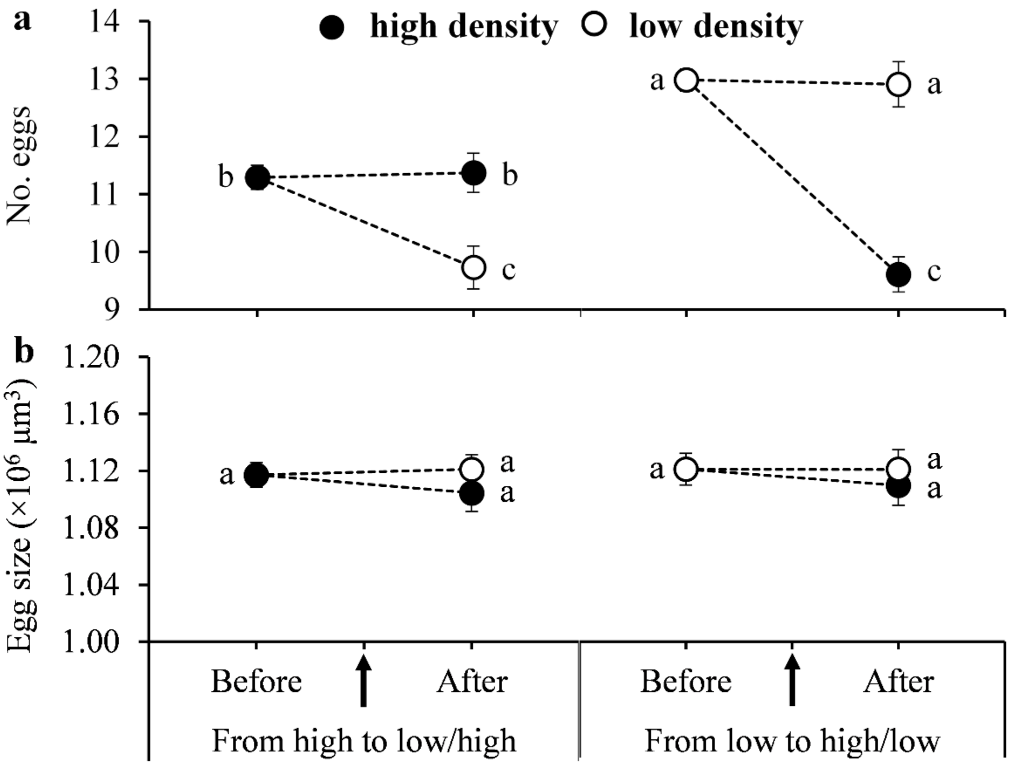


Figure 6.2 Effects of female population shift between high (●) and low (○) densities on the mean (± SE) number of eggs laid (**a**) and egg size (**b**) in *Tetranychus ludeni*. Closed and open circles with the same letters are not significantly different ($P > 0.05$). Female population density shift occurred on the fourth day of oviposition period.

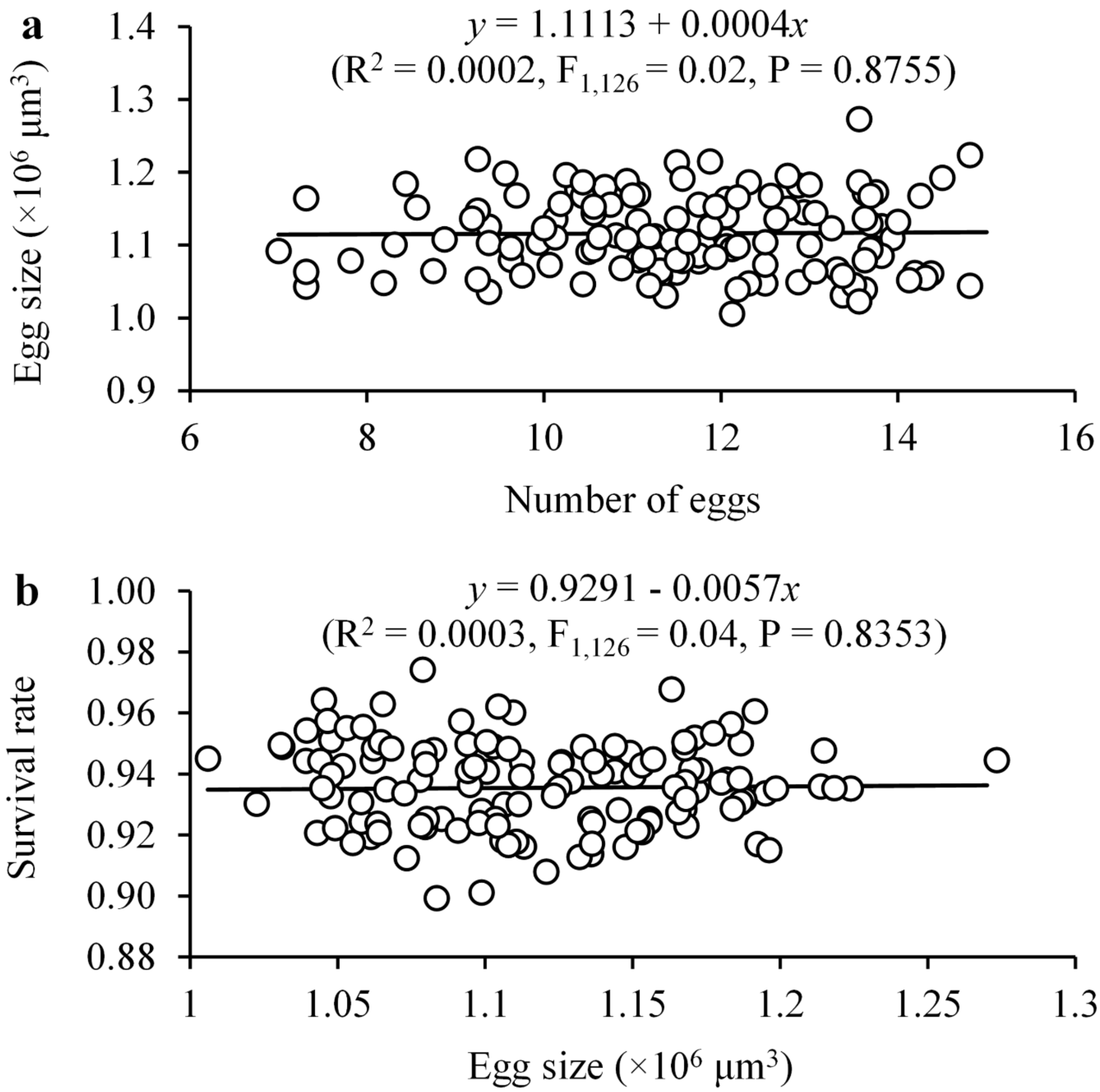


Figure 6.3 Relationships between egg size and number of eggs laid (**a**) and between egg size and immature survival rate (**b**) in *Tetranychus ludeni*.

6.3.2 Effect of population density shifts on sex allocation

I demonstrate that the sex ratio (proportion of daughters) was significantly higher at high density than at low density regardless of shifts; density shift from high to high, low to low or low to high significantly increased the sex ratio, and density shift from high to low significantly reduced the sex ratio ($F_{5,122} = 11.26$, $P < 0.0001$) (Figure 6.4). Egg size had no significant impact on sex ratio ($F_{1,126} = 0.03$, $P = 0.8614$) (Figure 6.5).

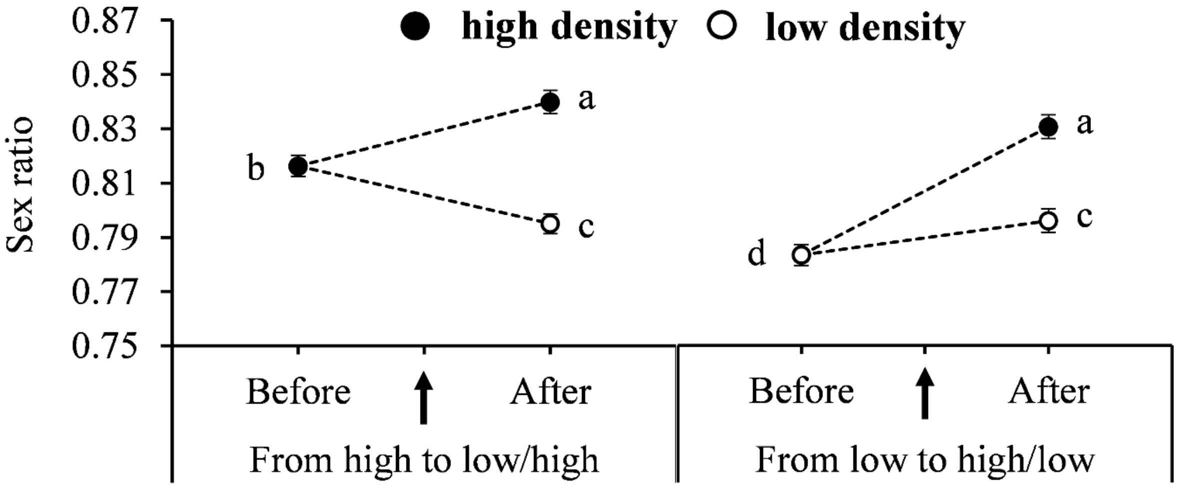


Figure 6.4 Effects of female population shift between high (●) and low (○) densities on the mean (± SE) sex ratio (proportion of females) in *Tetranychus ludeni*. Closed and open circles with the same letters are not significantly different ($P > 0.05$). Female population density shift occurred on the fourth day of oviposition period.

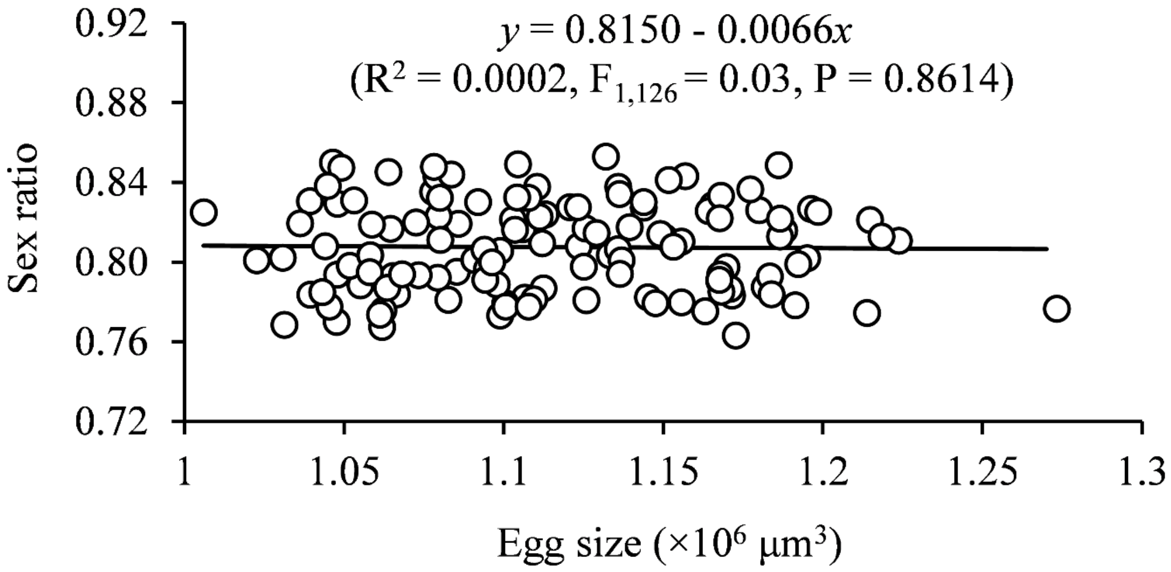


Figure 6.5 Relationships between sex ratio (proportion of female offspring) and egg size in *Tetranychus ludeni*.

6.4 Discussion

Our results indicate that *T. ludeni* females reduced their fecundity after the population density changed during their productive period (Figure 6.2a). I suggest that when the density quickly increases, they lower their fecundity to prevent the collapse of the local population due to the increase of resource competition and overexploitation of the host plants (Krips et al., 1998) or hostile interference or aggression among offspring for resource access (Estevez et al., 2007; Wong et al., 2013; Li and Zhang, 2021). Tetranychid mites construct silk webs (Saito, 1983; Mori and Saito, 2005; Clotuche et al., 2009; Le Goff et al., 2010) in the new habitats to protect themselves and their offspring from environmental hazards (Davis, 1952; McMurtry et al., 1970; Hazan et al., 1975; Ashley, 2003; Oku et al., 2003, 2004; Mori and Saito, 2005; Le Goff et al., 2010) but the silk consists of mainly proteins (Hazan et al., 1975), production of which incurs a considerable cost (Oku et al., 2009). Therefore, when they arrive in a new site of much larger area from a higher density population, they need to allocate more resources per female to produce enough silk to cover the area, leading to fecundity decline (Figure 6.2a).

