Life History Strategies of *Tetranychus ludeni* Zacher (Acari: Tetranychidae) with Special Reference to Biological Invasion

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Abstract

With the increase of worldwide trade and travel in recent decades, increasingly more arthropod species have become established outside their natural range of distribution, causing substantial ecological and economic impacts in novel habitats. Successful invaders may bear certain life history traits that can overcome various barriers such as mate and food shortage and inbreeding depression. Here, I investigated the life history strategies of a haplodiploid pest, *Tetranychus ludeni* Zacher (Acari: Tetranychidae), with special reference to its invasion success. It is native to Europe but now cosmopolitan. Virgin females laid larger eggs than mated females, giving rise to larger adults, and sons from virgin females produced more daughters at a higher rate than sons from mated females in their lifetime. Virgin females produced maximum number of sons in their early life to ensure subsequent mother-son mating but later saved resources to prolong longevity for potential future mating. Females maximised their resource allocation to egg production immediately after mating regardless of whether mating delay occurred to secure production of maximum number of both daughters and sons as early as possible. Mated females with mating delay increased proportion of daughters in offspring produced to compensate the loss of production of daughters during their virgin life. Neither mother-son mating nor sibling mating affected female reproductive output and longevity in any of the 11 successive inbred generations and neither sex showed inbreeding avoidance behaviour, suggesting that inbreeding has no negative impact on its invasion success at any points or generations. Mated females did not trade off their survival and lifetime reproductive output with dispersal. Long-distance dispersers invested more in dispersal in their early life while resident mites and short-distance dispersers invested more in reproduction during their early life, which may allow long-distance dispersers to explore the novel environment more effectively without compromising lifetime reproductive fitness. Older females with more mature eggs were more likely to disperse and move longer distances than younger ones with fewer eggs. Females increased dispersal probability and distance with the increase of population density. The synchronization of dispersal and reproduction and the positive density-dependent dispersal strategy may facilitate habitat colonization and invasion speed of *T. ludeni*. Findings from this study improve our understanding of the invasion mechanisms of *T. ludeni* and other haplodiploid species, providing knowledge for development of programmes for prediction and management of biological invasions.

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Chapter 1

Introduction

1.1 General introduction

Biological invasion may occur when an organism becomes established beyond its natural range (Williamson & Griffiths 1996). With the increase of worldwide trade and travel in recent decades, biological invasions have become more frequent, causing substantial ecological and economic impacts in novel habitats (Fournier et al. 2019). Successful invasion involves a sequence of steps such as introduction to a new site, population establishment and spreading in the new habitat. However, following the introductions, exotic species may experience barriers that may result in invasion failure (Heger & Trepl 2003; Blackburn et al. 2011). For example, small populations at the introduction front may suffer from mate shortage (Courchamp et al. 1999; South & Kenward 2001; Council 2002) and inbreeding depression (O'Grady et al. 2006; Estoup et al. 2016; Nonaka et al. 2019), which may reduce their chances to become established. Poor dispersal abilities may limit further range expansion and distribution of introduced populations (Jonsen et al. 2007; Brudvig et al. 2011; Widhalm et al. 2020). It is expected that successful invaders bear some characteristics that may help overcome these barriers (Heger & Trepl 2003; Ribeiro et al. 2007; Boubou et al. 2010; Navajas et al. 2013; Reitz et al. 2020). Therefore, investigations into the life history strategies of successful invaders facilitate our understanding of the invasion mechanisms and provide knowledge for development of programs for prediction and management of biological invasions.

Spider mites belong to the Acari family Tetranychidae consisting of over 1300 species (Migeon & Dorkeld 2022). Some of these mites are invasive pests, such as *Tetranychus urticae* Koch, *T. evansi* Baker & Pritchard, *T. kanzawai* Kishida, and *T. ludeni* Zacher, damaging many economic crops worldwide (Bolland et al. 1998; Zhang 2003), and they are actively spreading. For example, *T. ludeni* was recently detected in Italy in 2019 (Ragusa et al. 2019), and *T. evansi* was found the first time in New Zealand in 2020 during this study (MPI 2020). Several traits may have contributed to their invasion success. For example, (1) they can adapt to a wide range of host plants and temperatures (Zhang 2003; Gotoh et al. 2015; Ristyadi et al. 2019, 2021); (2) their haplodiploid nature allows virgin females to perform mother-son mating and start a population without being constrained by mate shortage (Adamson & Ludwig 1993); (3) they suffer less or little inbreeding depression (Henter 2003; Zhou et al. 2020, 2021a); (4) they usually have flexible reproductive strategies that increase the probability of encountering a mate in the future (Macke et al. 2012; Li & Zhang 2021a; Zhou et al. 2021a); (5) their females lay larger eggs under harsh conditions (Bitume et al. 2014; Dahirel et al. 2019) to increase the survival and fitness of offspring, and (6) they can perform aerial and ambulatory dispersal, greatly increasing their spreading ability (Li & Margolies 1993; Azandémè-Hounmalon et al. 2014).

Tetranychus ludeni attacks over 300 host plants and has been a serious pest of many economically important crops such as bean, papaya, guava, eggplant, pumpkin, and apple worldwide (Bolland et al. 1998; Zhang 2003; Kaimal & Ramani 2011a; Migeon & Dorkeld 2022) and has been predicted to become an even more serious pest in the future (Gotoh et al. 2015). This mite is native to Europe but has now invaded all continents except Antarctica (Bolland et al. 1998; CABI/EPPO 2020). Therefore, it may have evolved characteristics that facilitate invasion success. However, prior to my PhD studies, little was known about its reproductive strategies, inbreeding depression, inbreeding avoidance, and dispersal ability.

1.2 Relevance of research

Reproductive strategies, inbreeding depression, and inbreeding avoidance affect population establishment at newly invaded sites. Subsequent dispersal plays a crucial role in population expansion. Therefore, investigations into these biological features would help better understand biological invasions.

1.2.1 Reproductive strategies in relation to invasion

Population size is usually small at the newly invaded sites or front edge of population expansion where mates may be limited (Courchamp et al. 1999; South & Kenward 2001; Engen et al. 2003). Successful reproduction may therefore be difficult for species with sexual reproduction (Heger & Trepl 2003; Blackburn et al. 2011), leading to population extinction.

In haplodiploid animals, virgin females can produce haploid sons, providing an opportunity to overcome the barrier of mate shortage by performing mother-son mating (Adamson & Ludwig 1993). However, virgin mothers will have to be alive when their sons become sexually mature, and even if they eventually mate with their sons, their reproductive fitness could decrease significantly due to mating delay (e.g., Krainacker & Carey 1990a; McCulloch & Owen 2012). Therefore, successful invaders of haplodiploid species may have developed reproductive strategies to facilitate mother-son mating with limited impact on their reproductive fitness. For example, females may reduce their reproductive rate when virgin to extend their longevity (Bonato & Gutierrez 1996, 1999; Li & Zhang 2021a) and increase resource allocations to reproduction after mating (Schmidt et al. 2014). Prior to this study, little is known about the reproductive strategies of *T. ludeni* in response to mating and mating delay, knowledge of which is vital to the understanding of invasion success in this mite and prediction of invasive potential of other haplodiploid animals.

Mating success and reproductive capacity can greatly affect population growth when mate availability is low (Gascoigne et al. 2009; Fauvergue 2013). Therefore, virgin females should develop strategies to adjust resource allocations to promote their sons' reproductive success, increasing the chances of establishment. Two previous studies on the haplodiploid spider mite, *T. urticae*, show that virgin mothers produce larger male eggs than mated mothers (Macke et al. 2011b, 2012). However, prior to the current study it is not clear whether maternal mating status affects sons' mating success and reproductive outputs in any haplodiploid mite species.

1.2.2 Inbreeding depression and inbreeding avoidance in relation to invasion

1.2.2.1 Inbreeding depression

Small populations have lower genetic diversity and higher probability of inbreeding which may increase the homozygosity of deleterious recessive alleles (i.e., genetic load) and/or lead to a loss of heterozygote advantage, reducing fitness (i.e., inbreeding depression) (Charlesworth & Charlesworth 1987; Charlesworth & Willis 2009). These negative effects can reduce establishment probability and population growth, and thus invasion success (O'Grady et al. 2006; Estoup et al. 2016; Schrieber & Lachmuth 2017; Nonaka et al. 2019; Parrett et al. 2021). However, genetic load purging may occur in small populations, which may facilitate population establishment (Marchini et al. 2015; Trevisan et al. 2016; Schrieber & Lachmuth 2017). For example, in the invasive ant *Brachyponera chinensis* (Emery), generations of sibling mating have purged genetic load, reducing inbreeding depression and favouring successful invasion (Eyer et al. 2018). Therefore, examination of inbreeding depression is essential for evaluating population establishment of invasive species.

In haplodiploid species, deleterious alleles are subject to constant selection in haploid males (Atmar 1991; Antolin 1999; Smith 2000; Henter 2003), and thus inbreeding depression may be less severe and should only affect female-specific traits such as fecundity and offspring sex allocation (Henter 2003; Mori et al. 2005; de la Filia et al. 2015; Tien et al. 2015). However, inbreeding for numerous generations may still lead to serious inbreeding depression (Mori et al. 2005). Previous authors have studied inbreeding in haplodiploids for multiple generations, however, most of them have only examined inbreeding depression in one or a few generations (e.g., Hoy 1977; Ito et al. 2012; Tien et al. 2015; Atalay & Schausberger 2018; Çekin & Schausberger 2019). It is still not clear whether inbreeding in successive generations affects reproductive fitness, information of which is important for evaluation of whether inbreeding could increase the risk of population collapse in any generations and how inbreeding potentially affects long-term population growth.

1.2.2.2 Inbreeding avoidance

Animals may exhibit inbreeding avoidance behaviour when inbreeding depression is severe (Pusey & Wolf 1996; Nichols 2017), which may reduce the number of suitable mates and aggravate mate shortage at the invasion front (Tainaka & Itoh 1996; Jordal et al. 2001). However, animals do not need to avoid inbreeding if no inbreeding depression occurs (Tan et al. 2012). To date, whether inbreeding avoidance behaviour changes with the magnitude of inbreeding depression is largely unknown in haplodiploid animals. In addition, although inbreeding may affect female-specific traits, it is not clear whether inbreeding avoidance is sex-specific.

1.2.3 Dispersal in relation to invasion

Dispersal affects population size and thus the persistence and extinction of a population. For example, small populations may become extinct due to demographic stochasticity (Engen et al. 2003; Sol & Maspons 2016) or from Allee effects (Engen et al. 2003; Gascoigne et al. 2009; Fauvergue 2013), but immigrants can increase population size and maintain the persistence of these populations (rescue effect) (Etienne 2000; Buoro & Carlson 2014). Dispersal also allows individuals to establish in empty patches and thus affects colonisation rate/invasion speed (Bowler & Benton 2005; Kubisch et al. 2014). Some factors such as population density and internal state of individuals can influence dispersal (Bowler & Benton 2005; Clobert et al. 2009). Therefore, investigations into the effect of these factors on dispersal tendency, distance and subsequent reproductive fitness are warranted.

1.2.3.1 Reproduction and survival of dispersers

The reproductive performance and survival of dispersers at new invasion sites can affect population establishment and subsequent expansion (Kot et al. 1996; Phillips et al. 2010; Ronce & Clobert 2012; Renault et al. 2018; Williams et al. 2019). Because dispersal is costly, dispersers may allocate more resources for movement, trading off resources for fecundity and survival (Mole & Zera 1993; Bonte et al. 2012; Khuhro et al. 2014; Renault 2020; Nasu & Tokuda 2021). In addition, such costs may increase with dispersal distance (Rousset & Gandon 2002; Baker & Rao 2004; Samietz & Kohler 2012; Serrano & Tella 2012), resulting in distance-specific resource trade-offs. Therefore, the reproduction and survival of dispersers in relation to dispersal distance are closely related to population establishment at the invasion front and invasion speed of species.

1.2.3.2 Density- and age-dependent dispersal

Population persistence and invasion speed depend on dispersal strategies of species (Kot et al. 1996) such as negative or positive density-dependent dispersal. A negative density-dependent dispersal strategy means that more individuals move out from low density populations and more immigrants into high density populations. This strategy increases the extinction rate of small (low density) populations leading to a smaller metapopulation size and reducing the colonisation rate (Sæther et al. 1999). In contrast, a positive density-dependent dispersal strategy allows individuals to leave high density habitats and immigrate into low density habitats and/or colonize new habitats, leading to a lower extinction rate and faster invasion speed (Sæther et al. 1999).

Females at the age of high fecundity may have higher dispersal probability and longer dispersal distance to spread their eggs (Gu & Danthanarayana 1990; Fadamiro 1997; Ishizaki et al. 2011; Perez-Mendoza et al. 2011). Moreover, the reproductive output of dispersers at the age of dispersal can affect population growth and establishment success in novel habitats

(Dingle 1965; Williamson & Charlesworth 1976; Stewart & Gaylor 1991; Schumacher et al. 1997; Järemo & Bengtsson 2011). Therefore, investigations into age-dependent dispersal in relation to age-specific reproduction can help better understand population dynamics (Payo-Payo et al. 2018; Hoy et al. 2020) and invasion success.

1.3 Aim and objectives of this study

The aim of this research is to understand characteristics of *T. ludeni* that may have facilitated its invasion success, with four objectives:

1) To investigate resource allocations of virgin females to their sons and reproductive strategies of females in response to mating in *T. ludeni*;

2) To study inbreeding depression and inbreeding avoidance behaviour in *T. ludeni*;

3) To determine the relationship between dispersal and reproduction/survival in *T. ludeni,* and

4) To determine the effects of population density and age on dispersal probability and distance in *T. ludeni*.

1.4 Literature review

This section reviews the current knowledge on aspects of life history strategies relevant to my studies on *T. ludeni*.

1.4.1 Taxonomy and distribution of *T. ludeni*

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

This species was identified by Prof Zhi-Qiang Zhang, University of Auckland, New Zealand.

Tetranychus ludeni was recorded for the first time from specimens collected on *Cucurbit* sp. and *Salvia splendens* Sellow ex Schult in Germany (Zacher 1913). It has since been found in many regions of the world, including Africa, America, Asia, and Oceania (McGregor 1919; Manson, 1967; Bolland et al. 1998; Waite 2000; Zhang 2002; Adango et al. 2006; Hong et al. 2010; Mendonça et al. 2011; Soares et al. 2012; Arimoto et al. 2013; Gotoh et al. 2015; Mandal et al. 2016; Singh & Chauhan 2017; Zele et al. 2018; Ragusa et al. 2019; CABI/EPPO 2020; Chen et al. 2020; Migeon & Dorkeld 2022; Naves et al. 2021; Sierra-Monroy et al. 2021).

1.4.2 General biology of *T. ludeni*

The life cycle of *T. ludeni* consists of five developmental stages: egg, larva, protonymph, deutonymph, and adult (Figure 1.1). The larva, protonymph, and deutonymph stages pass through a quiescent phase before moulting into the next stage (Zhang 2003). The lower developmental threshold is 14.7ºC (Zhang 2003) and the upper threshold is 36ºC, with the optimal thermal condition for survival and development being between 22~28ºC (Ristyadi et al. 2019). Under 26 ± 1ºC and 65 – 85% RH, developmental duration from egg to adult is 10.12 ± 0.07 days with 4.19 ± 0.04, 1.98 ± 0.05, 1.82 ± 0.05 and 2.12 ± 0.07 days for egg, larva, protonymph and deutonymph, respectively, on African eggplant *Solanum macrocarpon* L. (Adango et al. 2006). Males develop faster and emerge earlier than females (e.g., 8.72 vs 9.03 days on beans under 25 ± 1 ºC and 60 – 70% RH) (Zhang 2003; Gotoh et al. 2015).

1.4.2.1 Egg

Tetranychus ludeni eggs are spherical and 110 ~ 140 μm in diameter (Zhou et al. 2018). There are two types of eggs: (1) haploid, giving rise to sons, and (2) diploid, developing to daughters. Mated mothers produce translucent haploid and diploid eggs whereas virgin mothers produce reddish haploid eggs (Figure 1.1A). Eggs laid by mated females are smaller than those by virgin females (Zhou et al. 2018).

1.4.2.2 Larva, protonymph, and deutonymph

The larvae are white and small and have three pairs of legs (Figure 1.1B). The protonymphs differ from larvae by their darker colour, larger size, and possession of four pairs of legs (Figure 1.1C). The appearance of deutonymphs is very similar to that of adults except that deutonymphs have smaller body size and shorter legs (Figure 1.1D-F). Sex can be determined at this stage (Figure 1.1D-E). The hysterosoma of the female is markedly enlarged because of ovarian development while that of the male is tapered towards the anal region (Kaimal & Ramani 2011b; see Figure 1.1F). The quiescent deutonymphs become silvery just before moulting.

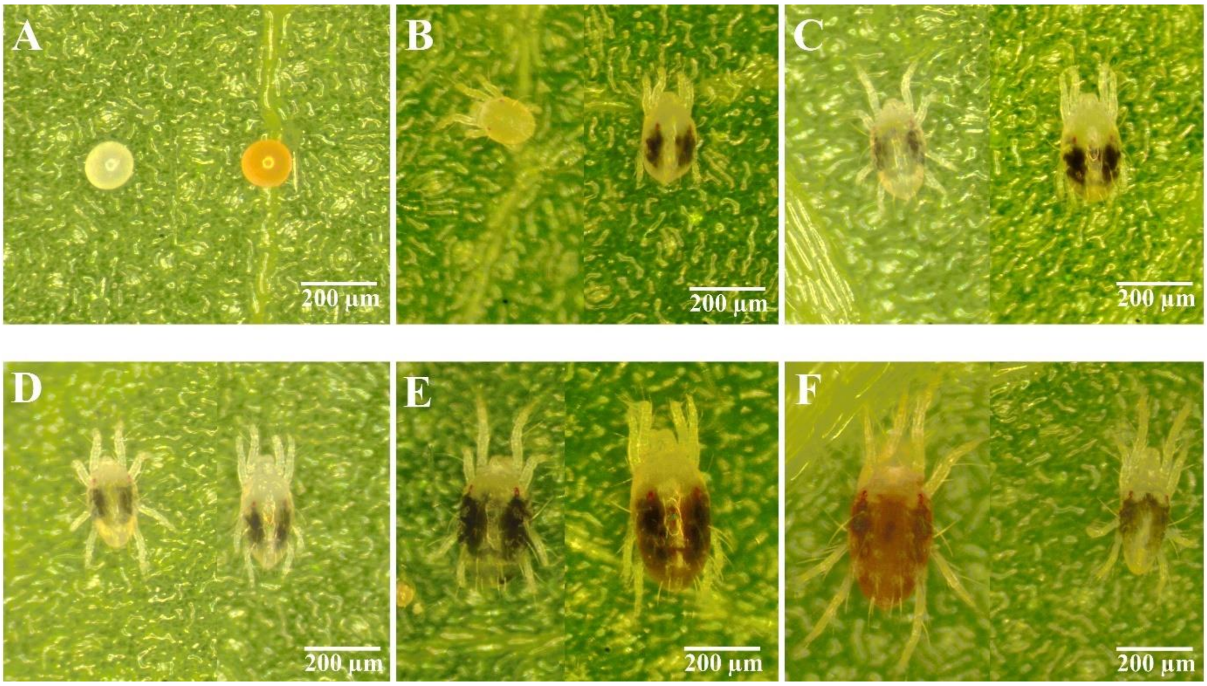

Figure 1.1 Life cycle and morphology of *T. ludeni*: (**A**) translucent (left) and reddish (right) eggs, (**B**) active (left) and quiescent (right) larvae, (**C**) active (left) and quiescent (right) protonymphs, (**D**) active (left) and quiescent (right) male deutonymphs, (**E**) active (left) and quiescent (right) female deutonymphs, and (**F**) female (left) and male (right) adults.

1.4.2.3 Adult

Adult females are dark red in colour and the body shape is more or less elliptical. Adult males have a wedge shaped hysterosoma and are smaller than females (Figure 1.1F). Newly emerged female is nearly three times larger than males (Ristyadi et al. 2019). Female body size keeps increasing to about 520 μm long and 300 μm wide in the first three or four days after emergence due to enlarged abdomen to accommodate eggs (increased 20% from 1 to 4 days old), whereas male body size remains similar across adult life. Both male and female can mate multiple times. Mated females produce an average of 84.29 ± 10.51 eggs with a female-biased offspring sex ratio (Adango et al. 2006). Males live longer than females (18 days vs 15 days, unpublished data).

1.4.3 Reproductive biology of Tetranychidae

1.4.3.1 Haplodiploidy

Haplodiploidy has three forms (reviewed in Normark 2003; Bachtrog et al. 2014; de la Filia et al. 2015). The most familiar and widespread form is arrhenotokous haplodiploidy, in which males develop from unfertilized eggs and thus lack paternal chromosomes at all development stages (Normark 2003; Bachtrog et al. 2014; de la Filia et al. 2015). Tetranychidae mites such as *T. urticae* and *T. ludeni* have this kind of haplodiploidy (de la Filia et al. 2015). In the other two forms, males begin life as diploid zygotes but their paternal chromosomes are either lost during embryonic development (embryonic elimination) or inactivated (Normark 2003; Bachtrog et al. 2014; de la Filia et al. 2015). These two phenomena are termed as paternal genome elimination (PGE). PGE is identical to arrhenotokous haplodiploidy in terms of genetic transmission.

1.4.3.2 Males

Adult males usually guard quiescent deutonymph females (Figure 1.2A) and mate soon after the latter emerge (Figure 1.2B) (Penman & Cone 1974; Potter 1978; Satoh et al. 2001). Mating lasts an average of two minutes (1.5 ~ 3.0 minutes). Males are attracted by the webs produced by female deutonymphs just prior to quiescence, and the colour and sex pheromones of quiescent female deutonymphs (Cone et al. 1971; Penman & Cone 1972; Penman & Cone 1974; Royalty et al. 1993; Rasmy & Hussein 1994). The quantity of emitted sex pheromones increases with the age of quiescent deutonymphal females, resulting in a significant increase in frequency of male guarding behaviour close to female moulting (Cone et al. 1971; Potter 1981).

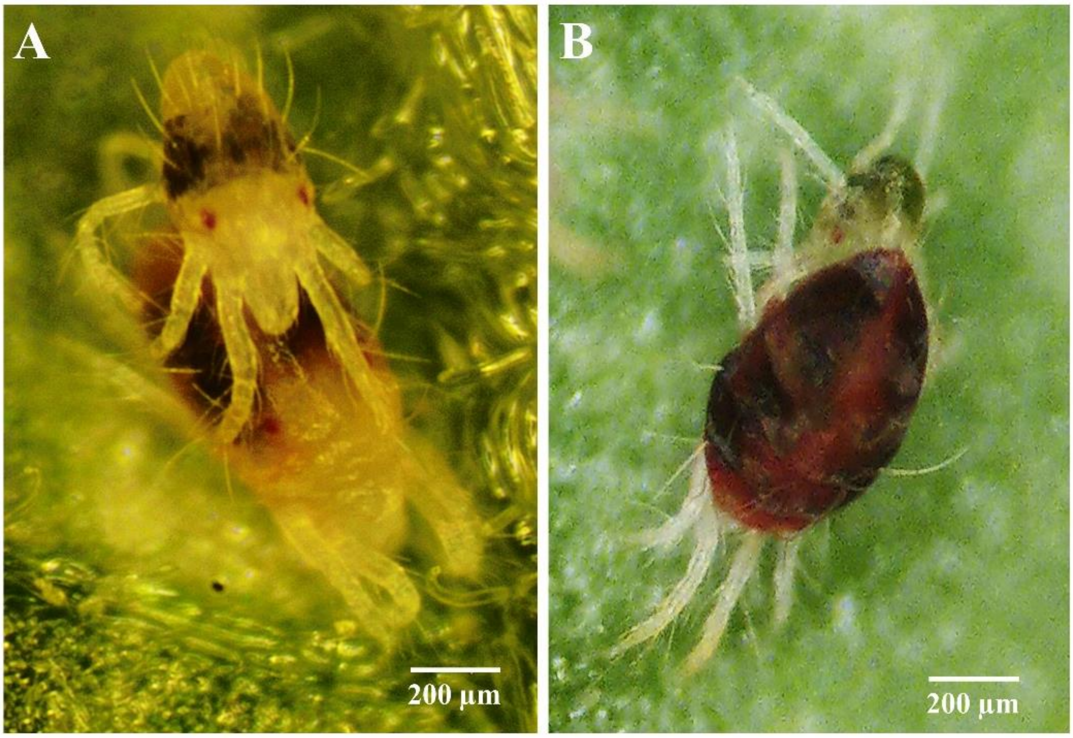

Figure 1.2 An adult male guarding a quiescent deutonymph female (**A**) and a mating pair of *T. ludeni* (**B**).

Being the first male to mate with females is important in spider mites because of the first-male sperm precedence (Helle 1967; Potter & Wrensch 1978; Morita et al. 2020; Rodrigues et al. 2020). Males thus often fight for guarding quiescent deutonymphal females using their first legs and stylets as weapons (Potter 1978; Satoh et al. 2001). Combats will happen when non-guarding (intruder) males attempt to approach females that are already guarded by conspecific males. The outcome of fighting is closely related to male body sizes and the position of males (invaders or guarders). For example, if the intruder is larger than the guarding male, the former has greater possibility to take over the quiescent deutonymph female and win the fight (Potter 1978; Enders 1993). In addition, guarding males have advantages when fighting against intruders (Potter 1978). In *T. urticae*, larger guarders have a probability of 89% to win; however, the probability drops to about 50% if they are intruders (Enders 1993).

The frequency of fighting increases when sex ratio is more male-biased (Enders 1993). Quiescent deutonymph females that are guarded by males may strategically release more chemical signals to attract more males, increasing combats between males to select better mates as indicated in the Kanzawa spider mite, *T. kanzawai* (Oku 2009). Male-biased sex ratio can also be caused by sex-specific mating frequency and dispersal. Adult females show decreasing attraction to males with age (Rasmy & Hussein 1994) whereas males are sexually capable throughout their adult live (Potter 1978), leading to male-biased operational sex ratio in a local population. In addition, adult females often disperse away after mating but males normally remain in their natal sites (Potter et al. 1976a).

Male spider mites have high reproductive capacity (Krainacker & Carey 1989; Krainacker & Carey 1990b; Li et al. 2017). For example, in *Amphitetranychus viennensis* (Zacher), one male can inseminate 26 to 37 females and produce 688 to 989 daughters in his lifetime (Li et al. 2017). A *T. urticae* male can copulate with 70 females and contribute to 1145 daughters in the first eight days of adulthood (Krainacker & Carey 1989). Male reproductive ability declines over age (Morita et al. 2021), for example, in *T. urticae*, 1-d-old males can inseminate an average of 15 females contributing to an average of 335.9 daughters while a 2-d-old male produces only half the number of daughters as compared to 1-d-old males, and a 4-d-old male produces only approximately 100 daughters (Krainacker & Carey 1989).

1.4.3.3 Females

Due to haplodiploid nature (Potter et al. 1976a), virgin spider mite females can produce haploid eggs and mated females produce both haploid and diploid eggs. Maternal mating status thus has profound effects on resource allocation to eggs. Haploid eggs produced by virgin females are larger than those by mated females in some species such as *T. urticae* (Macke et al. 2011b) and *T. ludeni* (Zhou et al. 2018). In *T. urticae*, one study shows that sons produced by virgin females appear to be able to find their mates more quickly than sons produced by mated females (Ohzora & Yano 2008). In another study, however, maternal mating status does not affect mate-searching behaviour of sons (Oku & van den Beuken 2017). Although larger haploid eggs develop to larger male adults (Macke et al. 2011b; Zhou et al. 2018), it is unknown whether larger males produced by virgin females have a fitness advantage over smaller ones produced by mated females, such as better mating success and higher reproductive output.

Mating also modifies female lifetime and age-specific reproductive investment. Compared to mated females, virgin females produce fewer lifetime number of eggs in *T. fijiensis* Hirst, *T. lambi* Pritchard and Baker, *T. marianae* McGregor, *T. neocaledonicus* André (Bonato & Gutierrez 1999) and *T. ludeni* (Zhou et al. 2021a). However, both virgin and mated females in *T. urticae* lay similar number of eggs in their lifetime (Li & Zhang 2021a). Compared to females without mating delay, females with mating delay lay fewer lifetime number of eggs in *T. ludeni* (Zhou et al. 2021a) but have similar fecundity in *T. urticae* (Morita et al. 2021). Delay-mated females produce offspring with a more female-biased sex ratio after mating in *T. ludeni* (Zhou et al. 2021a). In some species, mated females invest more in reproduction early in life, whereas virgin females spread reproductive investment over time and have a lower daily reproductive rate to prolong reproductive period and longevity (Macke et al. 2012; Li & Zhang 2021a; Zhou et al. 2021a). Females with mating delay can rapidly increase their investment in reproduction immediately after mating (Saitō 1987; Zhou et al. 2021a), suggesting that females can quickly adjust resource allocation in response to mating.

Females can also mate multiple times (Potter & Wrensch 1978). However, females predominantly use sperm of the first mated males to fertilize eggs regardless of mating interval and the number of matings (Helle 1967; Potter & Wrensch 1978; Rodrigues et al. 2020). They can mainly use sperm of the second male if their first mates are sperm depleted and the interval between the first and second mating is short (e.g., a few minutes) (Morita et al. 2021). Multiple mating can be harmful to females. For example, compared to once-mated females, multiply mated females lay fewer eggs and produce a less female-biased offspring sex ratio in *T. urticae* (Oku 2010; Macke et al. 2012; Rodrigues et al. 2020). Polyandry may benefit some males because mating with mated females can reduce the fitness of other males (Rodrigues et al. 2020).

Female-female interactions also affect their reproduction. In *T. urticae*, group-living females produce significantly more eggs on the fourth and fifth days of a five-day observation (Clotuche et al. 2010). However, the average daily fecundity and lifetime fecundity of group-living females are significantly lower than single-living ones, suggesting a cost of social interaction (Li & Zhang 2021b). In some species, such as *Stigmaeopsis longus* (Saito) and *S. miscanthi* (Saito), group living does not appear to affect lifetime fecundity (Sato & Saito 2007). In *T. ludeni*, group-living females reach reproductive peak earlier than single-living ones, suggesting a resource relocation strategy in response to conspecific competition (Weerawansha et al. 2020). The number of foundress females also affects offspring sex allocation. For example, group-living females produce more male-biased sex ratio than singly reared females in *T. urticae* (Roeder 1992; Macke et al. 2011a, 2012), *S. longus* and *S. miscanthi* (Sato & Saito 2007).

1.4.4 Inbreeding

1.4.4.1 Inbreeding depression

Mating with relatives often accumulate homozygous alleles, resulting in fitness reduction termed as inbreeding depression. Two main theories are proposed to account for inbreeding depression (Charlesworth & Charlesworth 1987; Charlesworth & Willis 2009): (1) the overdominance theory stresses that homozygotes lead to the loss of heterozygote advantage, and (2) the partial dominance theory emphasizes that homozygotes result in the expression of recessive or partially recessive deleterious alleles. Although inbreeding depression is assumed to be common across species, many studies report no inbreeding depression (Kureck et al. 2012; Trevisan et al. 2016; Quaglietti et al. 2017). The presence and severity of inbreeding depression are affected by many factors including inbreeding level, inbreeding history of populations/species, and haplodiploidy.

Inbreeding level (frequently referred to as inbreeding coefficient) describes the probability of homozygosity of alleles (including deleterious alleles) of a locus on the genome. Higher inbreeding level often leads to severer inbreeding depression, which has been illustrated theoretically (Keller & Waller 2002) and experimentally (Durel et al. 1996; Mori et al. 2005; Michalczyk et al. 2011; Ahlinder et al. 2021). For example, inbreeding for more generations leads to higher inbreeding level and severer depression in the spider mite, *S. miscanthi* (Mori et al. 2005). Mother-son mating has higher probability to produce homozygous offspring and may lead to severer depression than sibling mating. However, empirical evidence is largely lacking in this regard.

Frequent inbreeding may occur in small populations at the invasion front and in species with inbreeding mating system (Marchini et al. 2015) such as spider mites (Mitchell 1973). Theory predicts that frequent inbreeding could lead to the expression of homozygous deleterious recessive alleles and expose these alleles to selection and thus elimination from populations, for example, purging the genetic load (Glémin 2003). Therefore, populations or species with a long history of inbreeding may not suffer from inbreeding depression in the future, which has been widely illustrated in empirical studies (Crnokrak & Barrett 2002; Swindell & Bouzat 2006; Marchini et al. 2015; Tien et al. 2015; Roessler et al. 2019; Zhou et al. 2020, 2021a). For example, in *T. urticae*, inbreeding reduces female fecundity in the first generation, but such negative effect is diminished after several generations of inbreeding (Tien et al. 2015).

Studies show that haplodiploid animals have reduced inbreeding depression as compared to diploid ones. Two main reasons are generally assumed to account for this phenomenon (Werren 1993): (1) haplodiploid animals have lower effective mutation rate than diploids, and (2) deleterious recessive alleles responsible for inbreeding depression are expressed and purged in haploid males, reducing genetic load. However, genes controlling female-specific traits that cannot be expressed through males are free from immediate selection by haploid males and stay at high frequencies (Crozier 1976), and thus inbreeding is expected to affect female-specific traits in haplodiploids. Consistent with these predictions, empirical studies show no inbreeding depression in shared traits between males and females such as longevity and body size but detect substantial inbreeding depression in female-specific traits such as fecundity and offspring sex ratio in the wasps *Cotesia glomerata* (L.) (Zhou et al. 2007) and *Venturia canescens* (Gravenhorst) (Vayssade et al. 2014) and fecundity in the spider mite *T. urticae* (Tien et al. 2015).

1.4.4.2 Inbreeding avoidance

Given the negative effect of inbreeding, organisms are expected to have evolved mechanisms to avoid inbreeding. Inbreeding avoidance can be regarded as a special case of sexual selection. Sexual selection theory predicts that females that invest more in offspring than males choose mates that provide the most material benefits (direct selection) (Møller & Jennions 2001) or genetic benefits (indirect selection) (Andersson 1994). In the latter case, females may prefer mates with genes that are compatible with their own genotypes and reject mates with "incompatible genes" that may lead to deleterious allelic combinations that reduce offspring viability or fertility (genetic incompatibility hypothesis) (Brown 1997; Zeh & Zeh 1997; Neff & Pitcher 2005). Similarly, the inbreeding avoidance is to avoid the combination of deleterious alleles, a special case of genetic incompatibility avoidance (Neff & Pitcher 2005).

