

Interactions between Spider Mites and Predators in Systems with Dispersal Opportunities

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Abstract

The fitness of animals is largely affected by dispersal between habitat patches. Therefore, some important predator-prey interactions are detectable only in systems where prey can disperse between multiple patches, although most conventional studies on interactions between spider mites and their predators have observed them in closed systems without dispersal opportunities. By using connected patch systems, I studied predator avoidance of the spider mite *Tetranychus kanzawai*.

I examined lethal and non-lethal effects of predators on between-patch dispersal of spider mite females. A generalist ant *Pristomyrmex punctatus* effectively prevented dispersal of spider mite females by directly consuming dispersing individuals, and a generalist predatory mite *Euseius sojaensis* prevented dispersal of females by making them hesitate to disperse. In contrast, a specialist predatory mite *Neoseiulus womersleyi* allowed spider mite females to escape, promoting distant dispersal and increasing the number of colonized patches of spider mites within the system.

I then examined the within-patch oviposition site shifts onto webs by spider mites in response to predation risks. Although the benefits of the shifts have been addressed in previous studies using closed systems, the benefits would have been underestimated because predators cannot disperse from prey patches in the closed systems. By using connected patch systems, I showed that specialist predatory mite *N. womersleyi* left prey patches with more eggs unpredated when higher proportions of prey eggs were located on webs, and egg survival on webs was much higher than on leaf surfaces.

The above results imply that the stealthiness of predatory mites stalking spider mite females should largely determine the success of spider mite control. The stealthiness indices (the proportions of dispersed mites in response to predator intrusion, and the proportion of prey eggs laid on webs in response to predator eggs) of three predatory mites (*Phytoseiulus persimilis*, *Neoseiulus californicus*, and *N. womersleyi*) against spider mites (*Tetranychus urticae* and *T. kanzawai*) was measured. Both indices resulted in the same ranking across prey species and prey populations, with *P. persimilis* ranking highest and *N. womersleyi* lowest, suggesting that the indices could be used for evaluating predatory mites as biological control agents.

This study will contribute to the efficient use of biological control agents for successful sustainable management of spider mites. The results suggest that the generalist predators which prevent spider mite dispersal serve as important biological control agents under low spider mite densities, while specialist predators with high stealthiness are effective mainly under high spider mite densities.

Chapter 1.

General Introduction

Dispersal ecology aims to elucidate how ecological factors affect the dispersal processes of organisms. Dispersal consists of three distinct phases: departure, transfer, and settlement (Clobert et al. 2009). Actively dispersing individuals make decisions on whether to stay in or depart their natal habitat patches based on dispersal costs and benefits; once they have reached a potential patch, they must decide whether to settle or to continue searching for more suitable patches (Bonte et al. 2012). The primary benefit of dispersal is leaving a patch with relatively lower fitness expectations due to overcrowding (resource deterioration) and kin competition (Hamilton & May 1977), and predation risk (Lima & Dill 1990).

Prey individuals can reduce predation risk through habitat selection and dispersal decisions (Lima & Dill 1990). For example, prey avoids habitats with predator presence and/or predator cues (Chesson 1984; Resetarits & Wilbur 1989; Spieler & Linsenmair 1997; Kats & Dill 1998; Magalhães et al. 2002; Kiflawi et al. 2003; Nomikou et al. 2003; Blaustein et al. 2004). Moreover, predation risk within prey habitats promotes the departure of prey from the intruded habitats (Bernstein 1984; Sih et al. 1992; Peckarsky 1996; Hakkarainen et al. 2001; Cronin et al. 2004; McCauley & Rowe 2010). However, prey individuals dispersing through hostile environments may also suffer predation risk (Young & Lockley 1988; Bonnet et al. 1999; Hiddink et al. 2002). Prey killed or disturbed by predators during dispersal would lose the opportunity to establish new local populations. Because metapopulation persistence depends on local population dynamics (Levins 1969), the effects of predators either to promote or to depress prey dispersal should determine the metapopulation dynamics as well as the survival of prey individuals.