I did not observe a trade-off between the egg number and size in response to the density changes (Figure 6.3a), challenging theoretical assumptions (Smith and Fretwell, 1974; Parker and Begon, 1986; Roff, 1992, 2002; Stearns, 1992; Fox and Czesak, 2000; Fischer et al., 2011). The lack of such trade-offs has also been reported in some animal species (e.g., Doughty and Shine, 1997; Zera and Harshman, 2001; Jordan and Snell, 2002; Bowden et al., 2004; Uller and Olsson, 2005). My results show that increasing egg size did not significantly increase the proportion of daughters (Figure 6.5), contradictory to the previous assumption that sex allocation in spider mites is mediated by egg size (Macke et al., 2011b). These findings suggest that *T. ludeni* females only adjust their fecundity but not egg size in response to density dynamics as reported in some birds (Christians, 2002) because egg size has little impact on reproductive fitness, such as offspring survival (Figure 6.3b) and sex allocation (Figure 6.5). Therefore, egg size is not a reliable indicator of offspring fitness when future environmental conditions are uncertain or unpredictable (Wiklund and Persson, 1983; Karlsson and Wiklund, 1985; McEdward and Carson, 1987; Lalonde, 2005; Morrongiello et al., 2012).

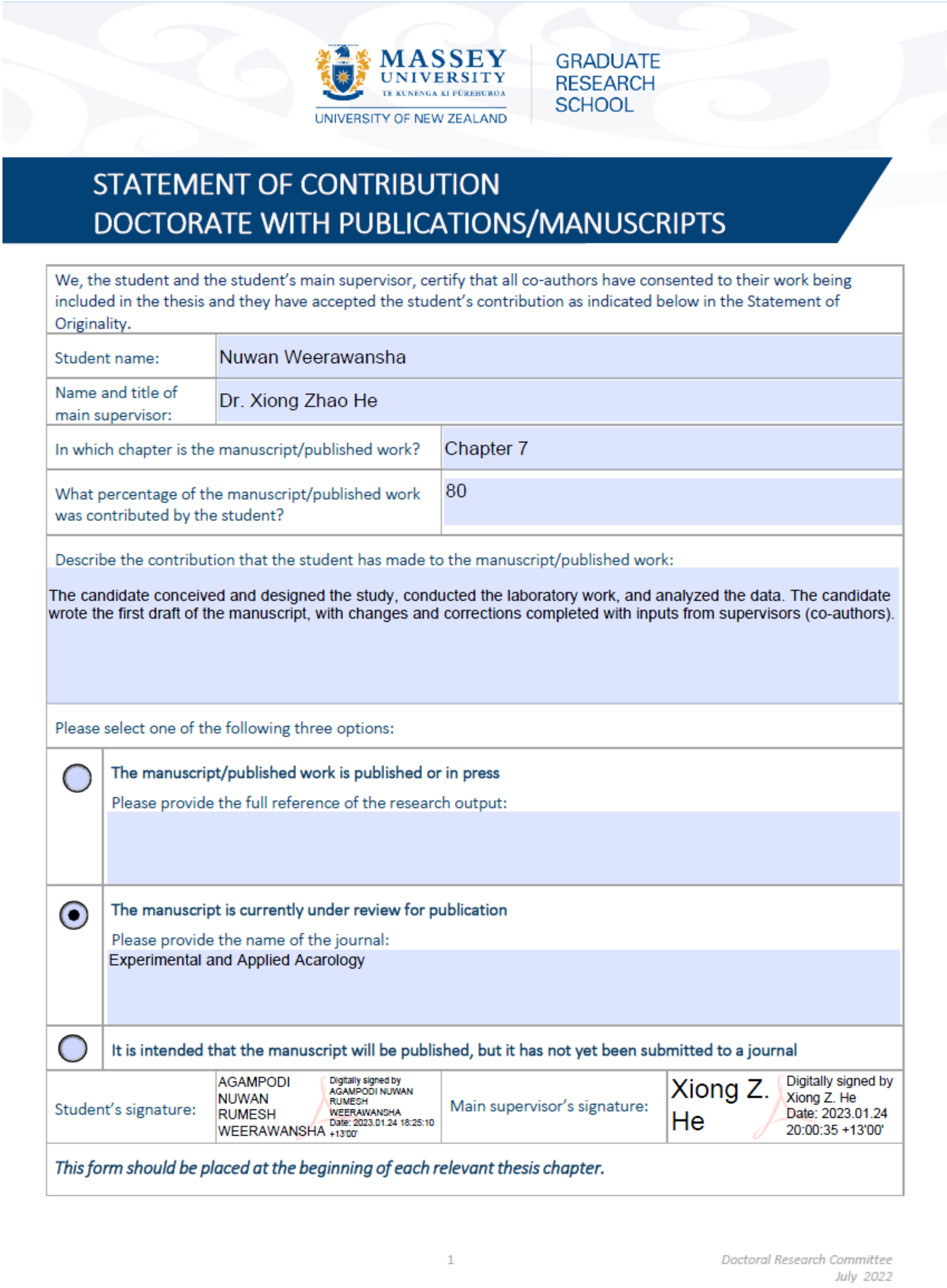
I demonstrate that regardless of density changes, offspring produced by females in high population density was significantly more female-biased than in low density (Figure 6.4). This could be due to sex-specific dispersal tendency in spider mites. Female spider mites usually disperse from dense conditions after mating (Suski and Naegele, 1968; McEnroe, 1969; Brandenburg and Kennedy, 1982; Li and Margolies, 1993) to establish new colonies (Mitchell, 1973; Brandenburg and Kennedy, 1982) and reduce future competition for food or space (Clark, 1978; Silk, 1983; Mari et al., 2008; Hjernquist et al., 2009; West, 2009; Visser et al., 2014; Song et al., 2016; Weerawansha et al., 2022a), resulting in production of more dispersing daughters in dense conditions. Compared to density shift from high to high or from low to low, that from low to high led to a faster increase in proportion of daughters produced (Figure 6.4). This suggests that *T. ludeni* females can quickly adjust their sex allocations in response to the change of social environment for optimal fitness of their offspring.

In the present study, I demonstrate that *T. ludeni* females could adjust their reproductive strategies in response to dynamic social environments during their reproductive period. Females reduce fecundity and produce more dispersive female offspring in dense environments, which will reduce the local resource competition. However, females do not adjust the egg size in response to the shift of population density, as egg size imposes no significant effect on fecundity and offspring sex ratio and survival. Therefore, *T. ludeni* females adapt to the shift of population density by manipulating the fecundity and offspring sex ratio but not the egg size. Whether *T. ludeni* females could manipulate sex allocation via adjusting egg size in response to the shift of population size remains unclear and is warranted for future investigations.

Chapter 7

Spider mite females adjust their life history traits in response to the changing group size during the breeding period

This chapter was submitted to *Experimental and Applied Acarology*.



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STATEMENT OF CONTRIBUTION
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We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Nuwan Weerawansha
Name and title of main supervisor:	Dr. Xiong Zhao He
In which chapter is the manuscript/published work?	Chapter 7
What percentage of the manuscript/published work was contributed by the student?	80

Describe the contribution that the student has made to the manuscript/published work:

The candidate conceived and designed the study, conducted the laboratory work, and analyzed the data. The candidate wrote the first draft of the manuscript, with changes and corrections completed with inputs from supervisors (co-authors).

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Doctoral Research Committee
July 2022

Abstract

Animals living in groups must adjust their reproductive strategies to adapt to the social environment. Yet, it is largely unknown how animals optimize their reproductive performance in response to the changing social environment during their breeding period. I used *Tetranychus ludeni* Zacher, a haplodiploid spider mite, to investigate whether the ovipositing females could modify their life history traits in response to the change of group size with a consistent group density (1♀/cm^2). I demonstrate that (1) after females were shifted from a large group (16 ♀) to small ones (1, 5 and 10 ♀), they laid fewer but larger eggs with a higher female-biased sex ratio; (2) after females were shifted from small groups to a large one, they laid fewer and smaller eggs with a higher female-biased sex ratio, and (3) increasing egg size significantly increased offspring sex ratio (daughters%), but increasing egg size did not increase immature survival. The results suggest that (1) shifting between populations of different sizes will alter the social environment, and ovipositing females may constrain their reproduction and produce more dispersers in response to such environmental fluctuations; (2) females fertilized more larger eggs laid in a small population but fertilized more smaller eggs laid in a large population by lowering the fertilization threshold, above which eggs were fertilized, and (3) the reproductive adjustments in terms of egg number and size may contribute more to minimize the mate competition among sons but not to increase the number of inhabitants in the next generation. The current study provides evidence that spider mites can manipulate their reproductive output and adjust offspring sex ratio in response to dynamic social environments.

Key words: dynamic social environments, reproductive performance, fertilization threshold, sex ratio

7.1 Introduction

Animals are predicted to live in groups when the benefits of group living outweigh the costs (Alexander, 1974; Bilde et al., 2007; Guindre-Parker et al., 2020; Tinsley Johnson et al., 2021). In group-living species, group size is an important component of social environment that influences individuals' behaviour (Prokopy and Duan, 1998; Majolo et al., 2008; Krams et al., 2009; Le Goff et al., 2010; Fryxell and Berdahl, 2018) and physiology (Lihoreau and Rivault, 2008; Clotuche et al., 2014; Markham et al., 2015; Markham and Gesquiere, 2017; Rudolph et al., 2019), altering their life history traits (Prokopy and Reynolds, 1998; Bilde et al., 2007;

Borries et al., 2008; Le Goff et al., 2010; Chapman and Valenta, 2015; Vanthournout et al., 2016; Tinsley Johnson et al., 2021). Some fitness components are expected to increase with increasing group size, for example, the reduction of predation risk (e.g., Spieler, 2003; Morrell and James, 2008; Yano, 2012; Saito and Zhang, 2017) and increase in reproductive success (e.g., Snead and Alcock, 1985; Prokopy and Reynolds, 1998; Le Goff et al., 2010; Pérez-González et al., 2010; Bonsignore and Jones, 2014). However, living in a large group may also reduce fitness because of the interference and food competition among group members (Bilde et al., 2007; Estevez et al., 2007; Grove, 2012; Wong et al., 2013; Li and Zhang, 2021; Tinsley Johnson et al., 2021).

In many empirical studies, researchers usually compare the effect of group size on the fitness by maintaining individuals in small and large groups of constant size during their reproductive period (e.g., Avileś and Tufinõ, 1998; Bilde et al., 2007; Le Goff et al., 2010; Li and Zhang, 2021). However, in nature, individuals often experience variations in population size due to frequent deaths, births, aggregation (immigration) and dispersal (emigration) (Roeder, 1992; Roff, 1992; Stearns, 1992; Price and Hunter, 1995; Bowman et al., 2002; Schausberger et al., 2021). As a result, animals must have developed reproductive plasticity to adapt to dynamic social environment to maximize the fitness of their offspring and their own (Ross et al., 2013; Radwan et al., 2014; De Roissart, 2015; Weerawansha et al., 2020, 2022a, 2022b; Tinsley Johnson et al., 2021).

The spider mites (Acari: Tetranychidae), such as invasive pests *Tetranychus ludeni* Zacher and *T. urticae* Koch, have been used as the model species to examine changes of reproductive strategies mediated by social environment (e.g., Le Goff et al., 2010; Macke et al., 2012a; Weerawansha et al., 2020, 2022a, 2022b, 2022c, 2022d). They live in patchy groups with varying group size and density and produce silk webs for dispersal and protection against predation and environmental hazards (Le Goff et al., 2010; Yano, 2012; Schausberger et al., 2021). Furthermore, group-living females elevate egg production compared to solitary ones (Le Goff et al., 2010; Weerawansha et al., 2022a). However, when the group size becomes too large (overcrowding), the costs of competition among individuals outweigh the benefits of aggregation and females may leave the oviposition site in search of favourable habitats (Azandémè-Hounmalon et al., 2014; Li and Zhang, 2021; Schausberger et al., 2021; Zhou et al., 2021a), reducing the local group size.

Like other haplodiploid species, mated spider mite females produce both haploid sons and diploid daughters (Young et al., 1986; Macke et al., 2011a; Zhou et al., 2018). They can manipulate offspring sex ratio in response to the social environment by adjusting egg size and fertilizing larger eggs that develop to daughters (Macke et al., 2011a, 2012a). Weerawansha et al. (2022a, 2022d) report that when *T. ludeni* females are maintained in groups of constant size during their lifetime, they lay fewer eggs but produce a higher female-biased offspring in small groups compared to the large ones. This may be an adaptive strategy to minimise the local mate competition between sons (Sato and Saito, 2007; Macke et al., 2012a, 2014; Weerawansha et al., 2022a, 2022b, 2022d). Moreover, larger eggs have a greater likelihood of developing to larger offspring that are more likely to reach maturity (Jackson and Martin, 2010; Macke et al., 2011b) and of higher fecundity (Zhou et al., 2018). To date, whether females could optimise their fecundity and manipulate offspring sex ratio in response to the change of group size due to aggregation or dispersal during their breeding period is largely unknown. Such knowledge is crucial for understanding the mechanisms of population dynamics in the changing social environment and assessing the invasion success in novel habitats.

In the present study, I used *T. ludeni* to investigate whether and how ovipositing females adjust their reproductive strategies in response to the temporal and spatial variations of group size. I simulated three scenarios of mite population size changes that frequently occur in nature, i.e., dispersal (decreasing population size), aggregation (increasing population size), and residence after settling in a habitat (consistent population size) during female reproductive period. I tested the hypothesis that compared to females in large groups, those in small groups lay fewer and larger eggs, giving rise to higher female-biased offspring with greater survival. My study provides insight into the mechanisms of reproductive adjustments by animals for optimizing their reproductive fitness gains in response to social environmental changes during breeding period.