To avoid inbreeding, individuals should be able to recognize their kin. Kin recognition is a manner of differentiating conspecifics as a function of their genetic relatedness, which can be inferred through cues that correlate with relatedness (Holmes & Sherman 1983; Mateo 2004). Individuals may recognize their kin by (1) spatial distribution, (2) familiarity, either through association or phenotype comparison between themselves and their mates, and (3) recognition of relatives that share the same alleles and express a unique phenotypic trait (reviewed in Holmes & Sherman 1983; Mateo 2004). Inbreeding avoidance has been reported in several species such as *T. urticae* (Tien et al. 2011, 2015; Yoshioka & Yano 2014) and *Tribolium castaneum* (Herbst) (Michalczyk et al. 2011). However, deleterious alleles could have been purged due to frequent inbreeding (Tan et al. 2012; Nichols 2017; Zhou et al. 2020, 2021a), and in some cases parents may even gain fitness through inbreeding because mating between relatives helps spread identical beneficial genes by descent (Kokko & Ots 2006; Szulkin et al. 2013; Nichols 2017). For example, in *Pelvicachromis taeniatus* (Boulenger) both sexes prefer to mate with kin, and related parents are more cooperative and invest more in parental care than unrelated parents (Thunken et al. 2007). Under these circumstances, animals may not need to avoid inbreeding (Tan et al. 2012; Zhou et al. 2020). Selection for inbreeding avoidance can thus only be favoured if the costs of inbreeding exceed the benefits (Kokko & Ots 2006; Szulkin et al. 2013). However, inbreeding avoidance in haplodiploid animals is still largely unknown. It is also unclear whether inbreeding avoidance changes with inbreeding level.

1.4.5 Dispersal

Dispersal is the movement of individuals or populations from the natal habitat to breeding habitat (natal dispersal) or from one breeding habitat to another (breeding dispersal), which is the primary mechanisms leading to gene flow within and among populations (Renault 2020). Dispersal includes three distinct stages: emigration, transfer, and immigration (or departure, transience, and settlement) (Clobert et al. 2009; Bonte & Dahirel 2017). Each stage is affected by many factors (Clobert et al. 2009; Matthysen 2012), and factors that affect one stage may (Bitume et al. 2013) or may not (Hewison et al. 2021) have the same effect on other stages (Matthysen 2012). Investigation into the causal factors of each stage helps better understand the dispersal process (Matthysen 2012).

Dispersal can be selected if the benefits of dispersing exceed the costs (Bowler & Benton 2005; Matthysen 2012). Four main evolutionary forces have been proposed as ultimate causes of dispersal (Bowler & Benton 2005; Matthysen 2012): (1) kin interaction, including the benefits of avoiding inbreeding and kin competition and the costs of losing kin cooperation and outbreeding, (2) bet hedging, the increase of the variance in expected fitness through distributing offspring from the same parents over different habitats/conditions, (3) leaving unfavourable local conditions, and (4) costs of dispersal. At the proximate level, the causes of dispersal can be categorized as the internal state of individuals and external conditions (Clobert et al. 2009; Matthysen 2012).

1.4.5.1 Internal factors

Difference in dispersal among individuals to a large extent can be explained by variations that are associated with their life history, morphological, behavioural, and/or physiological traits (Clobert et al. 2009; Matthysen 2012; Ronce & Clobert 2012). The covariation of these phenotypic traits with dispersal is termed as dispersal syndrome. Dispersal syndromes can exist under the following situations: (1) some specialized traits promote dispersal success or there are trade-offs between dispersal and other traits in resource investment; (2) environmental conditions affect the expression of dispersal and other traits simultaneously, and (3) genetic correlations between dispersal and other traits occur.

1.4.5.1.1 Dispersal in relation to reproduction and survival

Dispersal is costly and thus often has a resource competition with other life functions such as reproduction and survival (reviewed in Ronce & Clobert 2012; Renault 2020). For example, before emigration, insects may invest more in structures (e.g., longer wings, legs, and flight muscles) that facilitate dispersal, and less in reproduction and survival (Mole & Zera 1993; Renault 2020). In addition, dispersal is energetically costly during movement, which can also reduce fecundity in actively dispersing arthropods such as *Stenobothrus lineatus* (Panzer) (Samietz & Kohler 2012) and *Choristoneura conflictana* (Walker) (Elliott & Evenden 2012).

Some studies do not detect a reduction in fecundity or survival of dispersers (Tigreros & Davidowitz 2019; Renault 2020; Zhou et al. 2021b). This could result from several reasons: (1) dispersers of some insect species are able to reallocate resources such as from flight muscle histolysis to reproduction and survival after settlement (Johnson 1963), for example, *Modicogryllus confirmatus* (Walker) (Tanaka 1993) and *Gryllus bimaculatus* De Geer (Lorenz 2007); (2) some species are able to accumulate resources for reproduction throughout their adult life, and thus dispersers can still obtain enough resources for reproduction after settling in resource-abundant habitats (Tigreros & Davidowitz 2019); (3) dispersal does not necessarily trade off with reproduction and/or survival, for example, body condition (e.g., size) and dispersal can be co-evolved (Bonte & de la Pena 2009), where dispersers have greater body condition and higher fecundity (Saastamoinen & Hanski 2008); and (4) the cost of dispersal may increase with the distance a species moves, resulting in distance-specific resource trade-offs (Rousset & Gandon 2002; Baker & Rao 2004; Samietz & Köhler 2012; Serrano & Tella 2012), e.g., short-distance dispersal may not reduce reproduction and survival (Veenstra & Byrne 1999). However, further investigations into the reproduction and survival of dispersers in relation to dispersal distance are warranted.

1.4.5.1.2 Dispersal in relation to age

Dispersal and reproduction may be age-specific. For instance, dispersers may trade off resources for early life reproduction to facilitate dispersal (Zhou et al. 2021b). In a parasitoid wasp *Melittobia australica* Girault, long-winged dispersers have fewer mature eggs at emergence and produce fewer eggs in the first clutch than the short-winged residents (Innocent et al. 2010). Dispersers are often founders of new populations and may experience different environmental conditions from residents, which may greatly affect the evolution of dispersers' life history such as reproductive effort (Phillips et al. 2010). For example, dispersers invest more in reproduction when young compared to residents in the common lizard, *Zootoca vivipara* (Lichtenstein), which could be selected by low competition intensity in newly settled sites, favoring larger investment in reproduction at early age (Cotto et al. 2015). Examining age-specific reproduction can therefore help better understand the cost of dispersal and/or reproductive strategies of dispersers.

Difference in dispersal tendency among age classes can be caused by different constraints or costs (Bowler & Benton 2005; Bowler & Benton 2009; Benton & Bowler 2012; Matthysen 2012). For example, in the soil mite *Sancassania berlesei* (Michael), immatures and old adults have higher dispersal mortality than young adults, explaining the more frequent dispersal of young adult mites (Bowler & Benton 2009). Different age classes may have different pressures or sensitivity to disperse. For example, in many birds, younger individuals are weak competitors and forced to find territory elsewhere (Bowler & Benton 2005; Benton & Bowler 2012) such as the magpie, *Pica pica* (L.) (Eden 1987). In reproducing females, age-specific reproductive values may also affect their sensitivity to environmental cues and dispersal decisions (Clobert et al. 2004, 2009).

Spider mites mainly disperse as adult females (Brandenburg & Kennedy 1982). Immatures do disperse, but only when living conditions are extremely poor such as lack of food (Smitley & Kennedy 1985; Clotuche et al. 2013). In *T. urticae* young adult females (≤ 2 days old) perform aerial dispersal much more frequently than old females (≥ 3 days old), probably because young females are smaller and easier to be carried aloft by air current (Li & Margolies 1993). With regards to ambulatory dispersal, Suiter and Gould (1992) show that adult females of different ages have similar dispersal tendency in *T. urticae*. However, the purpose of their study was to test female dispersal in response to a pyrethroid insecticide that is known to stimulate dispersal behaviour. So far, the age-specific ambulatory dispersal tendency of female spider mites is not clear.

1.4.5.2 External factors

Many environmental factors have been identified to affect dispersal such as density, habitat quality and sex ratio (Bowler & Benton 2005; Clobert et al. 2009). Population density and habitat quality can affect individual fitness and dispersal tendency in similar ways, for example, high density and poor host quality increase dispersal tendency in *T. urticae* (Li & Margolies 1993; Bitume et al. 2013). Biased sex ratio may entail intrasexual and intersexual competition, leading to dispersal (Odendaal et al. 1989; Steifetten & Dale 2012; Trochet et al. 2013). Here, I briefly review the effect of population density on dispersal, which is relevant to my study.

1.4.5.2.1 Effect of density on dispersal

Density is an extensively studied factor affecting dispersal (reviewed in Bowler & Benton 2005; Clobert et al. 2009; Matthysen 2012). High population density can increase both exploitative competition (which reduces *per capita* resources) and interference competition (direct effects of density itself), reducing individual fitness (Bowler & Benton 2005). Therefore, dispersal tendency is most often positively correlated with density, for instance, in spider mites, *T. evansi* and *T. urticae* (Li & Margolies 1993; Azandémè-Hounmalon et al. 2014), the spider *Erigone atra* Blackwall (De Meester & Bonte 2010), and the land snail *Cornu aspersum* (Müller) (Dahirel et al. 2016). However, negative correlations between density and dispersal probability have also been found in some species (Fronhofer et al. 2015; Ventura et al. 2017; Puzin et al. 2018), probably due to the benefits of group living, such as avoidance of predation risk and foraging facilitation (Ventura et al. 2017), Allee effects (reduced fitness at low densities) (Fronhofer et al. 2015), and/or higher cost of dispersal relative to a lower cost of being resident (Bowler & Benton 2005; Clobert et al. 2009). So far, most studies have focused on how density affects emigration (e.g., Li & Margolies 1993; Hovestadt et al. 2014; Mishra et al. 2018; Plazio et al. 2020) but the relationship between population density and dispersal distance is less understood (Bitume et al. 2013).

Chapter 2

Sons from Virgin Mothers Produce More Daughters in a Haplodiploid Mite

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Abstract

Population size can be very small in the newly invaded/introduced site or front edge of population expansion where mates are difficult to find. This scenario can lead to extinction of a local population in many animal species. However, when it happens to a haplodiploid animal, individual virgin females may adjust their strategies to produce sons of greater reproductive success such as higher mating success and fertility, which may help increase the chance of establishment. Here I investigated how maternal mating status affected sons' reproductive success in a haplodiploid spider mite, *Tetranychus ludeni* Zacher, a cosmopolitan pest of many crops. I show that virgin females laid significantly larger eggs than mated females, giving rise to larger deutonymphs and adults, but mating status of mothers had no influence on mating success and longevity of their sons. My study provides the first empirical evidence in a haplodiploid mite that virgin mothers adjusted their resource allocations to yield sons that produced more daughters at a higher rate.

2.1 Introduction

In haplodiploid arthropods, mated females may lay unfertilised haploid eggs giving rise to sons and fertilised diploid eggs leading to daughters while virgin females can only produce haploid sons (de la Filia et al. 2015). When population size is small, it is likely that females are not able to find mates and produce daughters. This scenario may be particularly common in new environments such as recently invaded sites or front edges of population expansion (Engen et al. 2003). Therefore, females may have developed strategies to adjust their resource allocations in response to the situation. So far, whether and how virgin females adjust their resource allocations and whether and how such adjustment influences their sons' reproductive success are poorly understood in haplodiploid animals.

Several authors have investigated the effect of maternal mating status on offspring performance in a haplodiploid mite, *Tetranychus urticae* Koch. In one study, sons produced by virgin females appear to be able to find their mates more quickly than males produced by mated females (Ohzora & Yano 2008). In another study, however, maternal mating status does not affect mate-searching behaviour of males (Oku & van den Beuken 2017). Macke et al. (2011b, 2012) report that male eggs laid by virgin females are usually larger than male eggs produced by mated females. Although the effect of maternal mating status is not examined, larger males are found to be more likely to win male-male competition for mates (Potter et al. 1976a, 1976b) and better resist against attempted interference during mating (Enders 1993). However, it is not clear whether maternal mating status has any influence on sons' reproductive outputs in any haplodiploid mite species, knowledge of which is important for the better understanding of invasive potential of these animals.

Tetranychus ludeni Zacher is native to Europe and now globally distributed (Bolland et al. 1998; CABI 2011). It appears to be better adapted to hot weather and have a more subtropical distribution than the cosmopolitan congeneric *T. urticae* (Martin 2000; Gotoh et al. 2015). This species is a serious pest of bean, eggplant, hibiscus, pumpkin and other cucurbitaceous plants in warm regions and a common pest on greenhouse plants in temperate areas (Zhang 2003). It has denser webbing than *T. urticae*, which is believed to make its biological control by predatory mites less effective (Martin 2000; Zhang 2002). However, compared to *T. urticae, T. ludeni* has been much less studied. For example, nothing is known about the effect of mothers' mating status on traits of their sons in *T. ludeni*.

In the present study, I carried out a series of experiments to examine how maternal mating status affected sons' body size, mating success, longevity, and reproductive outputs in *T. ludeni*. Based on the knowledge outlined above, I proposed and tested three hypotheses: (1) virgin females produce larger eggs than mated females and larger eggs develop to larger adults; (2) sons from virgin females (SVF) have better mating success than sons from mated females (SMF), and (3) SVF have higher reproductive output and greater longevity than SMF.

2.2 Materials and Methods

2.2.1 Mite colony

A colony of *T. ludeni* was established from adults collected on *Passiflora mollissima* (Kunth) in September 2017 in Palmerston North, New Zealand, and reared on kidney bean plants (*Phaseolus vulgaris* L.). Three to 5-week-old bean plants were used for maintaining the colony, and the first expanded leaves of 1 to 2-week-old plants were used for experiments. The colony was maintained and the potted plants grown in two separate environmental rooms at 25 ± 1 ºC, 40 ± 10% RH and 14:10 h (L:D) in the Massey University Entomology and IPM Laboratory, Palmerston North, New Zealand. Experiments were carried out in the third environmental room with the same environmental conditions. Mites were reared on kidney bean plants for three generations before experiments.

2.2.2 Experimental mites

To obtain sons from virgin females (SVF) and mated females (SMF), I randomly selected 60 female and 45 male deutonymphs from the colony and transferred them onto small leaf squares (1.0 × 1.0 cm), one mite on each square in a Petri dish (9.5 cm diameter and 1.0 cm height), and allowed them to become quiescent deutonymphs (QD) and emerge. Leaf squares were placed on the wetted cotton wool. The Petri dish was covered with a lid which had a 1-cm-diameter hole covered with metal mesh (aperture size = 0.25 mm) in the centre.

I kept 15 1-d-old virgin females individually in their Petri dishes to produce SVF. To obtain SMF I individually paired 45 1-d-old virgin females with 45 1-d-old virgin males for 24 hours and then removed the males. Because virgin *T. ludeni* females lay reddish eggs whereas mated females lay translucent eggs, I examined the colour of all eggs laid by the females exposed to males for 2 days following removal of males. Females that laid translucent eggs were considered as mated and used for producing SMF and the remaining were discarded. On the third day, I transferred virgin and mated females individually onto new leaf squares, one mite on each leaf square, and allowed them to oviposit for 24 hours, after which time, I transferred them onto new leaf squares. This procedure was repeated for 3-10 days depending on the desired number of mites required for experiments. I transferred eggs from virgin and mated females individually onto new leaf squares, one egg on each square in a Petri dish, and allowed them to develop to deutonymphs or adults. All dishes were numbered so that eggs and resultant adults were matched.

2.2.3 Body size and mating success of sons of virgin (SVF) and mated females (SMF)

To investigate the size and size distribution of eggs from females of different mating status (Figure 2.1), I measured 51 eggs laid by virgin females and 247 eggs (including both fertilised and unfertilised eggs) laid by mated females. I measured many more eggs from mated females because the sex ratio of offspring produced by mated female *T. ludeni* was highly female-biased (Gotoh et al. 2015). Egg size (total area from top view) was determined using a digital camera (Olympus SC30, Japan) connected to a stereomicroscope (Leica MZ12, Germany) and a computer with adequate imaging software (CellSens® GS-ST-V1.7, Olympus, Japan) installed. Measured eggs were individually transferred onto new leaf squares (1.0 × 1.0 cm) using a fine brush and raised in Petri dishes. On the fifth day, each larva or nymph was provided with a new leaf square of the same size to avoid leaf quality deterioration. Eggs that successfully developed to QDs were used to determine the difference in size of male eggs laid by virgin (n = 31) and mated females (n = 24) (Table 2.1). QDs of 27 SVF and 20 SMF were photographed and their size (total area from top view) measured using the above device. After emergence, 26 1-d-old SVF and 19 1-d-old SMF adults were photographed and their size measured again as above.

To determine whether the mating success of SVF and SMF was different, I randomly selected female deutonymphs from experimental mites as described in the previous section. Mites were individually transferred onto leaf squares (1.0 × 1.0 cm), one mite on each square in a Petri dish. About 20 hours after transfer, the QD females turned silvery and were ready for test. For each replicate, I randomly selected a 1-d-old SVF and a 1-d-old SMF from the experimental mites and randomly marked one of them on his rear dorsal using blue baking colour (Hansells, Queen New Zealand Pty. Ltd) before releasing them to a QD female on a leaf square in a Petri dish. I recorded their behaviours using the device mentioned above for 10 minutes after the female emerged. A total of 68 replicates were recorded. Because the first released males might be more likely to locate, guard and mate with the QD females independent of the mating status of their mothers, I carried out a second set of release regime: I transferred a 1-d-old SVF and a 1-d-old SMF onto a leaf square about 2 hours before the introduction of the QD female. Other experimental procedures were the same as the above test. Fifty-one replicates were performed.

2.2.4 Reproductive success of sons of virgin (SVF) and mated females (SMF)

This experiment was designed to determine the reproductive success of SVF and SMF. To provide sufficient female adults for experiments, I randomly collected 300 female deutonymphs from the colony each day and transferred them onto leaf squares (5 × 5 cm) with 50 mites on each square for development into adults. On the first day of experiments, I provided a 1-d-old SVF or SMF with 10 1-d-old virgin females on a leaf square (2 × 2 cm) in a Petri dish for 24 hours. The next day, the male (2-d-old) was transferred onto a new leaf square of the same size with 10 1-d-old virgin adult females for 24 hours. This procedure was repeated until the male died.

After the male had been transferred onto a new leaf square, the females were allowed to oviposit on their leaf square for 5 days, and then transferred onto a new leaf square of the same size. This procedure was repeated until all females died. All eggs laid by these females were allowed to develop to deutonymphs. For each test male, the longevity, fertile period (period during which the male could fertilise eggs, verified by the production of at least one daughter) and total number of daughters produced were recorded. Fifteen SVF and 15 SMF were tested.

2.2.5 Statistical analysis

All analyses were done using SAS 9.4 with a rejection level set at $\alpha < 0.05$. Male mating success (Figure 2.2) was analysed using a Chi-square test (FREQ procedure). A Wilcoxon test (LIFETEST procedure) was used to compare the survival probability of SVF and SMF. A goodness-of-fit test (Shapiro-Wilk test; UNIVARIATE procedure) was used to test the distribution of data when mean comparison was performed. Data on the mean size of male eggs, QDs and adults (Table 2.1), and the fertile period and number of daughters (Figure 2.3) were normally distributed and thus analysed using an analysis of variance (ANOVA, GLM procedure) followed by Tukey’s Studentized range test. However, data on mean size of eggs laid by virgin females and mated females (both fertilised and unfertilised eggs) were not normally distributed, and thus analysed using a non-parametric ANOVA (GLM procedure). The size distributions of those eggs were compared by a Kolmogorov-Smirnov Test (NPAR1WAY procedure) (Figure 2.1).

I also developed a Power curve model (NLIN procedure) to fit the data on accumulation of insemination potential (number of daughters produced by SVF or SMF; Figure 2.4): cumulative daughters = $a + b \times (1 - c^{\text{age}})$, where a is the model constant, b is the maximum number of daughters produced during males’ lifetime reflecting the maximum potential of sperm transferred, and c is the constant increasing rate of daughters produced per day, and $a > 1$, $b > 1$, and $1 > c > 0$. The estimated parameters are significantly different from 0 if the 95% confidence limits (CL) do not include 0 (both upper and lower CL > 0 or < 0) (Julious 2001). Julious’ (2004) methods were used to compare parameters in Table 2.2, where there is no significant difference for a given parameter if the 83.4% CL overlap. The coefficient of determination (R^2) for Power curve model was calculated as: 1 – (residual sum of square/corrected total sum of square) (Tahriri et al. 2007).

2.3 Results

2.3.1 Body size and mating success of sons of virgin (SVF) and mated females (SMF)

My results show that eggs laid by virgin females were significantly larger (mean ± SE = 1.3262 ± 0.0147, $\times 10^4\ \mu m^2$) than those by mated females (1.2601 ± 0.0063, $\times 10^4\ \mu m^2$) ($F_{1,296} = 19.52$, $P < 0.0001$), and the size distributions of eggs from virgin and mated females were significantly different ($D = 0.3172$, $P < 0.0001$; Figure 2.1). SVF of all stages from eggs to adults were significantly larger than SMF (Table 2.1). However, SVF and SMF had similar mating success regardless of which sex was introduced into the arena first (Chi-square test: $x_1^2 = 0.15$, $P = 0.7008$ when males were introduced first; $x_1^2 = 0.02$, $P = 0.8759$ when QD females were introduced first) (Figure 2.2).

Table 2.1 Size (× 10^4 μm^2) of sons of virgin (SVF) and mated females (SMF).

Son	Egg	Quiescent deutonymph	Adult
SVF	1.3519 ± 0.0158 a	3.1048 ± 0.0483 a	2.9674 ± 0.0373 a
SMF	1.1385 ± 0.0280 b	2.8625 ± 0.0551 b	2.7544 ± 0.0520 b
$F_{(df)}$	49.84 $_{(1,53)}$	10.95 $_{(1,45)}$	11.66 $_{(1,43)}$
P	< 0.0001	0.0018	0.0013

Means (± SE) with the same letter in each column are not significantly different (P > 0.05).

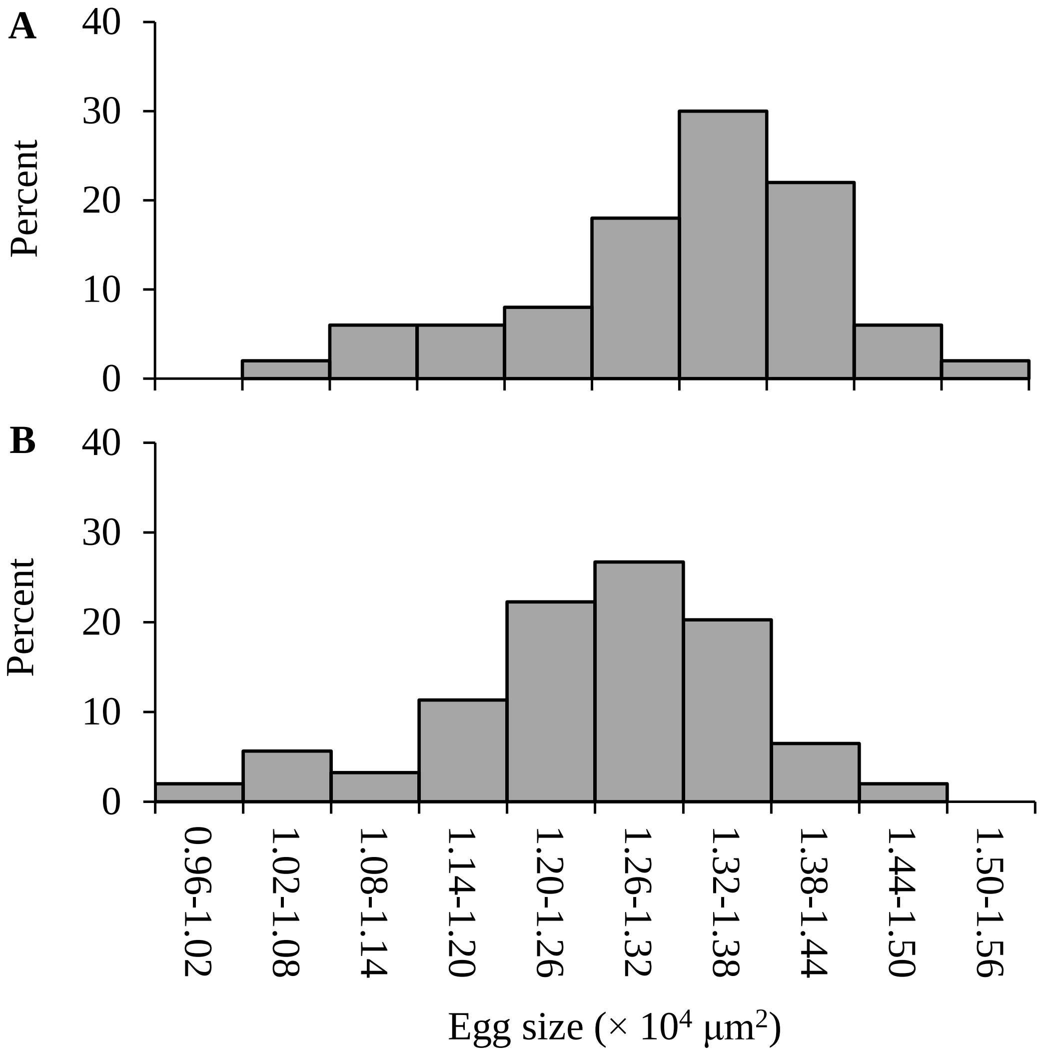

Figure 2.1 Size distribution of eggs laid by (**A**) virgin females (n = 51) and (**B**) mated females (n = 247).

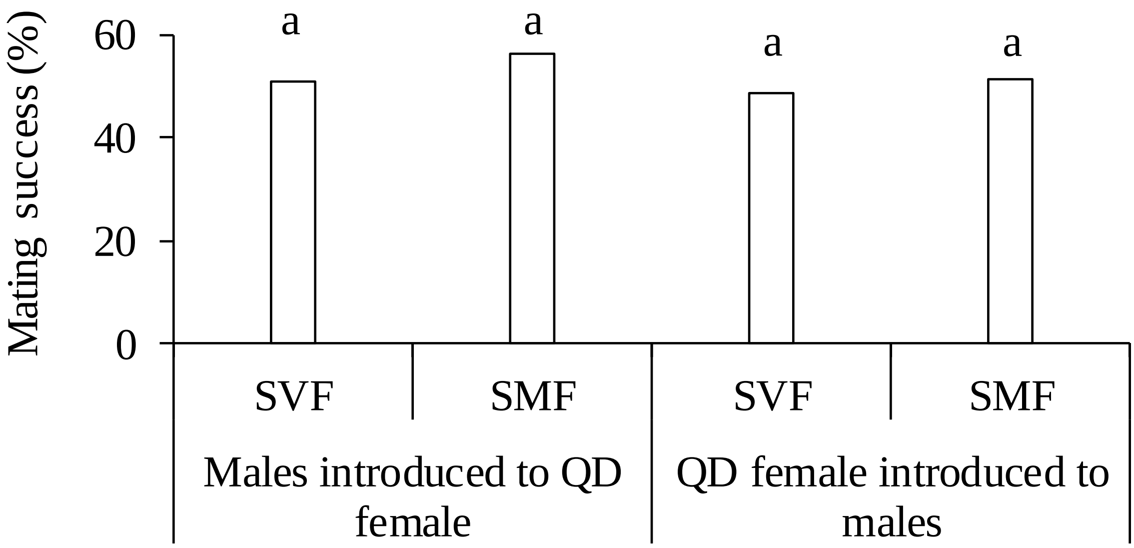

Figure 2.2 Mating success of sons of virgin (SVF) and mated females (SMF). Columns with the same letters in each case are not significantly different (Chi-square test: P > 0.05).

2.3.2 Reproductive success of sons of virgin (SVF) and mated females (SMF)

The fertile period was not significantly different between SVF and SMF ($F_{1,28}$ = 1.76, P = 0.1958; Figure 2.3A). SVF produced significantly more daughters (mean total ± SE = 1,407 ± 108) than SMF (mean total ± SE = 855 ± 78) ($F_{1,28}$ = 17.29, P = 0.0003; Figure 2.3B). Further analyses show that the estimated maximum number (b) of daughters produced was significantly greater in SVF than in SMF (83.4% CL did not overlap; Table 2.2), contributing to the faster accumulation of daughters produced (Figure 2.4). However, the longevity was not significantly different between SVF (17.73 ± 1.54 days) and SMF (18.07 ± 1.89 days) (x_1^2 = 0.13, P = 0.7182).

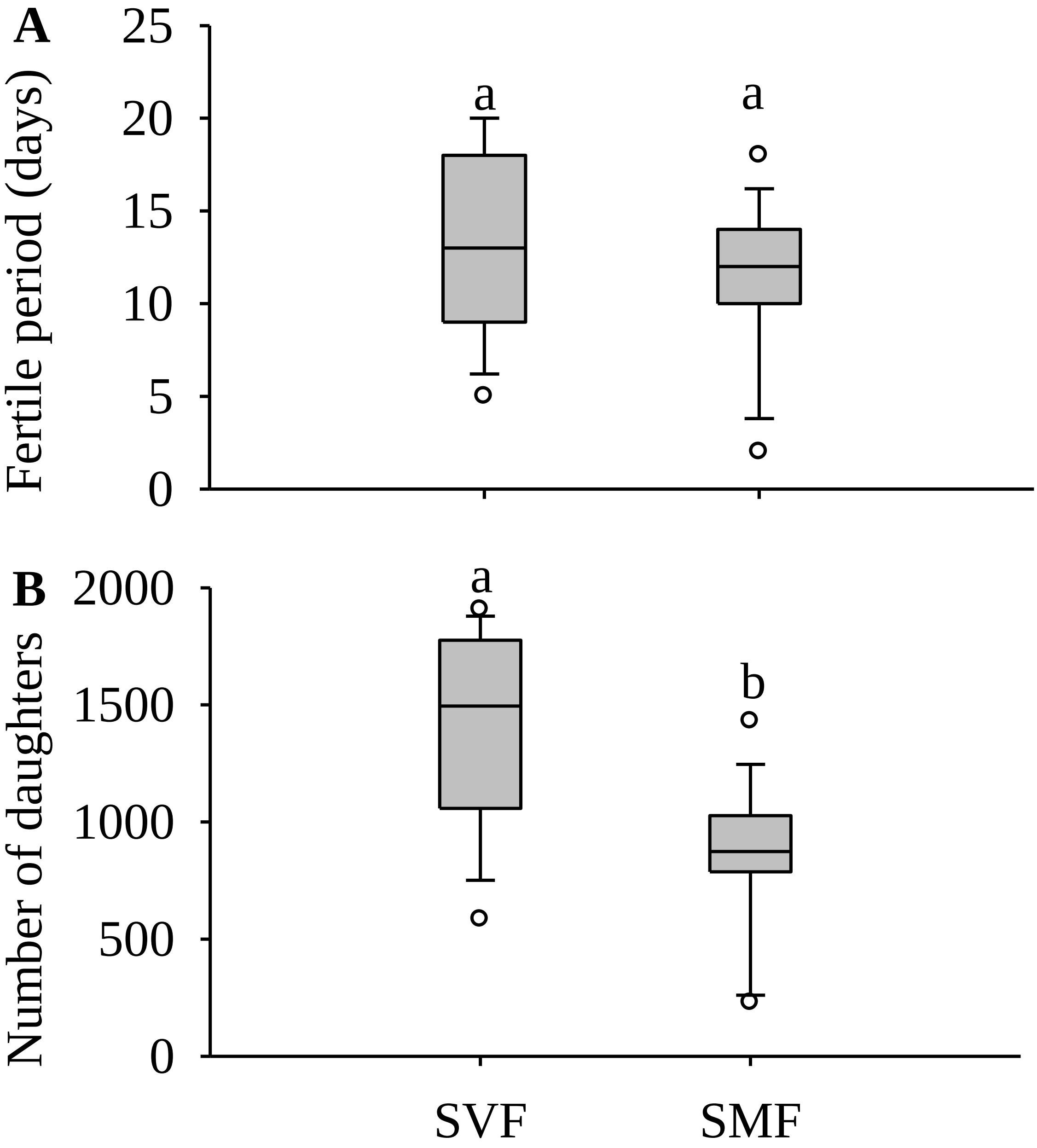

Figure 2.3 Fertile period (**A**) and daughters produced (**B**) in sons of virgin (SVF) and mated females (SMF). Columns with the same letters are not significantly different (ANOVA: $P > 0.05$).

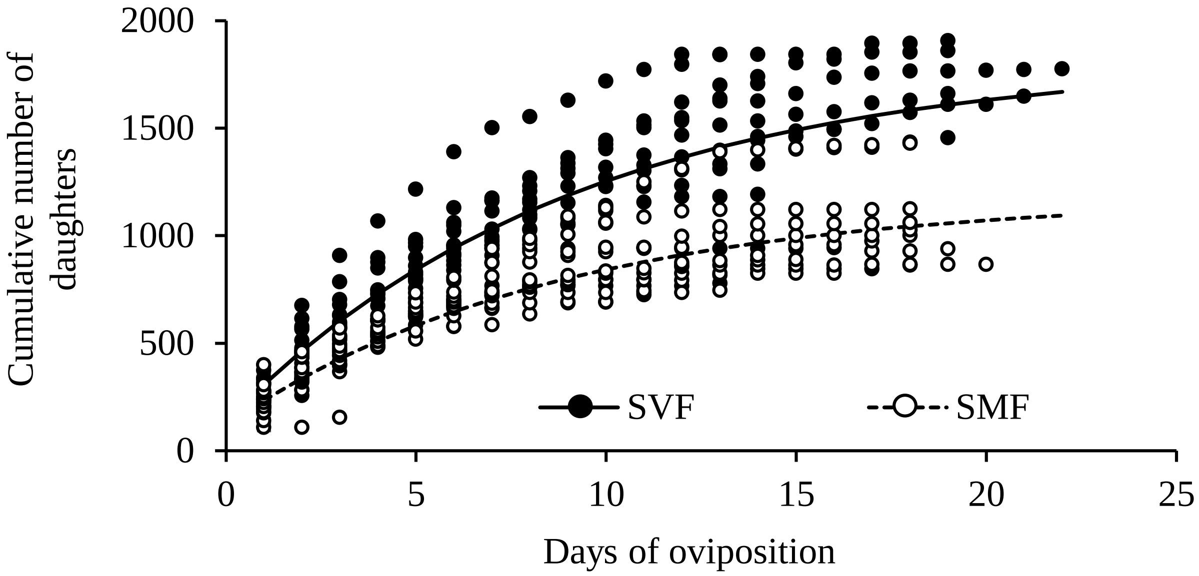

Figure 2.4 Lifetime reproductive potential of sons of virgin (SVF) and mated females (SMF). SVF: cumulative daughters = 134.10 + 1688.90 × (1 – 0.90^{age}) ($R^2 = 0.9639$, $F_{2,230} = 360.29$, $P < 0.0001$); SMF: cumulative daughters = 119.80 + 1056.80 × (1 – 0.89^{age}) ($R^2 = 0.9544$, $F_{2,206} = 232.61$, $P < 0.0001$).