Spider mites are tiny plant-infesting organisms that use patchy resources (Kennedy & Smitley 1985). Because they have a worldwide distribution and broad host range including crops, vegetables, and ornamental plants, spider mites have been major agricultural pests (Jeppson et al. 1975; Helle & Sabelis 1985; Johnson & Lyon 1988; Zhang 2003). Spider mites in the genus *Tetranychus* (Acari: Tetranychidae) produce silk threads and construct complicated protective webs on leaf surfaces and usually feed and oviposit on the leaf surface under these webs (Saito 1983). Hatched larvae feed and grow into adults, passing through two nymphal active stages and three quiescent stages, under

their mothers' webs (Saito 1983). In response to overcrowding and leaf deterioration, mated females disperse from their natal patches, mainly by walking (Brandenburg & Kennedy 1982; Kennedy & Smitley 1985; Margolies & Kennedy 1985), although they also passively disperse by wind (Margolies & Kennedy 1985; Smitley & Kennedy 1985). Because spider mites are haplodiploid species that suffer less inbreeding depression compared with diploid species (Hedrick & Parker 1997), even a single foundress can establish a new local population. Therefore, dispersal of spider mite females practically determines their population structure in agroecosystems.

Biological control is one of the main methods to sustainably suppress spider mites (Attia et al. 2013). Predatory mites (Acari: Phytoseiidae) are promising biological control agents against spider mites in both conservation and augmentative biological control strategies (e.g. McMurtry 1982, 1992). Although tetranychid mites can avoid many potential predators by staying in their protective webs (Ozawa & Yano 2009; Yano 2012; Otsuki & Yano 2014), predatory mite species that are specialized on spider mites can intrude into the webs (hereafter "specialist predatory mites") (McMurtry et al. 1970; Sabelis & Bakker 1992; McMurtry & Croft 1997; McMurtry et al. 2013), and some of the specialist predatory mite species have been commercialized for spider mite control. Considering the high ability of spider mites to establish new local populations, understanding the effects of predators on spider mite dispersal is crucially important for the successful control of spider mites.

Nevertheless, most previous conclusions concerning the ability of predators to suppress spider mites seem to have been drawn from observations of their interactions on a single patch or on heavily infested host plants, i.e. systems where spider mites could hardly disperse toward intact patches. Although spider mites that are aware of specialist predatory mites try to avoid predation by taking refuges on their webs (Oku et al. 2003b, 2004; Oku & Yano 2007), specialist predatory mites eventually consume all of the spider mites in these systems (e.g. Chant 1961; Hamamura 1986). Such consequences lead to the concepts that specialist predatory mites are effective biological control agents, whereas numerous other predators that cannot penetrate the protective webs of spider mites (hereafter "generalist predators") (Osakabe 1988; McMurtry & Croft 1997; Ozawa & Yano 2009) are ineffective.

I question the above well-accepted concepts for the following reasons. Spider mite females escape from the habitat patches intruded by specialist predatory mites when given a dispersal pathway (Bernstein 1984; Grostal & Dicke 1999; Oku et al. 2004; Škaloudová et al. 2007; Bowler et al. 2013). Moreover, because predators, in general, do not remain on a reward-less patch (Charnov 1976; Symondson et al. 2002), specialist predators with dispersal opportunities would leave spider mite patches before exhausting

prey. That is, the behaviors of spider mites and their predators observed in conventional setups without dispersal opportunities may have been artifacts.

This study sheds some light on predator-prey interactions of mites in systems where voluntary dispersal is possible. I tested three main questions:

Question 1. How do spider mites disperse between habitat patches against specialist or generalist predators? (Chapter 2):

Since generalist predators that are hindered by spider mite webs do prey on spider mites outside the webs (Ozawa & Yano 2009; Yano 2012; Otsuki & Yano 2014), I examined whether spider mites dispersing between patches are threatened or attacked by such generalist predators. On the other hand, specialist predatory mites increase the departure rate of spider mites (e.g. Bernstein 1984). Therefore, I further examined post-departure decision making, i.e. transfer (dispersal distance) and settlement (the number of colonized patches), by spider mites against specialist predators.

Question 2. Do within-patch defensive behaviors against specialist predatory mites benefit spider mites? (Chapter 3):

Spider mites shift oviposition sites within habitat patches in response to intrusion by specialist predatory mites (Oku & Yano 2007; Lemos et al. 2010). However, its benefit is unclear because predators may have excessively consumed spider mite eggs in setups with no dispersal opportunities, and also because it is unexplored whether spider mite females show within-patch oviposition site shifts when given dispersal opportunities. Murase et al. (2017) showed that predator-experienced spider mites learn to shift oviposition sites within predator-free habitats, suggesting certain benefits for spider mites. Therefore, I examined whether the within-patch oviposition site shifts decrease spider mite offspring predation when predatory mites are allowed to disperse from the patches.