7.2 Materials and methods

7.2.1 Mite colony

I started the colony of *Tetranychus ludeni* Zacher from adults collected on *Passiflora mollissima* (Kunth) in Palmerston North, New Zealand. I reared mites on the kidney bean plants (*Phaseolus vulgaris* L.) and used the first expanded leaves of 1 to 2-week-old plants for the experiment. I maintained the colony and carried out the experiment in two separate environmental rooms at 25 ± 1 ºC, 40 ± 10% RH, and a photoperiod of 14:8 h (light:dark).

7.2.2 Mite preparation

To obtain mated females, I randomly collected the quiescent female deutonymphs from the colony and individually transferred them onto a 1-cm^2 leaf square placed upside down on a wet cotton pad in a Petri dish (9.5 cm diameter × 1.0 cm height) with a mesh-sealed hole (1 cm diameter) in the middle of the lid. Before the female emerged (silvery in colour), I introduced a newly emerged male adult onto the leaf square. The male was produced by virgin females that developed from individually reared quiescent female deutonymphs randomly collected from the colony. The pair mated upon female emergence. The male was removed immediately after copulation ended. The newly mated females were used for the experiment. The mated females began to lay eggs on the emergence day, and some started to die six days after emergence (personal observation). I thus scheduled the shift of population size three days after emergence and used the data collected in the first six oviposition days for analysis.

7.2.3 Experimental design and data collection

To explore how group size changes during the breeding period of *T. ludeni* females altered their reproductive output and sex allocation, I kept the population density constant (1 ♀/cm^2 on leaf squares in Petri dishes mentioned above) during the experiment and set up three scenarios. (1) Dispersal – I simulated female dispersal during the experimental period by transferring the ovipositing females from a large group of 16 individuals on a 16-cm^2 leaf square to three small groups of 1, 5, and 10 females on 1-, 5-, and 10-cm^2 leaf squares, respectively, on the 4th day of oviposition. (2) Aggregation – I simulated female aggregation during the experimental period by transferring the females from three small groups of 1, 5, and 10 females ovipositing on 1-, 5-, and 10-cm^2 leaf squares, respectively, to a large group of 16 individuals on a 16-cm^2 leaf square on the 4th day of oviposition. (3) Residence – I simulated female residence by maintaining 1, 5, 10, and 16 females on 1-, 5-, 10-, and 16-cm^2 leaf squares, respectively, during the experimental period. Therefore, there were three treatments in scenario (1) or (2), and four treatments in scenario (3). Fifteen replicates were carried out for each treatment.

For each replicate in Dispersal and Aggregation, I transferred females onto a new leaf square of the same size daily for three consecutive days and then shifted them to leaf squares of desired population size on the 4th day. The shifted females were daily transferred onto a new leaf square of the same size for the following two days. For each replicate in Residence scenario, I transferred the females onto a new leaf square of the same size daily throughout the experimental period. Leaf squares were checked twice a day and any dead females were replaced immediately with that of the same age and reproductive history.

The number of eggs laid on each leaf square was recorded and the total number of eggs laid by individual females during days 1–3 and 4–6 were calculated. I measured the diameter of all, 10, 15 and 30 eggs on leaf squares with 1, 5, 10 and 16 females feeding on, respectively, under a stereomicroscope (Leica MZ12, Germany) connected to a digital camera (Olympus SC30, Japan) and imaging software (CellSens® GS-ST-v1.7, Olympus, Japan) installed in a computer. I then calculated the radius (r = diameter/2) and the egg size (volume = $4/3\pi r^3$). Egg hatching on each leaf square was recorded daily, and all alive individuals were transferred onto a clean leaf square of the same size once every five days. The number and sex of newly emerged adults were recorded. The immature survival rate was calculated as the number of eggs laid divided by the number of emerged adults.

7.2.4 Statistical analysis

I analysed data using SAS 9.4 with a reject level set at $\alpha < 0.05$. Data on the egg number and size were normally distributed (Shapiro-Wilk test; UNIVARIATE procedure). Thus, for the Dispersal or Aggregation scenario, a linear mixed model (MIXED procedure) was applied to compare egg number and size with group size as a fixed factor and replicate as a random factor, and a Tukey-Kramer test for multiple comparisons (Figures 7.1a–b and 7.2a–b). For each Residence treatment, I applied a paired t-test to compare the difference in the egg number and size between early (days 1–3) and late (days 4–6) reproductive episodes (Figures 7.1c and 7.2c). A generalized linear regression model (GLMMIX procedure) was used to analyse the data on offspring sex ratio (daughters%) (Figure 7.4) and immature survival rate with a binomial distribution and a *Logit* link function after the model, and a Tukey-Kramer test was applied for multiple comparisons. A generalized linear regression model (GLMMIX procedure) with a gamma distribution and a *Log* link function was applied to determine the relationships between number and size of eggs (Figure 7.3), between egg size and sex ratio (Figure 7.5), and between egg size and immature survival rate (Figure 7.6). The mean data for each female were calculated, and data of different treatments were pooled for regressions.

7.3 Results

7.3.1 Effect of population size changes on fecundity

Females laid significantly more eggs in the group of 16 ♀ before transferred to smaller groups of 1 and 5 ♀, where they laid significantly fewer eggs ($F_{3,42} = 17.86$, $P < 0.0001$) (Figure 7.1a). Before transferred to the group of 16 ♀, females laid significantly more eggs in the group of 10 ♀ than in groups of 1 and 5 ♀, but after transferred to the group of 16 ♀, they laid significantly fewer eggs ($F_{3,42} = 19.80$, $P < 0.0001$) (Figure 7.1b). In treatments of consistent group size, females in groups of 1 and 5 ♀ laid significantly more eggs during their late reproductive episode than the early one ($t_{14} = -2.82$ and -5.28 for 1 ♀ and 5 ♀, respectively; $P < 0.05$) (Figure 7.1c–d), whereas females in groups of 10 and 16 ♀ had similar fecundity in both reproductive episodes ($t_{14} = 0.43$ and -0.76 for 10 ♀ and 16 ♀, respectively; $P > 0.05$) (Figure 7.1e–f).

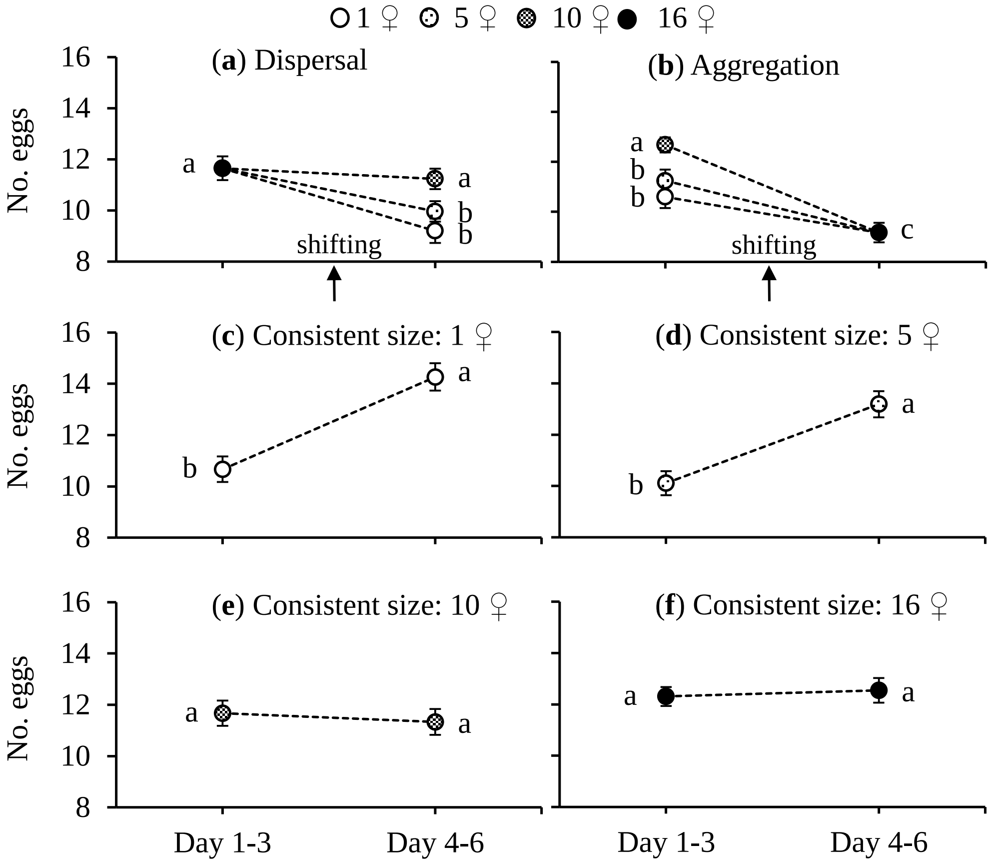

Figure 7.1 Mean (± SE) number of eggs laid by *Tetranychus ludeni* females during the early (days 1–3) and late reproductive episodes (days 4–6) when they dispersed from a large group to smaller groups (**a**), aggregated from smaller groups to a large group (**b**), or resided in groups of consistent size (**c**–**f**). The shift in group size under Dispersal and Aggregation was performed on the 4th day of oviposition. For each figure, cycles with the same letters are not significantly different ($P > 0.05$).

7.3.2 Effect of population size changes on egg size

Females laid significantly larger eggs after shifted from the group of 16 ♀ to groups of 1 and 5 ♀ but significantly smaller eggs after transferred from groups of 1 and 5 ♀ to the group of 16 ♀ ($F_{3,42}$ = 122.31 and 95.19 for Dispersal and Aggregation scenarios, respectively; $P < 0.0001$) (Figure 7.2a–b). If group size remained consistent, egg size did not change significantly during the two reproductive episodes (t_{14} = –0.43, 2.10, –2.13 and 1.44 for treatments of 1 ♀, 5 ♀, 10 ♀ and 16 ♀, respectively; $P > 0.05$) (Figure 7.2c–e). Furthermore, the egg size significantly decreased with the increase of egg numbers (Figure 7.3).

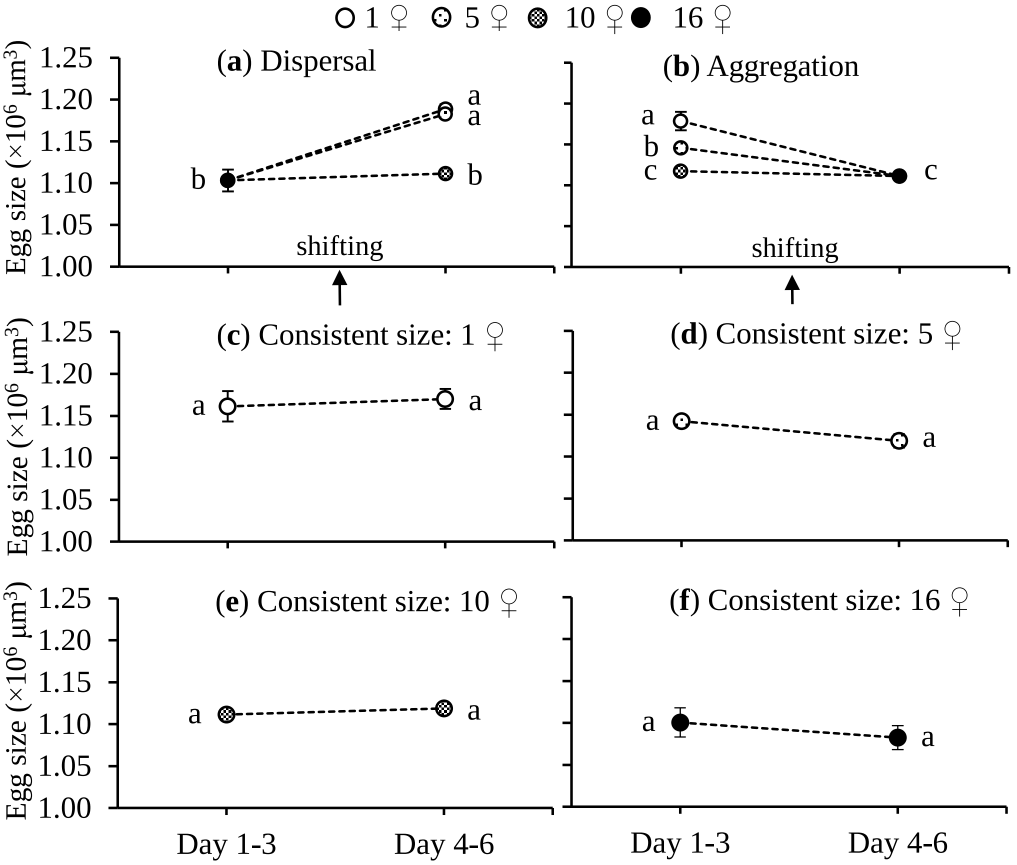

Figure 7.2 Mean (± SE) size of eggs laid by *Tetranychus ludeni* females during the early (days 1–3) and late reproductive episodes (days 4–6) when they dispersed from a large group to smaller groups (**a**), aggregated from smaller groups to a large group (**b**), or resided in groups of consistent size (**c**–**f**). The shift in group under Dispersal and Aggregation was performed on the 4th day of oviposition. For each figure, cycles with the same letters are not significantly different ($P > 0.05$).