Table 2.2 Comparison of estimated parameters of Power curve model in Figure 2.4 between sons of virgin (SVF) and mated females (SMF)*.

Parameter	Male	Estimate	SE	83.4% CL	
				lower	upper
a	SVF	134.10	60.25	50.42	217.90
	SMF	119.80	46.64	54.97	184.60
b	SVF	1688.90	80.31	1573.20	1804.70
	SMF	1056.80	65.19	966.20	1147.50
c	SVF	0.90	0.01	0.88	0.92
	SMF	0.89	0.02	0.86	0.92

* *a*, the model constant; *b*, the maximum number of daughters produced; and *c*, the constant daily increasing rate of cumulative daughters produced. All estimated parameters are significant at $P < 0.05$ level (95% CL > 0).

2.4 Discussion

Numerous studies have reported the positive relationships between egg size and offspring fitness across taxa including mites (e.g., Macke et al. 2011b), insects (e.g., Azevedo et al. 1997; Fox 1994, 2000; Fox & Czesak 2000; Torres-Vila & Rodriguez-Molina 2002; Fischer et al. 2002, 2003), fish (e.g., Hutchings 1991; Maruyama et al. 2003; Tamada & Iwata 2005), and birds (e.g., Price 1998; Ferrari et al. 2006). However, prior to the current study, little was known about strategic resource allocations to their sons by mothers of different mating status and the consequences of such strategies in a haplodiploid animal.

In *T. urticae*, virgin mothers produce significantly larger sons than mated mothers; however, mean egg size and egg size distribution are similar regardless of maternal mating status when both fertilised and unfertilised eggs are included in the analysis, suggesting that the differential egg size allocation takes place prior to fertilisation (Macke et al. 2011b). As a result, the fact that virgin females produce larger sons is not a resource allocation strategy in response to maternal mating status in that species. However, when both fertilised and unfertilised eggs were incorporated in analysis, I demonstrate that virgin females laid significantly larger eggs than mated females, and the size distributions of eggs from virgin and mated females were significantly different (Figure 2.1). My findings indicate that the egg size difference between virgin and mated *T. ludeni* females is indeed a result of strategic resource allocation in response to maternal mating status, with more resources being allocated to their sons when females do not have the chance to produce daughters.

When I compare male offspring produced by mothers of different mating status, my results show that virgin *T. ludeni* females laid significantly larger male eggs than mated females, and the resultant larger male eggs developed to larger deutonymphs and adults (Table 2.1). In principle, larger males have advantages in male-male competition (Andersson 1994; Emlen 2008; Hunt et al. 2009; Suzaki et al. 2013; Shelly 2018). Ohzora and Yano (2008) reveal that although size is not measured, sons from virgin mothers disperse faster and start guarding females sooner than those from mated mothers in *T. urticae*, suggesting that the former may have better mating success than the latter. However, my data from the two mate competition tests do not support this notion because larger SVF and smaller SMF had the same mating success when they were allowed to compete for a female (Figure 2.2). In an experiment on *T. urticae* with a design similar to mine, Oku and van den Beuken (2017) did not find any difference in mate-searching behaviour between SVF and SMF. These suggest that maternal mating status affects neither mating success nor mate-searching behaviour of their sons.

When I tested the lifetime fecundity of SVF and SMF, I found that SVF produced significantly more daughters than SMF (Figure 2.3B). Furthermore, compared to SMF, SVF contributed to a significantly faster increase of daughter population growth (Table 2.2, Figure 2.4). These data strongly suggest that when a female has no chance to mate, she produces sons that can yield more daughters at a higher speed. This strategy compensates the loss of producing daughters for virgin females through their sons' production of more daughters at a faster rate in the next generation. As a result, SVF may be able to found a colony faster as compared to SMF. The resource allocation strategy taken by virgin *T. ludeni* females and its consequences reported above may be attributed to the notion that large males generally have more sperm available for copulation (Wiernasz et al. 2001; Locatello et al. 2008; Anthes et al. 2014; O'Dea et al. 2014; Sturm 2014) and replenish their sperm reserves faster (O'Dea et al. 2014) than small males because testis size usually increases with body size (Gage 1994; Simmons 2012).

Mating may be costly to males, including expenditure on sperm and seminal fluid production (Van Voorhies 1992; Pitnick 1996), mate access (Barnes & Partridge 2003; Metzler et al. 2016), and immune defence against sexually transmitted pathogens (Schwenke et al. 2016), which could reduce resources for somatic maintenance (Vinogradov 1998). As a result, mating may cut males' future survival (Roff 1992; Stearns 1992; Cichoń 2001; Roff & Fairbairn 2007). Because SVF sire more eggs than SMF, the former is expected to spend more resources for reproduction and to live shorter lives. However, both SVF and SMF had similar longevity, suggesting that survival of *T. ludeni* males is not dependent on their reproductive expenditure or their mothers' mating status.

In conclusion, this study provides the first empirical evidence that the virgin females strategically allocate more resources to their sons, which can produce more daughters at a higher rate, in a haplodiploid mite. In a newly invaded scenario where population is small and females are more likely to be virgin, this feature may increase the chance for the species to become established.

Chapter 3

No Evidence for Inbreeding Depression and Inbreeding Avoidance in a Haplodiploid Mite *Tetranychus ludeni* Zacher

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Abstract

Mating between relatives often result in inbreeding depression, and substantial inbreeding depression may drive the evolution of inbreeding avoidance behaviour. However, it is suggested that inbreeding depression may be less severe in haplodiploid animals, and it is not clear whether they have evolved inbreeding avoidance. Here, I tested inbreeding depression and inbreeding avoidance using the haplodiploid spider mite, *Tetranychus ludeni* Zacher. I found that mother-son and brother-sister inbreeding had no negative influence on female reproductive output and longevity in the first and the 11th generations. Neither sex of *T. ludeni* had significant preference between siblings and unrelated mates in mate choice due to lack of inbreeding depression.

3.1 Introduction

Mating between relatives (inbreeding) may increase homozygosity of recessive or partially recessive deleterious alleles, resulting in inbreeding depression (Charlesworth & Charlesworth 1987; Charlesworth & Willis 2009). The cost of inbreeding may drive the evolution of inbreeding avoidance behaviour (Pusey & Wolf 1996; Nichols 2017). However, increased homozygosity due to inbreeding could expose recessive deleterious alleles to selection which purges them from the genome (Crnokrak & Barrett 2002; Keller & Waller 2002), resulting in little or no fitness reduction (Nichols 2017). Parents may even gain fitness through inbreeding because mating between relatives helps spread identical beneficial genes by descent (Kokko & Ots 2006; Szulkin et al. 2013; Nichols 2017) which will increase fitness (Hamilton 1972; Bai et al. 2005). Under these circumstances, animals may not need to avoid inbreeding (Tan et al. 2012). Therefore, whether inbreeding avoidance behaviour has evolved in an animal species may depend on the magnitude of inbreeding depression (Lande & Schemske 1985; Szulkin et al. 2013; Nichols 2017).

In haplodiploid animals where males are haploid from unfertilised eggs and females are diploid from fertilized eggs, inbreeding depression may be less severe and should only affect female-specific traits such as fecundity and offspring sex allocation (Henter 2003; Mori et al. 2005; de la Filia et al. 2015; Tien et al. 2015) because deleterious alleles are subject to selection in haploid males (Atmar 1991; Antolin 1999; Smith 2000; Henter 2003). However, whether inbreeding avoidance, if any, is sex-specific is unknown. Spider mites are a group of haplodiploid animals where frequent sibling and mother-son mating occurs (Mitchell 1973; Borgia 1980; Avilés & Purcell 2012) because mated mothers often lay haploid and diploid eggs closely together and brothers and sisters develop on the same spot and mate upon emergence (Mitchell 1973). Previous studies show that sibling mating causes no or limited inbreeding depression (Perrot-Minnot et al. 2004; Ito et al. 2012) whereas mother-son mating leads to substantial depression (Mori et al. 2005; Tien et al. 2015). However, most studies of effects of inbreeding on reproductive fitness in haplodiploids have only investigated one or a few generations, limiting our understanding of how inbreeding potentially affects long-term fitness.

Here I investigated aspects of inbreeding using the spider mite *Tetranychus ludeni* Zacher, nothing of which was known prior to this study. I carried out a series of experiments to determine (1) whether and to what extent inbreeding depression occurred in over 11 generations of sibling and mother-son inbreeding, and (2) whether the species performed sex-specific inbreeding avoidance.

3.2 Material and methods

3.2.1 Experimental Mites

I established a colony of *T. ludeni* from adults collected on *Passiflora mollissima* (Kunth) in Palmerston North, New Zealand, and reared it on 3- to 5-week-old kidney bean plants (*Phaseolus vulgaris* L.). I then split the colony into two colonies (A and B) and reared them on kidney bean plants in two separate climate rooms for 2.5 months (about 8 generations) before experiments, allowing to conduct inbreeding and outbreeding treatments (see below). I maintained the colonies and carried out experiments at 25 ± 1ºC, 40 ± 10% RH and 14:10 (L:D) photoperiod. I used the first expanded leaves of 1- to 2-week-old bean plants for all experiments. To prepare mites for inbreeding experiments, I randomly selected 40 male and 40 female deutonymphs from Colony A and maintained them individually until emergence. I allowed newly emerged virgin females to mate with newly emerged virgin males once and then transferred each mated female onto a leaf square (2.0 × 2.0 cm) placed on wet cotton wool in a Petri dish (9.5 cm diameter × 1.5 cm height) for oviposition for five days.

3.2.2 Effects of inbreeding on reproduction and survival in different generations

To determine whether and how inbreeding affected offspring fitness, I randomly selected three female deutonymphs that developed from the above eggs laid by each female for the following three treatments: (1) MS — mothers mated with their sons for 11 successive generations, (2) BS — brothers mated with their sisters for 11 successive generations, and (3) OB (outbreeding) — females mated with males from Colony B for 11 successive generations. As females in treatment MS were about 10 days old when their sons developed to adults, I used 10-day-old females for all three treatments in each generation to keep female age and oviposition experience consistent. In each generation I individually transferred female deutonymphs prepared as described above onto leaf squares (2.5 × 2.5 cm) for emergence. I allowed virgin females to reproduce for 10 days and then paired each of them with a newly emerged virgin male according to treatments until death. The leaf squares were replaced once every five days for each replicate. From each mated female, one to three female deutonymphs produced within five days after mother mating was randomly selected to start the next generation. I recorded the lifetime number of eggs laid, offspring survival, offspring sex ratio after mating, and longevity for each pair in the first and 11th generations. I obtained 30, 29 and 31 replicates in the first generation and 28, 27 and 29 replicates in the 11th generation for treatments MS, BS and OB, respectively.

3.2.3 Inbreeding avoidance

To test inbreeding avoidance behaviour, I used offspring from the 11th generation of the above experiment and carried out two experiments: (1) female mate choice — a female was allowed to choose between a brother and a male from Colony B, and (2) male mate choice — a male was allowed to choose between a sister and a female from Colony B. Female and male mate choice were tested for each of the MS, BS, and OB treatments, resulting in 6 combination choice treatments with 39–56 replicates for each treatment. To start the experiments, I introduced two virgin 1-d-old mates on a leaf square (1 × 1 cm) and then the test virgin 1-d-old female or virgin 1-d-old male at a point with the same distance from the two mates. I video-recorded each replicate for 15 minutes and recorded premating period, mating success, and mating duration. Mating was scored as successful when the male genital was connected with the tip of the female abdomen for over 30 seconds, during which time insemination occurs (Potter & Wrensch 1978).

3.2.4 Statistical analysis

The distribution of all data was tested using a Shapiro-Wilk test (UNIVARIATE Procedure) before analysis. Data on the number of eggs and daughters, male and female longevity in the inbreeding experiment, and premating duration in the female mate choice experiment were normally distributed and analysed using an analysis of variance (ANOVA, GLM Procedure) with a Tukey test for multiple comparison. Data on proportion of daughters and offspring survival in the inbreeding experiment, and premating period and mating duration in the male mate choice experiment, and mating duration in the female mate choice were not normally distributed and analysed using nonparametric ANOVA (GLM Procedure). Data on mate choice were analysed with a Chi-square test (FREQ Procedure). I conducted all analyses using SAS software (SAS 9.4, SAS Institute Inc., Cary, NC).

3.3 Results

3.3.1 Effects of inbreeding on reproduction and survival in different generations

My results show that offspring from MS, BS and OB had similar fitness in the first and the 11th generations (Table 3.1).

Table 3.1 Effects of inbreeding on reproduction and survival of *T. ludeni* in different generations.

Treatment	Number of eggs	Number of daughters	Daughters (%)	Offspring survival (%)	Longevity (days)	
					Female	Male
First generation						
MS	75.73 ± 6.78	36.50 ± 4.15	75.19 ± 2.15	92.29 ± 0.94 ab	22.59 ± 0.86	30.52 ± 1.57
BS	69.76 ± 4.48	35.24 ± 3.18	80.82 ± 1.84	91.57 ± 0.82 ab	22.07 ± 0.93	28.08 ± 1.52
OB	68.13 ± 4.37	34.26 ± 3.66	78.61 ± 1.92	93.83 ± 0.69 a	20.81 ± 0.63	26.96 ± 1.58
Eleventh generation						
MS	83.11 ± 6.98	40.50 ± 3.79	71.60 ± 3.16	87.54 ± 1.44 b	24.63 ± 1.33	24.58 ± 2.49
BS	72.89 ± 5.22	37.89 ± 3.88	73.52 ± 3.28	86.50 ± 3.06 b	22.89 ± 1.33	23.23 ± 2.77
OB	83.38 ± 5.70	47.21 ± 3.65	77.02 ± 1.64	88.25 ± 1.17 b	24.41 ± 1.04	24.41 ± 1.69
$F_{(df)}$	$1.1_{(5,168)}$	$1.63_{(5,168)}$	$1.68_{(5,168)}$	$5.6_{(5,168)}$	$1.86_{(5,163)}$	$1.93_{(5,154)}$
P	0.364	0.1536	0.1414	< 0.0001	0.104	0.0924

Means (± SE) of offspring survival (%) with the same letters are not significantly different (non-parametric ANOVA: P > 0.05). MS, mother-son mating; BS, brother-sister mating; OB, outbreeding.

3.3.2 Inbreeding avoidance

Inbred *T. ludeni* had no significant preference between siblings and unrelated mates in mate choice (For females: MS, $\chi^2 = 2.27$, P = 0.1317; BS, $\chi^2 = 1.26$, P = 0.2623; OB, $\chi^2 = 0.10$, P = 0.7576, Figure 3.1A; for males: MS, $\chi^2 = 2.57$, P = 0.1088; BS, $\chi^2 = 2.81$, P = 0.0934; OB, χ^2

= 0.00, P = 1.0000, Figure 3.1B). Furthermore, when mated with siblings or unrelated mates, *T. ludeni* had similar premating period (For females: $F_{5,119} = 1.18$, P = 0.3229, Figure 3.2A; for males, $F_{5,145} = 1.98$, P = 0.0842, Figure 3.2B) and mating duration (For females: $F_{5,119} = 0.24$, P = 0.9429, Figure 3.2C; for males, $F_{5,145} = 0.24$; P = 0.9444, Figure 3.2D).

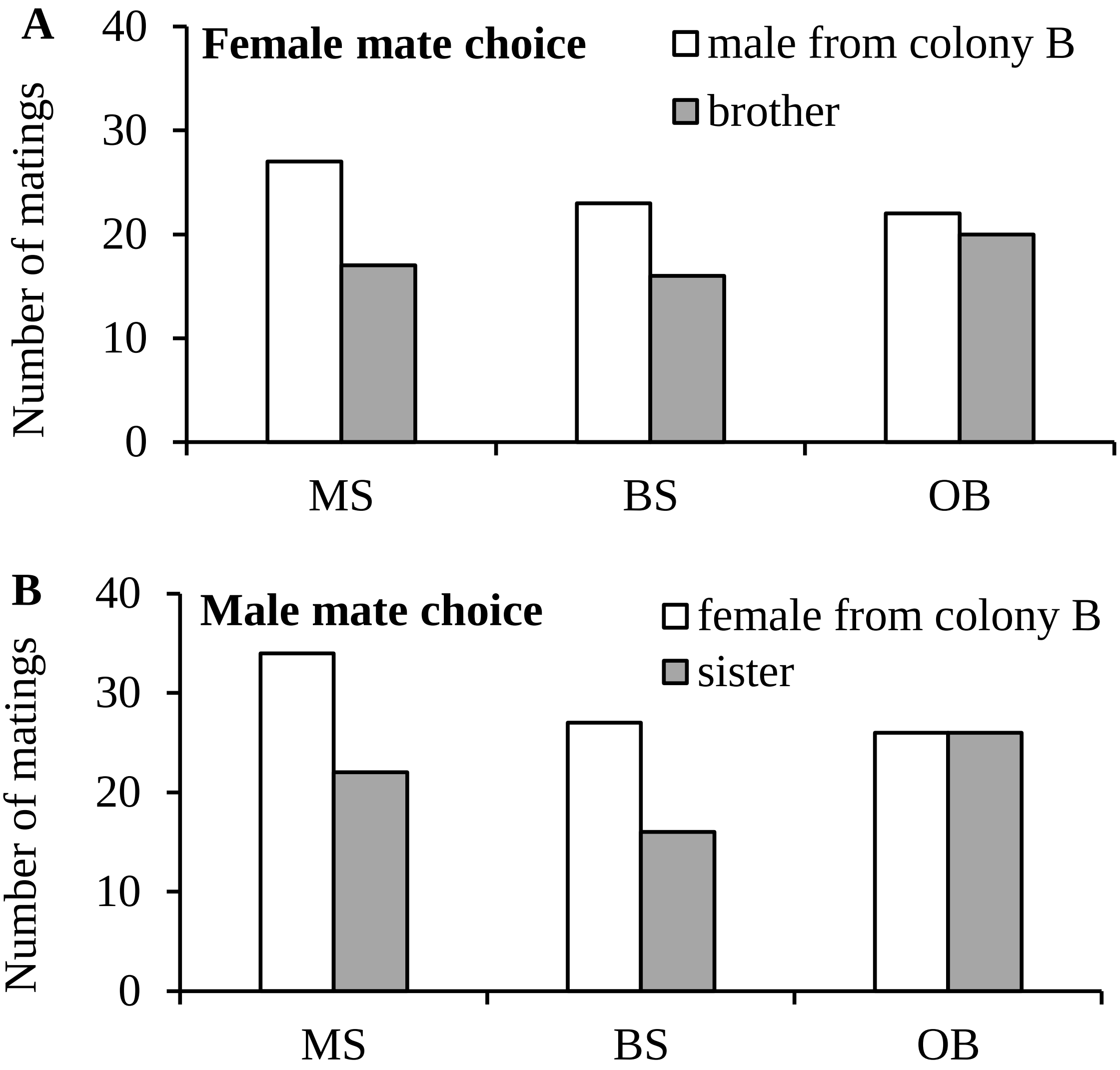

Figure 3.1 Female (**A**) and male (**B**) *Tetranychus ludeni* mate choice. MS, mother-son mating; BS, brother-sister mating; OB, outbreeding mating. There were 44, 39, 42 replicates for MS, BS, and OB, respectively, in female mate choice, and 56, 43, 52 replicates for MS, BS, and OB, respectively, in male mate choice.

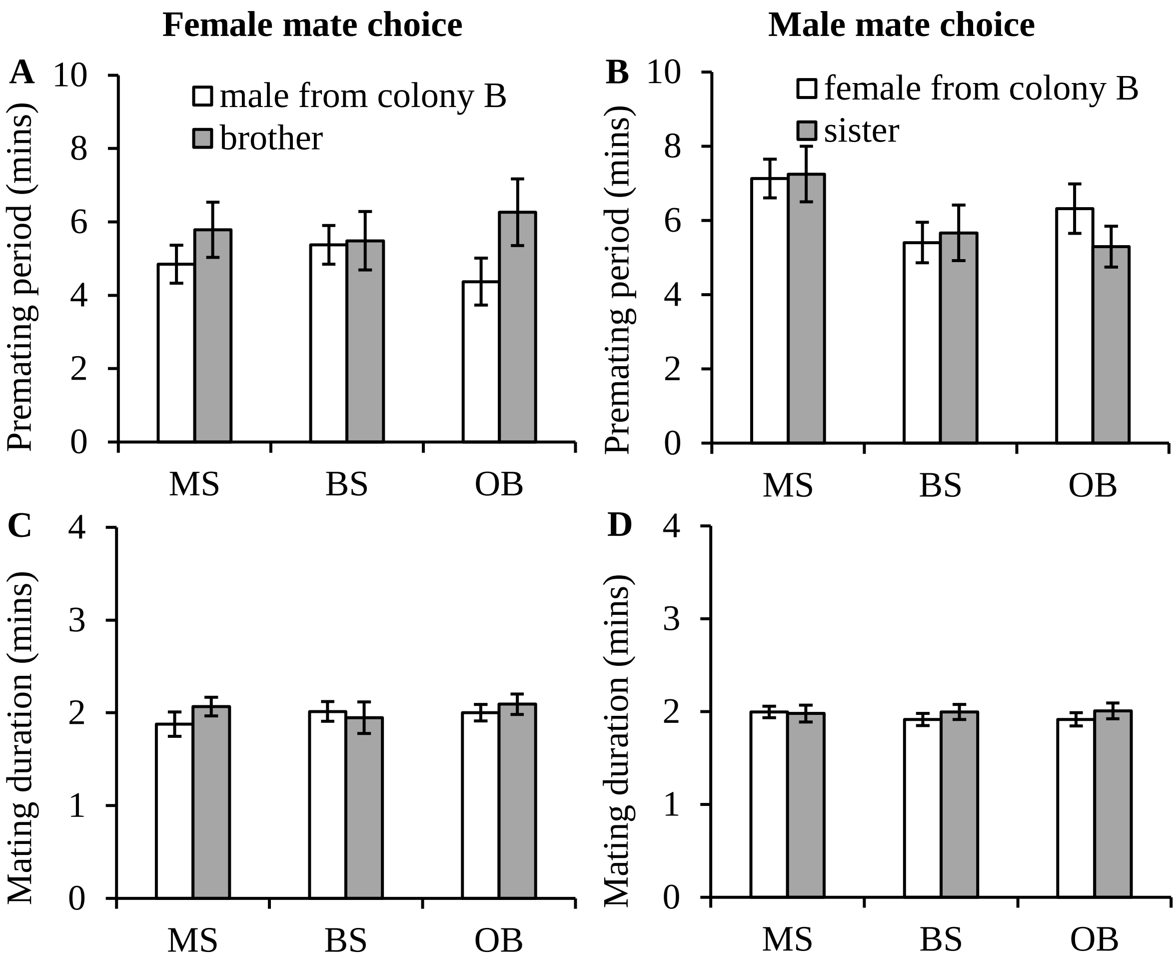

Figure 3.2 Premating period in female (**A**) and male (**B**) *Tetranychus ludeni* mate choice, and mating duration in female (**C**) and male (**D**) mate choice. MS, mother-son mating; BS, brother-sister mating; OB, outbreeding mating. Error bars are SE. There were 44, 39, 42 replicates for MS, BS, and OB, respectively, in female mate choice, and 56, 43, 52 replicates for MS, BS, and OB, respectively, in male mate choice.

3.4 Discussion

The present study shows that offspring from MS, BS and OB had similar fitness in the first and the 11th generations (Table 3.1), suggesting that neither mother-son mating nor sibling mating causes inbreeding depression in *T. ludeni* in both short- and long-term inbreeding. Similarly, Ito et al. (2012) report that sibling mating does not trigger inbreeding depression in *T. kanzawai* Kishida and Mori et al. (2005) reveal that mother-son mating results in no inbreeding depression in most tested populations of *Stigmaeopsis miscanthi* (Saito). The phenomenon could result from purging of deleterious alleles through haploid males (Atmar 1991; Antolin

1999; Henter 2003; Tien et al. 2015) and frequent inbreeding (Mitchell 1973) in haplodipoid mites. However, *T. urticae* Koch females suffer substantial inbreeding depression (Tien et al. 2015).

Theory predicts that individuals may avoid inbreeding when inbreeding depression is substantial but inbreeding avoidance may not occur when inbreeding depression is low or absent (Lande & Schemske 1985; Szulkin et al. 2013; Nichols 2017). Indeed, inbred *T. ludeni* had no significant preference between siblings and unrelated mates in mate choice (Figure 3.1). Furthermore, when mated with siblings or unrelated mates, *T. ludeni* had similar premating period and mating duration (Figure 3.2). My findings suggest that both sexes of *T. ludeni* do not avoid mating with kin at all inbreeding levels. Lack of inbreeding avoidance has also been reported in several other haplodiploid species (Bourdais & Hance 2009; de Souza et al. 2017; Bogo et al. 2018). However, *T. urticae* females prefer to mate with unrelated males (Tien et al. 2011) due to substantial inbreeding depression that occurs in this species (Tien et al. 2015).

In summary, I have found no evidence for inbreeding depression over eleven generations of sibling or mother-son mating in *T. ludeni*. However, my results do not support the prediction that parents may gain fitness through inbreeding. Due to lack of inbreeding depression neither sex of this species displays inbreeding avoidance behaviour in mate choice.

Chapter 4

Reproductive Strategies that May Facilitate Invasion Success: Evidence from a Spider Mite

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Abstract

With significant surge of international trade in recent decades, increasingly more arthropod species have become established outside their natural range of distribution, causing enormous damage in their novel habitats. However, whether a species can colonize its new environment depends on its ability to overcome various barriers which may result in establishment failure, such as inbreeding depression and difficulty to find mates. Here, I used a haplodiploid pest, *Tetranychus ludeni* Zacher (Acari: Tetranychidae), which is native to Europe but now cosmopolitan, to investigate whether its reproductive strategies have facilitated its invasion success, providing knowledge to develop programs for prediction and management of biological invasions. I show that inbreeding had no negative influence on female reproductive outputs and longevity over 11 successive generations, allowing mother-son and brother-sister mating to occur at the invasion front without adverse consequences in fitness. Virgin females produced maximum number of sons in their early life to ensure subsequent mother-son mating but later saved resources to prolong longevity for potential future mating. Females maximized their resource allocation to egg production immediately after mating to secure production of maximum number of both daughters and sons as early as possible. Furthermore, mated females with mating delay increased proportion of daughters in offspring produced to compensate the loss of production of daughters during their virgin life. I suggest that the lack of inbreeding depression in successive generations and the ability to adjust resource allocations depending on whether and when mating occurs may be the key features that have facilitated its invasion success.

4.1 Introduction

Investigation into reproductive strategies that successful invaders may have employed to survive and colonize uncertain environment can provide knowledge for prediction and management of invasion risks. The European native haplodiploid mite, *Tetranychus ludeni* Zacher (Acari: Tetranychidae), has now invaded all continents except Antarctica (Bolland et al. 1998; CABI/EPPO 2020) and become an important pest of bean, eggplant, hibiscus, pumpkin, and other cucurbitaceous plants globally (Reddy 2001; Zhang 2003; Kaimal & Ramani 2011a). To date, little is known about whether this mite has developed reproductive strategies that may have facilitated its invasion success, offering a good opportunity for the study of underlying mechanisms of biological invasions.

Biological invasion involves a series of stages such as introduction, establishment and spread, each of which has barriers that may result in invasion failure (Duncan et al. 2003; Heger & Trepl 2003; Blackburn et al. 2011). For example, in species with sexual reproduction, mates may be limited at the introduction front (Courchamp et al. 1999; South & Kenward 2001; Council 2002) where successful reproduction may be difficult (Heger & Trepl 2003; Blackburn et al. 2011), leading to population extinction. However, haplodiploid species may overcome the barrier of mate shortage at the invasion front (Baker 1955; Ward et al. 2012; Mazzolari et al. 2017), because females can produce haploid sons and then mate with their sons to produce both sons and daughters which can perform brother-sister mating thereafter (Cowan 1979; Adamson & Ludwig 1993; Peer & Taborsky 2005; McCulloch & Owen 2012; Schmidt et al. 2014). Mother-son mating could thus be potentially central to the invasion success of some haplodiploid species (Adamson & Ludwig 1993). Furthermore, mother-son and sibling mating may also reduce the cost and risk for mate search outside their natal habitat, increasing chances of successful colonization (Jordal et al. 2001).

Mother-son or sibling mating may result in inbreeding depression, reducing offspring fitness (Charlesworth & Willis 2009; Tien et al. 2015), and increasing extinction risks of small populations (Reed et al. 2003; O'Grady et al. 2006; Bozzuto et al. 2019; Nonaka et al. 2019). However, inbreeding depression does not appear to occur in some haplodiploid species after one (Peer & Taborsky 2005) or a few (Trevisan et al. 2016; Quaglietti et al. 2017) generations of inbreeding. Similarly, when I compare the reproductive fitness of the first generation with that of the 11th generation with different inbreeding levels in *T. ludeni*, I have not found any evidence of inbreeding depression (Zhou et al. 2020). Yet, it is not clear whether the lack of inbreeding depression remains consistent in successive generations, information of which is important for evaluation of whether inbred animals could have risk of population collapse in any generations.

For mother-son mating to occur in haplodiploid animals, virgin mothers would have to wait until their sons become sexually mature, by which time, even if they eventually mate with their sons, their reproductive fitness could decrease to the minimum due to mating delay, leading to extinction of the population. Therefore, females of successive haplodiploid invaders may have developed strategies to allow mother-son mating to take place with limited impact on their reproductive fitness. For example, females may reduce their reproductive rate when virgin to extend their longevity (Bonato & Gutierrez 1996, 1999) and increase resource allocations to reproduction after mating (Schmidt et al. 2014). However, it is still unclear how *T. ludeni* females adjust their reproductive strategies including resource allocation in response to mating delay, knowledge of which is vital to the understanding of invasion success in this mite and prediction of invasive potential of other haplodiploid animals.

In the present study, I carried out a series of experiments to determine whether inbreeding could increase risk of population collapse in successive generations and whether females could adjust their resource allocation in response to mating delay that facilitates mother-son mating in *T. ludeni*. First, I set up three breeding lines, namely mother-son mating, brother-sister mating and outbreeding, allowed mating to occur according to treatments for 11 successive generations, and recorded female reproductive outputs and longevity in each generation to evaluate the effect of inbreeding over generations. Because females are about 10 days old when their sons mature, all females were 10 days old when mated to make the data comparable. Second, I tested how females adjusted their resource allocation independent of inbreeding by comparing lifetime fecundity, daughter production, and longevity between females of different mating status, namely virgin, mated when one day old and mated when 10 days old.

4.2 Materials and Methods

4.2.1 Experimental mites

I established a laboratory colony of *T. ludeni* from field-collected mites on *Passiflora mollissima* (Kunth) (Malpighiales: Passifloraceae) in Palmerston North, New Zealand, and maintained it on 3- to 5-week-old common bean [*Phaseolus vulgaris* L. (Fabales: Fabaceae)] plants. I divided the colony into two (A and B) and kept them in two separate rooms for about eight generations before experiments. I used the first expanded leaves of *P. vulgaris* for all experiments. All colonies were maintained, and experiments carried out under the environmental conditions of 25 ± 1 ºC, 40 ± 10% RH, and photoperiod of 14:10 (L:D) h.

I randomly selected 40 male and 40 female deutonymphs from Colony A and individually transferred mites onto a clean leaf square (5.0 × 5.0 cm) placed on wet cotton wool in a Petri dish (9.5-cm diameter × 1.5-cm height) to ensure virginity. I then individually paired a 1-d-old female with a 1-d-old male on a clean leaf square (2.0 × 2.0 cm) to allow them to mate once and then transferred the mated female onto a new leaf square (2.0 × 2.0 cm) for oviposition for five days. I randomly selected three female deutonymphs that developed from the above eggs laid by each mated female for the following experiments.

4.2.2 Effect of inbreeding over generations on female reproductive fitness and survival

To examine whether and how inbreeding affected reproductive fitness and survival over generations, I performed three treatments: (1) MSM — mothers and their sons mated for 11 successive generations, (2) BSM — brothers and sisters mated for 11 successive generations, and (3) OBM (outbreeding) — females from Colony A and males from Colony B mated for 11 successive generations. Because females in treatment MSM were about 10 days old when their sons became adults, for all three treatments I allowed females in each generation to mate when they were 10 days old. I individually transferred female deutonymphs prepared as described above to leaf squares (2.5 × 2.5 cm) and allowed virgin female adults to lay eggs for 10 days and then individually paired them with 1-d-old virgin males according to treatments until death. I replaced the leaf squares once every five days and randomly selected one to three female deutonymphs produced by each mated female within the first five days after females mated to start the next generation. I recorded the number of eggs laid before and after mating and the number and percentage of daughters produced after mating by each mated female, once every five days until she died. I used the following number of mated females for data recording from the first to the 11th generations, respectively: 30, 24, 18, 27, 34, 30, 22, 33, 26, 30, and 28 for MSM; 29, 20, 15, 26, 27, 31, 24, 34, 27, 32, and 27 for BSM; and 31, 25, 18, 35, 34, 37, 25, 36, 26, 31, and 29 for OBM.