Question 3. Is predatory mite stealthiness consistent across spider mite species? (Chapter 4):

Because spider mites that are aware of specialist predatory mites can reduce predation by between-patch dispersal and within-patch oviposition site shifts, stealthiness of predatory mites to spider mites should partly represent the effectiveness of the predators as biological control agents. From this viewpoint, I compared stealthiness indices of three predatory mite species to two spider mite species and explored factors affecting different degrees of stealthiness.

To examine the above questions, I used the spider mite *Tetranychus kanzawai* Kishida, the specialist predatory mite *Neoseiulus womersleyi* Schicha, the generalist predatory mite *Euseius sojaensis* Ehara, and the generalist ant *Pristomyrmex punctatus* Mayr (Hymenoptera: Formicidae). *Tetranychus kanzawai* has been reported to induce different defensive behaviors against different predators: adult females avoid the specialist *N. womersleyi* by moving out of the protective webs (Oku et al. 2003b, 2004; Oku & Yano 2007; Otsuki & Yano 2014) or out of habitat patches (Oku et al. 2004), while they stay inside the webs against the generalists *E. sojaensis* (Ozawa & Yano 2009; Yano 2012) and *P. punctatus* (Otsuki & Yano 2014). These three predator species often co-occur with *T. kanzawai* on wild plants such as bushkiller (*Cayratia japonica*; Vitaceae) (Yano, personal observation). For the third question, I used two more specialist predatory mite species: *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* McGregor, both commercialized biological control agents used against tetranychid mites (Hussey & Bravenboer 1971; Copping 2001). Because all of the above species are wingless ambulatory dispersers, I can easily observe their interactions by using connected patch systems in the laboratory.

Based on the results, I discuss spider mite dispersal strategies in light of its predator avoidance and the effectiveness of the specialist and generalist predators for sustainable management of spider mites.

Chapter 2.

Between-Patch Dispersal of Spider Mites under Predation Risk

2.1. Introduction

In general, dispersing organisms adjust their behaviors based on the costs and benefits of dispersal (Bonte et al. 2012). That is, an individual has the option to remain in or to leave a habitat patch, and, after leaving the patch, the option to settle in a potential patch or to continue dispersing. Prey disperses when predation risks in habitat patches are high (Bernstein 1984; Sih et al. 1992; Peckarsky 1996; Hakkarainen et al. 2001; Cronin et al. 2004; McCauley & Rowe 2010). Although dispersers can reduce predation risks in the intruded patches, they are exposed to other predators during dispersal process (Young & Lockley 1988; Bonnet et al. 1999; Hiddink et al. 2002; Korb & Linsenmair 2002; Johnson et al. 2009; Pietrek et al. 2009) and some individuals postpone/give up dispersal to avoid such predators (Young & Lockley 1988; Korb & Linsenmair 2002; Pietrek et al. 2009). Predators not only directly consume prey but impose various indirect costs by making prey disperse or stay in its habitat. For example, aphids produce winged offspring which can aurally escape from risky habitats (Dixon & Agarwala 1999; Weisser et al. 1999) at the expense of prolonged developmental time (Dixon & Kindlmann 1999; Conway & Kring 2004). Prey individuals that stay in a refuge suffer reduced feeding time (Koivula et al. 1995; Dill & Fraser 1997), reduced mating opportunities (Sih 1994; Cooper 1999), and physiological costs from unfavorable conditions in refuges (Wolf & Kramer 1987; Martin & Lopez 1999). Such non-lethal effects of predators can sometimes be comparable to those of direct consumption (Lima 1998; Bolker et al. 2003; Dill et al. 2003; Werner & Peacor 2003; Nelson et al. 2004; Preisser et al. 2005; Creel & Christianson 2008). Since appropriate timing/distance of dispersal and successful settlement are crucial for prey organisms living in patchy habitats, the lethal and non-lethal effects of predators on dispersal should largely affect individual fitness and population structure of prey.