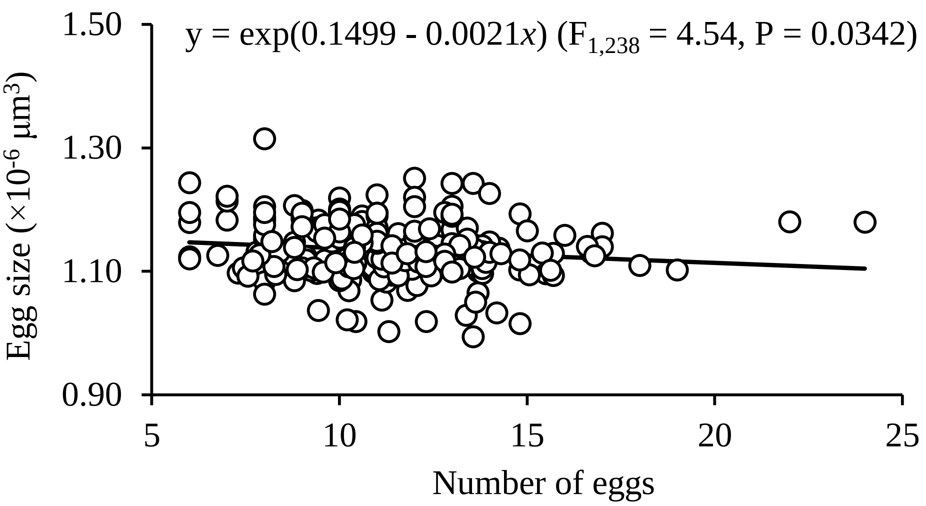

Figure 7.3 Relationship between the egg size and number in *T. ludeni*.

7.3.3 Effect of population size changes on sex ratio and survival of offspring

Offspring sex ratio was female-biased (77.5–93.5% daughters) regardless of mothers' group size (Figure 7.4). After transferred from large to small groups, females produced significantly more female-biased offspring ($F_{3,42} = 8.93$, $P < 0.0001$) (Figure 7.4a) but after transferred from groups of 5 and 10 ♀ to the group of 16 ♀, they generated significantly more female-biased offspring ($F_{3,42} = 5.80$, $P = 0.0021$) (Figure 7.4b). Regardless of the shifting scenarios, the sex ratio (daughters %) was significantly higher in group of 1 ♀ than in group of 10 ♀ (Figure 7.4a–b). If group size remained consistent, females produced significantly more female-biased offspring in the late reproductive episode than in the early one ($F_{1,27} = 4.39$, 7.07, 8.02 and 4.23 for 1, 5, 10 and 16 ♀, respectively; $P < 0.05$) (Figure 7.4c–f). A significant positive relationship was detected between the sex ratio and egg size (Figure 7.5). The mean immature survival rate ranged between 0.85 and 0.94 which was not significantly different between the early and late reproductive episodes regardless of the shifting scenarios ($F_{3,42} = 2.08$ and 1.95 for Dispersal and Aggregation scenarios, respectively; $P > 0.05$) or for groups of consistent size ($F_{1,27} = 1.76$, 0.03, 2.73 and 0.61 for 1, 5, 10 and 16 ♀, respectively; $P > 0.05$). Egg size had no significant impact on immature survival rate (Figure 7.6).

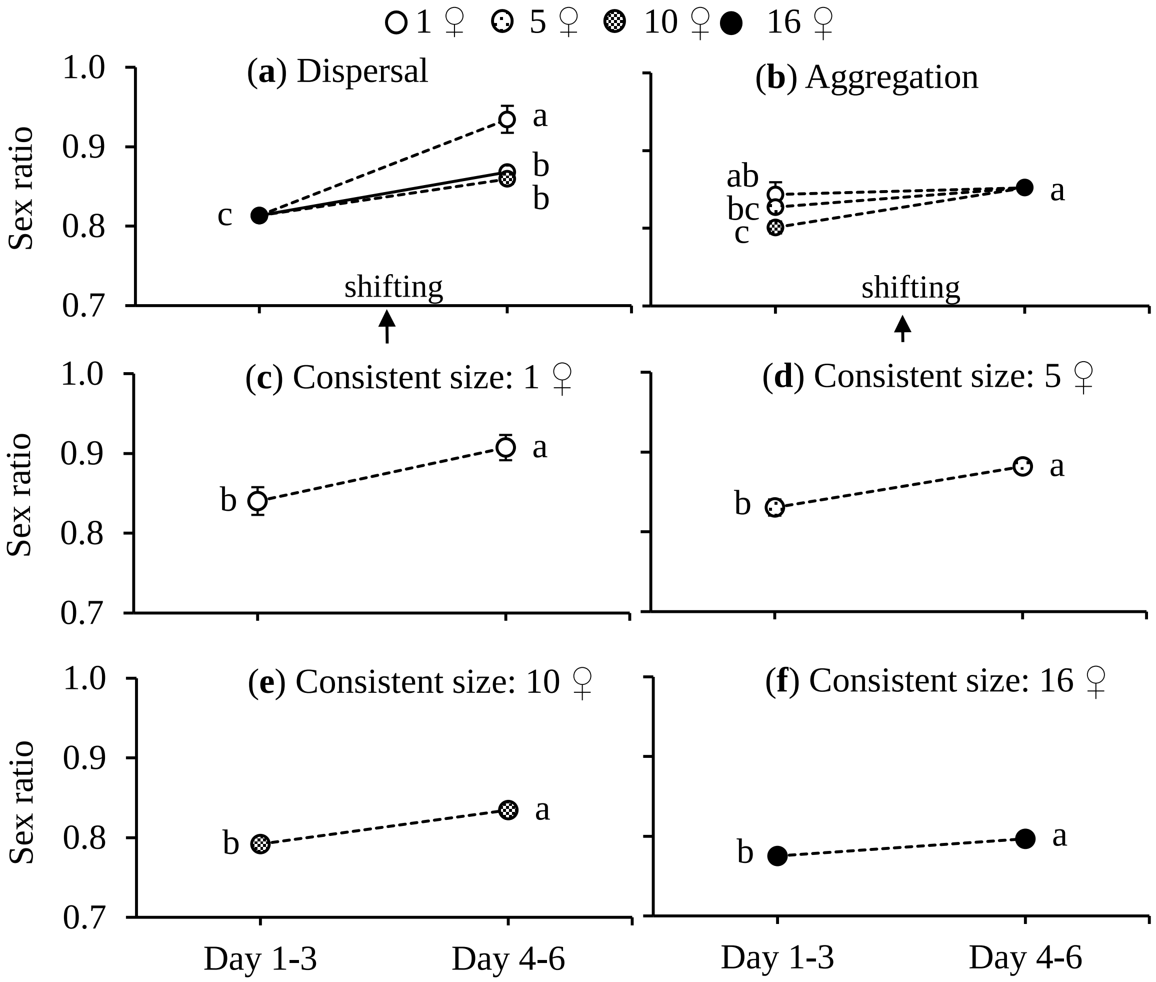

Figure 7.4 Mean (± SE) sex ratio (daughters%) of *Tetranychus ludeni* females during the early (day 1–3) and late reproductive episodes (day 4–6) when they dispersed from a large group to smaller groups (**a**), aggregated from smaller groups to a large group (**b**), or resided in groups of consistent size (**c**–**f**). The shift in group size under Dispersal and Aggregation was performed on the 4th day of oviposition. For each figure, cycles with the same letters are not significantly different ($P > 0.05$).

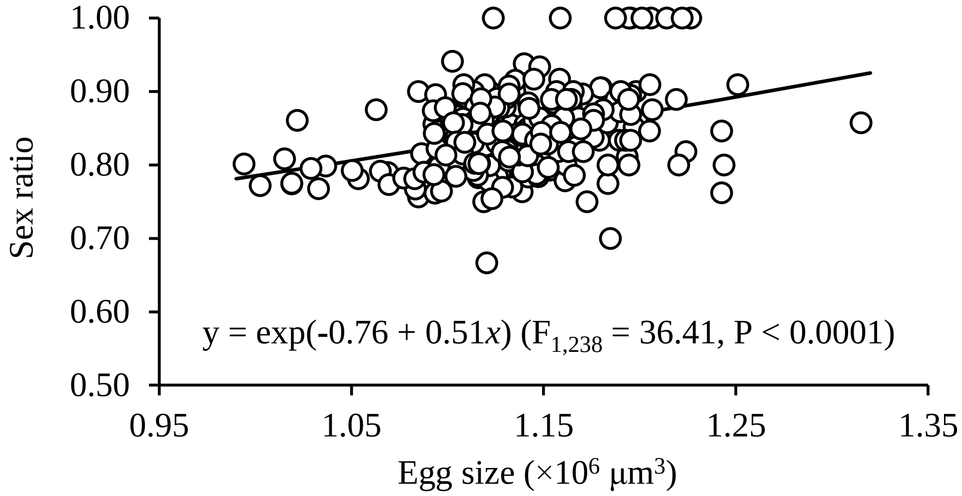

Figure 7.5 Relationship between the sex ratio (daughters%) and egg size in *Tetranychus ludeni*.

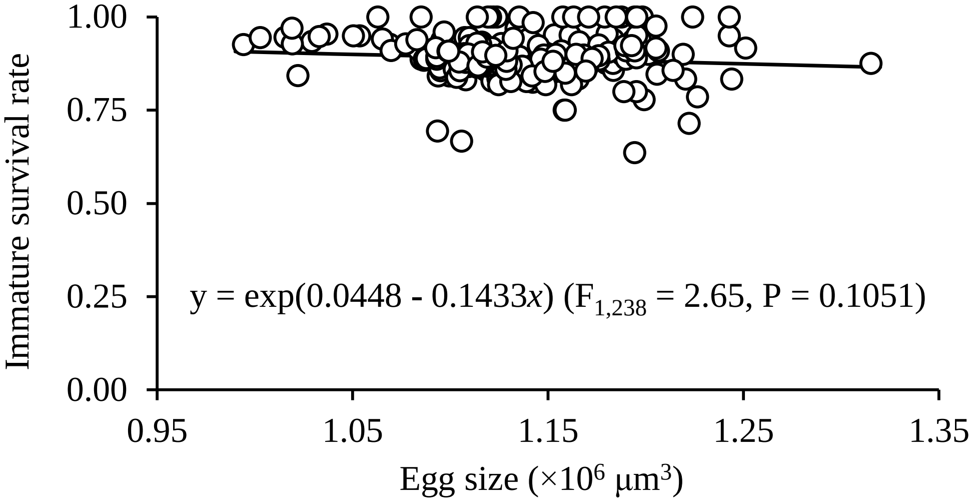

Figure 7.6 Immature survival rate in relation to egg size in *Tetranychus ludeni*.

7.4 Discussion

I demonstrate that *T. ludeni* females could manipulate their reproductive strategies in response to changes in group size during the breeding period. As expected, the ovipositing females laid significantly more eggs in a large group of 16 ♀ before they dispersed and settled in smaller groups of 1, 5, and 10 ♀ (Figure 7.2a). It is well known that spider mites collectively spin common silk webs, which help disperse and protect them from predation and environmental hazards (Le Goff et al., 2010; Yano, 2012; Schausberger et al., 2021). Nevertheless, silk is composed mainly of proteins, and thus silk production costs energy and nutrient reserved (Hazan et al., 1975; Oku et al., 2009). Living in a group may confer the advantage of sharing the spun webs or reducing the intensity (i.e., thinner, shorter, and/or fewer silks) of web production (Hazan et al., 1975) so that the energy and nutrient saved in web production can be invested in reproduction (Oku et al., 2009; Le Goff et al., 2010). In addition, group-living individuals could benefit from modifying plant biochemistry, such as breaking down the plant defense system, resulting in more favourable nutritional quality of the shared host plants (Kant et al., 2008; Rioja et al., 2017), which may also elevate female reproduction.

I further find that females in small groups of consistent size (i.e., 1 and 5 ♀) laid significantly more eggs in the late reproductive episode than in the early one, but those in large groups of consistent size (i.e., 10 and 16 ♀) had similar fecundity during the two reproductive episodes (Figure 7.2c–f). Following this line, it is expected that in the Dispersal and Aggregation scenarios, females would increase or at least maintain the fecundity in the late reproduction; however, contradicting to the expectation, females reduced reproduction after they were shifted from a large to small groups (i.e., 1 and 5 ♀) (Figure 7.2a) or from small groups to a large one (Figure 7.2b). I suggest that ovipositing females might constrain their reproduction in response to group size changes. Previous experimental and theoretical studies have also shown that a lower reproductive rate in response to unpredictable environments can confer a long-term selective advantage with regard to population persistence (Gilpin, 1975; Nathanson, 1975; Wade, 1980; Sober and Wilson, 1998; Reed et al., 2010).

In the present study, I show a consistent pattern of adjustment in egg size and offspring sex ratio of *T. ludeni* females in smaller groups, i.e., 1 ♀ > 5 ♀ > 10 ♀, regardless of their shifting scenarios (Figures 7.3a–b and 7.5a–b) and demonstrated a significantly positive relationship between the egg size and offspring sex ratio (Figure 7.6). These results agree to my hypothesis that *T. ludeni* females could promote the offspring fitness by producing larger eggs and more daughters, and thus reducing the intensity of mate competition between their sons in smaller groups (Macke et al., 2011a, 2012a; Weerawansha et al., 2022a, 2022b, 2022d). I further reveal that after aggregating into a large group, females laid significantly smaller eggs (Figure 7.3b) but produced a significantly higher female-biased sex ratio (Figure 7.5b), which may be attributed to the flexibility of egg fertilization in spider mites. Macke et al. (2011b) report that in spider mites, mated females will fertilize eggs when the size of those eggs exceeds a threshold value. Therefore, my results suggest that *T. ludeni* females could adjust the fertilization threshold to a lower level and fertilise relatively smaller eggs.