4.2.3 Effect of mating status on female lifetime reproductive fitness and survival

To determine whether mating status affected reproductive fitness and survival independent of inbreeding, I set up three treatments: 1) WMD (with mating delay) — virgin females from Colony A were allowed to lay eggs for 10 days and then mate with 1-d-old virgin males from Colony B, 2) NMD (without mating delay) — 1-d-old virgin females from Colony A were allowed to mate with 1-d-old virgin males from Colony B, and 3) VF (virgin females) — virgin females from Colony A were allowed to lay eggs for lifetime without mating. I recorded lifetime number of eggs and daughters and the percentage of daughters after mating every five days until females died as described above for one generation. For fair comparisons, data from females that lived over 10 days were used for analysis. I used 31, 26, and 21 females for WMD, NMD, and VF, respectively.

4.2.4 Statistical analysis

I used a logistic linear model to determine the effect of inbreeding on female reproductive fitness and longevity over 11 generations in three treatments, mother-son mating, brother-sister mating, and outbreeding: $y = \exp(a + bx)$, where y is the number of eggs and daughters produced, percentage of daughters, or female longevity, x is generation, and a and b are constant parameters of the model. I used a Negative Binomial distribution with a log link function for count data (number of eggs and daughters, and longevity), and a Gamma distribution with a log link function for percentage of daughters (GLIMMIX Procedure).

I applied a simple linear regression model to determine the egg-laying patterns over lifetime in virgin females, females with mating delay and females without mating delay (REG Procedure): $y = a + bx$, where y is the number of eggs, x is female age (days), and a is a constant and b the slope of regression. Because I recorded the number of eggs laid once every five days, virgin females and females with mating delay had the same status in the first 10 days of life and mating had a major impact on egg laying, I used three regression lines to fit each treatment when $x \leq 10$, $= 10$–15, and ≥ 15 days, respectively, and compared oviposition patterns between treatments. If 95% confidence limits (CLs) of slopes overlap, then there is no significant difference (Julious 2004). I compared the total number of eggs laid and female survival in all three treatments and the number and proportion of daughters produced by females with mating delay and without mating delay. Data on the total number and percentage of daughters were normally distributed (Shapiro-Wilk test, UNIVARIATE Procedure) and analyzed by an analysis of variance (ANOVA, GLM Procedure). The total number of eggs laid in all three treatments were not normally distributed and analyzed using nonparametric ANOVA (GLM Procedure). Female survival was analyzed using a Lifetest (LIFETEST Procedure). I performed all analyses using SAS software (SAS 9.4, SAS Institute Inc., Cary, NC).

4.3 Results

4.3.1 Effect of inbreeding over generations on female reproductive fitness and survival

My results indicate that inbreeding had little effect on reproductive output and female longevity of each of 11 successive generations (eggs: $F_{1,300} = 1.60$, $P = 0.2063$ for MSM; $F_{1,290} = 0.04$, $P = 0.8494$ for BSM; $F_{1,325} = 0.02$, $P = 0.8975$ for OBM; daughters: $F_{1,300} = 1.15$, $P = 0.2844$ for MSM; $F_{1,290} = 0.03$, $P = 0.8581$ for BSM; $F_{1,325} = 0.25$, $P = 0.6209$ for OBM; percentage of daughters: $F_{1,300} = 0.51$, $P = 0.4767$ for MSM; $F_{1,290} = 3.79$, $P = 0.0524$ for BSM; $F_{1,322} = 0.03$, $P = 0.8550$ for OBM; longevity: $F_{1,286} = 2.16$, $P = 0.1430$ for MSM; $F_{1,284} = 3.12$, $P = 0.0786$ for BSM; $F_{1,315} = 0.09$, $P = 0.7664$ for OBM; Figure 4.1). There was no significant difference in regression slopes between treatments for all parameters recorded (overlapping 95% CLs, Table S4.1).

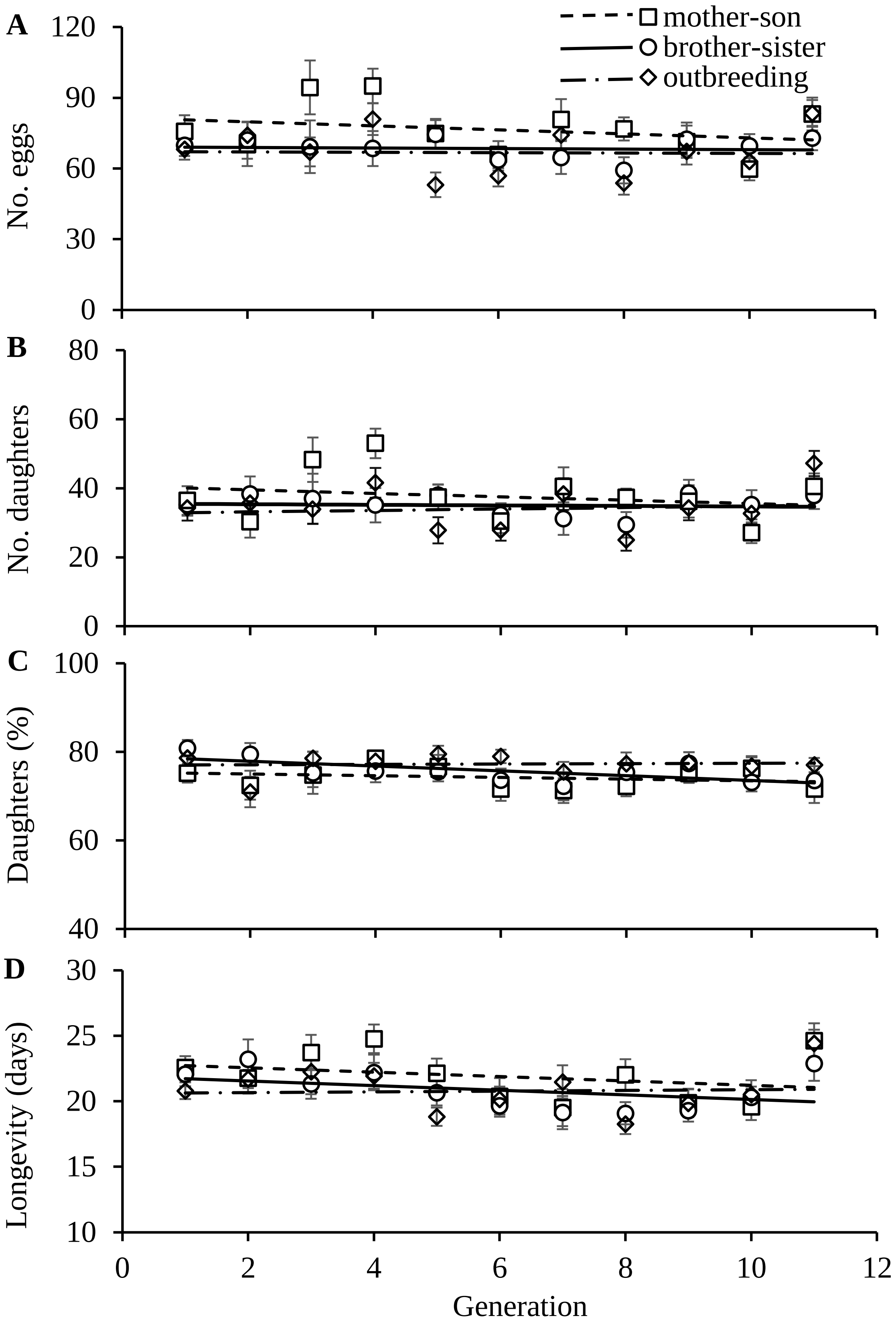

Figure 4.1 Number of eggs (**A**), number of daughters (**B**), percentage of female offspring after mating (**C**), and female longevity (**D**) over 11 generations in mother-son, brother-sister, and outbreeding treatments in *Tetranychus ludeni*.

4.3.2 Effect of mating status on female lifetime reproductive fitness and survival

Lifetime oviposition patterns are shown in Figure 4.2. Within the first 10 days of female life, the number of eggs laid by WMD and VF significantly decreased over time ($F_{1,60} = 56.21$, $P < 0.0001$ for WMD; $F_{1,40} = 59.56$, $P < 0.0001$ for VF) with similar decline rate (overlapping 95% CLs, Table S4.2) but that by NMD significantly increased during the same period ($F_{1,50} = 22.67$, $P < 0.0001$). When female age = 10-15 days, the number of eggs laid significantly increased in WMD ($F_{1,60} = 98.60$, $P < 0.0001$), significantly decreased in NMD ($F_{1,45} = 40.80$, $P < 0.0001$) and remained similar in VF ($F_{1,40} = 0.77$, $P = 0.3859$). Although oviposition significantly increased after mating in both WMD and NMD, it increased significantly faster in WMD than in NMD (nonoverlapping 95% CLs, Table S4.2). After female aged ⩾15 days, the number of eggs laid decreased over time in all treatments ($F_{1,58} = 43.15$, $P < 0.0001$ for WMD; $F_{1,33} = 1.64$, $P = 0.2092$ for NMD; $F_{1,66} = 17.52$, $P < 0.0001$ for VF), with the decrease in WMD being significantly faster than in NMD and VF (nonoverlapping 95% CLs, Table S4.2).

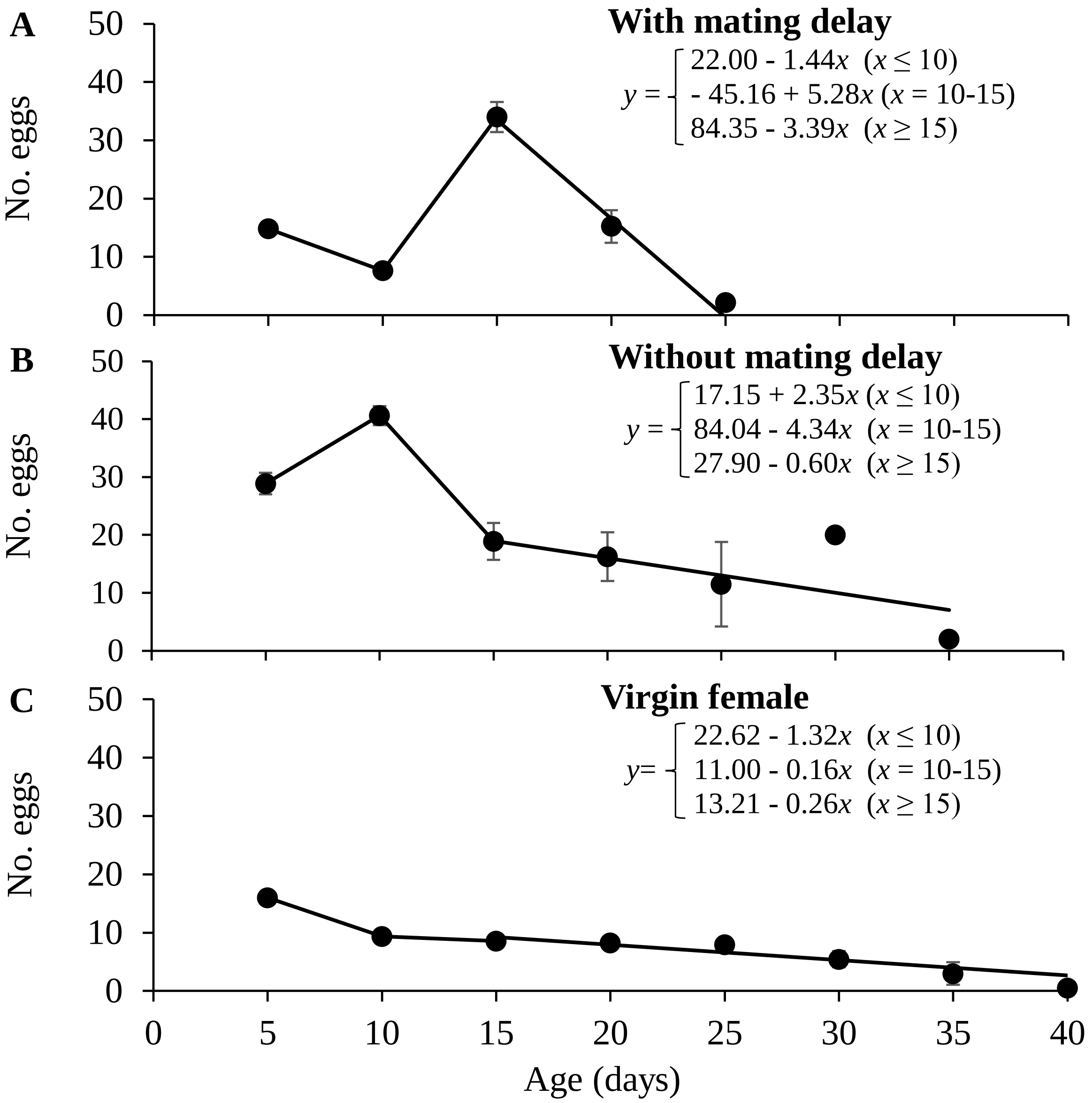

Figure 4.2 Lifetime oviposition patterns in *Tetranychus ludeni* females of different mating status: with mating delay (A), without mating delay (B), and virgin females (C).

Virgin females (VF) laid significantly fewer eggs than mated females (WMD and NMD) in their lifetime, and WMD females laid significantly fewer eggs than NMD ones ($F_{2,75} = 18.50$, $P < 0.0001$; Figure 4.3A). In mated treatments, WMD females produced significantly fewer number of daughters ($F_{1,55} = 7.57$, $P = 0.0080$; Figure 4.3B) but significantly higher proportion of daughters ($F_{1,55} = 7.15$, $P = 0.0098$; Figure 4.3C) in their after-mating life than NMD ones. The survival probability of females in different treatments was significantly different, with an order of VF > WMD > NMD ($x_2^2 = 22.02$, $P < 0.0001$; Figure 4.4).

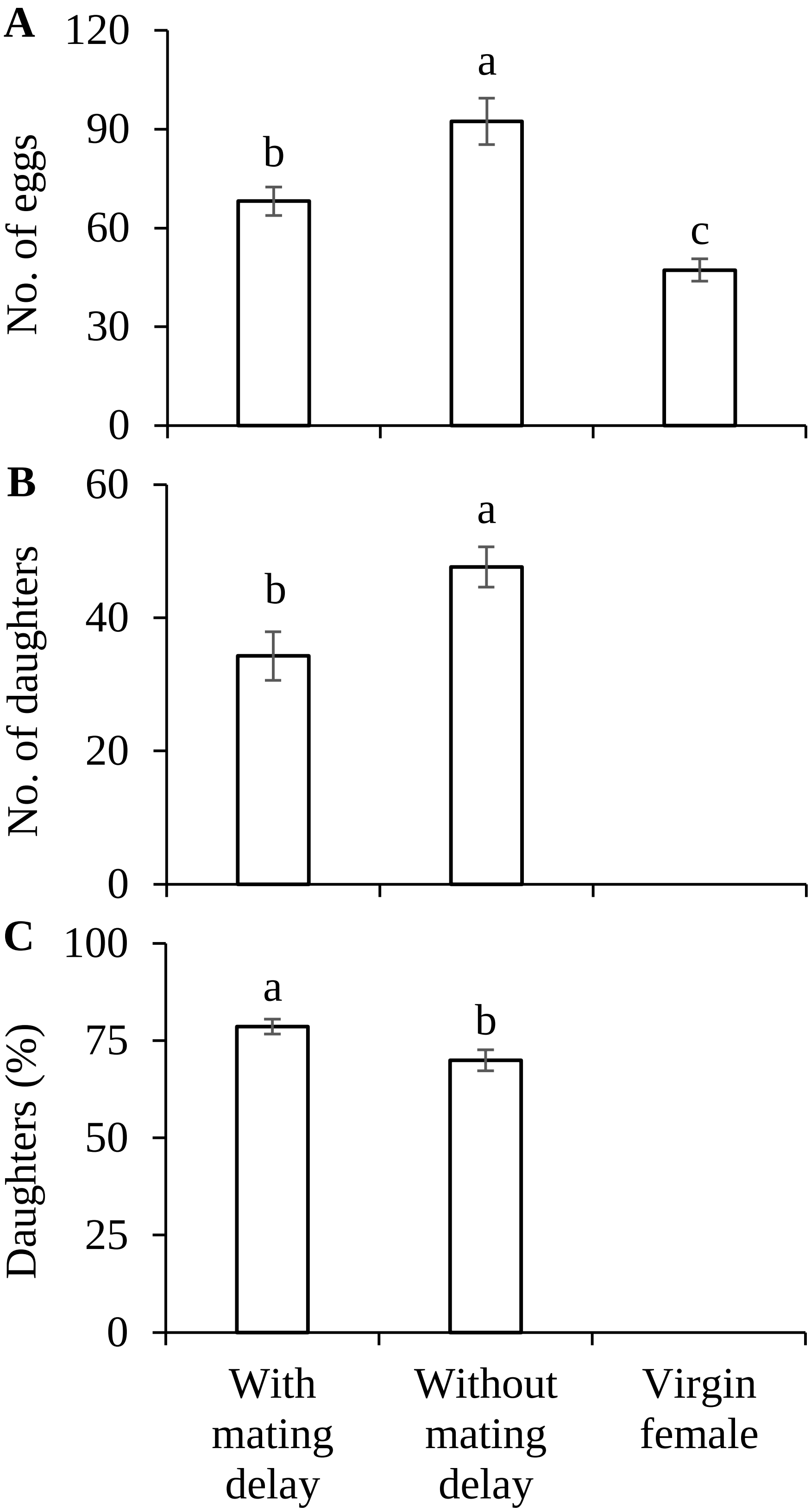

Figure 4.3 Lifetime number of eggs (**A**) and daughters (**B**) and percentage of daughters after mating (**C**) of *Tetranychus ludeni* females with different mating status. Treatments with different letters are significantly different ($P < 0.05$).

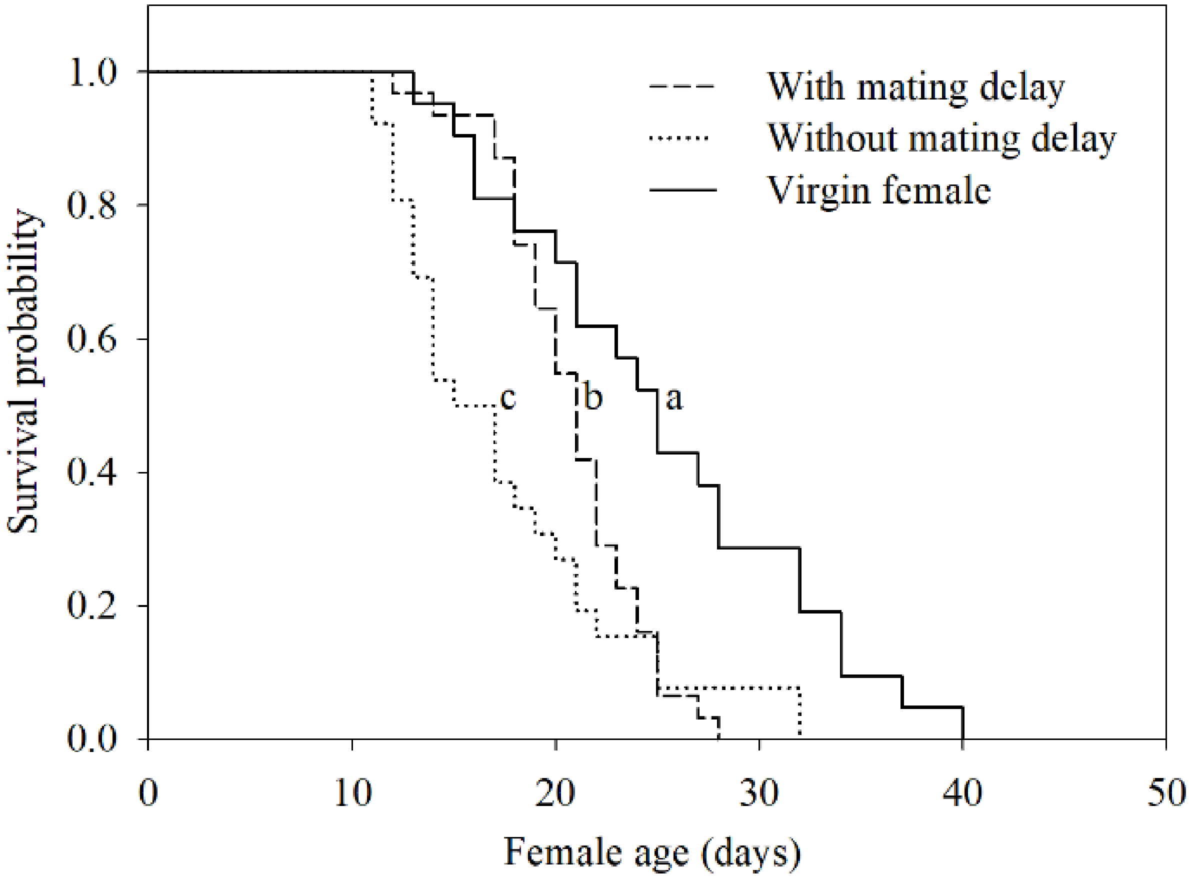

Figure 4.4 Survival probability of *Tetranychus ludeni* females of different mating status. Lines with different letters are significantly different (P < 0.05).

4.4 Discussion

Inbreeding depression may reduce offspring fitness (Charlesworth & Willis 2009; Tien et al. 2015), leading to extinction of small populations (Reed et al. 2003; O'Grady et al. 2006; Bozzuto et al. 2019; Nonaka et al. 2019). Previous studies show that the inbreeding depression can occur in any generation of inbred animals, which could cause invasion failure. For example, in the haplodiploid *Stigmaeopsis miscanthi* (Saito) (Acari: Tetranychidae) (Mori et al. 2005) and *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) (Çekin & Schausberger 2019), inbreeding depression does not occur in the first few generations but takes place in later generations whereas in *T. urticae* Koch (Acari: Tetranychidae), it is only detected in the first few generations (Tien et al. 2015). In the present study, I found no evidence for inbreeding depression in any of the 11 inbred generations in *T. ludeni* (Figure 4.1), suggesting that inbreeding has no negative impact on its invasion success at any points or generations. I attribute this phenomenon to the notion that the expression of deleterious recessive alleles from inbreeding may be selected out in small populations of some haplodiploid animals (e.g., Quaglietti et al. 2017; Eyer et al. 2018). The mechanism allows them to undertake mother-son and brother-sister mating at the invasion front without adverse consequences in fitness (Adamson & Ludwig 1993; Kronauer et al. 2012; McCulloch & Owen 2012; Schmidt et al. 2014; Lantschner et al. 2020; Queffelec et al. 2020).

My results demonstrate that *T. ludeni* had developed strategies to adjust resource allocations in response to uncertain environment. For example, virgin females produced highest number of sons in their early life (Figure 4.2A and C) to secure subsequent mother-son mating. This feature can ensure continuous population growth in uncertain situations, such as no males available at the invasion front. However, if there were still no males to mate after this reproductive episode, virgin females saved resources by reducing egg production (Figure 4.2A and C) to prolong longevity (Figure 4.4) for potential future mating. Some other spider mites, such as *T. marianae* McGregor (Acari: Tetranychidae), *Mononychellus progresivus* Doreste (Acari: Tetranychidae) (Bonato & Gutierrez 1996, 1999), and *T. urticae* (Li & Zhang 2021a), appear to share similar reproductive strategies with *T. ludeni*, that may contribute to their invasion success.

Immediately after receiving ejaculates, females sharply increased their resource investment in egg production regardless of whether mating delay occurred (Figure 4.2A and B) to ensure production of maximum number of both daughters and sons as early as possible. This resource allocation strategy may reduce risks of potential reproductive failure in a new and unstable environment (Stearns 1992; Stearns et al. 2000), facilitating establishment of a newly invaded population (Dangremond & Feller 2016; Fetters & McGlothlin 2017; James et al. 2017). The very reproductive strategy also occurs in haplodiploid mite *Schizotetranychus celarius* (Banks) (Acari: Tetranychidae) (Saitō 1987) and ant *Cardiocondyla argyrotricha* (Hymenoptera: Formicidae) (Schmidt et al. 2014). My results show that both after-mating increase and after-peak decline of egg laying were faster in females with mating delay than in those without mating delay (Figure 4.2A and B). This suggests that *T. ludeni* females can perform a clear resource allocation trade-off (Waelti & Reyer 2007; Billman & Belk 2014), i.e., higher resource investment in eggs by delay-mated females as compared to undelay-mated ones results in faster decrease of resource available for future egg production. The ability to quickly adjust resource allocation depending on when mating occurs may provide more flexibility for the species to establish in a new environment.

Although virgin females laid fewer eggs in their lifetime than mated ones (Figure 4.3A), their sons are larger which produce more daughters after mating than those of mated ones (Zhou et al. 2018). This life history strategy should also contribute to future population growth and invasion success (Wiernasz et al. 2001; De Jesus & Reiskind 2016; Zhou et al. 2018). My results demonstrate that females with mating delay produced fewer number of eggs and daughters as compared to those without mating delay. However, the reduction was less than 25% (Figure 4.3A and B), which may not be enough to lead to establishment failure. Furthermore, females with mating delay produced higher proportion of daughters than those without mating delay (Figure 4.3C), which may help compensate the loss of production of daughters in their earlier life and catch up population growth.

In the present study, I show that inbreeding has no negative influence on female reproductive outputs and longevity throughout 11 inbred generations, which allows mother-son and brother-sister mating to occur at the invasion front without adverse consequences in fitness. Virgin females lay maximum number of sons in their early life to ensure subsequent mother-son mating but later save resources to prolong longevity for potential future mating. Females maximize their resource allocation to egg production immediately after mating to secure production of maximum number of both daughters and sons as early as possible, reducing risks of potential reproductive failure in a new environment. Finally, mated females with mating delay quickly increase proportion of daughters in offspring produced to compensate the loss of production of daughters during their virgin life. These reproductive strategies of *T. ludeni* coupled with its adaptation to wide ranges of hosts (Gotoh et al. 2015) and temperatures (Gotoh et al. 2015; Ristyadi et al. 2019) may have facilitated its invasion success. My findings may be also useful for prediction of invasive potential of other haplodiploid pests.

Table S4.1 Estimated regression coefficients and their 95% confidence limits (CLs) of regression models on the changes of number of eggs and daughters, percentage of daughters, and female longevity over 11 generations in mother-son (MSM), brother-sister (BSM) and outbreeding mating (OBM) treatments in Figure 4.1.

Treatment	*a*	95% CLs	*b*	95% CLs	$F_{(df)}$	P
No. eggs						
MSM	4.40	(4.28 ~ 4.52)	-0.0113	(-0.0287 ~ 0.0062)	$1.60_{(1,300)}$	0.2063
BSM	4.24	(4.12 ~ 4.36)	-0.0016	(-0.0187 ~ 0.0154)	$0.04_{(1,290)}$	0.8494
OBM	4.21	(4.09 ~ 4.32)	-0.0011	(-0.0179 ~ 0.0157)	$0.02_{(1,325)}$	0.8975
No. daughters						
MSM	3.70	(3.54 ~ 3.87)	-0.0132	(-0.0374 ~ 0.0110)	$1.15_{(1,300)}$	0.2844
BSM	3.57	(3.39 ~ 3.75)	-0.0024	(-0.0282 ~ 0.0235)	$0.03_{(1,290)}$	0.8581
OBM	3.49	(3.32 ~ 3.66)	0.0061	(-0.0182 ~ 0.0305)	$0.25_{(1,325)}$	0.6209
Daughters (%)						
MSM	-0.28	(-0.33 ~ -0.23)	-0.0026	(-0.0096 ~ 0.0045)	$0.51_{(1,300)}$	0.4767
BSM	-0.24	(-0.29 ~ -0.18)	-0.0072	(-0.0145 ~ 0.0001)	$3.79_{(1,290)}$	0.0524
OBM	-0.26	(-0.30 ~ -0.22)	0.0006	(-0.0054 ~ 0.0065)	$0.03_{(1,322)}$	0.8550
Longevity (days)						
MSM	3.13	(3.06 ~ 3.20)	-0.0077	(-0.0179 ~ 0.0026)	$2.16_{(1,286)}$	0.1430
BSM	3.09	(3.02 ~ 3.15)	-0.0085	(-0.0180 ~ 0.0010)	$3.12_{(1,284)}$	0.0786
OBM	3.03	(2.96 ~ 3.09)	0.0014	(-0.0077 ~ 0.0105)	$0.09_{(1,315)}$	0.7664

Data were fitted with a logistic linear regression model: $y = \exp(a + bx)$.

Table S4.2 Estimated regression coefficients and their 95% confidence limits (CLs) of regression models on the changes of number of eggs over female age (days) in treatments with mating delay (WMD), without mating delay (NMD), and virgin female (VF) in Figure 4.2.

Treatment	*a*	95% CLs	*b*	95% CLs	$F_{(df)}$	P
Age ≤ 10						
WMD	22.00	(18.97 ~ 25.03)	-1.44	(-1.82 ~ -1.05)	$56.21_{(1,60)}$	<0.0001
NMD	17.15	(9.33 ~ 24.98)	2.35	(1.36 ~ 3.34)	$22.67_{(1,50)}$	<0.0001
VF	22.62	(19.88 ~ 25.36)	-1.32	(-1.67 ~ -0.98)	$59.56_{(1,40)}$	<0.0001
Age = 10-15						
WMD	-45.16	(-58.71 ~ -31.61)	5.28	(4.21 ~ 6.34)	$98.60_{(1,60)}$	<0.0001
NMD	84.04	(66.94 ~ 101.13)	-4.34	(-5.71 ~ -2.97)	$40.80_{(1,45)}$	<0.0001
VF	11.00	(6.24 ~ 15.76)	-0.16	(-0.54 ~ 0.21)	$0.77_{(1,40)}$	0.3859
Age ≥ 15						
WMD	84.35	(65.53 ~ 103.18)	-3.39	(-4.42 ~ -2.36)	$43.15_{(1,58)}$	<0.0001
NMD	27.90	(9.97 ~ 45.83)	-0.60	(-1.54 ~ 0.35)	$1.64_{(1,33)}$	0.2092
VF	13.21	(10.21 ~ 16.20)	-0.26	(-0.39 ~ -0.14)	$17.52_{(1,66)}$	<0.0001

Data were fitted with a general linear regression model: $y = a + bx$.

Chapter 5

Dispersal in Relation to Resource Relocation, Age and Density in *Tetranychus ludeni*

Abstract

Life history strategies of a species may affect its invasion success. Here, I used a haplodiploid pest, *Tetranychus ludeni* Zacher (Acari: Tetranychidae), which is native to Europe but now cosmopolitan, to investigate its dispersal tendency and distance in relation to its resource relocation, age, and density. First, I tested whether and how dispersal influenced survival, lifetime fecundity, age-specific reproduction, and life table parameters of mated females. I show that mated females did not trade off their survival and lifetime reproductive output with dispersal but adjusted their resource allocations during different reproductive stages depending on whether and how far they dispersed. Long-distance dispersers invested more in dispersal in their early life while resident mites and short-distance dispersers invested more in reproduction during their early life. These resource allocation strategies may allow long-distance dispersers to explore the novel environment more effectively without compromising lifetime reproductive fitness, contributing to the invasion success of *T. ludeni*. Second, I examined the effect of age and density on ambulatory dispersal probability and distance of mated females. My results show that older females (3-12 days old) with more mature eggs were more likely to disperse and moved longer distances than younger ones (1 day old) with fewer eggs. Females significantly increased dispersal probability and distance with the increase of population density. The synchronization of dispersal and reproduction and the positive density-dependent dispersal strategy may facilitate habitat colonization and invasion speed of *T. ludeni*.

5.1 Resource relocations in relation to dispersal in *Tetranychus ludeni* Zacher

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5.1.1 Introduction

Biological invasions have become more frequent in the recent decades due to the increase of international trade and travel, causing significant economic losses worldwide. Following introduction of exotic species to a new habitat by man, their dispersal plays a crucial role in population expansion (Clobert et al. 2009). Because dispersers' life history traits such as fecundity and survival can affect their population establishment at the invasion front and subsequent expansion (Duckworth & Badyaev 2007; Renault et al. 2018; Williams et al. 2019), understanding the reproductive performance of dispersers can provide knowledge for prediction and management of invasion risks.

Compared with residents, dispersers often have lower fecundity and higher mortality (Mole & Zera 1993; Bonte et al. 2012; Khuhro et al. 2014; Renault 2020; Nasu & Tokuda 2021), leading to slower population growth (Baguette & Schtickzelle 2006; Keenan & Cornell 2021). In addition, the cost of dispersal may increase with the distance a species moves, resulting in distance-specific resource trade-offs (Rousset & Gandon 2002; Baker & Rao 2004; Samietz & Köhler 2012; Serrano & Tella 2012), the magnitude of which can affect expansion speed. For example, dispersers in some species may allocate less resource to reproduction at their early life to facilitate dispersal (Roff 1984; Roff & Fairbairn 1991; Tanaka 1993; Innocent et al. 2010). Therefore, examining age-specific reproduction helps better understand the cost of dispersal and resource allocation strategies of dispersers.

Tetranychus ludeni Zacher (Acari: Tetranychidae) is a spider mite from Europe but has now invaded all continents except Antarctica (Bolland et al. 1998; CABI/EPPO 2020), providing an opportunity to study the underlying mechanisms of its invasion success. It attacks over 300 plant species, including economically important crops such as bean, papaya, guava, eggplant, pumpkin, and apple (Bolland et al. 1998; Zhang 2003; Migeon et al. 2010). In spider mites, females usually disperse after mating (Mitchell 1973), especially when the population is crowded or when food is insufficient or poor in quality (Mcenroe 1969; Bitume et al. 2013).

Although its flexible reproductive strategies (Zhou et al. 2018, 2021) and good adaptation to external environment (Zhang 2003; Gotoh et al. 2015; Ristyadi et al. 2019, 2021) may have helped *T. ludeni* establish in novel habitats, it is still unknown whether dispersers adjust their reproductive strategies to facilitate invasion success.

In the present study, I examined whether dispersal tendency and distance affected reproductive output, survival, age-specific reproduction, and life table parameters in *T. ludeni*. I allowed mites to disperse along a dispersal system, sampled residents and dispersers, followed up their daily reproduction and survival, and calculated their life table parameters. I hypothesized that compared with residents, dispersers should have lower lifetime fecundity, shorter longevity, and slower population growth.