In an applied context, dispersal of spider mites in response to their biological control agents should affect the success of the control, because only a single spider mite female can establish a new local population through sib-mating among its offspring (Hussey & Parr 1963). However, conventional studies that used setups without dispersal

opportunities have inevitably overlooked the decisions to disperse by the prey. In systems without dispersal opportunities, specialist predatory mites that can penetrate spider mite webs (Sabelis & Bakker 1992) can easily suppress the spider mites (e.g. Chant 1961; Hamamura 1986), while generalist predators that are hindered by the protective webs of spider mites (Osakabe 1988; McMurtry & Croft 1997; Ozawa & Yano 2009) seem ineffective for suppressing spider mites and thus their effects on spider mite dispersal remains unexplored.

In this chapter, I examine between-patch dispersal of the spider mite *T. kanzawai* in the presence of (i) generalist and (ii) specialist predators. (i) I used the predatory mite *E. sojaensis* and the ant *P. punctatus* as generalist predators. Since both predators readily prey on *T. kanzawai* females outside their webs (Ozawa & Yano 2009; Yano 2012; Otsuki & Yano 2014), spider mites may be consumed by the predators during dispersal or hesitate to disperse under the predation risk. (ii) The specialist predatory mite *N. womersleyi* increase the departure rate of *T. kanzawai* females (Oku et al. 2004) and thus may increase the spread of spider mite local populations. These putative lethal and non-lethal effects of generalist and specialist predators on spider mites are detectable only in experimental systems where dispersal of mites toward intact patches is allowed.

The tested hypotheses and the corresponding subsections are as follows:

- i. Do spider mite females refrain from dispersal in the presence of generalist predators? (2.2.3 and 2.2.4)
- ii. Do spider mite females disperse farther and establish more patches in the presence of specialist predatory mites? (2.2.5)

2.2. Materials and methods

2.2.1. Animals

Spider mites

I maintained mite study populations on expanded primary leaves of kidney bean (*Phaseolus vulgaris*; Fabaceae), which were cut from the petiole and pressed onto water-saturated cotton in Petri dishes (90 mm in diameter, 14 mm in depth; hereafter “leaf discs”). Kidney bean (hereafter “bean”) is one of the most preferred hosts for *T. kanzawai* (Yano et al. 1998). The water-saturated cotton served as a barrier to prevent mites from escaping. All experimental setups below were placed on water-saturated cotton.

I used *T. kanzawai* population collected from narrow-leaved vetch (*Vicia sativa* subsp. *nigra*; Fabaceae) in 2012 in Kyoto, Japan, for the experiment to test dispersal distance (2.2.5); for the other experiments, I used the population collected from bindweed (*Calystegia japonica*; Convolvulaceae) in 2005 in Kyoto. To initiate study population, I introduced ten 2- to 4-day-old adult females (i.e. reproductive and dispersal stage) of *T. kanzawai* (hereafter “*T. kanzawai* females”) onto one leaf disc together with two male adults. To minimize inbreeding depression of the study populations, I transferred the same numbers of female and male adults from several leaf discs onto several fresh discs for every generation.

Leaf discs were placed in transparent plastic containers maintained at $25 \pm 1^\circ\text{C}$, $50 \pm 1\%$ relative humidity, and a 16L (7:00–23:00): 8D photoperiod. All rearing and experiments were conducted under these conditions.

Predatory mites

The *N. womersleyi* study population was collected from rose (*Rosa centifolia*; Rosaceae) in 2010 in Nara, Japan. The population was reared on leaf discs that were infested with *Tetranychus urticae* as prey (30–50 adult females and individuals of other stages per leaf). The *E. sojaensis* population was collected from kudzu vines (*Pueraria lobata*; Fabaceae) in 2009 in Kyoto and was reared on tea pollen on leaf discs (see Shiotsuka & Yano 2011 for detailed methods).

Ants

I collected 10,000 to 20,000 *P. punctatus* ants from a decayed tree on Mt. Yoshida in Kyoto and divided them into colonies of ca. 500 ants each. Since *P. punctatus* does not have a queen, and the workers can reproduce thelytokously (Mizutani 1980; Itow et al. 1984), I can consider ant individuals collected from one colony as an inbred strain that has minimal genetic variation, and I can easily replicate ant colonies with a fixed number of workers in individual microcosms. Each colony was reared in a microcosm constructed from a transparent plastic container (220 × 300 × 60 mm; Fig. 1). I coated the interior walls of the container with talc powder to prevent ants from escaping. A Petri dish (85 mm in diameter, 11 mm in depth) with a 6-mm plaster layer on the bottom was used as an artificial ant nest. The dish cover was painted with red pigment to encourage settlement. I added water on the plaster twice a week to maintain moisture. *Pristomyrmex punctatus* in the wild preferentially forages on aphid honeydew and extrafloral nectaries (Yamawo et al. 2017), but also needs protein sources to raise their offspring. The ants were fed water and honey ad libitum, and freshly killed mealworms every week as a protein source to promote worker reproduction and to promote their prey searching.