In consideration of the fact that mothers have finite resources partitioned to their offspring (Bernardo, 1996; Fox and Czesak, 2000), they can either produce many small or a few large offspring with a balance or trade-off between egg number and size (Roff, 1992; Einum and Fleming, 2000; Krist, 2011; Morrongiello et al., 2012). In spider mites, as females allocate more resources to fertilised eggs (Macke et al., 2011b, 2012a), it is not surprising that increasing egg size induced a significant decrease of egg number in this study (Figure 7.4). However, increasing egg size had no significant impact on immature survival (Figure 7.7), suggesting that females increasing egg provision may contribute to the female-biased offspring sex ratio (Figure 7.6) rather than the survival of offspring (Figure 7.7). Therefore, in response to the variations of group size, the reproductive adjustments in terms of egg number and size will minimise the mate competition among sons (i.e., high female-biased offspring sex ratio), rather than increase the number of inhabitants in the next generation.

In summary, I show that *T. ludeni* females lay more eggs in a large group before they are shifted to the small groups probably due to the enhanced cooperation in spinning webs so that the conserved energy and resources could be invested into reproduction. I first demonstrate that the ovipositing females constrain reproduction in response to the alternation of social environment during their reproductive period. I further reveal that females trade off egg number for size and fertilise larger eggs that are more likely to give rise to daughters, and they could lower the fertilization threshold to fertilise more eggs when egg size is small in response to the alternation of social environment. However, increasing egg size does not increase immature survival, thus females increasing provisioning in egg size is to promote the female-biased sex ratio rather than the survival of offspring. My study provides insight into the adaptive responses of haplodiploid animals to varying social environments.

Chapter 8

General Discussion

8.1 Introduction

The local population size and density of animals are two key elements of social environment that regulate population dynamics in a local habitat (Kunin, 1997a; Estevez et al., 2007; Danko et al., 2018; Webber and Wal, 2018; Ausband and Mitchell, 2021). However, few studies have distinguished the different effects of population size and density on animal reproductive performance due to their high inter-correlations in most natural populations (Amsalem and Hefetz, 2011). In this thesis, I have carried out a series of experiments to investigate how *T. ludeni* females maximise the fitness of their own and their offspring by adjusting their life history strategies in response to the consistent and varying social environments. Knowledge generated is essential for understanding the mechanisms behind oviposition, sex allocation and thus local population dynamics. In this chapter, I summarise my main findings, discussed their ecological implications, and made recommendations for future studies.

8.2 Effects of population size and density on oviposition, sex allocation and population growth in *Tetranychus ludeni*

My work provides the first empirical evidence that the haplodiploid female mites adjust their reproductive performance in response to the local population size and density. As reported in many animal species (e.g., Sinclair, 1989; Kempenaers and Dhondt, 1992; Murdoch, 1994; Harrison and Cappuccino, 1995; Turchin, 1995; Both, 1998b; Turchin, 1999; Sibly et al., 2005), *T. ludeni* females lower their reproductive output in large and dense populations (Figures 2.2, 2.3, 2.4a and 3.1b). Spider mites often live in groups (Dhooria, 2016). This feature may result inscramble competition (Krips et al., 1998) and increase direct interference among individuals (Post et al., 1997). Therefore, when conspecific competition for resources increases, *T. ludeni* females reduce their reproduction (Krebs 1971; Rodenhouse et al., 1997; Leips and Travis, 1999; Creighton, 2005; Goubault et al., 2007). Lowering fecundity under stressful conditions can be an adaptive strategy of ovipositing females to increase the amount of food available to their offspring (Bonduriansky and Head, 2007; De Roissart et al., 2016) and minimise the possibility of local population extinction.

I further reveal that although the negative density-dependent reproduction is detected only in larger populations (Figures 2.2, 2.3, 2.4a and 3.1b), females increase the number of eggs laid and daughters produced much earlier and faster in large populations regardless of the population density (Figures 2.2 and 2.3). The results have two implications. First, *T. ludeni* females may adjust reproductive strategies to optimise their own and their offspring's fitness, as producing more daughters early would enable more dispersing individuals to complete development, reducing future food competition intensity (Osakabe et al., 2008; Yano, 2008; Le Goff et al., 2010; Azandémè-Hounmalon et al., 2014) and promoting population growth (i.e., higher intrinsic rate of increase but shorter doubling time) (Figure, 2.4b and 2.4d). Second, population size may have overweighed population density as a major socio-environmental factor regulating future population dynamics in *T. ludeni*.

It is predicted that females should bias their investment to one sex over the other, when selection favours the facultative adjustment of offspring sex ratios to the sex with the highest inclusive fitness return (Charnov, 1982; West, 2009). My results confirm that ovipositing females could skew the sex ratio towards more female biased in response to the decrease in population size (Figure 3.2), reducing the local mate competition (LMC) among sons (Hamilton, 1967; Sato and Saito, 2006; Macke et al., 2011a, 2012a, 2014). There is a wide array of taxa that have shown female-biased sex ratios consistent with LMC prediction (Hamilton, 1967; Maynard-Smith, 1978; Taylor, 1981; Charnov, 1982; Frank, 1985; Herre, 1985; West, 2009).

I also demonstrate a significant positive interaction of population size and density, which results in an increase of female-biased sex ratio in large and dense populations (Figure 3.2b), although population density alone has no significant effect on offspring sex ratio (Figure 3.2a). These results suggest that in small populations, *T. ludeni* females produce more daughters to reduce mate competition between sons while in large and dense populations they give birth to more dispersing daughters to lower the local resource competition (LRC) (Clark, 1978). The findings indicate that *T. ludeni* females are capable of shifting the offspring sex allocation in response to the social environment they live in. My results also reveal that population size mediates the population density-dependent sex allocation (Figure 3.2b) and fecundity (Figure 3.1b) in *T. ludeni*. Therefore, we may predict the pest population dynamics more precisely when both population size and density as well as their interaction are accounted for analysis.

8.3 Extremely high female-biased sex ratio in *Tetranychus ludeni*

My findings demonstrate that the predictions of LMC models only fitted the observed sex ratios (proportion of male offspring) in small populations with intensive inbreeding, but the observed sex ratio in the large populations was significantly lower than that of the predictions (Table 4.1). The extremely high female-biased sex ratio under LMC is frequently reported in haplodiploid insects such as ambrosia beetles, parasitoid wasps, and fig wasps (Herre, 1985; Molbo and Parker, 1996; Peer and Taborsky, 2004; Burton-Chellew et al., 2008). However, the causes for such extremities are mostly species-specific (Peer and Taborsky, 2004). In haplodiploid species, optimal sex ratio further depends on the level of inbreeding, because inbreeding increases the relatedness asymmetry between a mother and her sons and daughters. Females are always equally related to their sons, but with increasing levels of inbreeding they become more related to their daughters. Therefore, inbreeding should be included in modelling sex allocation when animals live in groups.

The extremely female-biased sex ratio in *T. ludeni* may be attributed to the life history characteristics of spider mites. First, as a haplodiploid species, both virgin and mated females are able to found next generation [i.e., virgin females mate with their sons and produce granddaughters and grandsons (Zhou et al., 2018)] but males have much higher fecundity (fertilising 850–1400 eggs; Zhou et al., 2018)] than females (up to 60 eggs; Figure 2.2). Therefore, mothers increasing investment in daughters should have greater fitness return (Seger and Stubblefield, 2002). Second, although daughters are more likely to compete with their mothers or siblings for local resource due to their large body size, they usually disperse from the dense conditions after mating (Li and Margolies, 1993). Thus, production of more dispersing daughters will eventually reduce LRC as well as LMC. Third, spider mite females may cooperate as a form of local resource enhancement (LRE) in spinning silk webs which are crucial for dispersal and protection against environmental hazards (Le Goff et al., 2010). In the scenario of LRE, females are the helpers and thus production of more daughters may help improve the local living environment. My results suggest that under varying social environments, LMC, LRC and LRE could be the mechanisms behind the extremely female-biased sex ratio in *T. ludeni*.

8.4 Effects of social environments and life history traits on sex allocation in *Tetranychus ludeni*

I develop a novel methodology to compare the direct and indirect effects of social environments and life history traits on sex allocation in a haplodiploid spider mite. I deliver evidence that the effects of life history traits on *T. ludeni* sex allocation outweigh that of social environments, and social environments influencing offspring sex ratios are mediated through life history traits (i.e., egg number and egg size) (Table 5.2). I further confirm that population size affecting *T. ludeni* sex allocation is implemented with population density and *vice versa* (Table 5.2) even though the life history traits are excluded for analysis (Figure 3.2).

I demonstrate a significant positive effects of egg size on sex ratio, suggesting that *T. ludeni* females could adjust offspring sex ratio in response to the varying social environments by altering the egg size and fertilizing larger eggs that develop to daughters. Similar findings are also reported in other haplodiploid species, such as the two-spotted spider mite *T. urticae* (Macke et al., 2011b, 2012a) and thrip *P. kellyanus* (Katlav et al., 2021). My results reveal that the number of eggs produced has a significant positive effect on sex ratio. This is because the production of daughters and sons increases significantly with increasing egg number, but the number of daughters produced increases significantly faster than that of sons, entailing a strong female-biased sex ratio in a population.

My PhD work shows that among the test socio-environmental factors and life history traits, maternal age imposes a significantly greater effect on sex ratio and explains more variance (Figure 5.2, Table 5.2). These may be attributed to two reasons: (1) the mated mothers do not experience any sperm depletion, as single mating is sufficient to fertilize eggs produced by a female during its life (Figure 3.2b), and (2) the mothers tend to increase fecundity as well as daughter production during their early life (Table 5.3), resulting in a significant positive indirect effect on sex ratio (Figure 5.2). However, my results indicate that a large amount of variance (> 70%) in sex ratio remains unexplained by the test social environments and life history traits, suggesting that sex allocation in *T. ludeni* is more complex than expected. Further experiments integrating more socio-environmental factors and life history traits may help disclose the complexity of sex allocation strategies in haplodiploid organisms.

8.5 Effects of shifting social environments on reproductive performance in *Tetranychus ludeni*

I establish that *T. ludeni* females adjust their reproductive strategies in response to the dynamics of population density and size during their reproductive period differently. Females lay significantly fewer eggs in a dense population before they are shifted to a sparse population (Figure 6.2a) but more eggs in a large population before they disperse to smaller populations (Figure 7.1a). Females reducing reproduction in a dense habitat (Figure 6.2a) should be an adaptive strategy to minimise the intensity of food competition, interference and aggression (Post et al., 1997; Krips et al., 1998; Grove, 2012; Li and Zhang, 2021). However, in a large population, females may strengthen cooporation in shared tasks, e.g., collective settlement and web building (Saito, 1977; Gutierrez and Helle, 1985; Sabelis and Bakker, 1992; Saito, 2010; Clotuche, 2011; Clotuche et al., 2012, 2014), so that the energy and nutrients saved in web production can be invested in reproduction (Oku et al., 2009; Le Goff et al., 2010).

I further reveal that females reduce reproduction regardless of the shifts of ovipositing females between the dense and sparse populations or between the small and large ones (Figures 6.2a and 7.1a). The results suggest that shifting between populations of different densities or sizes will alter the social environment, and ovipositing females may constrain their reproduction in response to such environmental fluctuations. Previous experimental and theoretical studies have also shown that a lower reproductive rate in response to unpredictable environments can confer a long-term selective advantage in favour of population persistence (Gilpin, 1975; Nathanson, 1975; Wade, 1980; Sober and Wilson, 1998; Reed et al., 2010).

Egg size is a crucial life history trait that has profound impacts on offspring fitness in animals (Krist, 2011; Morrongiello et al., 2012; Wiernasz and Cole, 2018; Katlav et al., 2021), especially in the haplodiploid species, such as the spider mites, in which the mated females could manipulate offspring sex ratio by adjusting egg size and fertilizing relatively larger eggs (Macke et al., 2011b, 2012a, 2014). However, I find that females do not manipulate egg size in response to the shift of population density (Figure 6.2.b), as females receive little fitness return from having a large egg size, i.e., egg size has no significant impact on fecundity, offspring survival (Figure 6.3) and offspring sex ratio (Figure 6.5). Females produce a more female-biased sex ratio in a population of high density than in that of low density (Figure 6.4). This would reduce future competition for food or space (Clark, 1978; Silk, 1983; Mari et al., 2008; Hjernquist et al., 2009; West, 2009; Visser et al., 2014; Song et al., 2016; Weerawansha et al., 2022a, 2022c) because daughters usually disperse from dense conditions after mating (Suski and Naegele, 1968; McEnroe, 1969; Brandenburg and Kennedy, 1982; Li and Margolies, 1993).