5.1.2 Materials and Methods

5.1.2.1 Mite colony and experimental conditions

I collected *T. ludeni* adults on *Passiflora mollissima* (Kunth) (Malpighiales: Passifloraceae) in Palmerston North, New Zealand, and started and maintained the colony using 3- to 5-week-old common bean plants *Phaseolus vulgaris* L. (Fabales: Fabaceae) grown in pots. I used the first expanded leaves cut from 1- to 2-week-old plants grown in pots for all experiments. The colony was kept, and experiments carried out under environmental conditions of 25 ± 1 ℃, 40 ± 10% RH and 14:10 h (light:dark) photoperiod.

5.1.2.2 Preparation of mated females for experiments

To prepare male adults for mating, I transferred 50 female deutonymphs randomly selected from the colony onto a clean fresh leaf square (4 × 4 cm) on wet cotton wool in a Petri dish (9 cm in diameter × 1 cm in height) and allowed them to develop to virgin adult females and lay unfertilised eggs for three days. I then removed those adult females and allowed the eggs to develop to virgin male adults. I prepared three such dishes. I used 1- to 3-d-old virgin adult males to mate with females prepared below.

I randomly selected 50 adult females from the colony and transferred them onto a clean fresh leaf square (4 × 4 cm) on wet cotton wool in a Petri dish for egg laying for 24 hours. I then removed the female adults and allowed eggs to develop to the quiescent deutonymphal stage. I set up a total of 10 such dishes. I introduced 15–20 virgin males prepared above to female deutonymphs in each dish and allowed them to stay with the newly emerged females for 5 h, ensuring all females mated at or soon after emergence. I transferred 50 newly emerged (< 24 hours old), mated females from the above dishes onto a new leaf square (4 × 4 cm) in a dish. I prepared a total of 10 such leaf squares. I used 3-d-old, mated females for experiments because females of this age were most likely to disperse (unpublished data).

5.1.2.3 Experimental setup and data collection

I set up a dispersal system consisting of an array of 21 leaf squares (2 cm × 2 cm for the first leaf square and 2 cm × 1 cm for the rest) (Figure 5.1) placed on wet cotton wool in a tray (45 cm in length × 36 cm in width × 1.5 cm in height). To facilitate analysis of mite dispersal distance and subsequent reproductive fitness, I divided the array into three sections for data collection: (1) resident leaf square (R) — the first leaf square where I released the mites; (2) median distance of dispersal (M) — leaf squares 2–11, and (3) long distance of dispersal (L) — leaf squares 12–21.

I transferred 50 3-d-old, mated females onto R leaf square and allowed them to settle for 40 minutes, after which time, all leaf squares were connected by Parafilm bridges (4.5 cm in length × 1.5 cm in width; Parafilm®, USA). Twenty-four hours after bridge connection, I sampled mites on R, M, and L leaf squares. I repeated the above procedure eight times. I sampled a total of 15, 20, and 13 mites from sections R, M, and L, respectively, for measurement of reproductive fitness in relation to dispersal distance: 15 mites from the 1st leaf square for section R; 1, 2, 3, 5, 6, 1 and 2 mites from the 2nd, 4th, 6th, 7th, 8th, 9th and 11th leaf squares, respectively, for section M, and 1, 2, 1, 5, 2, 1, and 1 mites from the 12th 13th, 15th, 16th, 17th, 19th and 21st leaf squares, respectively, for section L. I individually transferred sampled mites onto new leaf squares (1 × 1 cm) placed on wet cotton wool in Petri dishes, replaced leaf squares once every 24 hours until the mites died, and recorded their longevity. I counted the number of eggs laid on each leaf square, allowed them to develop to adult stage on the same leaf squares and recorded the number and sex of newly emerged adults.

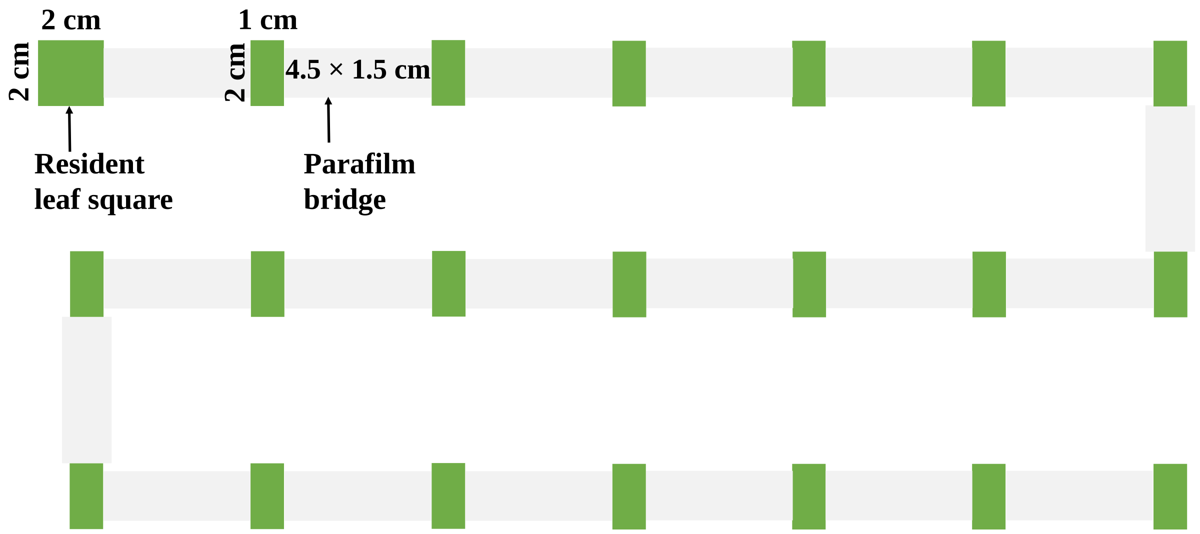

Figure 5.1 Dispersal system for experiments. The resident leaf square was used to introduce experimental mites. Parafilm bridges were placed on the top of leaf squares with approximately 1 mm overlapping to reduce the influence of water between leaf squares and bridges on mite dispersal.

5.1.2.4 Statistical analysis

I analyzed all data using SAS software (SAS 9.4, SAS Institute Inc., Cary, NC). To estimate how dispersal affected population growth, I calculated the life table parameters (Jervis et al. 2005) using the data on daily survival and daughter production of each female. The intrinsic rate of natural increase (r) was calculated by solving the Lotka-Euler equation: $\sum e^{-rx} l_x m_x = 1$, where x is the pivotal age, l_x is the proportion of females surviving to age x, and m_x is the number of daughters produced per female at age x. I also calculated the net reproductive rate ($R_0 = \sum l_x m_x$, daughters/female/generation), doubling time [$D_t = \log_e(2)/r$, days], and generation time [$T = \log_e(R_0)/r$, days]. I used the bootstrap method (Huang & Chi 2012; Yu et al. 2013) with 50,000 bootstrap samples to calculate the pseudo-values of a given parameter and employed the paired-bootstrap test (Efron & Tibshirani 1993; Mou et al. 2015; Reddy & Chi 2015) for multiple comparisons between any two dispersal distances (TTEST Procedure). The significance was determined according to the 95% t-based confidence limits (95% CLs), i.e., if 95% CLs > 0 or < 0, the mean difference between the two dispersal distances is significantly different.

Data distribution was tested using Shapiro-Wilk test (UNIVARIATE Procedure). Data on female longevity were normally distributed and analyzed using ANOVA with Tukey test for multiple comparison (GLM procedure). Data on the number of eggs laid were not normally distributed and thus analyzed with non-parametric ANOVA followed by Bonferroni test for multiple comparison (GLM procedure).

I modified a Gaussian functional model (Archontoulis & Miguez 2015) to fit the daily number of eggs and daughters with two reproductive peaks: $y = p_1*\exp\{-0.5*[(x - x_1)/w_1]^2\} + p_2*\exp\{-0.5*[(x - x_2)/w_2]^2\}$, where p_1 and p_2 are the maximum y value of the first and second peaks, respectively; w_1 and w_2 are coefficients controlling the width of the first and second peaks, respectively; and x_1 (= 4) and x_2 are the age of mites at first and second peaks, respectively. I compared reproductive parameters of mites sampled in different sections (R, M, and L) and p_1 and p_2 within each section according to Julious (2004): if the 83.4% CLs do not overlap, then there is a significant difference.

5.1.3 Results

Resident (R) and dispersed females (M and L) laid similar number of eggs in their lifetime ($F_{2,45}$ = 1.06, P = 0.3566) and had similar longevity ($F_{2,45}$ = 1.68, P = 0.1987) (Figure 5.2). However, life table parameters changed with the distance females dispersed (Table 5.1): (1) the longer the dispersal distances were, the lower net reproductive rate (R_0) and intrinsic rate of increase (r) occurred, and (2) the opposite was the case for the generation time (T) and doubling time (D_t).

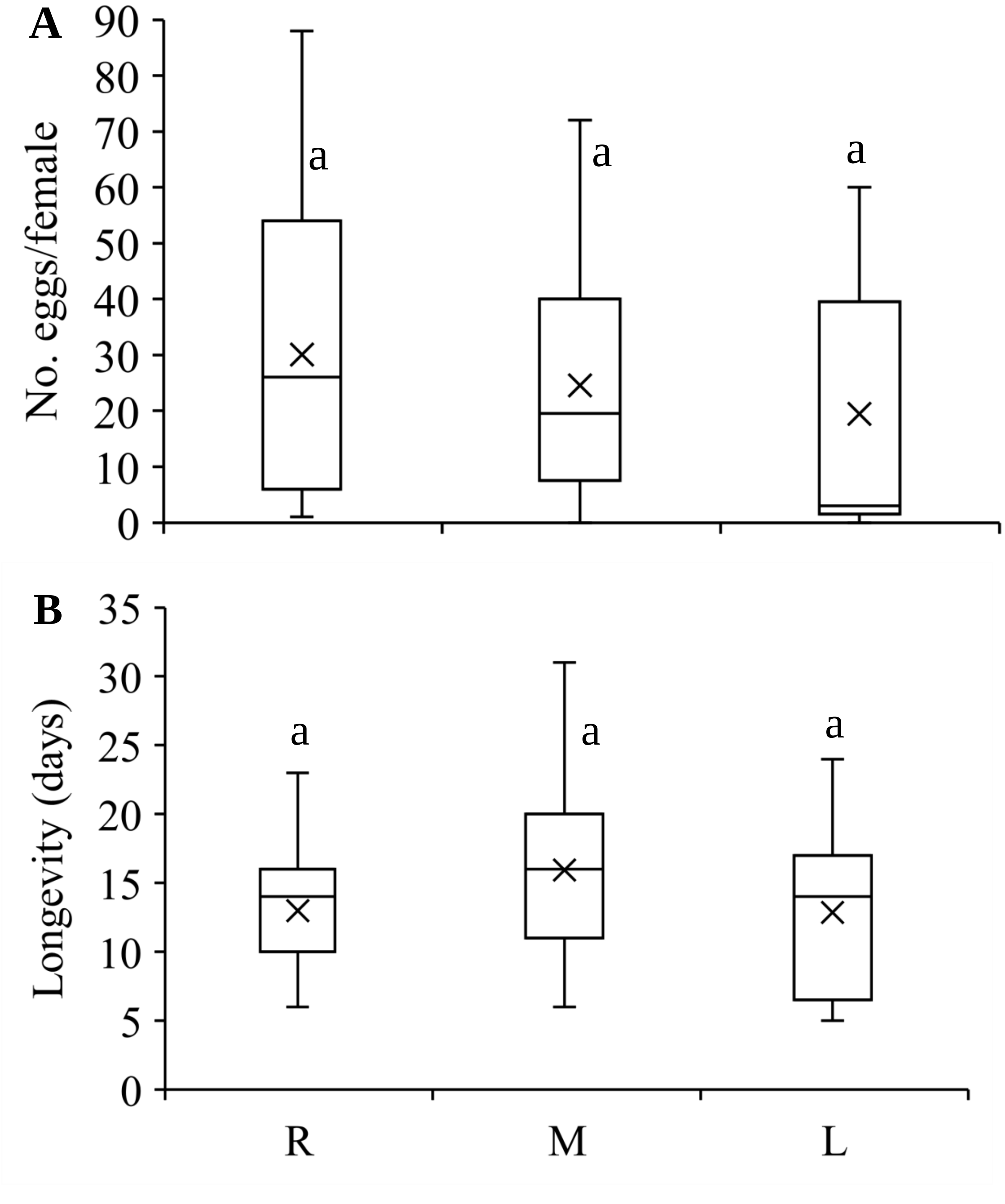

Figure 5.2 The total mean number of eggs laid per female (**A**) and longevity (**B**) of resident (R) and dispersed females (M and L) in *T. ludeni*. Each box plot shows the mean (×), median line, the upper and lower quartiles (the range where 25% of scores fall above and 25% fall below the median), and maximum ‘⊤’ and minimum ‘⊥ ’ scores. Boxes with the same letters are not significantly different ($P > 0.05$).

Table 5.1 The estimated mean (± SE) life table parameters at different dispersal distances in *T. ludeni*.

Distance	R_0	r	T	D_t
R	18.31 ± 0.30 a	0.1789 ± 0.0010 a	16.25 ± 0.06 c	3.88 ± 0.02 c
M	17.35 ± 0.18 b	0.1628 ± 0.0010 b	17.16 ± 0.08 b	4.17 ± 0.03 b
L	13.16 ± 0.36 c	0.1453 ± 0.0017 c	17.71 ± 0.09 a	4.78 ± 0.06 a
CLs $_{\text{(R vs M)}}$	0.2701~1.6599	0.0097~0.0154	-1.1138~-0.7141	-0.3623~-0.2264
CLs $_{\text{(R vs L)}}$	4.2272~6.0751	0.0295~0.0375	-1.6854~-1.2398	-1.0271~-0.7751
CLs $_{\text{(M vs L)}}$	3.4022~4.9701	0.0170~0.0249	-0.7897~-0.3076	-0.7336~-0.4799

Estimated values in columns followed by different letters are significantly different ($P < 0.05$). For each parameter, 95% CLs > 0 or < 0 indicates a significant difference between dispersal distances.

Daily number of eggs and daughters produced had two peaks of similar shape regardless of dispersal distance, with one occurring immediately after their transfer to new leaf squares and the other taking place 5–7 days after their transfer (Figure 5.3). The first peak of daily fecundity and daughter production was significantly lower in L females than in R and M females (non-overlapping 83.4% CLs). The first peak was significantly higher than the second one in R and M females (nonoverlapping 83.4% CLs) but the two peaks had similar height in L females (overlapping 83.4% CLs) (Figure 5.3).

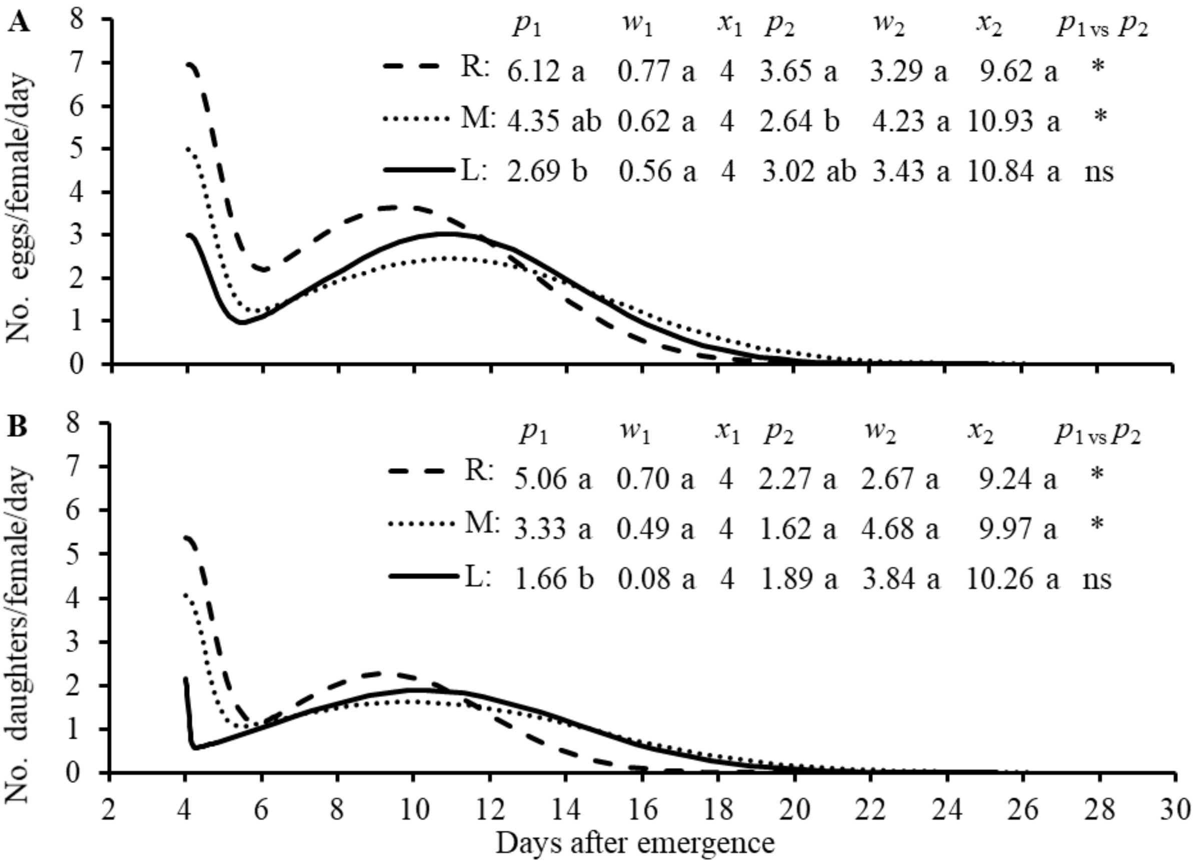

Figure 5.3 The estimated patterns of daily number of eggs (**A**) and daughters (**B**) produced by resident (R) and dispersed females (M and L) of *T. ludeni*. Raw data were fitted to the two-peak model for each parameter. Parameters with the same letters in column are not significantly different (overlapping 83.4% CLs) between R, M, and L. 'p_1 vs p_2' is the comparison in peak height between the first and second peaks within each line, where * indicates significantly different and 'ns' not significantly different.

5.1.4 Discussion

Theoretically, dispersal has energetic and survival costs (Bonte et al. 2012; Travis et al. 2012; Weigang & Kisdi 2015), often resulting in lower fecundity and higher mortality. However, my current study shows that females had similar lifetime fecundity and longevity regardless of whether and how far they dispersed (Figure 5.2). It is thus suggested that mated females may not trade off their lifetime fecundity and survival with dispersal in *T. ludeni*. There is also no evidence for trade-off between dispersal and fecundity in a damselfly (Therry et al. 2015). I argue that the lack of such trade-offs may contribute to invasion success observed in *T. ludeni* (Zhou et al. 2021a).

My results indicate that dispersal tendency and distance had significant effects on life table parameters (Table 5.1). For example, dispersal significantly reduced the net reproductive rate (R_0) and intrinsic rate of increase (r) and increased the generation time (T) and doubling time (D_t). These findings suggest that the mites adjust their resource allocation to reproduction and movement depending on whether and how far they intend to disperse. In another word, although dispersed and resident mites had similar lifetime fecundity, dispersed mites spread their resources for reproduction in a wider range, prolonging generation time and population doubling time (Weerawansha et al. 2020). Similar phenomenon has also been reported in butterflies *Proclossiana eunomia* Esper, *Boloria aquilonaris* Stichel, *Euphydryas editha bayensis* Sternitsky, *E. aurinia* (Rottemburg), and *Lopinga achine* (Scopoli) (Lepidoptera: Nymphalidae) (Baguette & Schtickzelle 2006).

As shown in Figure 5.3, mated *T. ludeni* females had two reproductive episodes in terms of daily number of eggs and daughters produced regardless of dispersal distance. However, females that dispersed a long distance (L) laid fewer eggs and produced fewer daughters than those that did not disperse (R) or only dispersed a median distance (M) during the first episode. These findings strongly suggest that L females allocate more resources for dispersal and reduce investment in reproduction in their early life (Zera & Denno 1997; Bonte et al. 2012). This kind of resource relocation has also been reported in other invertebrates (Roff 1984; Roff & Fairbairn 1991; Tanaka 1993). For example, in a haplodiploid insect *Melittobia australica* Girault (Hymenoptera: Eulophidae), dispersers have fewer number of eggs developed at emergence and produce fewer eggs in their first clutch than residents (Innocent et al. 2010).

When I compared the two reproductive peaks within mites that dispersed similar distances, I found that the second peaks in R and M females were significantly lower than the first ones while the two peaks had similar height in L females (Figure 5.3). My results further suggest that in their early life R and M females invest more resources in reproduction than in dispersal while L females allocate more resources to dispersal than to reproduction. This strategy may allow L females to explore the novel environment more effectively and help invasion success (Sol et al. 2012; Sol & Maspons 2016) without compromising lifetime reproductive fitness.

In conclusion, mated *T. ludeni* females do not trade off dispersal with their lifetime reproductive output and survival. Rather, they adjust their resource allocation depending on whether and how far they disperse, with long-distance dispersers investing more in dispersal in their early life while resident mites and short-distance dispersers investing more in reproduction in their early life. These strategies may facilitate invasion success of *T. ludeni*.

5.2 Effect of density and age on dispersal probability and distance in *Tetranychus ludeni*

This section will be submitted to a journal for publication

5.2.1 Introduction

Investigation into how different factors affect dispersal probability and distance helps better understand the dispersal process (Matthysen 2012), knowledge of which is important for population management and invasion evaluation (Bowler & Benton 2005; Clobert et al. 2009; Azandémè-Hounmalon et al. 2014). Population density and individual age are two important factors that can influence dispersal (Bowler & Benton 2005; Matthysen 2012). However, most studies have focused on how these factors affect dispersal probability (e.g., Suiter & Gould 1992; Li & Margolies 1993; Bowler & Benton 2005; Hovestadt et al. 2014; Mishra et al. 2018; Plazio et al. 2020). To date, the effect of density and age on dispersal distance is still poorly understood.

High population density often leads to competition, reducing individual fitness (Bowler & Benton 2005) and increasing dispersal probability (Li & Margolies 1993; Bowler & Benton 2005; De Meester & Bonte 2010; Azandémè-Hounmalon et al. 2014) and distance (Poethke et al. 2011; Bitume et al. 2013). Females at the age of high fecundity may have higher dispersal probability and longer dispersal distance to spread their eggs (Gu & Danthanarayana 1990; Fadamiro 1997; Ishizaki et al. 2011; Perez-Mendoza et al. 2011). Moreover, the reproductive output of dispersers at the age of dispersal can affect population growth and establishment success at new sites (Dingle 1965; Williamson & Charlesworth 1976; Stewart & Gaylor 1991; Schumacher et al. 1997; Järemo & Bengtsson 2011).

The European native spider mite *Tetranychus ludeni* Zacher (Acari: Tetranychidae) has now invaded all continents except Antarctica (Bolland et al. 1998; CABI/EPPO 2020). It attacks over 300 hosts, including many economically important crops such as bean, eggplant, hibiscus, apple, pumpkin, and other cucurbitaceous plants (Bolland et al. 1998; Zhang 2003; Migeon et al. 2010). Adult females of spider mites usually disperse after mating (Mitchell 1973) through ballooning and walking (or aerial and ambulatory dispersal) (Suiter & Gould 1992; Li & Margolies 1993), especially under high population density or poor habitat quality (Mcenroe 1969; Bitume et al. 2013). Young females disperse more frequently than older females by ballooning (Li & Margolies 1993). However, the age-specific ambulatory dispersal is not clear (Suiter & Gould 1992).

I used *T. ludeni* to investigate the effect of density and age on dispersal (ambulatory) probability and distance. For age experiment, I set up five treatments (1, 3, 6, 9, and 12 days old with a fixed density of 12.5 mated females/cm^2). I allowed females to disperse for 24 hours and then recorded their dispersal probability, dispersal distance, and number of eggs laid within 24 hours. For the density experiment, I set up four treatments (2.5, 12.5, 25, and 37.5 mated females/cm^2 with a fixed age of one day old), allowed them to disperse for 24 hours and then recorded dispersal probability and distance. I hypothesized that (1) females at the age of high fecundity would have higher dispersal probability and longer dispersal distance, and (2) high density would increase dispersal probability and distance.

5.2.2 Materials and Methods

5.2.2.1 Mite colony and experimental conditions

I collected *T. ludeni* adults on *Passiflora mollissima* Kunth (Malpighiales: Passifloraceae) in Palmerston North, New Zealand and used 3- to 5-week-old common bean *Phaseolus vulgaris* L. (Fabales: Fabaceae) plants grown in pots to maintain the colony. I used leaf squares cut from the first expanded leaves of 1- to 2-week-old bean plants grown in pots for all experiments. Environmental conditions for the colony and experiments were set at 25 ± 1 ºC, 40 ± 10% RH, and 14:10 h (L:D) photoperiod.

5.2.2.2 Preparation of mated females for experiment

To obtain virgin males to inseminate females, I randomly selected 50 female deutonymphs from the colony and transferred them onto a clean fresh leaf square (4 × 4 cm) on wet cotton wool in a Petri dish (9 cm in diameter × 1 cm in height). I allowed these deutonymphs to develop into virgin adult females and lay unfertilised eggs for three days. I then removed adult females and allowed the eggs to develop to virgin male adults. I prepared a total of 46 Petri dishes for this purpose. I used 1-d-old virgin adult males to mate with females prepared below.

I randomly selected 50 adult females from the colony and transferred them onto a clean fresh leaf square (4 × 4 cm) on wet cotton wool in a Petri dish for egg laying for 24 hours. I then removed the female adults and allowed eggs to develop to the quiescent deutonymphal stage. I set up approximately 230 Petri dishes for this purpose. I transferred 15 ~ 20 virgin males prepared above to female quiescent deutonymphs in each Petri dish, allowing them to stay with the newly emerged females for 5 hours to ensure that all females mated at or soon after emergence. I transferred 50 newly emerged (< 24 hours old) and mated females from an above dish onto a new leaf square (4 × 4 cm) on wet cotton wool in a Petri dish. I prepared 230 such dishes and I replaced all leaf squares once a day to obtain mated females of 1, 3, 6, 9, and 12 days old for experiment. To keep female density on each leaf square consistent prior to experiment, I checked the leaf square every day and replaced dead females with live ones of the same age.

5.2.2.3 Dispersal and reproduction of females of different ages

I established a dispersal arena consisting of 21 leaf squares (2 cm × 2 cm for the first leaf square and 2 cm × 1 cm for others) placed on wet cotton in a tray (45 cm long × 36 cm wide × 1.5 cm high) (Figure 5.1, Zhou et al. 2021). I performed 17, 14, 14, 14, and 16 replicates for treatments of 1, 3, 6, 9, and 12 days old, respectively. For each replicate, I transferred 50 mated females of a desired age onto the first leaf square (primary leaf square) and allowed them to settle for 40 minutes. I then connected all leaf squares using Parafilm bridges (4.5 cm long × 1.5 cm wide; Parafilm®, USA) and allowed mites to disperse freely for 24 hours, after which time, I removed all bridges, counted the number of adults on each leaf square, and removed all adults and counted the number of eggs on each leaf square. I defined the dispersal probability as the percentage of mites that dispersed out of the primary leaf square. I used the number of leaf squares from the primary leaf square as dispersal distance and regarded the primary leaf square as zero distance. I recorded the mean dispersal distance of a replicate by averaging the dispersal distance of all individuals in the replicate and the total number of eggs laid in a replicate by summing up the number of eggs on all leaf squares.

5.2.2.4 Dispersal of females at different densities

Because spider mite density in nature ranges from 0.1 to 50 individuals/cm^2 (Helle & Sabelis 1985; Geroh et al. 2010), I set up four densities: 2.5, 12.5, 25 and 37.5 females/cm^2, with 10, 50, 100 and 150 mated females on the primary leaf square, respectively. There were 15, 15, 16, and 16 replicates, respectively, for above density treatments. For each replicate, I transferred 1-d-old mated females of a desired number according to treatments on the primary leaf square and allowed them to settle for 40 minutes. I then connected all leaf squares as above and allowed mites to disperse freely for 24 hours, after which time, I removed all bridges and counted the number of adults on each leaf square. I calculated dispersal probability and distance of a replicate as described above.

5.2.2.5 Statistical analysis

I analyzed all data using SAS software (SAS 9.4, SAS Institute Inc., Cary, NC). All data were normally distributed (dispersal distance in density experiment were normally distributed after square-root transformation) (Shapiro-Wilk test, UNIVARIATE Procedure) and analyzed using ANOVA with Tukey's test for multiple comparison (GLM procedure).

5.2.3 Results

5.2.3.1 Dispersal and reproduction of females of different ages

My results show that within 24 h experimental period, 1-d-old females were significantly less likely to disperse as compared to older females ($F_{4,70} = 15.51$, $P < 0.0001$) (Figure 5.4A), and they dispersed significantly shorter distance than older females ($F_{4,70} = 7.55$, $P < 0.0001$) (Figure 5.4B). Furthermore, 1-d-old females laid significantly fewer eggs than older females within 24 h ($F_{4,70} = 25.57$, $P < 0.0001$) (Figure 5.4C).

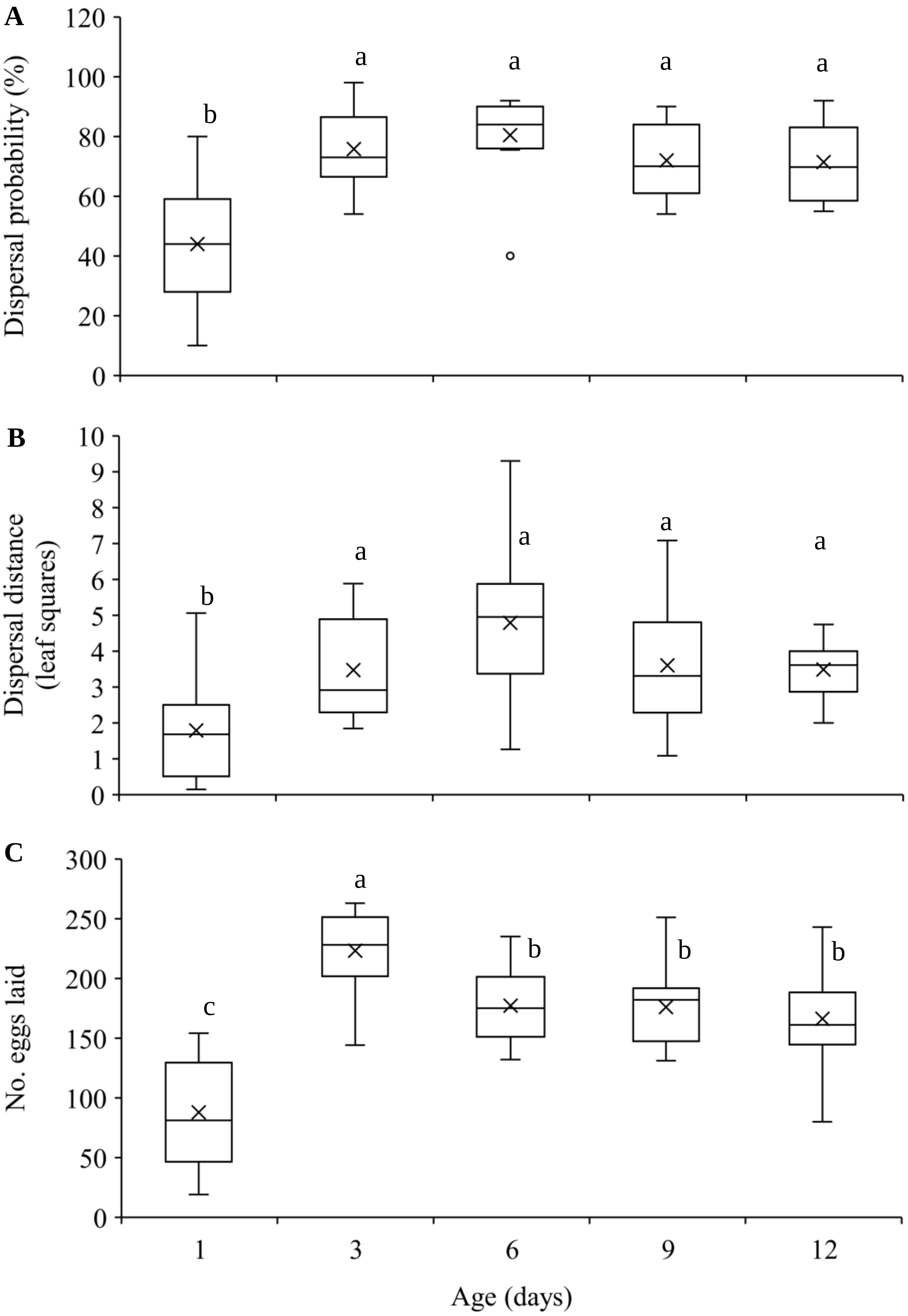

Figure 5.4 Mean dispersal probability (**A**), dispersal distance (**B**), and total number of eggs laid (**C**) within 24 h in *T. ludeni* females of different ages. Each box plot shows the maximum (⊤) and minimum (⊥) scores, the range of upper and lower quartiles (the box), and the mean (×) and median (line in the box) scores; the circle is an outlier of minimum score. For each parameter, boxes with the same letters are not significantly different (P > 0.05).

5.2.3.2 Dispersal of females at different densities

Mites were significantly more likely to disperse with the increase of population density ($F_{3,58} = 15.33$, $P < 0.0001$) (Figure 5.5A). Similarly, they dispersed significantly longer distance within 24 h when population density was higher ($F_{3,58} = 14.06$, $P < 0.0001$) (Figure 5.5B).