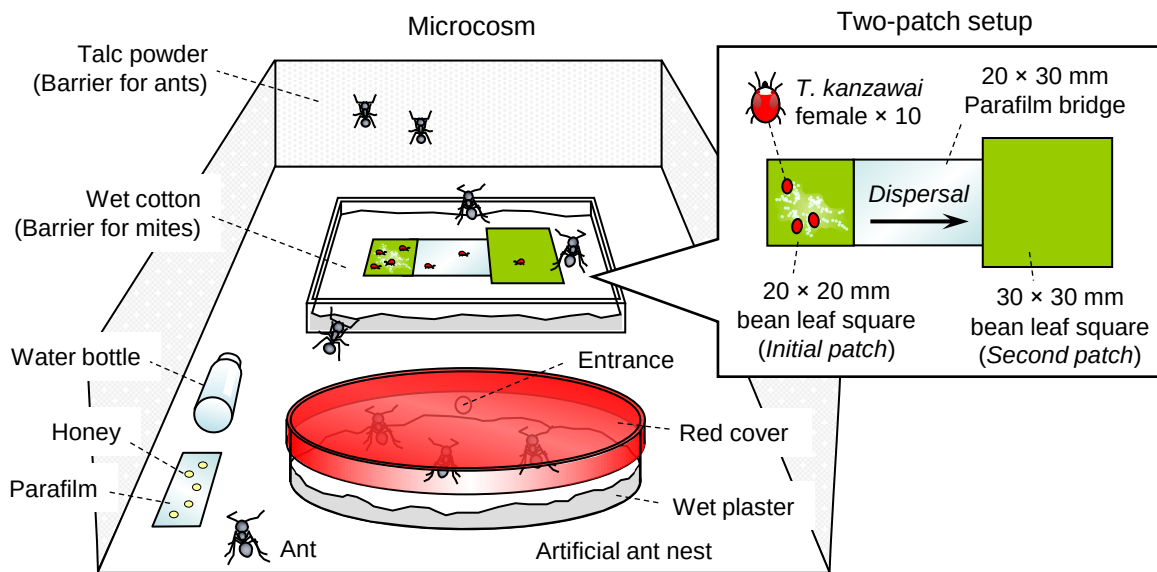


Fig. 1 Setup of microcosms for testing between-patch dispersal of *T. kanzawai* females in the presence or absence of the generalist ant *P. punctatus*

2.2.2. Setups to study between-patch dispersal of mites

Spider mite females that disperse from habitat patches move through a hostile environment without webs. I simulated ambulatory mite dispersal from an infested patch to an intact remote patch by connecting bean leaves with Parafilm (Parafilm M; American National Can, Chicago, IL, USA), a non-food flat substrate on which spider mites cannot construct protective webs. An example of a connected patch system is shown in Fig. 1. Because connected patches were placed on wet cotton barrier, and because mites cannot disperse aurally in a windless laboratory, they could disperse only by walking across the bridges.

The following experiments are based on a premise that mites departing their patches would not be aware of the next available patch in the wild. To confirm that adult female spider mites were not attracted to bean leaves at a certain distance, I connected an uninfested leaf and a Parafilm square (10 × 10 mm each) with a T-shaped Parafilm pathway (Fig. 2). Each square was at the same distance (30 mm) from the T-junction. I introduced one *T. kanzawai* female at the bottom of the pathway using a fine brush. The number of females that moved in each direction from the T-junction did not significantly differ from equality ($n = 60$, leaf:control, 28:32; binomial test, $p = 0.70$). Therefore, dispersing *T. kanzawai* females to patches at a distance of 30 mm were considered to have abandoned the previous patch, as opposed to having been attracted to adjacent

patches. That is, the adjacent patches in connected patch systems in this study were not considered to have affected mite dispersal decisions.