In contrast, I demonstrate a consistent pattern of adjustment in egg size and offspring sex ratio in smaller groups regardless of the shifts of population size (Figures 7.2a–b and 7.4a–b), and a significant positive relationship between the egg size and offspring sex ratio (Figure 7.5). These results agree to the general sex allocation strategy of spider mites that females could promote the offspring fitness by producing larger eggs and more daughters, and thus reducing the intensity of mate competition between their sons in smaller groups (Macke et al., 2011a, 2012a, 2014). I also show that after being shifted from small populations to a large one, females lay significantly smaller eggs (Figure 7.2b) but have a significantly higher female-biased sex ratio (Figure 7.4b). The results suggest that *T. ludeni* females could adjust the fertilization threshold to a lower level and fertilise relatively smaller eggs, as spider mite females fertilize eggs only when the size of those eggs exceeds a certain threshold value (Macke et al., 2011b).

8.6 Conclusion

In this study, I demonstrate that *T. ludeni* females adjust their reproductive performance in response to the consistant and dynamic social environments in terms of population density and size. Females adopt unique reproductive strategies, including differential resource allocations, reproductive output and offspring sex allocation, to optimize the fitness of the offspring and their own. Knowledge generated in this thesis is crucial for predicting the pest population dynamics. However, the social environment in nature is much more complex, for example, *T. ludeni* may co-exist with other sibling species such as *T. urticae* and *T. evansi* as well as their natural enemies such as the predatory mites. Future studies on the impacts of social cues from the sibling species and their predatory mites on dispersal, habitat selection and subsequent reproduction, sex allocation, and survival of *T. ludeni* may provide insights into the mechanisms of *T. ludeni* population dynamics in a complex ecosystem.

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Appendix: Published papers from my PhD studies

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Article

Effect of foundress population density and size on reproduction and population growth of a haplodiploid mite

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Abstract

Tetranychus ludeni Zacher (Acari: Tetranychidae) is an invasive polyphagous haplodiploid pest mite of many vegetable crops in many parts of the world. This study investigated the effects of simultaneous variations in the local foundress population density and size on the reproduction and population growth of *T. ludeni*. We show that negative population-density and -size dependency limited the reproduction of foundresses, where the effects of population size on population growth overweighed that of population density. We further demonstrate that ovipositing females could accelerate population growth rate (intrinsic rate of increase, r_m) at higher population sizes by producing more daughters early during their lifespan, which can be an adaptive strategy to allow more daughters to complete development and disperse to reduce future food competition intensity. This study provided knowledge on population dynamics in response to foundress population density and size in general and *T. ludeni* population forecast and precise timing of pest management in particular.

Key words: *Tetranychus ludeni*, daughter production, disperse, resource competition, life table parameter

Introduction

The ability of organisms to increase their populations is crucial to their establishment in a new habitat especially for the invasive species. Local foundress population density and size are two important socio-environmental factors that regulate reproduction and population growth (Kunin 1997a; Danko *et al*. 2018). The former represents a function of spacing between neighbouring individuals in a finite area and the latter is the number of individuals in a local population (Kunin 1997a). In nature, resources are unevenly partitioned among individuals, and this inequality increases when resources become scarce with increasing population density and size (Krebs 1971; Łomnicki 1978; Rodenhouse *et al*. 1997; Leips & Travis 1999; Creighton 2005; Goubault *et al*. 2007). Therefore, it is fundamentally important to understand the nature and extent of population regulation by density- and size-dependent processes in population ecology (Levins 1979; Newton 1994, 1998; Ferrer & Donazar 1996; Rodenhouse *et al*. 1997; Penteriani *et al*. 2003).

It is widely reported that population growth is a decreasing function of population density (Harrison & Cappuccino 1995; Turchin 1995; Sibly *et al*. 2005), inducing a negative density-dependent reproduction associated with present and/or past population densities (Adler & Levins 1994; Sinervo *et al*. 2000; Rotem & Agrawal 2003; Meylan *et al*. 2007; Allen *et al*. 2008; Creighton 2005; Leips *et al*. 2009). The negative density-dependent reproduction could arise either through decreasing territory quality and resource scarcity which limit the individuals to low provisioning rates at high population densities (Andrewartha & Birch 1954; Pulliam & Danielson 1991; Dhondt

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Article

Adjustment of fecundity and sex ratio in response to social environments in a haplodiploid mite

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Abstract

Animals can adjust reproductive strategies in favour of corporation or competition in response to local population size and density, the two key factors of social environments. However, previous studies usually focus on either population size or density but ignore their interactions. Using a haplodiploid spider mite, *Tetranychus ludeni Zacher*, we carried out a factorial experiment in the laboratory to examine how ovipositing females adjust their fecundity and offspring sex ratio during their early reproductive life under various population size and density. We reveal that females laid significantly more eggs with increasing population size and significantly fewer eggs with increasing population density. This suggests that large populations favour cooperation between individuals and dense populations increase competition. We demonstrate a significant negative interaction of population size and density that resulted in significantly fewer eggs laid in the large and dense populations. Furthermore, we show that females significantly skewed the offspring sex ratio towards female-biased in small populations to reduce the local mate competition among their sons. However, population density incurred no significant impact on offspring sex ratio, while the significant positive interaction of population size and density significantly increased the proportion of female offspring in the large and dense populations, which will minimise food or space competition as females usually disperse after mating at crowded conditions. These results also suggest that population density affecting sex allocation in *T. ludeni* is intercorrelated with population size. This study provides evidence that animals can manipulate their reproductive output and adjust offspring sex ratio in response to various social environments, and the interactions of different socio-environmental factors may play significant roles.

Key words: population size, population density, interaction, spider mite, *Tetranychus ludeni*, reproduction, sex allocation

Introduction

Animals in patchy habitats often face intraspecific competitions (Iritani *et al.* 2020; Ausband & Mitchell 2021; Li & Zhang 2021) and adjust their reproductive strategies in response to social environments (Lihoreau & Rivault 2008; Ruan & Wu 2008; Krams *et al.* 2009; Schausberger *et al.* 2017; Conroy & Roff 2018; Crocker & Hunter 2018; Li & Zhang 2021). The number of individuals in a local population(population size) and the number of individuals per unit area in a habitat (population density) are two key socio-environmental forces that drive population regulations (Estevez *et al.* 2007; West 2009; Sprenger *et al.* 2011; Weerawansha *et al.* 2020; Ausband & Mitchell 2021). Fluctuations in population size and density alter the resources available for local individuals so that they must change their fecundity and offspring sex ratio accordingly to adapt (Wauters & Lens 1995; Estevez *et al.* 2007; Webber & Wal 2018; Burant *et al.* 2020; Weerawansha

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Letter to the Editor

Local mate competition model alone cannot predict the offspring sex ratio in large and dense populations of a haplodiploid arthropod

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Key words: competition, cooperation, population size and density, sex allocation.

Sex ratio in animals has profound impacts on the fitness of mothers and their offspring. When the local competition intensity is sex-specific, selection favors the sex that may mitigate such negative impacts (West 2009). This may occur under 1) local mate competition (LMC)—in a discrete population established by one or a few foundresses, they tend to produce female-biased offspring sex ratio to reduce the LMC between related males (Hamilton 1967); 2) local resource competition (LRC)—in a dense population where related individuals compete for resources, mothers are selected to produce more offspring of dispersive sex to decrease resource competition (Clark 1978; Wild and Taylor 2004; Iritani et al. 2021), and 3) local resource enhancement (LRE)—when one sex helps one another rather than compete with one another, selection favors an excess production of the more helpful sex (Taylor 1981). These scenarios may take place simultaneously in the same species (West 2013). However, theoretical models for prediction of sex ratio only consider LMC (Hamilton 1967, 1979; Herre 1985; West 2009).

Under the assumption that mated females disperse to found new colonies, Hamilton (1967) proposes the first theoretical model to predict the evolutionary outcome of offspring sex ratio (proportion of male offspring, s) for diploid species under LMC: $s = (N-1)/(2N)$ (Equation 1), where N is the number of females in a population whose offspring develop and mate on the patch. The model predicts that an increase of LMC (i.e., decrease of foundress population size) reduces the proportion of sons from 50% for a large population to 0% for a single foundress, and the 0% of sons could be interpreted as the minimum proportion of sons needed for fertility insurance even though this minimum is unknown. In haplodiploid species, however, inbreeding is common and females can control the sex of their offspring in response to the social environment through arrhenotoky (fertilized eggs give rise to diploid female offspring and unfertilized eggs to haploid male offspring) (West 2009). The LMC with intensive inbreeding in small local populations will promote a female-biased sex ratio (Herre 1985). Therefore, the effect of inbreeding should be incorporated into Hamilton's (1967) model when predicting sex ratio in this group of animals. If the probability of inbreeding in a population of constant size (N) is fixed, that is, $k = 1/N$ $(1 \leq k \leq 0)$ (West 2009), then $s = [(N-1)\ (2N-1)]/[N\ (4N-1)]$ (Equation 2). If N varies across patches in a population and there is an average level of inbreeding k $(1 \leq k \leq 0)$ (Herre 1985; West 2009), then $s = [(N-1)\ (2-k)]/[N\ (4-k)]$ (Equation 3) (when $k = 0$, Equation 3 is the same as Equation 1).

In numerous haplodiploid taxa, including many wasps, beetles, thrips, and mites, females may produce extremely female-biased offspring sex ratios (West 2009) which cannot be explained by LMC theory alone, probably because of impacts from LRC and/or LRE (Iritani et al. 2021). However, it is still not clear how LMC, LRC, and LRE affect sex allocations in haplodiploid animals. Here, we used a haplodiploid mite *Tetranychus ludeni* Zacher to test 1) how females adjusted the sex allocation strategies in response to different levels of LMC and LRC and 2) whether the LMC theoretical models could predict the offspring sex ratio under different levels of LMC and LRC. *Tetranychus ludeni* is an invasive pest of horticultural crop and inhabits discrete patches of various population sizes and densities with varying inbreeding intensity. We designed a factorial experiment by keeping each of the three test population sizes constant but allowing the population density to alter at three levels, and *vice versa* (Table 1) and recorded the sex ratio.

The sex ratio of *T. ludeni* under all tested social environments was low (0.13–0.21) (Figure 1 and Table 1). Results show that when population size was large (5 or 10 females), offspring produced were significantly more female biased at higher population densities (1

Table 1. Comparisons of observed sex ratios (proportion of male offspring) at different social environments with predictions of LMC models

Leaf area (cm^2)	Population size (females)	Population density (females/cm^2)	Observation n mean (SE)	Prediction (Equation 2)	Prediction (Equation 3)		
					$k = 0$ (Equation 1)^a	$k = 0.5$	$k = 1$
				0.2143	0.2500^b	0.2142	0.1667
1.5	1	0.67	25 0.1497 (0.0144)	4.47^{c****}	6.94^{****}	4.46^{***}	1.18^{ns}
1	1	1	18 0.1544 (0.0193)	3.11^{**}	4.96^{****}	3.10^{**}	0.64^{ns}
0.5	1	2	24 0.1534 (0.0067)	9.14^{****}	14.51^{****}	9.13^{****}	1.99^{ns}
				0.3789	0.4000	0.3429	0.2667
7.5	5	0.67	25 0.2065 (0.0080)	21.54^{****}	24.18^{****}	17.05^{****}	7.53^{****}
5	5	1	22 0.1484 (0.0106)	21.83^{****}	23.83^{****}	18.42^{****}	11.21^{****}
2.5	5	2	16 0.1520 (0.0089)	25.36^{****}	27.72^{****}	21.43^{****}	12.82^{****}
				0.4385	0.4500	0.3857	0.3000
15	10	0.67	18 0.1934 (0.0086)	28.36^{****}	29.69^{****}	22.25^{****}	12.33^{****}
10	10	1	16 0.1325 (0.0086)	35.76^{****}	37.11^{****}	29.59^{****}	19.58^{****}
5	10	2	21 0.1482 (0.0126)	23.20^{****}	23.93^{****}	18.83^{****}	12.04^{****}

ns indicates no significant difference between the observed and predicted sex ratios., [a] When $k = 0$ (i.e., no inbreeding) in Equation (3), the predicted sex ratios are the same as that in Equation (1)., [b] When $N = 1$ in Equations (1) and (3), the sex ratio $s = 0$ which is logically incorrect, thus the predicted sex ratio $s = 0.25$ when $N = 2$., [c] t-Value for the one-sample t-test., **, ***, and **** indicate a significantly lower observed sex ratio than the predicted ones at $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively.