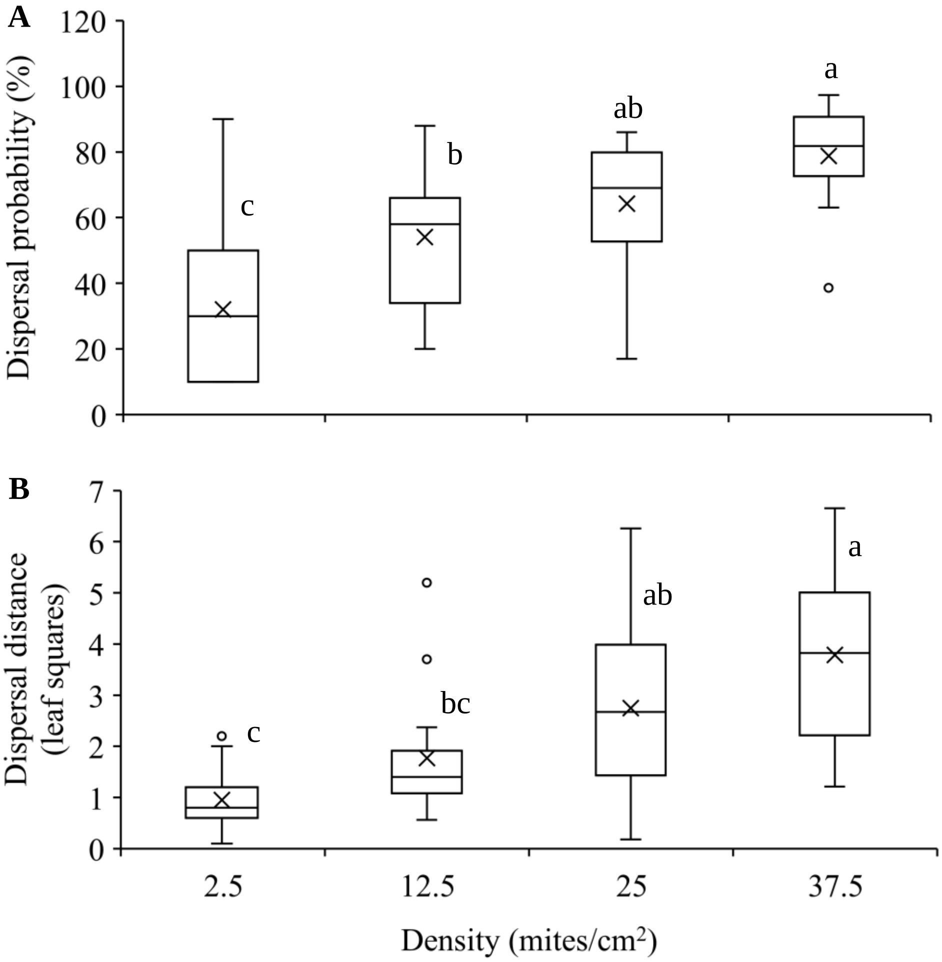

Figure 5.5 Mean dispersal probability (**A**) and dispersal distance (**B**) within 24 h in *T. ludeni* females of different densities. Each box plot shows the maximum (⊤) and minimum (⊥) scores, the range of upper and lower quartiles (the box), and the mean (×) and median (line in the box) scores; the circles are outliers of minimum or maximum scores. For each parameter, boxes with the same letters are not significantly different ($P > 0.05$).

5.2.4 Discussion

My results show that as compared to 1-d-old females, older ones had significantly higher dispersal probability, greater dispersal distance and laid more eggs in *T. ludeni* (Figure 5.4). These findings strongly suggest that the age-dependent dispersal is caused by reproductive readiness when older females carry more mature eggs (Adango et al. 2006). In general, females with more eggs tend to disperse and spread their eggs in wider areas (Matthysen 2012). The synchronization of dispersal and reproduction has also been reported in many insects such as the light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) (Gu & Danthanarayana 1990), the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) (Stewart & Gaylor 1991; Stewart & Gaylor 1994), the rice bug, *Leptocorisa chinensis* Dallas (Hemiptera: Alydidae) (Ishizaki et al. 2011), and the butterfly, *Maculinea* (*Phengaris*) *teleius* Bergsträsser (Lepidoptera: Lycaenidae) (Plazio et al. 2020).

Higher dispersal probability and longer dispersal distance of females ladened with more eggs may contribute to fast population growth at new sites, facilitating pest outbreak and range expansion (Dingle 1965; Stewart & Gaylor 1994; Schumacher et al. 1997). Furthermore, the similarly high dispersal probability and long dispersal distance among older females (3-12 days old) (Figure 5.4) suggest that *T. ludeni* females can quickly disperse and infest new host plants in most of their adult lives. In their study on dispersal behaviour of *T. urticae*, Li and Margolies (1993) reveal that the aerial dispersal is much more frequent in younger females (≤ 2 days old) than in older females (≥ 3 days old) because young females are smaller in body size and may be more easily carried aloft by air currents. However, I have not tested the effect of aerial ballooning on dispersal in the present study.

The present study indicates that *T. ludeni* females significantly increased dispersal probability and distance with the increase of population density (Figure 5.5). This could be attributed to the fact that high population density reduces individual and population fitness (Bowler & Benton 2005; Weerawansha et al. 2020; Li & Zhang 2021b). The density-dependent dispersal strategy has been reported in *T. urticae* (Li & Margolies 1993; Bitume et al. 2013) and the brown garden snail *Cornu aspersum* (Müller) (Gastropoda: Helicidae) (Dahirel et al. 2016). This strategy allows individuals to leave high density habitats and immigrate into low density habitats and/or colonize new habitats (Sæther et al. 1999; Bowler & Benton 2005; Hovestadt & Poethke 2006), promoting the invasion speed of *T. ludeni*.

In conclusion, I show that increasing age and density promote dispersal probability and distance in *T. ludeni*. Females with more mature eggs are more likely to disperse and move longer distances. These dispersal strategies help to spread eggs and reduce competition, which may facilitate pest outbreak and invasion success of *T. ludeni*.

Chapter 6

General Discussion and Conclusion

6.1 Introduction

The overall aim of this PhD thesis was to investigate the life history strategies of *T. ludeni* with special reference to invasion success. I have made extensive review of literature relevant to my PhD studies, including general biology of *T. ludeni*, reproductive strategies, inbreeding depression, and dispersal. I have then conducted a series of experiments to explore female life history strategies of *T. ludeni* in response to mating status, inbreeding, and dispersal. Knowledge generated here is important for understanding its life history traits and the mechanisms behind its invasion success. In this chapter, I summarize and discuss my main findings and recommend future research.

6.2 Resource allocations by virgin females

My work delivers the first empirical evidence that the virgin females strategically allocate more resources to their sons, producing larger male eggs which develop to larger adults, than mated females, in a haplodiploid mite. I also demonstrate that larger sons produced by virgin mothers yield more daughters at a higher speed in *T. ludeni*, supporting previous findings in other animals that larger males generally have more sperm available (Wiernasz et al. 2001; Locatello et al. 2008; Anthes et al. 2014; O’Dea et al. 2014; Sturm 2014) and replenish their sperm reserves faster (O’Dea et al. 2014) than small males because testis size usually increases with body size (Gage 1994; Simmons 2012).

These are important features that may enhance the chance for *T. ludeni* to become established in new habitats because the population is usually small, and females are more likely to be virgin at the beginning of an invasion. This resource allocation strategy compensates the loss of daughter production for virgin females through their sons’ production of more daughters at a faster rate in the next generation and helps found a colony faster. Previous studies report that larger males are usually better than smaller ones in male-male competition (Andersson 1994; Emlen 2008; Hunt et al. 2009; Suzaki et al. 2013; Shelly 2018). However, my study does not support this notion because sons from virgin mothers have the same chance to mate as those from mated ones, suggesting that male body size resulting from their mothers’ mating status does not contribute to mating success in *T. ludeni*.

My findings suggest that male survival is not dependent on their reproductive expenditure or their mothers' mating status in *T. ludeni*. This study does not support previous reports where mating is costly to males, reducing resources for somatic maintenance (Vinogradov 1998) and shortening males' longevity (Roff 1992; Stearns 1992; Cichoń 2001; Roff & Fairbairn 2007). The reported costs of mating include expenditure on sperm and seminal fluid production (Van Voorhies 1992; Pitnick 1996), mate access (Barnes & Partridge 2003; Metzler et al. 2016), and immune defence against sexually transmitted pathogens (Schwenke et al. 2016). However, the current study has not examined spending on ejaculates and immune defence in males produced by virgin and mated mothers, which could a topic for future studies.

6.3 Inbreeding depression and inbreeding avoidance behaviour

Theories predict that inbreeding may increase homozygosity of recessive alleles, causing inbreeding depression (Charlesworth & Charlesworth 1987; Charlesworth & Willis 2009) and evolution of inbreeding avoidance behaviour (Pusey & Wolf 1996; Nichols 2017). In some cases, however, inbreeding may purge recessive deleterious alleles from the genome (Crnokrak & Barrett 2002; Keller & Waller 2002), resulting in little or no fitness reduction (Nichols 2017). Furthermore, inbreeding may even benefit parents because mating between relatives helps spread identical beneficial genes by descent (Kokko & Ots 2006; Szulkin et al. 2013; Nichols 2017). When inbreeding has benefits or no negative impact, inbreeding avoidance may not occur (Tan et al. 2012). My study has not detected inbreeding depression and inbreeding avoidance behaviour or any fitness gain through inbreeding in *T. ludeni*.

In the current study, I have examined the fitness of offspring from parents at different mating settings (mother-son mating, brother-sister mating, and outbreeding) in the first and the 11th generations. I show that neither mother-son mating nor sibling mating causes inbreeding depression in *T. ludeni* in both short- and long-term inbreeding. Similar results are also reported in *T. kanzawai* (Ito et al. 2012) and *S. miscanthi* (Mori et al. 2005). I suggest that these findings could result from purging of deleterious alleles through haploid males (Atmar 1991; Antolin 1999; Henter 2003; Tien et al. 2015) and frequent inbreeding (Mitchell 1973) in haplodiploid mites. However, *T. urticae* Koch females suffer substantial inbreeding depression (Tien et al. 2015). The reasons behind the difference in consequences from inbreeding between *T. ludeni* and *T. urticae* warrant further investigations.

I have also investigated the mating behaviour of offspring from parents experiencing inbreeding to determine whether *T. ludeni* adults present inbreeding avoidance behaviour. I have not detected any evidence for that because inbred *T. ludeni* have no significant preference between siblings and unrelated mates in mate choice. Similarly, no inbreeding avoidance occurs in several other haplodiploid species (Bourdais & Hance 2009; de Souza et al. 2017; Bogo et al. 2018). However, due to substantial inbreeding depression in *T. urticae* (Tien et al. 2015), its females prefer to mate with unrelated males (Tien et al. 2011). These findings in different species support the theoretical predictions that individuals may avoid inbreeding when inbreeding depression is substantial but inbreeding avoidance may not occur when inbreeding depression is low or absent (Lande & Schemske 1985; Szulkin et al. 2013; Nichols 2017).

6.4 Female reproductive strategies in response to inbreeding and mating delay

Based on my findings in Chapters 2 and 3, I have carried out further experiments to determine whether inbreeding could increase risk of population collapse in successive generations and whether females could adjust their resource allocation in response to mating delay that may facilitate mother-son mating in *T. ludeni*. My results indicate that inbreeding has no effect on female reproductive outputs and longevity throughout 11 successive inbred generations. This should allow mother-son and brother-sister mating to occur at the invasion front without adverse consequences in fitness.

My results do not support previous reports on the haplodiploid mites where the inbreeding depression can occur in any generation of inbred animals, which could cause invasion failure. For example, inbreeding depression takes place in later generations but not in the first few generations in *S. miscanthi* (Mori et al. 2005) and *P. persimilis* (Çekin & Schausberger 2019) while it is detected in the first few generations in *T. urticae* (Tien et al. 2015). I suggest that the expression of deleterious recessive alleles from inbreeding may be selected out in small populations in *T. ludeni* as reported in some other haplodiploid animals (e.g., Quaglietti et al. 2017; Eyer et al. 2018). This mechanism may allow mother-son and brother-sister mating to occur at the invasion front without adverse consequences in fitness (Adamson & Ludwig 1993; Kronauer et al. 2012; McCulloch & Owen 2012; Schmidt et al. 2014; Lantschner et al. 2020; Queffelec et al. 2020). Future studies on genetics in relation to potential purging of deleterious recessive alleles in *T. ludeni* are recommended.

I show that virgin *T. ludeni* females have developed a strategy to secure subsequent mother-son mating by producing highest number of sons in their early life, allowing continuous population growth in uncertain situations including unavailability of mates at the invasion front. If virgin *T. ludeni* still have no chance to mate after this reproductive episode, they will save resources by reducing egg production for potential future mating. Several other spider mites appear to share similar strategies with *T. ludeni* (Bonato & Gutierrez 1996, 1999; Li & Zhang 2021a). However, females increase their resource allocation to egg production immediately after mating to ensure production of maximum number of both daughters and sons as early as possible. Mated females with mating delay quickly increase proportion of daughters in offspring produced to compensate the loss of production of daughters during their virgin life. This resource allocation strategy may reduce risks of potential reproductive failure in a new and unstable environment (Stearns 1992; Stearns et al. 2000), facilitating establishment of a newly invaded population (Dangremond & Feller 2016; Fetters & McGlothlin 2017; James et al. 2017).

6.5 Dispersal in relation to reproduction, survival, age, and population density

I have also conducted experiments to examine *T. ludeni* female dispersal in relation to reproduction, survival, age, and population density. My results reveal that mated *T. ludeni* females do not trade off dispersal with their lifetime reproductive output and survival, but they adjust their resource allocation depending on whether and how far they disperse. Furthermore, both female age and population density influence dispersal probability and distance in *T. ludeni*.

Contrary to theoretical predictions (Bonte et al. 2012; Travis et al. 2012; Weigang & Kisdi 2015), *T. ludeni* females have similar lifetime fecundity and longevity regardless of whether and how far they disperse. However, they adjust their resource allocation to different life functions depending on whether and how far they disperse. All females have two reproductive episodes, but long-distance dispersers produce fewer eggs and fewer daughters during the first episode. Similar findings are also reported in other invertebrates (Roff 1984; Roff & Fairbairn 1991; Tanaka 1993; Innocent et al. 2010). Looking into the two reproductive peaks, I find that the second peaks in residents and short-distance dispersers are lower than the first ones while the two peaks have similar height in long-distance dispersers. These results suggest that in their early life residents and short-distance dispersers invest more resources in reproduction than in dispersal while long-distance dispersers allocate more resources to dispersal than to reproduction.

My results suggest that reproductive readiness (Adango et al. 2006) and tendency to spread their eggs in wider areas (Matthysen 2012) promote dispersal which facilitates pest outbreak and range expansion (Dingle 1965; Stewart & Gaylor 1994; Schumacher et al. 1997). In their study on *T. urticae*, Li and Margolies (1993) show that the aerial dispersal is much more frequent in younger females (≤ 2 days old) than in older females (≥ 3 days old) because young females are smaller in body size and may be more easily carried aloft by air currents. However, I have not tested the effect of aerial ballooning on dispersal in the present study, which warrants future investigations. Furthermore, *T. ludeni* females in denser populations are more likely to disperse and to disperse greater dispersal distance, probably because high population density reduces individual and population fitness (Bowler & Benton 2005; Weerawansha et al. 2020; Li & Zhang 2021b). The density-dependent dispersal strategy may allow individuals to leave high density habitats and immigrate into low density habitats and/or colonize new habitats (Sæther et al. 1999; Bowler & Benton 2005; Hovestadt & Poethke 2006), promoting the invasion speed of *T. ludeni*.

6.6 Conclusion

This study provides new insight into life history strategies of *T. ludeni* females that may help overcome various barriers following introduction to a new habitat and contribute to its invasion success. These strategies include adjustment of resource allocations and behaviour in response to availability of mates, inbreeding depression, and dispersal. Knowledge generated in this thesis is vital for appraising prospects for the development of programmes for prediction and management of biological invasions and for further investigations of the invasion mechanisms of this mite and other haplodiploid species. Further studies could investigate (1) the costs of mating in males produced by virgin and mated mothers including expenditure on ejaculates and immune defence against sexually transmitted pathogens; (2) reasons behind the difference in inbreeding depression between *T. ludeni* and *T. urticae*; (3) genetics in relation to potential purging of deleterious recessive alleles in *T. ludeni*; and (4) aerial dispersal of *T. ludeni* in relation to age and body size.

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Appendix 1: Four Published Papers from My PhD Studies

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Article

Sons from virgin mothers produce more daughters in a haplodiploid mite

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Abstract

Population size can be very small in the newly invaded/introduced site or front edge of population expansion where mates are difficult to find. This scenario can lead to extinction of a local population in many animal species. However, when it happens to a haplodiploid animal, individual virgin females may adjust their strategies to produce sons of greater reproductive success such as higher mating success and fertility, which may help increase the chance of establishment. Here we investigated how maternal mating status affected sons' reproductive success in a haplodiploid spider mite, *Tetranychus ludeni* Zacher, a cosmopolitan pest of many crops. We show that virgin females laid significantly larger eggs than mated females, giving rise to larger deutonymphs and adults, but mating status of mothers had no influence on mating success and longevity of their sons. We provide the first empirical evidence in a haplodiploid mite that virgin mothers adjusted their resource allocations to yield sons that produced more daughters at a higher rate.

Key words: Spider mite, female mating status, male mating success, male reproductive success

Introduction

In haplodiploid arthropods, mated females may lay unfertilised haploid eggs giving rise to sons and fertilised diploid eggs leading to daughters while virgin females can only produce haploid sons (Filia *et al.* 2015). When population size is small, it is likely that females are not able to find mates and produce daughters. This scenario may be particularly common in new environment such as recently invaded site or front edge of population expansion (Engen *et al.* 2003). Therefore, females may have developed strategies to adjust their resource allocations in response to the situation. So far, whether and how virgin females adjust their resource allocations and whether and how such adjustment influences their sons' reproductive success are poorly understood in haplodiploid animals.

Several authors have investigated the effect of maternal mating status on offspring performance in a haplodiploid mite, *Tetranychus urticae* Koch. In one study, sons produced by virgin females appear to be able to find their mates more quickly than males produced by mated females (Ohzora & Yano 2008). In another study, however, maternal mating status does not affect mate-searching behaviour of males (Oku & van den Beuken 2017). Macke *et al.* (2011, 2012) report that male eggs laid by virgin females are usually larger than male eggs produced by mated females. Although the effect of maternal mating status is not examined, larger males are found to be more likely to win male-male competition for mates (Potter *et al.* 1976) and better resist against attempted interference during mating (Enders 1993). However, it is not clear whether maternal mating status has any influence on sons' reproductive outputs in any haplodiploid mite species, knowledge of which is important for the better understanding of invasive potential of these animals.

Tetranychus ludeni Zacher is native to Europe and now globally distributed (Bolland *et al*. 1998; CABI 2011). It appears to be better adapted to hot weather and have a more subtropical distribution than the cosmopolitan congeneric *T. urticae* (Martin 2000; Gotoh *et al*. 2015). This species is a serious pest of bean, eggplant, hibiscus, pumpkin and other cucurbitaceous plants in warm regions and a common pest on greenhouse plants in temperate areas (Zhang 2003). It has denser webbing than *T. urticae*, which is believed to make its biological control by predatory mites less effective (Martin 2000; Zhang 2002). However, compared to *T. urticae*, *T. ludeni* has been much less studied. For example, nothing is known about the effect of mothers' mating status on traits of their sons in *T. ludeni*.

In the present study, we carried out a series of experiments to examine how maternal mating status affected sons' body size, mating success, longevity, and reproductive outputs in *T. ludeni*. Based on the knowledge outlined above, we proposed and tested three hypotheses: (1) virgin females produce larger eggs than mated females and larger eggs develop to larger adults; (2) sons from virgin females (SVF) have better mating success than sons from mated females (SMF), and (3) SVF have higher reproductive output and greater longevity than SMF.

Materials and Methods

Mite colony

A colony of *T. ludeni* was established from adults collected on *Passiflora mollissima* (Kunth) in September 2017 in Palmerston North, New Zealand, and reared on kidney bean plants (*Phaseolus vulgaris* L.). Three to 5-week-old bean plants were used for maintaining the colony, and the first expanded leaves of 1 to 2-week-old plants were used for experiments. The colony was maintained and the potted plants grown in two separate environmental rooms at 25 ± 1 °C, 40 ± 10% RH and 14:10 h (L:D) in the Massey University Entomology and IPM Laboratory, Palmerston North, New Zealand. Experiments were carried out in the third environmental room with the same environmental conditions. Mites were reared on kidney bean plants for three generations before experiments.

Experimental mites

To obtain sons from virgin females (SVF) and mated females (SMF), we randomly selected 60 female and 45 male deutonymphs from the colony and transferred them onto small leaf squares (1.0 × 1.0 cm), one mite on each square in a Petri dish (9.5 cm diameter and 1.0 cm height), and allowed them to become quiescent deutonymphs (QD) and emerge. Leaf squares were placed on the wetted cotton wool. The Petri dish was covered with a lid which had a 1-cm-diameter hole covered with metal mesh (aperture size = 0.25 mm) in the centre.

We kept 15 1-d-old virgin females individually in their Petri dishes to produce SVF. To obtain SMF we individually paired 45 1-d-old virgin females with 45 1-d-old virgin males for 24 hours and then removed the males. Because virgin *T. ludeni* females lay reddish eggs whereas mated females lay translucent eggs, we examined the colour of all eggs laid by the females exposed to males for 2 days following removal of males. Females that laid translucent eggs were considered as mated and used for producing SMF and the remaining were discarded. On the third day, we transferred virgin and mated females individually onto new leaf squares, one mite on each leaf square, and allowed them to oviposit for 24 hours, after which time, we transferred them onto new leaf squares. This procedure was repeated for 3–10 days depending on the desired number of mites required for experiments. We transferred eggs from virgin and mated females individually onto new leaf squares, one egg on each square in a Petri dish, and allowed them to develop to deutonymphs or adults. All dishes were numbered so that eggs and resultant adults were matched.

Body size and mating success of SVF and SMF

To investigate the size and size distribution of eggs from females of different mating status (Figure 1), we measured 51 eggs laid by virgin females and 247 eggs (including both fertilised and unfertilised eggs) laid by mated females. We measured many more eggs from mated females because the sex ratio of offspring produced by mated female *T. ludeni* was highly female-biased (Gotoh *et al.* 2015). Egg size (total area from top view) was determined using a digital camera (Olympus SC30, Japan) connected to a stereomicroscope (Leica MZ12, Germany) and a computer with adequate imaging software (CellSens® GS-ST-V1.7, Olympus, Japan) installed. Measured eggs were individually transferred onto new leaf squares (1.0 × 1.0 cm) using a fine brush and raised in Petri dishes. On the fifth day, each larva or nymph was provided with a new leaf square of the same size to avoid leaf quality deterioration. Eggs that successfully developed to QDs were used to determine the difference in size of male eggs laid by virgin (n = 31) and mated females (n = 24) (Table 1). QDs of 27 SVF and 20 SMF were photographed and their size (total area from top view) measured using the above device. After emergence, 26 1-d-old SVF and 19 1-d-old SMF adults were photographed and their size measured again as above.

TABLE 1. Size (× $10^4\ \mu m^2$) of SVF and SMF.

	Egg	Quiescent deutonymph	Adult
SVF	1.3519 ± 0.0158 a	3.1048 ± 0.0483 a	2.9674 ± 0.0373 a
SMF	1.1385 ± 0.0280 b	2.8625 ± 0.0551 b	2.7544 ± 0.0520 b
$F_{(df)}$	49.84 $_{(1,53)}$	10.95 $_{(1,45)}$	11.66 $_{(1,43)}$
P	< 0.0001	0.0018	0.0013

Means (± SE) with the same letter in each column are not significantly different (P > 0.05).

To determine whether the mating success of SVF and SMF was different, we randomly selected female deutonymphs from experimental mites as described in the previous section. Mites were individually transferred onto leaf squares (1.0 × 1.0 cm), one mite on each square in a Petri dish. About 20 hours after transfer, the QD females turned silvery and were ready for test. For each replicate, we randomly selected a 1-d-old SVF and a 1-d-old SMF from the experimental mites and randomly marked one of them on his rear dorsal using blue baking colour (Hansells, Queen New Zealand Pty. Ltd) before releasing them to a QD female on a leaf square in a Petri dish. We recorded their behaviours using the device mentioned above for 10 minutes after the female emerged. A total of 68 replicates were recorded. Because the first released males might be more likely to locate, guard and mate with the QD females independent of the mating status of their mothers, we carried out a second set of release regime: we transferred a 1-d-old SVF and a 1-d-old SMF onto a leaf square about 2 hours before the introduction of the QD female. Other experimental procedures were the same as the above test. Fifty-one replicates were performed.

Reproductive success of SVF and SMF

This experiment was designed to determine the reproductive success of SVF and SMF. To provide sufficient female adults for experiments, we randomly collected 300 female deutonymphs from the colony each day and transferred them onto leaf squares (5 × 5 cm) with 50 mites on each square for development into adults. On the first day of experiments, we provided a 1-d-old SVF or SMF with 10 1-d-old virgin females on a leaf square (2 × 2 cm) in a Petri dish for 24 hours. The next day, the male (2-d-old) was transferred onto a new leaf square of the same size with 10 1-d-old virgin adult females for 24 hours. This procedure was repeated until the male died.

After the male had been transferred onto a new leaf square, the females were allowed to oviposit on their leaf square for 5 days, and then transferred onto a new leaf square of the same size. This procedure was repeated until all females died. All eggs laid by these females were allowed to develop to deutonymphs. For each test male, the longevity, fertile period (period during which the male could fertilise eggs, verified by the production of at least one daughter) and total number of daughters produced were recorded. Fifteen SVF and 15 SMF were tested.

Statistical analysis

All analyses were done using SAS 9.4 with a rejection level set at $\alpha < 0.05$. Male mating success (Figure 2) was analysed using a Chi-square test (FREQ procedure). A Wilcoxon test (LIFETEST procedure) was used to compare the survival probability of SVF and SMF. A goodness-of-fit test (Shapiro-Wilk test; UNIVARIATE procedure) was used to test the distribution of data when mean comparison was performed. Data on the mean size of male eggs, QDs and adults (Table 1) and the fertile period and number of daughters (Figure 3) were normally distributed and thus analysed using an analysis of variance (ANOVA, GLM procedure) followed by Tukey's Studentized range test. However, data on mean size of eggs laid by virgin females and mated females (both fertilised and unfertilised eggs) were not normally distributed, and thus analysed using a non-parametric ANOVA (GLM procedure). The size distributions of those eggs were compared by a Kolmogorov-Smirnov Test (NPAR1WAY procedure) (Figure 1).

We also developed a Power curve model (NLIN procedure) to fit the data on accumulation of insemination potential (number of daughters produced by SVF or SMF; Figure 4): accumulative daughters = $a + b\ (1 - c^{age})$, where a is the model constant, b is the maximum number of daughters produced during males' lifetime reflecting the maximum potential of sperm transferred, and c is the constant increasing rate of daughters produced per day, and $a > 1$, $b > 1$, and $1 > c > 0$. The estimated parameters are significantly different from 0 if the 95% confidence limits (CL) do not include 0 (both upper and lower CL > 0 or < 0) (Julious 2001). Julious' (2004) methods were used to compare parameters in Table 2, where there is no significant difference for a given parameter if the 83.4% CL overlap. The coefficient of determination (R^2) for Power curve model was calculated as: 1 – (residual sum of square/corrected total sum of square) (Tahriri *et al.* 2007).

TABLE 2. Comparison of estimated parameters of Power curve model between SVF and SMF*.

Parameter	Male	Estimate	SE	83.4% CL	
				lower	upper
a	SVF	134.10	60.25	50.42	217.90
	SMF	119.80	46.64	54.97	184.60
b	SVF	1688.90	80.31	1573.20	1804.70
	SMF	1056.80	65.19	966.20	1147.50
c	SVF	0.90	0.01	0.88	0.92
	SMF	0.89	0.02	0.86	0.92

* *a*, the model constant; *b*, the maximum number of daughters produced; and *c*, the constant daily increasing rate of accumulative daughters produced. All estimated parameters are significant at $P < 0.05$ level (95% CL > 0).

Results

Body size and mating success of SVF and SMF

Our results show that eggs laid by virgin females were significantly larger (mean ± SE = 1.3262 ± 0.0147, × 10^4 μm^2) than those by mated females (1.2601 ± 0.0063, × 10^4 μm^2) ($F_{1,296} = 19.52$, P <

0.0001), and the size distributions of eggs from virgin and mated females were significantly different (D = 0.3172, P < 0.0001; Figure 1). SVF of all stages from eggs to adults were significantly larger than SMF (Table 1). However, SVF and SMF had similar mating success regardless of which sex was introduced into the arena first (Chi-square test: x_1^2 = 0.15, P = 0.7008 when males were introduced first; x_1^2 = 0.02, P = 0.8759 when QD females were introduced first) (Figure 2).

Reproductive success of SVF and SMF

The fertile period was not significantly different between SVF and SMF ($F_{1,28}$ = 1.76, P = 0.1958; Figure 3A). SVF produced significantly more daughters (mean total ± SE = 1,407 ± 108) than SMF (mean total ± SE = 855 ± 78) ($F_{1,28}$ = 17.29, P = 0.0003; Figure 3B). Further analyses show that the estimated maximum number (*b*) of daughters produced was significantly greater in SVF than in SMF (83.4% CL did not overlap; Table 2), contributing to the faster accumulation of daughters produced (Figure 4). However, the longevity was not significantly different between SVF (17.73 ± 1.54 days) and SMF (18.07 ± 1.89 days) (x_1^2 = 0.13, P = 0.7182).

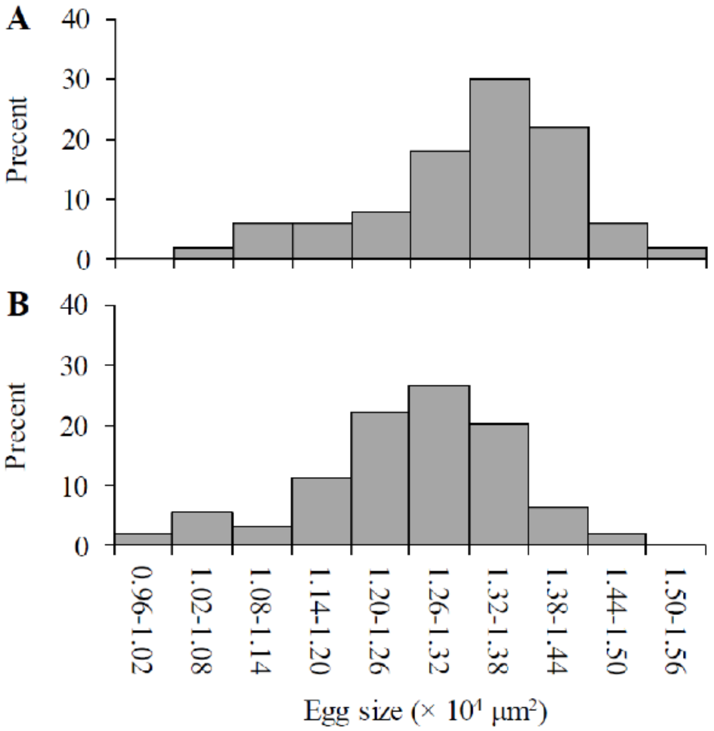

FIGURE 1. Size distribution of eggs laid by (A) virgin females (n = 51) and (B) mated females (n = 247).

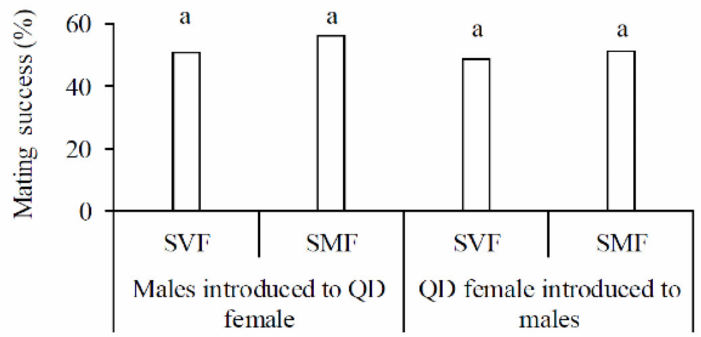

FIGURE 2. Mating success of SVF and SMF. Columns with the same letters in each case are not significantly different (Chi-square test: P > 0.05).

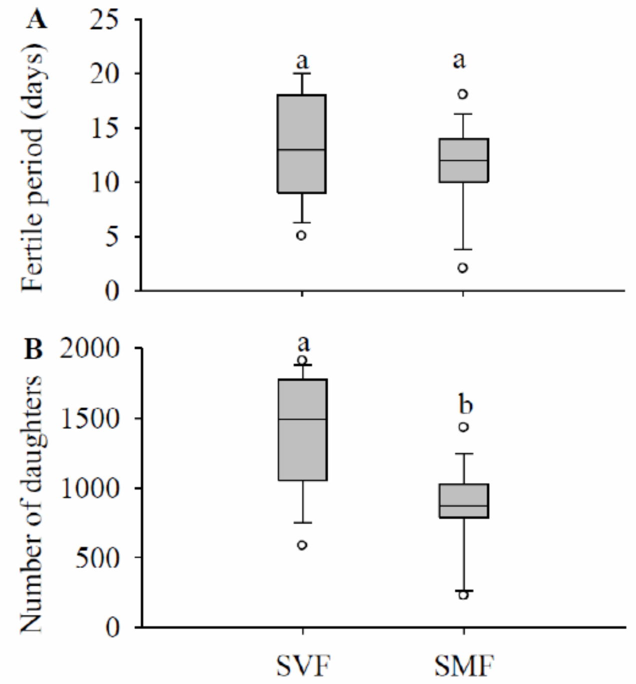

FIGURE 3. Fertile period (A) and daughters produced (B) in SVF and SMF. Columns with the same letters are not significantly different (ANOVA: $P > 0.05$).

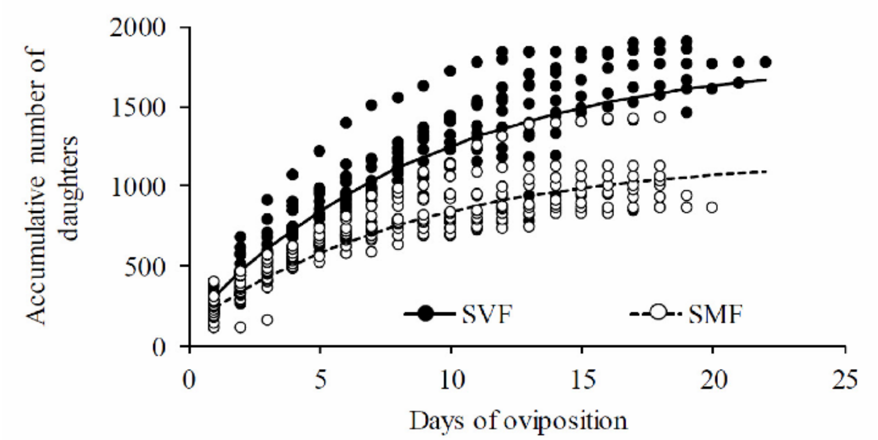

FIGURE 4. Lifetime reproductive potential of SVF and SMF. SVF: accumulative daughters = $134.10 + 1688.90(1 - 0.90^{age})$ ($R^2 = 0.9639$, $F_{2,230} = 360.29$, $P < 0.0001$); SMF: accumulative daughters = $119.80 + 1056.80(1 - 0.89^{age})$ ($R^2 = 0.9544$, $F_{2,206} = 232.61$, $P < 0.0001$).