2.2.3. Do spider mites refrain from dispersal in the presence of ants?

I replicated ant microcosms using two-patch setups. I introduced ten *T. kanzawai* females onto uninfested bean leaf squares (20×20 mm; “initial patches”), and allowed them to build webs and oviposit. Since preliminary tests showed that *T. kanzawai* females in the absence of ants start dispersing from the setup 24 h after the introduction and that webs build by ten females for 24 h are effective against ant predation (also see Otsuki & Yano 2014), I connected each initial patch to an uninfested leaf square (30×30 mm; “second patches”) with a Parafilm bridge (20×30 mm; Fig. 1) after 24 h of the introduction. The second patch was larger than the initial one because a preliminary test showed that the larger patch size was necessary to retain dispersed females on second patches during the experimental period. Each setup was placed on wet cotton in a square dish ($87 \times 125 \times 8$ mm).

For the ant treatment, I placed each dish in the microcosm 50 mm away from an artificial ant nest (Fig. 1). For the non-ant treatments, I placed each dish in the microcosm with an empty artificial ant nest, talc powder, and water to control for possible environmental biases. Ants could easily access the spider mite leaves by walking across the wet cotton in the dish. About 10% of the ants were active and out of the nest in each colony (Otsuki, personal observation).

After 3 days, when the differences between treatments were most conspicuous after the placement in microcosms, I recorded the number of surviving *T. kanzawai* females on each patch and the bridge. I obtained the data at 19:00 because *T. kanzawai* females mainly exhibited dispersal behaviors between 16:00 and 18:00 under the laboratory conditions; the same applies to the following experiments to test mite dispersal unless otherwise stated. I calculated the proportion of surviving females in the microcosm (“survival rate”), the proportion of surviving females on the second patch (“dispersal rate”), and the proportion of surviving females on the initial patch (“remaining rate”).

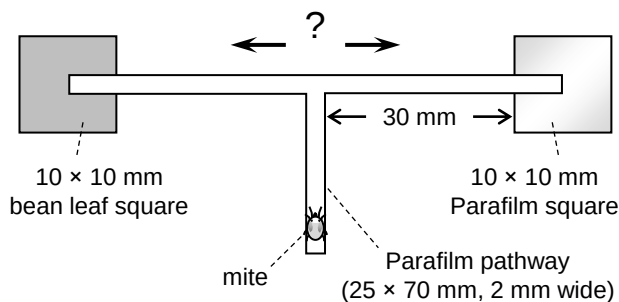


Fig. 2 Experimental setup to confirm that *T. kanzawai* females are not attracted to bean leaves at a distance of 30 mm

I also recorded the state of dead females as either drowning on the surrounding cotton or consumed by predators. Because *P. punctatus* always took the prey mite away from the setup, i.e. never leaving prey corpses on the setup, and because spider mites could not escape the setup (Otsuki & Yano 2014), I considered missing *T. kanzawai* females as having been consumed by *P. punctatus*.

The numbers of replications were 17 (ant present) and 16 (ant absent), respectively. To eliminate the possible effect of learning, I used each colony only once. I compared the survival, dispersal, and remaining rates between treatments using the generalized linear model with binomial error distribution. The Holm-Bonferroni correction (Holm 1979) was used to adjust p-values. All of the statistical analyses in this chapter were performed using JMP 9.0 (SAS Institute Inc 2010).

2.2.4. Do spider mites refrain from dispersal in the presence of generalist predatory mites?

I created two-patch setups which differed in size from the above experiment, since webs build by one *T. kanzawai* female effectively protect the female from *E. sojaensis* (Ozawa & Yano 2009; Yano 2012), and the leaf square used in the above experiment (20×20 mm) was too large to be exhausted by a *T. kanzawai* female. I introduced *T. kanzawai* females singly onto uninfested leaf squares (10×10 mm; “initial patches”). Since preliminary tests showed that *T. kanzawai* females in the absence of *E. sojaensis* start dispersing from the setup on day 3 and that webs build for 3 days are effective against the predators, I connected each initial patch to an uninfested leaf square (10×10 mm; “second patches”) with a Parafilm bridge (10×30 mm) after 3 days of the introduction. I then introduced one mated female *E. sojaensis* onto each setup, while setups without predators served as controls. I did not introduce more than one predatory mite female on each setup because they do not live in a group as a rule (Yano, unpublished). The predatory mite females were isolated in 1.5 mL microtubes (Bioramo Microtube; As One, Osaka, Japan) with a water droplet for the previous 48 h, as starved predators remain on the setups longer than predators with previous access to food (Yano, unpublished). The tubes had sufficient ventilation for mite survival. Because *E. sojaensis* females cannot penetrate or walk on the complicated spider mite webs (Osakabe 1988), they prowled on the bridges and on the second patches where spider mites could disperse.