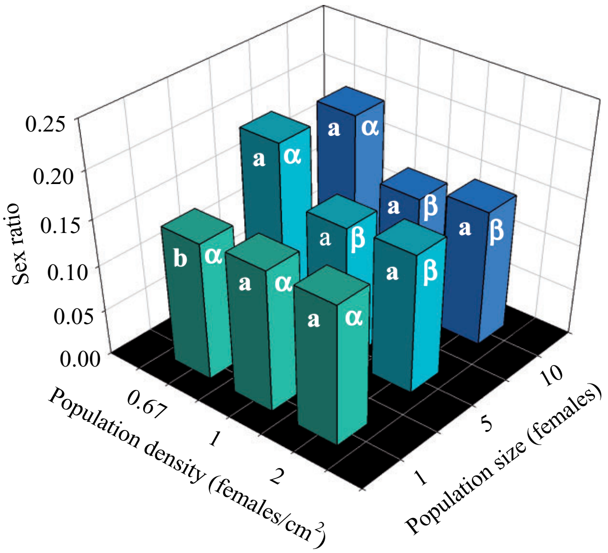

Figure 1. Sex ratio (proportion of male offspring) of *T. ludeni* under various social environments. For a given population density, columns with the same English letters are not significantly different between population sizes; for a given population size, columns with the same Greek letters are not significantly different between population densities (Tukey–Kramer test: $P > 0.05$).

and 2 females/cm^2) than at lower population density (0.67 female/cm^2) ($F_{2,60} = 13.55$, $P < 0.0001$ for 5 females; $F_{2,52} = 26.95$, $P < 0.0001$ for 10 females). However, when population size was small (1 female), population density had no effect on offspring sex ratio ($F_{2,64} = 0.01$, $P = 0.9879$) (Figure 1). Furthermore, we detected a significantly lower sex ratio at the small population size of 1 female than at large population sizes (5 and 10 females) with a low population density (0.67 female/cm^2) ($F_{2,65} = 3.17$, $P = 0.0488$) but population size had no significant effect on sex ratio when population density was high (1 or 2 females/cm^2) ($F_{2,53} = 1.35$, $P = 0.2689$ for 1 female/cm^2; $F_{2,58} = 0.15$, $P = 0.8609$ for 2 females/cm^2) (Figure 1). When comparing the observed sex ratios with the predictions of the three LMC models, we found that the predictions of LMC models only fitted the observed sex ratios when the population size was 1 female with intensive inbreeding ($k = 1$, in Equation 3), but the observed sex ratio in the larger populations was significantly lower than that predicted by LMC models (Table 1).

Our results only support LMC theory (Hamilton 1967) when population size is small where mated mothers produce more daughters to reduce LMC between their sons (Figure 1). Regardless of population size, females may be selected to produce more dispersing daughters in the dense populations (Figure 1) to reduce resource competition in the local populations, agreeing to the LRC prediction (Clark 1978; Wild and Taylor 2004; Iritani et al. 2021). We suggest that females change their sex allocation strategies in response to social environment. In small populations, they produce more daughters to reduce mate competition between sons while in large and dense populations they give birth to more dispersing daughters to reduce resource competition.

The extremely female-biased sex ratio in *T. ludeni* may be attributed to the life history characteristics of spider mites. First, if the increase in fitness return per unit investment is greater in daughters than in sons, an optimal sex allocation is expected to be female-biased (Seger and Stubblefield 2002). In *T. ludeni*, females are larger in size than males, thus investing more resources to daughters is advantageous for the mothers. Second, although daughters are more likely to compete for local food due to their large body size, they usually disperse from the dense conditions after mating. Thus, production of more dispersing daughters will eventually reduce LRC as well as LMC. Third, spider mite females may cooperate as a form of LRE in spinning silk webs which are crucial for dispersal and protection against environmental hazards, and group-living females produce more silks per mite than single females (Le Goff et al. 2010). In the scenario of LRE, females are the helpers and thus production of more daughters may reduce both local mate and resource competition. Although production of more mates or helping and dispersing daughters may potentially intensify LRC, the benefit gained from more daughters produced under LMC, LRC, or LRE outweighs the costs of resource competition among females. We suggest that under varying social environments, LMC, LRC, and LRE could be the main mechanisms leading to the extremely female-biased sex ratio in *T. ludeni*.

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Authors' Contribution

N.W., X.Z.H., and Q.W. conceived and designed the study. N.W. collected the data. N.W. and X.Z.H. analyzed the data. All authors contributed to manuscript preparation.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

Conflict of Interest

The authors declare no conflicts of interest to any other institutions.

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Comparing the effects of social environments and life history traits on sex allocation in a haplodiploid spider mite

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Social environment refers to an individual's physical surroundings, community resources and social relationships, including population size, population composition, fission-fusion dynamics, and the social interactions among group members (Webber *et al*. 2022). Population size and density are two key socio-environmental factors that affect the offspring sex ratio in group-living animals. For example, in small populations founded by one or a few foundresses, females tend to produce female-biased offspring to reduce the local mate competition between related males (Hamilton 1967; Krainacker & Carey 1991; Roeder 1992; Sato & Saito 2006; West 2009; Macke *et al*. 2012, 2014), whereas in dense populations mothers incline to produce more offspring of dispersive sex to reduce resource competition (Clark 1978; Mari *et al*. 2008; Hjernquist *et al*. 2009; Iritani *et al*. 2021; Weerawansha *et al*. 2022a, b). Social environments may affect offspring sex ratio directly by altering mothers' sex allocation strategies (Clark 1978; Iritani *et al*. 2021; Weerawansha *et al*. 2022a, b) or indirectly through mediating their other life history traits such as clutch size (West 2009) and egg size (West 2009; Macke *et al*. 2011, 2012).

Moreover, previous studies reveal that in haplodiploid species (i.e., whose fertilized eggs give rise to diploid female offspring and unfertilized eggs to haploid male offspring), life history traits such as the maternal age can also directly affect sex allocation due to the age-specific fertilization (i.e., more daughters produced at specific ages) (Roy *et al*. 2003; Ueno 2014; Weerawansha *et al*. 2020). Nevertheless, maternal age may also affect offspring sex ratio indirectly through age-specific investment in clutch size (Weerawansha *et al*. 2020) and egg size (Reviewed in Mousseau & Fox 1998). Therefore, multiple factors of social environments and life history traits may simultaneously act on offspring sex ratio during the females' reproductive period. However, studies usually test the influence of social environments and life history traits on sex allocation separately and focus on the direct effect only (West 2009; Macke *et al*. 2011, 2012; Katlav *et al*. 2021). Due to the lack of appropriate methodology, few studies have investigated (1) whether social environments and maternal age have both direct and indirect effects on sex allocation, and (2) whether social environments impose weaker or stronger effects on offspring sex ratio than the life history traits. Here we used a haplodiploid spider mite *Tetranychus ludeni* Zacher (Acari: Tetranychidae) as a model species to estimate and compare the effects of social environments and life history traits on sex allocation.

We established a colony of *T. ludeni* Zacher from adults collected on *Passiflora mollissima* (Kunth) in Palmerston North, New Zealand. The colony was maintained, and the experiment was carried out at 25 ± 1°C and 40–50% RH with a photoperiod of 16L:8D hrs. We used the first

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A haplodiploid mite adjusts fecundity and sex ratio in response to density changes during the reproductive period

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Abstract

Population density is one of the main socio-environmental factors that have critical impacts on reproduction of animals. Consequently, they need to adjust their reproductive strategies in response to changes of local population density. In this study we used a haplodiploid spider mite, *Tetranychus ludeni* Zacher (Acari: Tetranychidae), to test how population density dynamics during the reproductive period altered female reproductive performance. We demonstrate that females produced fewer eggs with a significantly higher female-biased sex ratio in dense populations. Reducing fecundity and increasing daughter production in a dense environment could be an advantageous strategy to minimise the intensity of local food competition. However, females also reduced their fecundity after arrival in a new site of larger area from a dense population, which may be associated with higher web production costs because females need to produce more webs to cover the larger area. There was no trade-off between egg number and size, and egg size had little impact on reproductive fitness. Therefore, *T. ludeni* females could adapt to the shift of population density during their reproductive period by manipulating the fecundity and offspring sex ratio but not the egg size.

Keywords Spider mite · Fecundity · Sex ratio · Resource competition · Social environment

Introduction

Population density is one of the major components of social environments that can affect population dynamics. A local population density may vary over time due to aggregation (immigration), dispersal (emigration), or mortality (Roeder 1992; Roff 1992; Stearns 1992; Bowman et al. 2002; Schausberger et al. 2021). Animals may aggregate in a habitat to increase reproduction opportunities (e.g., Snead and Alcock 1985; Bengtsson

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2008; Wheeler and Jr Welsh 2008; Le Goff et al. 2010; Pérez-González et al. 2010; Bonsignore and Jones 2014; DeVries et al. 2017. Dar et al. 2021) or to reduce predation risk (e.g., Spieler 2003; Morrell and James 2008; Yano 2012; Clotuche et al. 2014). However, aggregation may raise population density in the habitat, leading to intensive resource competition and reducing reproductive fitness (Li and Zhang 2021; Weerawansha et al. 2020, 2022). In this scenario, the reproductive females would disperse to seek new habitats for the next generation (Schaub and von Hirschheydt 2009; Azandémè-Hounmalon et al. 2014; Lutz et al. 2015; Kingma et al. 2017; Kusch et al. 2020; Manguette et al. 2020; Vaishali and Krushnamegh 2020; Schausberger et al. 2021; Zhou et al. 2021). With a few exceptions (e.g., Roeder 1992; Fox et al. 1997; Matsuura and Kobayashi 2010; Maenoa et al. 2020), studies on the effects of population density on reproductive plasticity have been carried out under constant population densities during female breeding time (e.g., Wrensch and Young 1978; Fischer et al. 2011; Weerawansha et al. 2020, 2022). To date, it is still unclear how females alter their reproductive strategies in response to the varying population density during their reproductive life.

After settling in new habitats, females are expected to adjust their reproductive strategies to optimize their fitness (Roff 1992; Stearns 1992; West et al. 2005; Fischer et al. 2011; Bowers et al. 2017; Maenoa et al. 2020; Weerawansha et al. 2022). For example, if the population is dense, females may lay fewer (van Noordwijk and de Jong 1986; Khan et al. 2018; Li and Zhang 2021) but larger eggs (Parker and Begon 1986; Sibly et al. 1988; Fischer et al. 2011), trading-offs the number with size of eggs to make best utilization of limited resources and maximise offspring fitness (Smith and Fretwell 1974; Parker and Begon 1986; Stearns 1992; Fox and Czesak 2000; Fischer et al. 2011; Macke et al. 2012; Walzer and Schausberger 2015; Maenoa et al. 2020). In species with sexual size dimorphism, resource-deficient females either reduce the egg size of the larger sex (Fox and Czesak 2000; Walzer and Schausberger 2013, 2015) or produce fewer eggs of the larger sex (Trivers and Willard 1973; Charnov 1982; Walzer and Schausberger 2015) to optimize their reproductive fitness returns. Moreover, if one sex is dispersive and the other is philopatric, females often skew investment towards philopatric offspring when local resources are abundant but allocate more resources to the dispersive sex when local resources are deficient (Clark 1978; Silk 1983, 1984; West et al. 2005; Hjernquist et al. 2009; West 2009).

Spider mites (Acari: Tetranychidae) are phytophagous invertebrates, often living as groups (Helle and Sabelis 1985; Le Goff et al. 2010; Schausberger et al. 2021) in discrete patches (Mitchell 1973; Nachappa et al. 2011; Sarwar 2013). Female adults are larger than male adults (Mitchell 1973) and thus more likely to compete for food with their mothers or siblings (Young et al. 1986). However, female adults, rather than male adults and immature nymphs, may disperse to found new colonies (Mitchell 1973; Brandenburg and Kennedy 1982) especially when the populations are crowded or when food is insufficient or poor in quality (Suski and Naegele 1968; McEnroe 1969). As spider mites are haplodiploid, mated females can manipulate offspring sex ratio by fertilizing relatively larger eggs that develop to daughters (Young et al. 1986; Roeder et al. 1996; Macke et al. 2011). It has been reported that females produce fewer eggs with more dispersing daughters in large and dense populations to reduce local competition for food (Weerawansha et al. 2022). Moreover, spider mites aggregate and cooperate in spinning silk webs for dispersal and protection against environmental hazards (Le Goff et al. 2010; Yano 2012), and group-living females produce more silk and lay more eggs per mite than single females (Le Goff et al. 2010). Therefore, spider mites should be able to adjust offspring sex ratio in response to the social environments.

Here, we used an invasive pest spider mite, *Tetranychus ludeni* Zacher (Zhang 2003), to examine how changes in population density during female reproductive life altered egg production and sex allocation. We simulated the aggregation by moving females from low to high population density and the dispersal by shifting females from high to low population density. We recorded the number and size of eggs laid and offspring sex ratio (i.e., proportion of daughters) before and after density changes. Based on the knowledge outlined above, we hypothesize that (1) females lay fewer but larger eggs and produce offspring with a more female-biased sex ratio in response to the aggregation scenario, and (2) the opposite case occurs in response to the dispersal scenario. This study provides insight into the mechanisms behind the adjustment of fecundity and sex ratio in response to the varying social environments.

Materials and methods

Mite colony

We maintained a colony of *T. ludeni* on kidney bean plants (*Phaseolus vulgaris* L.) in the laboratory—and carried out the experiment—at 25 ± 1 °C, 40 ± 10% RH and L16:D8 h photoperiod. We used the first expanded leaves of 1- to 2-week-old plants for the experiment.