Discussion

Numerous studies have reported the positive relationships between egg size and offspring fitness across taxa including mites (e.g., Macke *et al.* 2011), insects (e.g., Azevedo *et al.* 1997; Fox 1994, 2000; Fox & Czesak 2000; Torres-Vila & Rodriguez-Molina 2002; Fischer *et al.* 2002, 2003), fish (e.g., Hutchings 1991; Maruyama *et al.* 2003; Tamada & Iwata 2005), and birds (e.g., Price 1998; Ferrari *et al.* 2006). However, prior to the current study, little was known about strategic resource allocations to their sons by mothers of different mating status and the consequences of such strategies in a haplodiploid animal.

In *T. urticae*, virgin mothers produce significantly larger sons than mated mothers; however, mean egg size and egg size distribution are similar regardless of maternal mating status when both fertilised and unfertilised eggs are included in the analysis, suggesting that the differential egg size allocation takes place prior to fertilisation (Macke *et al.* 2011). As a result, the fact that virgin females produce larger sons is not a resource allocation strategy in response to maternal mating status in that species. However, when both fertilised and unfertilised eggs were incorporated in our analysis, we demonstrate that virgin females laid significantly larger eggs than mated females, and the size distributions of eggs from virgin and mated females were significantly different (Figure 1). Our findings indicate that the egg size difference between virgin and mated *T. ludeni* females is indeed a result of strategic resource allocation in response to maternal mating status, with more resources being allocated to their sons when females do not have the chance to produce daughters.

When we compare male offspring produced by mothers of different mating status, we show that virgin *T. ludeni* females laid significantly larger male eggs than mated females, and the resultant larger male eggs developed to larger deutonymphs and adults (Table 1). In principle, larger males have advantages in male-male competition (Andersson 1994; Emlen 2008; Hunt *et al.* 2009; Suzaki *et al.* 2013; Shelly 2018). Ohzora and Yano (2008) reveal that although size is not measured, sons from virgin mothers disperse faster and start guarding females sooner than those from mated mothers in *T. urticae*, suggesting that the former may have better mating success than the latter. However, our data from the two mate competition tests do not support this notion because larger SVF and smaller SMF had the same mating success when they were allowed to compete for a female (Figure 2). In an experiment on *T. urticae* with a design similar to ours, Oku and van den Beuken (2017) did not find any difference in mate-searching behaviour between SVF and SMF. These suggest that maternal mating status affects neither mating success nor mate-searching behaviour of their sons.

When we tested the lifetime fecundity of SVF and SMF, we found that SVF produced significantly more daughters than SMF (Figure 3B). Furthermore, compared to SMF, SVF contributed to a significantly faster increase of daughter population growth (Table 2, Figure 4). These data strongly suggest that when a female has no chance to mate, she produces sons that can yield more daughters at a higher speed. This strategy compensates the loss of producing daughters for virgin females through their sons' production of more daughters at a faster rate in the next generation. As a result, SVF may be able to found a colony faster as compared to SMF. The resource allocation strategy taken by virgin *T. ludeni* females and its consequences reported above may be attributed to the notion that large males generally have more sperm available for copulation (Wiernasz *et al.* 2001; Locatello *et al.* 2008; Anthes *et al.* 2014; O'Dea *et al.* 2014; Sturm 2014) and replenish their sperm reserves faster (O'Dea *et al.* 2014) than small males because testis size usually increases with body size (Gage 1994; Simmons 2012).

Mating may be costly to males, including expenditure on sperm and seminal fluid production (Voorhies 1992; Pitnick 1996), mate access (Barnes & Partridge 2003; Metzler *et al.* 2016), and immune defence against sexually transmitted pathogens (Schwenke *et al.* 2016), which could reduce resources for somatic maintenance (Vinogradov 1998). As a result, mating may cut males' future survival (Roff 1992; Stearns 1992; Cichoń 2001; Roff & Fairbairn 2007). Because SVF sire more eggs than SMF, the former are expected to spend more resources for reproduction and to live shorter lives. However, both SVF and SMF had similar longevity, suggesting that survival of *T. ludeni* males is not dependent on their reproductive expenditure or their mothers' mating status.

In conclusion, this study provides the first empirical evidence that the virgin females strategically allocate more resources to their sons, which can produce more daughters at a higher rate, in a haplodiploid mite. In a newly invaded scenario where population is small and females are more likely to be virgin, this feature may increase the chance for the species to become established.

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No evidence for inbreeding depression and inbreeding avoidance in a haplodiploid mite *Tetranychus ludeni* Zacher

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Mating between relatives (inbreeding) may increase homozygosity of recessive or partially recessive deleterious alleles, resulting in inbreeding depression (Charlesworth & Charlesworth 1987; Charlesworth & Willis 2009). The cost of inbreeding may drive the evolution of inbreeding avoidance behavior (Pusey & Wolf 1996; Nichols 2017). However, increased homozygosity due to inbreeding could expose recessive deleterious alleles to selection which purges them from the genome (Crnokrak & Barrett 2002; Keller & Waller 2002), resulting in little or no fitness reduction (Nichols 2017). Parents may even gain fitness through inbreeding because mating between relatives helps spread identical beneficial genes by descent (Kokko & Ots 2006; Szulkin *et al.* 2013; Nichols 2017) which will increase fitness (Hamilton 1972; Bai *et al.* 2005). Under these circumstances, animals may not need to avoid inbreeding (Tan *et al.* 2012). Therefore, whether inbreeding avoidance behaviour has evolved in an animal species may depend on the magnitude of inbreeding depression (Lande & Schemske 1985; Szulkin *et al.* 2013; Nichols 2017).

In haplodiploid animals where males are haploid from unfertilised eggs and females are diploid from fertilized eggs, inbreeding depression may be less severe and should only affect female-specific traits such as fecundity and offspring sex allocation (Henter 2003; Mori *et al.* 2005; de la Filia *et al.* 2015; Tien *et al.* 2015) because deleterious alleles are subject to selection in haploid males (Atmar 1991; Antolin 1999; Smith 2000; Henter 2003). However, whether inbreeding avoidance, if any, is sex-specific is unknown. Spider mites are a group of haplodiploid animals where frequent sibling and mother-son mating occurs (Mitchell 1973; Borgia 1980; Avilés & Purcell 2012) because mated mothers often lay haploid and diploid eggs closely together and brothers and sisters develop on the same spot and mate upon emergence (Mitchell 1973). Previous studies show that sibling mating causes no or limited inbreeding depression (Perrot-Minnot *et al.* 2004; Ito *et al.* 2012) whereas mother-son mating leads to substantial depression (Mori *et al.* 2005; Tien *et al.* 2015). However, most studies of effects of inbreeding on reproductive fitness in haplodiploids have only investigated one or a few generations, limiting our understanding of how inbreeding potentially affects long-term fitness.

Here we investigated aspects of inbreeding using the spider mite *Tetranychus ludeni* (Zacher), nothing of which was known prior to this study. We carried out a series of experiments to determine (1) whether and to what extent inbreeding depression occurred in over 11 generations of sibling and mother-son inbreeding and (2) whether the species performed sex-specific inbreeding avoidance.

We established a colony of *T. ludeni* from adults collected on *Passiflora mollissima* (Kunth) in Palmerston North, New Zealand, and reared it on 3- to 5-week-old kidney bean plants (*Phaseolus vulgaris* L.). We then split the colony into two colonies (A and B) and reared them on kidney bean plants in two separate climate rooms for 2.5 months (about 8 generations) before experiments, allowing us to conduct inbreeding and outbreeding treatments (see below). We maintained the colonies and carried out experiments at 25 ± 1°C, 40 ± 10% RH and 14:10 (L:D) photoperiod. We used the first expanded leaves of 1- to 2-week-old bean plants for all experiments. To prepare mites for inbreeding experiments, we randomly selected 40 male and 40 female deutonymphs from Colony A and maintained them individually until emergence. We allowed newly emerged virgin females to mate with newly emerged virgin males once and then transferred each mated female onto a leaf square (2.0 × 2.0 cm) placed on wet cotton wool in a Petri dish (9.5 cm diameter × 1.5 cm height) for oviposition for five days.

To determine whether and how inbreeding affected offspring fitness, we randomly selected three female deutonymphs that developed from the above eggs laid by each female for the following three treatments: (1) MS — mothers mated with their sons for 11 successive generations, (2) BS — brothers mated with their sisters for 11 successive generations, and (3) OB (outbreeding) — females mated with males from Colony B for 11 successive generations. As females in treatment MS were about 10 days old when their sons developed to adults, we used 10-day-old females for all three treatments in each generation to keep female age and oviposition experience consistent. In each generation we individually transferred female deutonymphs prepared as described above onto leaf squares (2.5 × 2.5 cm) for emergence. We allowed virgin females to reproduce for 10 days and then paired each of them with a newly emerged virgin male according to treatments until death. The leaf squares were replaced once every five days for each replicate. From each mated female, one to three female deutonymphs produced within five days after mother mating was randomly selected to start the next generation. We recorded the lifetime number of eggs laid, offspring survival, offspring sex ratio after mating, and longevity for each pair in the first and 11th generations. We obtained 30, 29 and 31 replicates in the first generation and 28, 27 and 29 replicates in the 11th generation for treatments MS, BS and OB, respectively.

To test inbreeding avoidance behaviour, we used offspring from the 11th generation of the above experiment and carried out two experiments: (1) female mate choice — a female was allowed to choose between a brother and a male from Colony B, and (2) male mate choice — a male was allowed to choose between a sister and a female from Colony B. Female and male mate choice were tested for each of the MS, BS, and OB treatments, resulting in 6 combination choice treatments with 39–56 replicates for each treatment. To start the experiments, we introduced two virgin 1-d-old mates on a leaf square (1 × 1 cm) and then the test virgin 1-d-old female or virgin 1-d-old male at a point with the same distance from the two mates. We video-recorded each replicate for 15 minutes and recorded premating period, mating success, and mating duration. Mating was scored as successful when the male genital was connected with the tip of the female abdomen for over 30 seconds, during which time insemination occurs (Potter & Wrensch 1978).

The distribution of all data was tested using a Shapiro-Wilk test (UNIVARIATE Procedure) before analysis. Data on the number of eggs and daughters, male and female longevity in the inbreeding experiment, and premating duration in the female mate choice experiment were normally distributed and analysed using an analysis of variance (ANOVA, GLM Procedure) with a Tukey test for multiple comparison. Data on proportion of daughters and offspring survival in the inbreeding experiment, and premating period and mating duration in the male mate choice experiment, and mating duration in the female mate choice were not normally distributed and analysed using non-parametric ANOVA (GLM Procedure). Data on mate choice were analysed with a Chi-square test (FREQ Procedure). We conducted all analyses using SAS software (SAS 9.4, SAS Institute Inc., Cary, NC).

We show that offspring from MS, BS and OB had similar fitness in the first and the 11th generations (Table 1), suggesting that neither mother-son mating nor sibling mating causes inbreeding depression in *T. ludeni* in both short and long term inbreeding. Similarly, Ito *et al.* (2012) report that sibling mating does not trigger inbreeding depression in *T. kanzawai* (Kishida) and Mori *et al.* (2005) reveal that mother-son mating results in no inbreeding depression in most tested populations of *Stigmaeopsis miscanthi* (Saito). The phenomenon could result from purging of deleterious alleles through haploid males (Atmar 1991; Antolin 1999; Henter 2003; Tien *et al.* 2015) and frequent inbreeding (Mitchell 1973) in haplodipoid mites. However, *T. urticae* (Koch) females suffer substantial inbreeding depression (Tien *et al.* 2015).

TABLE 1. Effects of inbreeding on reproduction and survival of *T. ludeni* in different generations.

Treatment	Number of eggs	Number of daughters	Daughters (%)	Offspring survival (%)	Longevity (days) Female	Longevity (days) Male
First generation						
MS	75.73 ± 6.78	36.50 ± 4.15	75.19 ± 2.15	92.29 ± 0.94 ab	22.59 ± 0.86	30.52 ± 1.57
BS	69.76 ± 4.48	35.24 ± 3.18	80.82 ± 1.84	91.57 ± 0.82 ab	22.07 ± 0.93	28.08 ± 1.52
OB	68.13 ± 4.37	34.26 ± 3.66	78.61 ± 1.92	93.83 ± 0.69 a	20.81 ± 0.63	26.96 ± 1.58
Eleventh generation						
MS	83.11 ± 6.98	40.50 ± 3.79	71.60 ± 3.16	87.54 ± 1.44 b	24.63 ± 1.33	24.58 ± 2.49
BS	72.89 ± 5.22	37.89 ± 3.88	73.52 ± 3.28	86.50 ± 3.06 b	22.89 ± 1.33	23.23 ± 2.77
OB	83.38 ± 5.70	47.21 ± 3.65	77.02 ± 1.64	88.25 ± 1.17 b	24.41 ± 1.04	24.41 ± 1.69
$F_{(df)}$	$1.1_{(5,168)}$	$1.63_{(5,168)}$	$1.68_{(5,168)}$	$5.6_{(5,168)}$	$1.86_{(5,163)}$	$1.93_{(5,154)}$
P	0.364	0.1536	0.1414	< 0.0001	0.104	0.0924

MS, mother-son mating; BS, brother-sister mating; OB, outbreeding. Means (± SE) of offspring survival (%) with the same letters are not significantly different (non-parametric ANOVA: P > 0.05).

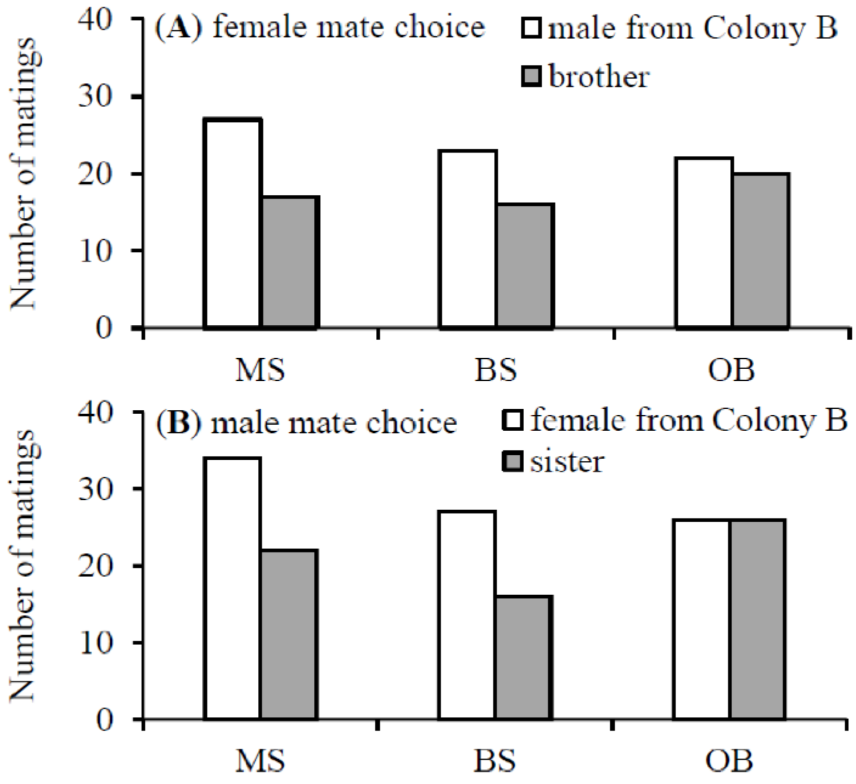

FIGURE 1. Female (A) and male (B) *Tetranychus ludeni* mate choice. MS, mother-son mating; BS, brother-sister mating; OB, outbreeding mating.

Theory predicts that individuals may avoid inbreeding when inbreeding depression is substantial but inbreeding avoidance may not occur when inbreeding depression is low or absent (Lande & Schemske 1985; Szulkin *et al.* 2013; Nichols 2017). Indeed, inbred *T. ludeni* had no significant preference between siblings and unrelated mates in mate choice (For females: MS, $\chi^2 = 2.27$, $P = 0.1317$; BS, $\chi^2 = 1.26$, $P = 0.2623$; OB, $\chi^2 = 0.10$, $P = 0.7576$, Figure 1A; for males: MS, $\chi^2 = 2.57$, $P = 0.1088$; BS, $\chi^2 = 2.81$, $P = 0.0934$; OB, $\chi^2 = 0.00$, $P = 1.0000$, Figure 1B). Furthermore, when mated with siblings or unrelated mates, *T. ludeni* had similar premating period (For females: $F_{5,119} = 1.18$, $P = 0.3229$, Figure 2A; for males, $F_{5,145} = 1.98$, $P = 0.0842$, Figure 2B) and mating duration (For females: $F_{5,119} = 0.24$, $P = 0.9429$, Figure 2C; for males, $F_{5,145} = 0.24$; $P = 0.9444$, Figure 2D). Our findings suggest that both sexes of *T. ludeni* do not avoid mating with kin at all inbreeding levels. Lack of inbreeding avoidance has also been reported in several other haplodiploid species (Bourdais & Hance 2009; de Souza *et al.* 2017; Bogo *et al.* 2018). However, *T. urticae* females prefer to mate with unrelated males (Tien *et al.* 2011) due to substantial inbreeding depression that occurs in this species (Tien *et al.* 2015).

In summary, we have found no evidence for inbreeding depression over eleven generations of sibling or mother-son mating in *T. ludeni*. However, our results do not support the prediction that parents may gain fitness through inbreeding. Due to lack of inbreeding depression neither sex of this species displays inbreeding avoidance behaviour in mate choice.

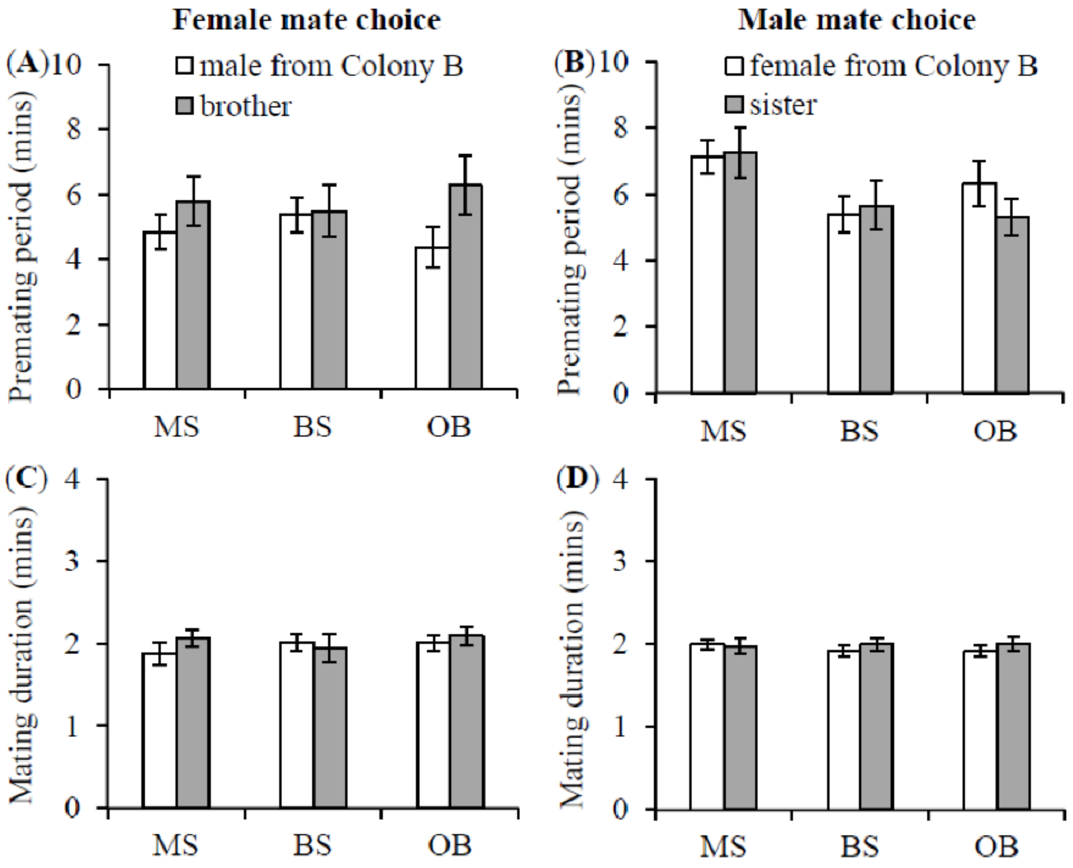

FIGURE 2. Premating period in female (A) and male (B) *Tetranychus ludeni* mate choice, and mating duration in female (C) and male (D) mate choice. MS, mother-son mating; BS, brother-sister mating; OB, outbreeding mating. Error bars are SE.

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Reproductive Strategies That May Facilitate Invasion Success: Evidence From a Spider Mite

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Abstract

With significant surge of international trade in recent decades, increasingly more arthropod species have become established outside their natural range of distribution, causing enormous damage in their novel habitats. However, whether a species can colonize its new environment depends on its ability to overcome various barriers which may result in establishment failure, such as inbreeding depression and difficulty to find mates. Here, we used a haplodiploid pest, *Tetranychus ludeni* Zacher (Acari: Tetranychidae), which is native to Europe but now cosmopolitan, to investigate whether its reproductive strategies have facilitated its invasion success, providing knowledge to develop programs for prediction and management of biological invasions. We show that inbreeding had no negative influence on female reproductive outputs and longevity over 11 successive generations, allowing mother-son and brother-sister mating to occur at the invasion front without adverse consequences in fitness. Virgin females produced maximum number of sons in their early life to ensure subsequent mother-son mating but later saved resources to prolong longevity for potential future mating. Females maximized their resource allocation to egg production immediately after mating to secure production of maximum number of both daughters and sons as early as possible. Furthermore, mated females with mating delay increased proportion of daughters in offspring produced to compensate the loss of production of daughters during their virgin life. We suggest that the lack of inbreeding depression in successive generations and the ability to adjust resource allocations depending whether and when mating occurs may be the key features that have facilitated its invasion success.

Key words: invasive pest, inbreeding depression, mating delay, resource allocation

Investigation into reproductive strategies that successful invaders may have employed to survive and colonize uncertain environment can provide knowledge for prediction and management of invasion risks. The European native haplodiploid mite, *Tetranychus ludeni* Zacher (Acari: Tetranychidae), has now invaded all continents except Antarctica (Bolland et al. 1998, CABI/EPPO 2020) and become an important pest of bean, eggplant, hibiscus, pumpkin, and other cucurbitaceous plants globally (Reddy 2001, Zhang 2003, Kaimal and Ramani 2011). To date, little is known about whether this mite has developed reproductive strategies that may have facilitated its invasion success, offering a good opportunity for the study of underlying mechanisms of biological invasions.

Biological invasion involves a series of stages such as introduction, establishment and spread, each of which has barriers that may result in invasion failure (Duncan et al. 2003, Heger and Trepl 2003, Blackburn et al. 2011). For example, in species with sexual reproduction, mates may be limited at the introduction front (Courchamp et al. 1999, South and Kenward 2001, Council 2002) where successful reproduction may be difficult (Heger and Trepl 2003, Blackburn et al. 2011), leading to population extinction. However, haplodiploid species may overcome the barrier of mate shortage at the invasion front (Baker 1955, Ward et al. 2012, Mazzolari et al. 2017), because females can produce haploid sons and then mate with their sons to produce both sons and daughters which can perform brother-sister mating thereafter (Cowan 1979, Adamson and Ludwig 1993, Peer and Taborsky 2005, McCulloch and Owen 2012, Schmidt et al. 2014). Mother-son mating could thus be potentially central to the invasion success of some haplodiploid species (Adamson and Ludwig 1993). Furthermore, mother-son and sibling mating may also reduce the cost and risk for mate search outside their natal habitat, increasing chances of successful colonization (Jordal et al. 2001).

Mother-son or sibling mating may result in inbreeding depression, reducing offspring fitness (Charlesworth and Willis 2009, Tien et al. 2015), and increasing extinction risks of small populations (Reed et al. 2003, O'Grady et al. 2006, Bozzuto et al. 2019, Nonaka et al.

2019). However, inbreeding depression does not appear to occur in some haplodiploid species after one (Peer and Taborsky 2005) or a few (Trevisan et al. 2016, Quaglietti et al. 2017) generations of inbreeding. Similarly, when we compare the reproductive fitness of the first generation with that of the 11th generation with different inbreeding levels in *T. ludeni*, we have not found any evidence of inbreeding depression (Zhou et al. 2020). Yet, it is not clear whether the lack of inbreeding depression remains consistent in successive generations, information of which is important for evaluation of whether inbred animals could have risk of population collapse in any generations.

For mother-son mating to occur in haplodiploid animals, virgin mothers would have to wait until their sons become sexually mature, by which time, even if they eventually mate with their sons, their reproductive fitness could decrease to the minimum due to mating delay, leading to extinction of the population. Therefore, females of successive haplodiploid invaders may have developed strategies to allow mother-son mating to take place with limited impact on their reproductive fitness. For example, females may reduce their reproductive rate when virgin to extend their longevity (Bonato and Gutierrez 1996, 1999) and increase resource allocations to reproduction after mating (Schmidt et al. 2014). However, it is still unclear how *T. ludeni* females adjust their reproductive strategies including resource allocation in response to mating delay, knowledge of which is vital to the understanding of invasion success in this mite and prediction of invasive potential of other haplodiploid animals.

In the present study, we carried out a series of experiments to determine whether inbreeding could increase risk of population collapse in successive generations and whether females could adjust their resource allocation in response to mating delay that facilitates mother-son mating in *T. ludeni*. First, we set up three breeding lines, namely mother-son mating, brother-sister mating and outbreeding, allowed mating to occur according to treatments for 11 successive generations, and recorded female reproductive outputs and longevity in each generation to evaluate the effect of inbreeding over generations. Because females are about 10-d old when their sons mature, all females were 10-d old when mated to make the data comparable. Second, we tested how females adjusted their resource allocation independent of inbreeding by comparing lifetime fecundity, daughter production, and longevity between females of different mating status, namely virgin, mated when 1-d old and mated when 10-d old.

Materials and Methods

Experimental Mites

We established a laboratory colony of *T. ludeni* from field-collected mites on *Passiflora mollissima* (Kunth) (Malpighiales: Passifloraceae) in Palmerston North, New Zealand, and maintained it on 3- to 5-wk-old common bean [*Phaseolus vulgaris* L. (Fabales: Fabaceae)] plants. We divided the colony into two (A and B) and kept them in two separate rooms for about eight generations before experiments. We used the first expanded leaves of *P. vulgaris* for all experiments. All colonies were maintained, and experiments carried out under the environmental conditions of 25 ± 1°C, 40 ± 10% RH, and photoperiod of 14:10 (L:D) h.

We randomly selected 40 male and 40 female deutonymphs from Colony A and individually transferred mites onto a clean leaf square (5.0 × 5.0 cm) placed on wet cotton wool in a Petri dish (9.5-cm diameter × 1.5-cm height) to ensure virginity. We then individually paired an 1-d-old female with an 1-d-old male on a clean leaf square (2.0 × 2.0 cm) to allow them to mate once and then transferred the mated female onto a new leaf square (2.0 × 2.0 cm) for oviposition for 5 d. We randomly selected three female deutonymphs that developed from the above eggs laid by each mated female for the following experiments.

Effect of Inbreeding Over Generations on Female Reproductive Fitness and Survival

To examine whether and how inbreeding affected reproductive fitness and survival over generations, we performed three treatments: 1) MSM—mothers and their sons mated for 11 successive generations, 2) BSM—brothers and sisters mated for 11 successive generations, and 3) OBM (outbreeding) —females from Colony A and males from Colony B mated for 11 successive generations. Because females in treatment MSM were about 10-d old when their sons became adults, for all three treatments we allowed females in each generation to mate when they were 10-d old. We individually transferred female deutonymphs prepared as described above to leaf squares (2.5 × 2.5 cm) and allowed virgin female adults to lay eggs for 10 d and then individually paired them with 1-d-old virgin males according to treatments until death. We replaced the leaf squares once every 5 d and randomly selected one to three female deutonymphs produced by each mated female within the first 5 d after females mated to start the next generation. We recorded the number of eggs laid before and after mating and the number and percentage of daughters produced after mating by each mated female, once every 5 d until she died. We used the following number of mated females for data recording from the first to the 11th generations, respectively: 30, 24, 18, 27, 34, 30, 22, 33, 26, 30, and 28 for MSM; 29, 20, 15, 26, 27, 31, 24, 34, 27, 32, and 27 for BSM; and 31, 25, 18, 35, 34, 37, 25, 36, 26, 31, and 29 for OBM.

Effect of Mating Status on Female Lifetime Reproductive Fitness and Survival

To determine whether mating status affected reproductive fitness and survival independent of inbreeding, we set up three treatments: 1) WMD (with mating delay)—virgin females from Colony A were allowed to lay eggs for 10 d and then mate with 1-d-old virgin males from Colony B, 2) NMD (without mating delay)—1-d-old virgin females from Colony A were allowed to mate with 1-d-old virgin males from Colony B, and 3) VF (virgin females)—virgin females from Colony A were allowed to lay eggs for lifetime without mating. We recorded lifetime number of eggs and daughters and the percentage of daughters after mating every 5 d until females died as described above for one generation. For fair comparisons, data from females that lived over 10 d were used for analysis. We used 31, 26, and 21 females for WMD, NMD, and VF, respectively.

Statistical Analysis

We used a logistic linear model to determine the effect of inbreeding on female reproductive fitness and longevity over 11 generations in three treatments, mother-son mating, brother-sister mating, and outbreeding: $y = \exp(a + bx)$, where y is the number of eggs and daughters produced, percentage of daughters, or female longevity, x is generation, and a and b are constant parameters of the model. We used a Negative Binomial distribution with a log link function for count data (number of eggs and daughters, and longevity), and a Gamma distribution with a log link function for percentage of daughters (GLIMMIX Procedure).

We applied a simple linear regression model to determine the egg-laying patterns over lifetime in virgin females, females with mating delay and females without mating delay (REG Procedure):

$y = a + bx$, where y is the number of eggs, x is female age (days), and a is a constant and b the slope of regression. Because we recorded the number of eggs laid once every 5 d, virgin females and females with mating delay had the same status in the first 10 d of life and mating had a major impact on egg laying, we used three regression lines to fit each treatment when $x \leq 10$, = 10–15, and ≥ 15 d, respectively, and compared oviposition patterns between treatments. If 95% confidence limits (CLs) of slopes overlap, then there is no significant difference (Julious 2004). We compared the total number of eggs laid and female survival in all three treatments and the number and proportion of daughters produced by females with mating delay and without mating delay. Data on the total number and percentage of daughters were normally distributed (Shapiro–Wilk test, UNIVARIATE Procedure) and analyzed by an analysis of variance (ANOVA, GLM Procedure). The total number of eggs laid in all three treatments were not normally distributed and analyzed using nonparametric ANOVA (GLM Procedure). Female survival was analyzed using a Lifetest (LIFETEST Procedure). We performed all analyses using SAS software (SAS 9.4, SAS Institute Inc., Cary, NC).

Results

Effect of Inbreeding Over Generations on Female Reproductive Fitness and Survival

Our results indicate that inbreeding had little effect on reproductive output and female longevity of each of 11 successive generations (eggs: $F_{1,300}$ = 1.60, P = 0.2063 for MSM; $F_{1,290}$ = 0.04, P = 0.8494 for BSM; $F_{1,325}$ = 0.02, P = 0.8975 for OBM; daughters: $F_{1,300}$ = 1.15, P = 0.2844 for MSM; $F_{1,290}$ = 0.03, P = 0.8581 for BSM; $F_{1,325}$ = 0.25, P = 0.6209 for OBM; percentage of daughters: $F_{1,300}$ = 0.51, P = 0.4767 for MSM; $F_{1,290}$ = 3.79, P = 0.0524 for BSM; $F_{1,322}$ = 0.03, P = 0.8550 for OBM; longevity: $F_{1,286}$ = 2.16, P = 0.1430 for MSM; $F_{1,284}$ = 3.12, P = 0.0786 for BSM; $F_{1,315}$ = 0.09, P = 0.7664 for OBM; Fig. 1). There was no significant difference in regression slopes between treatments for all parameters recorded (overlapping 95% CLs).

Effect of Mating Status on Female Lifetime Reproductive Fitness and Survival

Lifetime oviposition patterns are shown in Fig. 2. Within the first 10 d of female life, the number of eggs laid by WMD and VF significantly decreased over time ($F_{1,60}$ = 56.21, P < 0.0001 for WMD; $F_{1,40}$ = 59.56, P < 0.0001 for VF) with similar decline rate (overlapping 95% CLs) but that by NMD significantly increased during the same period ($F_{1,50}$ = 22.67, P < 0.0001). When female age = 10–15 d, the number of eggs laid significantly increased in WMD ($F_{1,60}$ = 98.60, P < 0.0001), significantly decreased in NMD ($F_{1,45}$ = 40.80, P < 0.0001) and remained similar in VF ($F_{1,40}$ = 0.77, P = 0.3859). Although oviposition significantly increased after mating in both WMD and NMD, it increased significantly faster in WMD than in NMD (nonoverlapping 95% CLs). After female aged ≥15 d, the number of eggs laid decreased over time in all treatments ($F_{1,58}$ = 43.15, P < 0.0001 for WMD; $F_{1,33}$ = 1.64, P = 0.2092 for NMD; $F_{1,66}$ = 17.52, P < 0.0001 for VF), with the decrease in WMD being significantly faster than in NMD and VF (nonoverlapping 95% CLs).