After 24 h, I recorded the state of *T. kanzawai* females. I determined predation by the sucked and shriveled corpses of females left on the setup. The survival, dispersal, and remaining rates as described above were compared between treatment groups using Fisher’s exact test with Holm-Bonferroni correction. The numbers of replications were 74 (predator present) and 73 (predator absent), respectively.

2.2.5. Do spider mites spread in the presence of specialist predatory mites?

Dispersal distance

I created setups with three patches as shown in Fig. 3. I introduced *T. kanzawai* females singly onto uninfested bean leaf squares (10×10 mm; “initial patches”) and allowed them to build webs and oviposit for 24 h. I then introduced one mated female *N. womersleyi* onto each initial patch, while setups without predators served as controls. I did not introduce more than one predatory mite on each setup because of the same reason as described above (2.2.4). The predators had been starved for the previous 48 h in the same manner as described above since starved predators remain on the initial patch containing abundant spider mite eggs longer than predators with previous access to food (Otsuki, unpublished). After allowing the predators 30 min of acclimation, I connected each initial patch in all setups to two uninfested consecutive leaf squares (10×10 mm each; “second/third patches”) linearly with Parafilm bridges (10×30 mm; Fig. 3).

I recorded the location and state of *T. kanzawai* females every 24 h until each had dispersed to either of the two consecutive patches. I identified which patch each spider mite first settled; I considered a patch to be settled if it contained webs, injury scars, feces, and eggs, regardless of female presence. *Neoseiulus womersleyi* stayed under the webs on the initial patch (Otsuki, personal observation). Although the predators oviposited in the initial patch, all *T. kanzawai* females had dispersed before predator offspring hatched.

I compared the dispersal timing (days after introduction) between treatment groups using Mann-Whitney *U*-test, and compared the dispersal distance (the proportion of female settlement in second to third patches) between groups using the Fisher’s exact test. The numbers of replications were 18 (predator present) and 30 (predator absent), respectively.

Patch colonization

I created setups as shown in Fig. 4. I introduced five *T. kanzawai* females onto uninfested bean leaf squares (10×10 mm; “initial patches”) and allowed them to build webs and

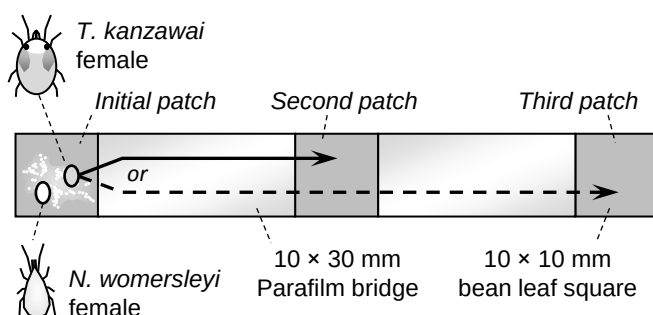


Fig. 3 Experimental setup to investigate whether *T. kanzawai* females settle in adjacent (second; solid arrow) patches or continue to distant (third; dashed arrow) patches in the presence or absence of the specialist predatory mite *N. womersleyi*

oviposit for 24 h. I then introduced one starved mated female *N. womersleyi* onto each initial patch, while setups without predators served as controls. After allowing the predators 30 min of acclimation, I connected each initial patch in all setups to four uninfested leaf squares (10×10 mm each; “consecutive patches”) linearly with Parafilm bridges (10×30 mm; Fig. 4).

After 2 days, when the differences between treatments were most conspicuous after predator introduction, I recorded the number and state of *T. kanzawai* females on each patch and the bridge. I calculated the survival rate as described above, and dispersal rate (the proportion of surviving females on the consecutive patches). I also recorded the number of newly colonized patches out of the four consecutive patches; I considered a patch as colonized in the same manner as described above (for settlement). No predator offspring hatched during the period.

The survival and dispersal rates and the number of colonized patches were compared between treatment groups using the generalized linear model with binomial error distribution adjusted using Holm-Bonferroni correction. The numbers of replications were 15 (predator present) and 24 (predator absent), respectively.