Experiment

To determine how females adjusted their fecundity and sex allocation in response to population density dynamics in *T. ludeni*, we set up two treatments, each with 32 leaf squares as replicates. Treatments 1 and 2 tested the effects of density changes from high to low (Fig. 1a) and from low to high (Fig. 1b), respectively. Briefly, we randomly selected the quiescent female deutonymphs just before emergence (silvery in colour) from the colony. We individually transferred them onto 1-cm^2 leaf squares placed upside down on a water-saturated cotton pad in a Petri dish (9.5 cm diameter, 1 cm high) with a hole (1 cm diameter) in the middle of the lid covered by a fine metal mesh (aperture size 0.25 × 0.25 mm). We then introduced a newly emerged virgin male adult produced by virgin females onto each square. We monitored the pair until the end of copulation, after which time, we removed the male. For each replicate in Treatment 1, we introduced 16 newly mated females onto

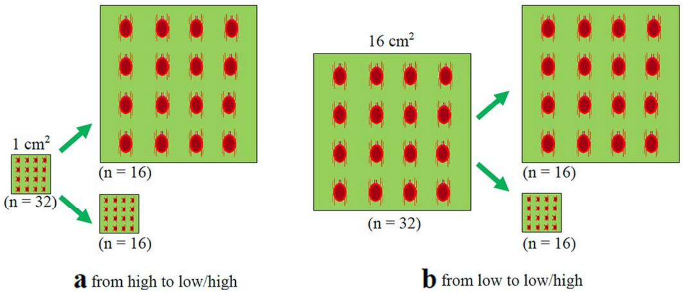

Fig. 1 Diagram of experimental design to test the effect of population density shift on reproduction in *Tetranychus ludeni*: **a** from high to low/high density (Treatment 1) and **b** from low to low/high density (Treatment 2). *n* number of leaf discs (replicates)

a 1-cm^2 clean leaf square and allowed them to stay on the square for 1 day. We then transferred them to a new square daily for two consecutive days. On the 4th day, we randomly selected 16 of the 32 leaf squares and transferred mites from each leaf square onto a new 16-cm^2 leaf square (from high to low density) and those from each of the remaining 16 leaf squares onto a new 1-cm^2 leaf square (from hight to high density as control) daily for three consecutive days. The same procedure was carried out for Treatment 2 except that we transferred mites from low to high density and from low to low density (as control).

We checked each leaf square twice a day during the six oviposition days and replaced any dead females immediately with females of the same age and social experience. We recorded the number of eggs laid on each leaf square. To determine the egg size, we randomly selected 30 eggs from each leaf square and individually measured their diameter under a stereomicroscope (Leica MZ12, Germany) connected to a digital camera (Olympus SC30, Japan) and imaging software (CellSens GS-ST-v.1.7, Olympus, Japan). We calculated the egg radius ($r = \text{diameter}/2$) and egg size ($\text{volume} = 4/3\pi r^3$). After eggs hatched, we transferred all live individuals onto a clean leaf square of the same size once every 5 days and recorded the sex of newly emerged adults.

Statistical analysis

We analysed all data using SAS v.9.4 with a rejection threshold set at $\alpha = 0.05$. Data on the number of eggs laid and egg size were normally distributed (Shapiro-Wilk test; UNIVARIATE procedure). We analysed the data on egg number and size using a linear mixed model (GLM procedure) with treatment (i.e., density shift) as a main factor and replicate as a random factor, and a Tukey-Kramer test for multiple comparisons. The mean egg size and number for each female before and after density shift were calculated and used for analysis. The data on sex ratio (proportion of daughters) were analysed by a generalized linear model (GLIMMIX procedure) with a binomial distribution and a *Logit* link function after the model, and a Tukey-Kramer test was applied for multiple comparisons. A general linear regression model (GLM procedure) was applied to determine the relationships between egg size and number, between immature survival rate and egg size, and between sex ratio and egg size. The mean egg size and number, immature survival rate, and sex ratio for each female were used for regressions.

Results

Effect of population density shifts on fecundity, egg size and immature survival

Our results show that females at low density laid significantly more eggs than at high density before density shift but laid significantly fewer eggs after the shift regardless of whether it was from high to low or from low to high ($F_{5,107} = 28.79$, $P < 0.0001$) (Fig. 2a). If the shift occurred at the same density levels, females produced similar numbers of eggs before and after shift (Fig. 2a). However, population density and its shift had no significant impact on egg size ($F_{5,107} = 0.37$, $P = 0.87$) (Fig. 2b). Moreover, increasing number of eggs laid did not significantly reduce the egg size (Fig. 3a) and egg size had no significant impact on immature survival rate (Fig. 3b).

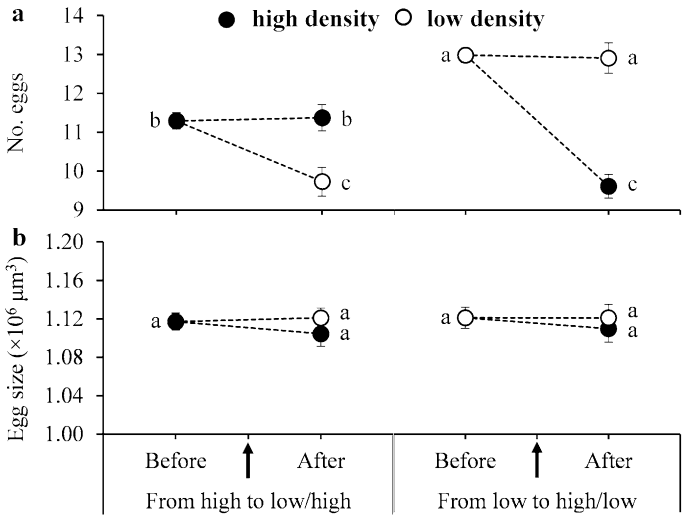

Fig. 2 Effects of female population shift between high (black dots) and low (white dots) densities on the mean (± SE) number of eggs laid (**a**) and egg size (**b**) in *Tetranychus ludeni*. Means within a panel with the same letters are not significantly different (Tukey-Kramer test: $P > 0.05$). Female population density shift occurred on the 4th day of oviposition period

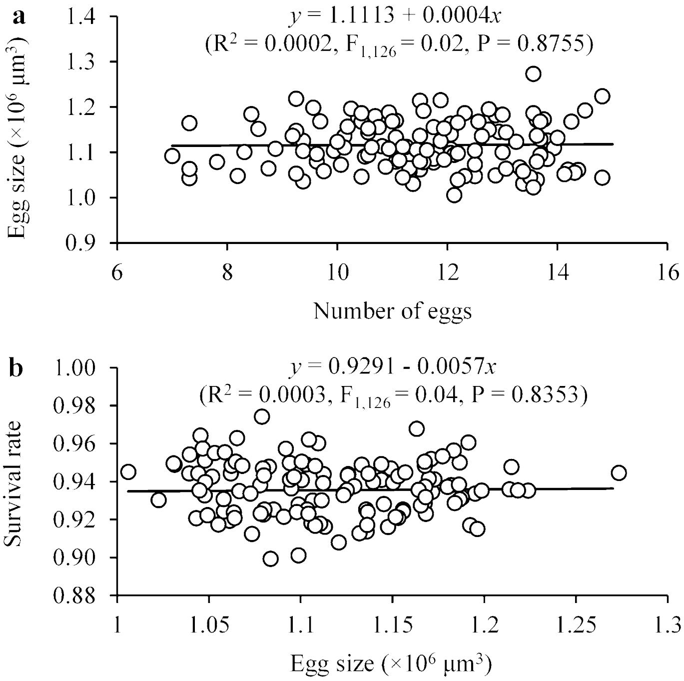

Fig. 3 Relationships between egg size and number of eggs laid (**a**) and between egg size and immature survival rate (**b**) in *Tetranychus ludeni*

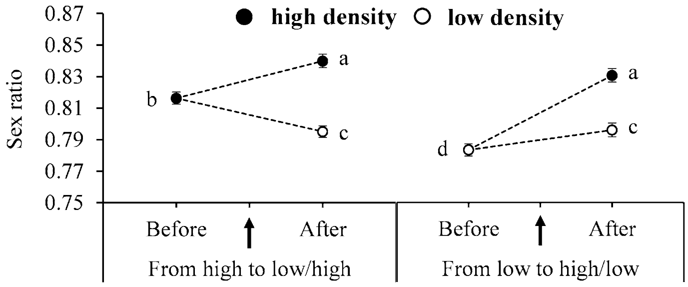

Fig. 4 Effects of female population shift between high (black dots) and low (white dots) densities on the mean (±SE) sex ratio (proportion of females) in *Tetranychus ludeni*. Means with the same letters are not significantly different (Tukey-Kramer test: $P > 0.05$). Female population density shift occurred on the 4th day of oviposition period

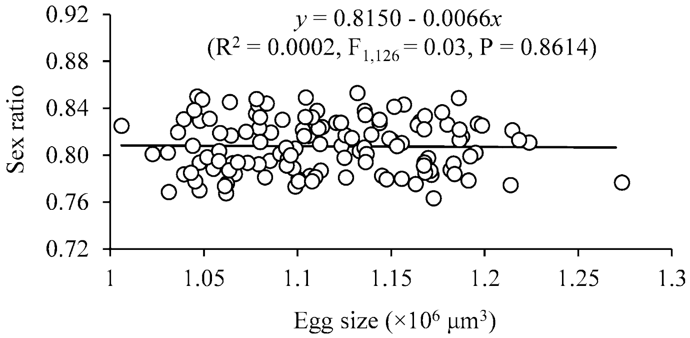

Fig. 5 Relationships between sex ratio (proportion of female offspring) and egg size in *Tetranychus ludeni*

Effect of population density shifts on sex allocation

We demonstrate that the sex ratio (proportion of daughters) was significantly higher at high density than at low density regardless of shifts; density shift from high to high, from low to low, or from low to high significantly increased the sex ratio, and density shift from high to low significantly reduced the sex ratio ($F_{5,122} = 11.26$, $P < 0.0001$) (Fig. 4). Egg size had no significant impact on sex ratio ($F_{1,126} = 0.03$, $P = 0.86$) (Fig. 5).

Discussion

Our results indicate that *T. ludeni* females reduced their fecundity after the population density changed during their productive period (Fig. 2a). We suggest that when the density quickly increases, they lower their fecundity to prevent the collapse of the local population due to the increase of resource competition and overexploitation of the host plants (Krips et al. 1998) or hostile interference or aggression among offspring for resource access (Estevez et al. 2007; Wong et al. 2013; Li and Zhang 2021). Tetranychid mites construct silk webs (Saito 1983; Mori and Saito 2005; Clotuche et al. 2009; Le

Goff et al. 2010) in the new habitats to protect themselves and their offspring from environmental hazards (Davis 1952; McMurtry et al. 1970; Hazan et al. 1975; Ashley 2003; Oku et al. 2003, 2004; Mori and Saito 2005; Le Goff et al. 2010) but the silk consists of mainly proteins (Hazan et al. 1975), the production of which incurs a considerable cost (Oku et al. 2009). Therefore, when they arrive in a new site of much larger area from a higher density population, they need to allocate more resources per female to produce enough silk to cover the area, leading to fecundity decline (Fig. 2a).

We did not observe a trade-off between the egg number and size in response to the density changes (Fig. 3a), challenging theoretical assumptions (Smith and Fretwell 1974; Parker and Begon 1986; Roff 1992, 2002; Stearns 1992; Fox and Czesak 2000; Fischer et al. 2011). The lack of such trade-offs has also been reported in some animal species (e.g., Doughty and Shine 1997; Zera and Harshman 2001; Jordan and Snell 2002; Bowden et al. 2004; Uller and Olsson 2005). Our results show that increasing egg size did not significantly increase the proportion of daughters (Fig. 5), contradictory to the previous assumption that sex allocation in spider mites is mediated by egg size (Macke et al. 2011). These findings suggest that *T. ludeni* females only adjust their fecundity but not egg size in response to density dynamics as reported in some birds (Christians 2002) because egg size has little impact on reproductive fitness, such as offspring survival (Fig. 3b) and sex allocation (Fig. 5). Therefore, egg size is not a reliable indicator of offspring fitness when future environmental conditions are uncertain or unpredictable (Wiklund and Persson 1983; Karlsson and Wiklund 1985; McEdward and Carson 1987; Lalonde 2005; Morrongiello et al. 2012).

We demonstrate that regardless of density changes, offspring produced by females in high population density was significantly more female-biased than in low density (Fig. 4). This could be due to sex-specific dispersal tendency in spider mites. Female spider mites usually disperse from dense conditions after mating (Suski and Naegele 1968; McEnroe 1969; Brandenburg and Kennedy 1982; Li and Margolies 1993) to establish new colonies (Mitchell 1973; Brandenburg and Kennedy 1982) and reduce future competition for food or space (Clark 1978; Silk 1983; Mari et al. 2008; Hjernquist et al. 2009; West 2009; Visser et al. 2014; Song et al. 2016; Weerawansha et al. 2022), resulting in production of more dispersing daughters in dense conditions. Compared to density shift from high to high or from low to low, that from low to high led to a faster increase in proportion of daughters produced (Fig. 4). This suggests that *T. ludeni* females can quickly adjust their sex allocation in response to the change of social environment for optimal fitness of their offspring.

In the present study, we demonstrate that *T. ludeni* females could adjust their reproductive strategies in response to dynamic social environments during their reproductive period. Females reduce fecundity and produce more dispersive female offspring in dense environments, which will reduce the local resource competition. However, females do not adjust the egg size in response to the shift of population density, as egg size imposes no significant effect on fecundity and offspring sex ratio and survival. Therefore, *T. ludeni* females adapt to the shift of population density by manipulating the fecundity and offspring sex ratio but not the egg size. Whether *T. ludeni* females could manipulate sex allocation via adjusting egg size in response to the shift of population size remains unclear and is warranted for future investigations.

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Author contributions NW, XZH, and QW conceived and designed the study. NW collected the data. NW and XZH analysed the data. All authors contributed to manuscript preparation.

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Declarations

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