Virgin females (VF) laid significantly fewer eggs than mated females (WMD and NMD) in their lifetime, and WMD females laid significantly fewer eggs than NMD ones ($F_{2,75}$ = 18.50, P < 0.0001; Fig. 3A). In mated treatments, WMD females produced significantly fewer number of daughters ($F_{1,55}$ = 7.57, P = 0.0080; Fig. 3B)

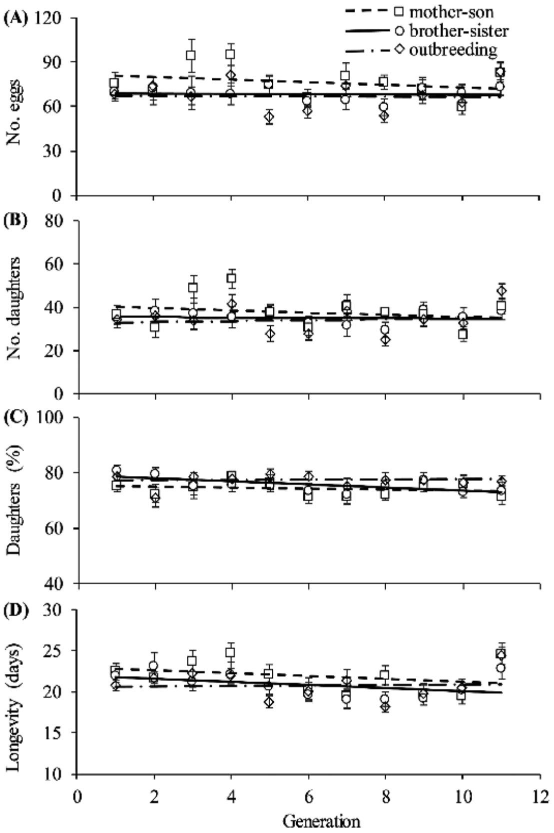

Fig. 1. Number of eggs (A), number of daughters (B), percentage of female offspring after mating (C), and female longevity (D) over 11 generations in mother-son, brother-sister, and outbreeding treatments in *Tetranychus ludeni*.

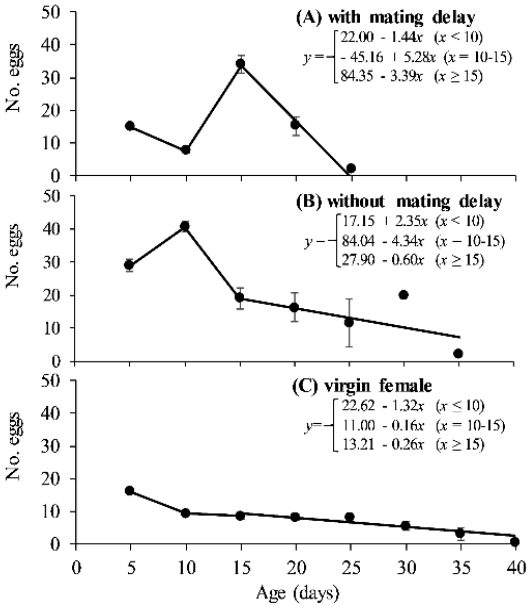

Fig. 2. Lifetime oviposition patterns in *Tetranychus ludeni* females of different mating status: with mating delay (A), without mating delay (B), and virgin females (C).

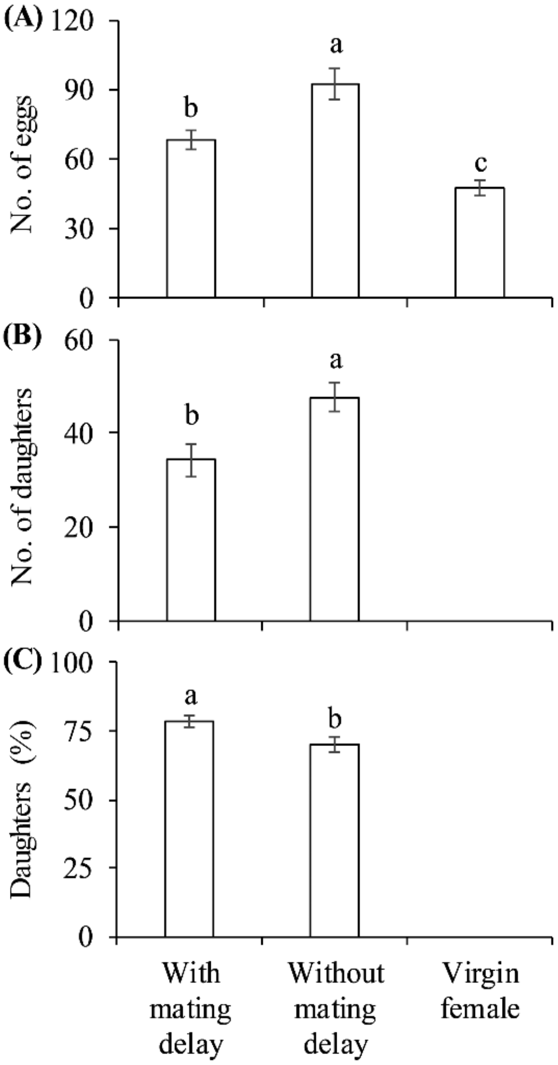

Fig. 3. Lifetime number of eggs (A) and daughters (B) and percentage of daughters after mating (C) of *Tetranychus ludeni* females with different mating status. Treatments with different letters are significantly different ($P < 0.05$).

but significantly higher proportion of daughters ($F_{1,55}$ = 7.15, P = 0.0098; Fig. 3C) in their after-mating life than NMD ones. The survival probability of females in different treatments was significantly different, with an order of VF > WMD > NMD ($\chi_2^2 = 22.02$, $P < 0.0001$; Fig. 4).

Discussion

Inbreeding depression may reduce offspring fitness (Charlesworth and Willis 2009, Tien et al. 2015), leading to extinction of small populations (Reed et al. 2003, O'Grady et al. 2006, Bozzuto et al. 2019, Nonaka et al. 2019). Previous studies show that the inbreeding depression can occur in any generation of inbred animals, which could cause invasion failure. For example, in the haplodiploid *Stigmaeopsis miscanthi* (Saito) (Acari: Tetranychidae) (Mori et al. 2005) and *Phytoseiulus persimilis* (Athias-Henriot) (Acari: Phytoseiidae) (Çekin and Schausberger 2019), inbreeding depression does not occur in the first few generations but takes place in later generations whereas in *T. urticae* Koch (Acari: Tetranychidae), it is only detected in the first few generations (Tien et al. 2015). In the present study, we found no evidence for inbreeding depression in any of the 11 inbred generations in *T. ludeni* (Fig. 1), suggesting that inbreeding has no negative impact on its invasion success at any points or generations. We attribute this phenomenon to the notion that the expression of deleterious recessive alleles from inbreeding may be selected out in small populations of some haplodiploid animals (e.g., Quaglietti et al. 2017, Eyer et al. 2018). The mechanism allows them to undertake mother-son and brother-sister mating at the invasion front without adverse consequences in fitness (Adamson and Ludwig 1993, Kronauer et al. 2012, McCulloch and Owen 2012, Schmidt et al. 2014, Lantschner et al. 2020, Queffelec et al. 2020).

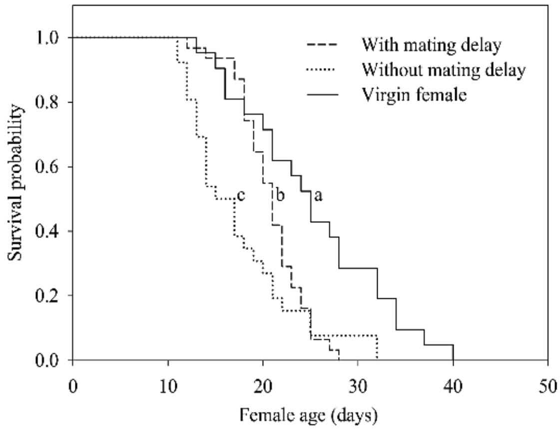

Fig. 4. Survival probability of *Tetranychus ludeni* females of different mating status. Lines with different letters are significantly different ($P < 0.05$).

Our results demonstrate that *T. ludeni* had developed strategies to adjust resource allocations in response to uncertain environment. For example, virgin females produced highest number of sons in their early life (Fig. 2A and C) to secure subsequent mother-son mating. This feature can ensure continuous population growth in uncertain situations, such as no males available at the invasion front. However, if there were still no males to mate after this reproductive episode, virgin females saved resources by reducing egg production (Fig. 2A and C) to prolong longevity (Fig. 4) for potential future mating. Some other spider mites, such as *T. marianae* McGregor (Acari: Tetranychidae), *Mononychellus progresivus* Doreste (Acari: Tetranychidae) (Bonato and Gutierrez 1996, 1999), and *T. urticae* (Li and Zhang 2020), appear to share similar reproductive strategies with *T. ludeni*, that may contribute to their invasion success.

Immediately after receiving ejaculates, females sharply increased their resource investment in egg production regardless of whether mating delay occurred (Fig. 2A and B) to ensure production of maximum number of both daughters and sons as early as possible. This resource allocation strategy may reduce risks of potential reproductive failure in a new and unstable environment (Stearns 1992, Stearns et al. 2000), facilitating establishment of a newly invaded population (Dangremond and Feller 2016, Fetters and McGlothlin 2017, James et al. 2017). The very reproductive strategy also occurs in haplodiploid mite *Schizotetranychus celarius* (Banks) (Acari: Tetranychidae) (Saitō 1987) and ant *Cardiocondyla argyrotricha* (Hymenoptera: Formicidae) (Schmidt et al. 2014). Our results show that both after-mating increase and after-peak decline of egg laying were faster in females with mating delay than in those without mating delay (Fig. 2A and B). This suggests that *T. ludeni* females can perform a clear resource allocation trade-off (Waelti and Reyer 2007, Billman and Belk 2014), i.e., higher resource investment in eggs by delay-mated females as compared to undelay-mated ones results in faster decrease of resource available for future egg production. The ability to quickly adjust resource allocation depending on when mating occurs may provide more flexibility for the species to establish in a new environment.

Although virgin females laid fewer eggs in their lifetime than mated ones (Fig. 3A), their sons are larger which produce more daughters after mating than those of mated ones (Zhou et al. 2018). This life history strategy should also contribute to future population growth and invasion success (Wiernasz et al. 2001, De Jesus and Reiskind 2016, Zhou et al. 2018). Our results demonstrate that females with mating delay produced fewer number of eggs and daughters as compared to those without mating delay. However, the reduction was less than 25% (Fig. 3A and B), which may not be enough to lead to establishment failure. Furthermore, females with mating delay produced higher proportion of daughters than those without mating delay (Fig. 3C), which may help compensate the loss of production of daughters in their earlier life and catch up population growth.

In the present study, we show that inbreeding has no negative influence on female reproductive outputs and longevity throughout 11 inbred generations, which allows mother-son and brother-sister mating to occur at the invasion front without adverse consequences in fitness. Virgin females lay maximum number of sons in their early life to ensure subsequent mother-son mating but later save resources to prolong longevity for potential future mating. Females maximize their resource allocation to egg production immediately after mating to secure production of maximum number of both daughters and sons as early as possible, reducing risks of potential reproductive failure in a new environment. Finally, mated females with mating delay quickly increase proportion of daughters in offspring produced to compensate the loss of production of daughters during their virgin life. These reproductive strategies of *T. ludeni* coupled with its adaptation to wide ranges of hosts (Gotoh et al. 2015) and temperatures (Gotoh et al. 2015, Ristyadi et al. 2019) may have facilitated its invasion success. Our findings may be also useful for prediction of invasive potential of other haplodiploid pests.

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Article

Resource relocations in relation to dispersal in *Tetranychus ludeni* Zacher

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Abstract

Life history strategies of a species may affect its invasion success. Here, we used a haplodiploid pest, *Tetranychus ludeni* Zacher (Acari: Tetranychidae), which is native to Europe but now cosmopolitan, to investigate its life history strategies in relation to its dispersal tendency and distance. We tested whether and how dispersal influenced survival, lifetime fecundity, age-specific reproduction, and life table parameters of mated females. We show that mated females did not trade off their survival and lifetime reproductive output with dispersal but adjusted their resource allocations during different reproductive stages depending on whether and how far they dispersed. Long-distance dispersers invested more in dispersal in their early life while resident mites and short-distance dispersers invested more in reproduction during their early life. These resource allocation strategies may allow long-distance dispersers to explore the novel environment more effectively without compromising lifetime reproductive fitness, contributing to the invasion success of *T. ludeni*.

Key words: dispersers, fecundity, longevity, dispersal distance, resource allocation, spider mite

Introduction

Biological invasions have become more frequent in the recent decades due to the increase of international trade and travel, causing significant economic losses worldwide. Following introduction of exotic species to a new habitat by man, their dispersal plays a crucial role in population expansion (Clobert *et al.* 2009). Because dispersers' life history traits such as fecundity and survival can affect their population establishment at the invasion front and subsequent expansion (Duckworth & Badyaev 2007; Renault *et al.* 2018; Williams *et al.* 2019), understanding the reproductive performance of dispersers can provide knowledge for prediction and management of invasion risks.

Compared with residents, dispersers often have lower fecundity and higher mortality (Mole & Zera 1993; Bonte *et al.* 2012; Khuhro *et al.* 2014; Renault 2020; Nasu & Tokuda 2021), leading to slower population growth (Baguette & Schtickzelle 2006; Keenan & Cornell 2021). In addition, the cost of dispersal may increase with the distance a species moves, resulting in distance-specific resource trade-offs (Rousset & Gandon 2002; Baker & Rao 2004; Samietz & Köhler 2012; Serrano & Tella 2012), the magnitude of which can affect expansion speed. For example, dispersers in some species may allocate less resource to reproduction at their early life to facilitate dispersal (Roff 1984; Roff & Fairbairn 1991; Tanaka 1993; Innocent *et al.* 2010). Therefore, examining age-specific reproduction helps better understand the cost of dispersal and resource allocation strategies of dispersers.

Tetranychus ludeni Zacher (Acari: Tetranychidae) is a spider mite from Europe but has now invaded all continents except Antarctica (Bolland *et al.* 1998; CABI 2020), providing an opportunity to study the underlying mechanisms of its invasion success. It attacks over 300 plant species, including economically important crops such as bean, papaya, guava, eggplant, pumpkin, and apple (Bolland *et al.* 1998; Zhang 2003; Migeon *et al.* 2010). In spider mites, females usually disperse after mating (Mitchell 1973), especially when the population is crowded or when food is insufficient or poor in quality (Mcenroe 1969; Bitume *et al.* 2013). Although its flexible reproductive strategies (Zhou *et al.* 2018, 2021) and good adaptation to external environment (Zhang 2003; Gotoh *et al.* 2015; Ristyadi *et al.* 2019, 2021) may have helped *T. ludeni* establish in novel habitats, it is still unknown whether dispersers adjust their reproductive strategies to facilitate invasion success.

In the present study, we examined whether dispersal tendency and distance affected reproductive output, survival, age-specific reproduction, and life table parameters in *T. ludeni*. We allowed mites to disperse along a dispersal system, sampled residents and dispersers, followed up their daily reproduction and survival, and calculated their life table parameters. We hypothesized that compared with residents, dispersers should have lower lifetime fecundity, shorter longevity, and slower population growth.

Materials and Methods

Mite colony and experimental conditions

We collected *T. ludeni* adults on *Passiflora mollissima* Kunth (Malpighiales: Passifloraceae) in Palmerston North, New Zealand, and started and maintained the colony using 3- to 5-week-old common bean plants *Phaseolus vulgaris* L. (Fabales: Fabaceae) grown in pots. We used the first expanded leaves cut from 1- to 2-week-old plants grown in pots for all experiments. The colony was kept, and experiments carried out under environmental conditions of 25 ± 1 ºC, 40 ± 10% RH and 14:10 h (light:dark) photoperiod.

Preparation of mated females for experiments

To prepare male adults for mating, we transferred 50 female deutonymphs randomly selected from the colony onto a clean fresh leaf square (4 × 4 cm) on wet cotton wool in a Petri dish (9 cm in diameter × 1 cm in height) and allowed them to develop to virgin adult females and lay unfertilised eggs for three days. We then removed those adult females and allowed the eggs to develop to virgin male adults. We prepared three such dishes. We used 1- to 3-day-old virgin adult males to mate with females prepared below.

We randomly selected 50 adult females from the colony and transferred them onto a clean fresh leaf square (4 × 4 cm) on wet cotton wool in a Petri dish for egg laying for 24 hours. We then removed the female adults and allowed eggs to develop to the quiescent deutonymphal stage. We set up a total of 10 such dishes. We introduced 15–20 virgin males prepared above to female deutonymphs in each dish and allowed them to stay with the newly emerged females for 5 h, ensuring all females mated at or soon after emergence. We transferred 50 newly emerged (< 24 hours old), mated females from the above dishes onto a new leaf square (4 × 4 cm) in a dish. We prepared a total of 10 such leaf squares. We used 3-d-old, mated females for experiments because females of this age were most likely to disperse (unpublished data).

Experimental setup and data collection

We set up a dispersal system consisting of an array of 21 leaf squares (2 cm × 2 cm for the first leaf square and 2 cm × 1 cm for the rest) (Figure 1) placed on wet cotton wool in a tray (45 cm in length × 36 cm in width × 1.5 cm in height). To facilitate analysis of mite dispersal distance and subsequent reproductive fitness, we divided the array into three sections for data collection: (1)

resident leaf square (R)—the first leaf square where we released the mites; (2) median distance of dispersal (M)—leaf squares 2–11, and (3) long distance of dispersal (L)—leaf squares 12–21.

We transferred 50 3-d-old, mated females onto R leaf square and allowed them to settle for 40 minutes, after which time, all leaf squares were connected by Parafilm bridges (4.5 cm in length × 1.5 cm in width; Parafilm®, USA). Twenty-four hours after bridge connection, we sampled mites on R, M, and L leaf squares. We repeated the above procedure eight times. We sampled a total of 15, 20, and 13 mites from sections R, M, and L, respectively, for measurement of reproductive fitness in relation to dispersal distance: 15 mites from the 1st leaf square for section R; 1, 2, 3, 5, 6, 1 and 2 mites from the 2nd, 4th, 6th, 7th, 8th, 9th and 11th leaf squares, respectively, for section M, and 1, 2, 1, 5, 2, 1, and 1 mites from the 12th 13th, 15th, 16th, 17th, 19th and 21st leaf squares, respectively, for section L. We individually transferred sampled mites onto new leaf squares (1 × 1 cm) placed on wet cotton wool in Petri dishes, replaced leaf squares once every 24 hours until the mites died, and recorded their longevity. We counted the number of eggs laid on each leaf square, allowed them to develop to adult stage on the same leaf squares and recorded the number and sex of newly emerged adults.

Statistical analysis

We analyzed all data using SAS software (SAS 9.4, SAS Institute Inc., Cary, NC). To estimate how dispersal affected population growth, we calculated the life table parameters (Jervis *et al.* 2005) using the data on daily survival and daughter production of each female. The intrinsic rate of natural increase (r) was calculated by solving the Lotka-Euler equation: $\sum e^{-rx} l_x m_x = 1$, where x is the pivotal age, l_x is the proportion of females surviving to age x, and m_x is the number of daughters produced per female at age x. We also calculated the net reproductive rate ($R_0 = \sum l_x m_x$, daughters/female/generation), doubling time [$D_t = \log_e(2)/r$, days], and generation time [$T = \log_e(R_0)/r$, days]. We used the bootstrap method (Huang & Chi 2012; Yu *et al.* 2013) with 50,000 bootstrap samples to calculate the pseudo-values of a given parameter and employed the paired-bootstrap test (Efron & Tibshirani 1993; Mou *et al.* 2015; Reddy & Chi 2015) for multiple comparisons between any two dispersal distances (TTEST Procedure). The significance was determined according to the 95% t-based confidence limits (95% CLs), i.e., if 95% CLs > 0 or < 0, the mean difference between the two dispersal distances is significantly different ($P < 0.05$).

Data distribution was tested using Shapiro-Wilk test (UNIVARIATE Procedure). Data on female longevity were normally distributed and analyzed using ANOVA with Tukey test for multiple comparison (GLM procedure). Data on the number of eggs laid were not normally distributed and thus analyzed with non-parametric ANOVA followed by Bonferroni test for multiple comparison (GLM procedure).

We modified a Gaussian functional model (Archontoulis & Miguez 2015) to fit the daily number of eggs and daughters with two reproductive peaks: $y = p_1 * \exp\{-0.5*[(x - x_1)/w_1]^2\} + p_2 * \exp\{-0.5*[(x - x_2)/w_2]^2\}$, where p_1 and p_2 are the maximum y value of the first and second peaks, respectively; w_1 and w_2 are coefficients controlling the width of the first and second peaks, respectively; and x_1 (= 4) and x_2 are the age of mites at first and second peaks, respectively. We compared reproductive parameters of mites sampled in different sections (R, M, and L) and p_1 and p_2 within each section according to Julious (2004): if the 83.4% CLs do not overlap, then there is a significant difference.

Results

Resident (R) and dispersed females (M and L) laid similar number of eggs in their lifetime ($F_{2,45} = 1.06$, $P = 0.3566$) and had similar longevity ($F_{2,45} = 1.68$, $P = 0.1987$) (Figure 2). However, life table parameters changed with the distance females dispersed (Table 1): (1) the longer the dispersal distances were, the lower net reproductive rate (R_0) and intrinsic rate of increase (r) occurred, and (2) the opposite was the case for the generation time (T) and doubling time (D_t).

TABLE 1. The estimated mean (± SE) life table parameters at different dispersal distances in *T. ludeni*.

Distance	R_0	r	T	D_t
R	18.31 ± 0.30 a	0.1789 ± 0.0010 a	16.25 ± 0.06 c	3.88 ± 0.02 c
M	17.35 ± 0.18 b	0.1628 ± 0.0010 b	17.16 ± 0.08 b	4.17 ± 0.03 b
L	13.16 ± 0.36 c	0.1453 ± 0.0017 c	17.71 ± 0.09 a	4.78 ± 0.06 a
CLs $_{\text{(R vs M)}}$	0.2701~1.6599	0.0097~0.0154	-1.1138~-0.7141	-0.3623~-0.2264
CLs $_{\text{(R vs L)}}$	4.2272~6.0751	0.0295~0.0375	-1.6854~-1.2398	-1.0271~-0.7751
CLs $_{\text{(M vs L)}}$	3.4022~4.9701	0.0170~0.0249	-0.7897~-0.3076	-0.7336~-0.4799

Estimated values in columns followed by different letters are significantly different ($P < 0.05$). For each parameter, 95% CLs > 0 or < 0 indicates a significant difference between dispersal distances ($P < 0.05$).

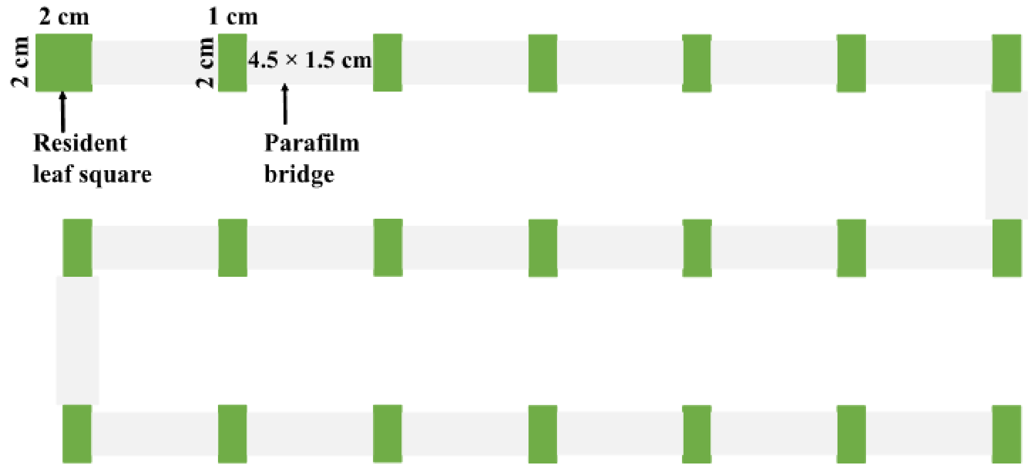

FIGURE 1. Dispersal system for experiments. The resident leaf square was used to introduce experimental mites. Parafilm bridges were placed on the top of leaf squares with approximately 1 mm overlapping to reduce the influence of water between leaf squares and bridges on mite dispersal.

Daily number of eggs and daughters produced had two peaks of similar shape regardless of dispersal distance, with one occurring immediately after their transfer to new leaf squares and the other taking place 5–7 days after their transfer (Figure 3). The first peak of daily fecundity and daughter production was significantly lower in L females than in R and M females (non-overlapping 83.4% CLs). The first peak was significantly higher than the second one in R and M females (non-overlapping 83.4% CLs) but the two peaks had similar height in L females (overlapping 83.4% CLs) (Figure 3).

Discussion

Theoretically, dispersal has energetic and survival costs (Bonte *et al.* 2012; Travis *et al.* 2012; Weigang & Kisdi 2015), often resulting in lower fecundity and higher mortality. However, our current study shows that females had similar lifetime fecundity and longevity regardless of whether and how far they dispersed (Figure 2). It is thus suggested that mated females may not trade off their lifetime fecundity and survival with dispersal in *T. ludeni*. There is also no evidence for trade-off between dispersal and fecundity in a damselfly (Therry *et al.* 2015). We argue that the lack of such trade-offs may contribute to invasion success observed in *T. ludeni* (Zhou *et al.* 2021).

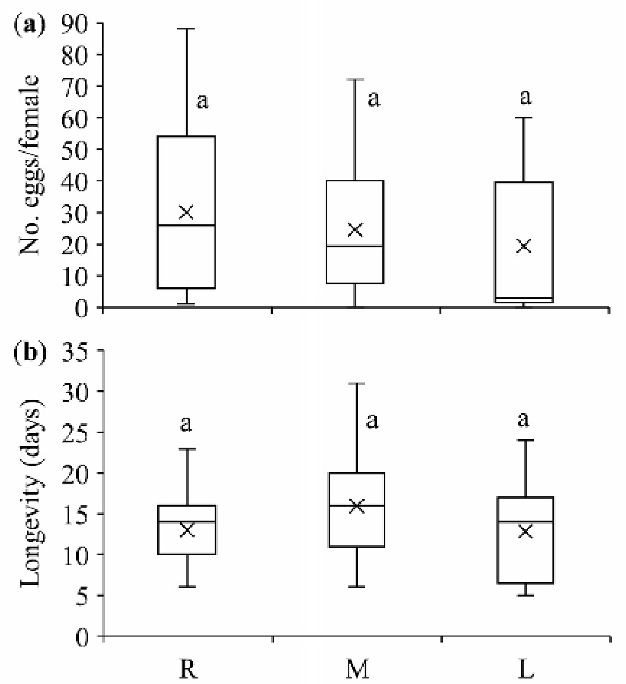

FIGURE 2. The total mean number of eggs laid per female (**a**) and longevity (**b**) of resident (R) and dispersed females (M and L) in *T. ludeni*. Each box plot shows the mean (×), median line, the upper and lower quartiles (the range where 25% of scores fall above and 25% fall below the median), and maximum '⊤' and minimum '⊥' scores. Boxes with the same letters are not significantly different ($P > 0.05$).

Our results indicate that dispersal tendency and distance had significant effects on life table parameters (Table 1). For example, dispersal significantly reduced the net reproductive rate (R_0) and intrinsic rate of increase (r) and increased the generation time (T) and doubling time (D_t). These findings suggest that the mites adjust their resource allocation to reproduction and movement depending on whether and how far they intend to disperse. In another word, although dispersed and resident mites had similar lifetime fecundity, dispersed mites spread their resources for reproduction in a wider range, prolonging generation time and population doubling time (Weerawansha *et al.* 2020). Similar phenomenon has also been reported in butterflies *Proclossiana eunomia* Esper, *Boloria aquilonaris* Stichel, *Euphydryas editha bayensis* Sternitsky, *E. aurinia* Rottemburg, and *Lopinga achine* Scopoli (Lepidoptera: Nymphalidae) (Baguette & Schtickzelle 2006).

As shown in Figure 3, mated *T. ludeni* females had two reproductive episodes in terms of daily number of eggs and daughters produced regardless of dispersal distance. However, females that dispersed a long distance (L) laid fewer eggs and produced fewer daughters than those that did not disperse (R) or only dispersed a median distance (M) during the first episode. These findings strongly suggest that L females allocate more resources for dispersal and reduce investment in reproduction in their early life (Zera & Denno 1997; Bonte *et al.* 2012). This kind of resource relocation has also been reported in other invertebrates (Roff 1984; Roff & Fairbairn 1991; Tanaka 1993). For example, in a haplodiploid insect *Melittobia australica* Girault (Hymenoptera: Eulophidae), dispersers have fewer number of eggs developed at emergence and produce fewer eggs in their first clutch than residents (Innocent *et al.* 2010).

When we compared the two reproductive peaks within mites that dispersed similar distances, we found that the second peaks in R and M females were significantly lower than the first ones while the two peaks had similar height in L females (Figure 3). Our results further suggest that in their early life R and M females invest more resources in reproduction than in dispersal while L females allocate more resources to dispersal than to reproduction. This strategy may allow L females to explore the novel environment more effectively and help invasion success (Sol *et al.* 2012; Sol & Maspons 2016) without compromising lifetime reproductive fitness.

In summary, we show that mated *T. ludeni* females do not trade off dispersal with their lifetime reproductive output and survival. Rather, they adjust their resource allocation depending on whether and how far they disperse, with long-distance dispersers investing more in dispersal in their early life while resident mites and short-distance dispersers investing more in reproduction in their early life. These strategies may facilitate invasion success of *T. ludeni*.

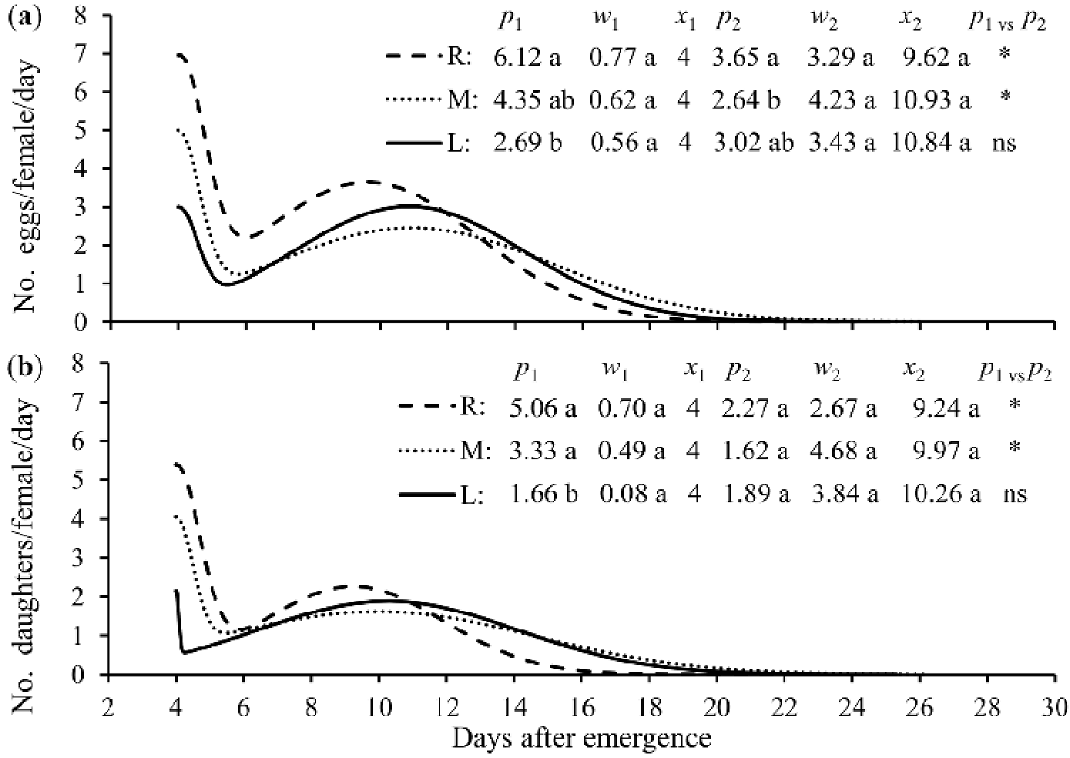

FIGURE 3. The estimated patterns of daily number of eggs (**a**) and daughters (**b**) produced by resident (R) and dispersed females (M and L) of *T. ludeni*. Raw data were fitted to the two-peak model for each parameter. Parameters with the same letters in column are not significantly different (overlapping 83.4% CLs, $P > 0.05$) between R, M, and L. 'p_1 $_{vs}$ p_2' is the comparison in peak height between the first and second peaks within each line, where * indicates significantly different and 'ns' not significantly different.

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Appendix 2: A List of Other Published Papers I Co-authored during My PhD Studies at Massey University

Chen, C., He, X.Z., Zhou, P. & Wang, Q. (2022) Diets for *Tamarixia triozae* adults before releasing in augmentative biological control. *BioControl*. https://doi.org/10.1007/s10526-022-10136-8

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Appendix 3: Statement of Contribution

MASSEY UNIVERSITY
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STATEMENT OF CONTRIBUTION
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Student name:	Peng Zhou
Name/title of main supervisor:	Professor Qiao Wang
In which chapter is the manuscript/published work?	Chapter 2

Please select one of the following three options:

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Zhou, P., He, X.Z. & Wang, Q. (2018) Sons from virgin mothers produce more daughters in a haplodiploid mite. Systematic and Applied Acarology, 23, 1869–1878.

○ The manuscript is currently under review for publication – please indicate:

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Please select one of the following three options:

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- Please provide the full reference of the research output:

Zhou, P., He, X.Z., Chen, C. & Wang, Q. (2020) No evidence for inbreeding depression and inbreeding avoidance in a haplodiploid mite Tetranychus ludeni Zacher. Systematic and Applied Acarology, 25, 1723–1728.

○ The manuscript is currently under review for publication – please indicate:

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In which chapter is the manuscript/published work?	Chapter 5, section 5.1

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- Please provide the full reference of the research output:

Zhou, P., He, X.Z., Chen, C. & Wang, Q. (2021) Resource relocations in relation to dispersal in Tetranychus ludeni Zacher. Systematic and Applied Acarology, 26, 2018–2026.

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- The name of the journal:
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