2.3. Results

2.3.1. Do spider mites refrain from dispersal in the presence of ants?

In the presence of ants, significantly fewer *T. kanzawai* females survived and dispersed to the second patch than in the absence of ants (Fig. 5a, b). All of the dead *T. kanzawai* females in the presence of ants were preyed upon. On the other hand, the number of *T. kanzawai* females that remained on the initial patch with the protective webs did not differ significantly between the treatments (Fig. 5c), indicating that *T. kanzawai* females did not hesitate to move out of the refuges and disperse.

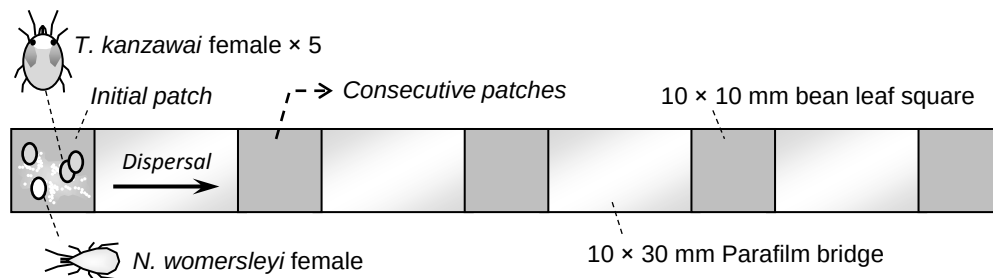


Fig. 4 Experimental setup to investigate the dispersal and new patch foundation of *T. kanzawai* females in the presence or absence of the specialist predatory mite *N. womersleyi*

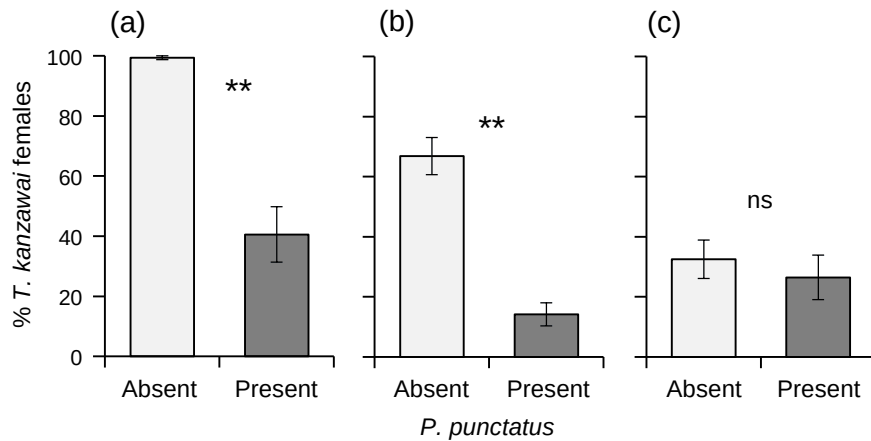


Fig. 5 Effects of the generalist ant *P. punctatus* on (a) survival, (b) dispersal and (c) remaining rates (mean $\pm$ SE) of *T. kanzawai* females. ** $p < 0.01$ (GLM with binomial error distribution adjusted using Holm-Bonferroni correction)

2.3.2. Do spider mites refrain from dispersal in the presence of generalist predatory mites?

In both treatments, nearly all *T. kanzawai* females survived, and the survival rate did not differ significantly between the treatments (Fig. 6a). All dead females in the presence of *E. sojaensis* were drowned, not preyed upon by *E. sojaensis*. In the presence of *E. sojaensis*, significantly fewer *T. kanzawai* females dispersed to the second patch than in the absence of *E. sojaensis* (Fig. 6b). The proportion of *T. kanzawai* females that remained on the initial patch did not differ significantly between the treatments (Fig. 6c), suggesting that *T. kanzawai* females that had moved out of the initial patch could not access or settle in the second patch in the presence of *E. sojaensis*.

2.3.3. Do spider mites spread in the presence of specialist predatory mites?

Dispersal distance

In the presence of *N. womersleyi*, the mean time (day $\pm$ SE) before *T. kanzawai* females began dispersing was significantly shorter (1.3 ± 0.11) than in the absence of *N. womersleyi* (5.8 ± 0.30 ; Mann-Whitney *U*-test, $p < 0.0001$). No female was preyed upon by *N. womersleyi*. Most *T. kanzawai* females dispersing in the absence of predators settled in adjacent (second) patches, whereas the majority of those dispersing in the presence of predators settled in distant (third) patches (Fig. 7).

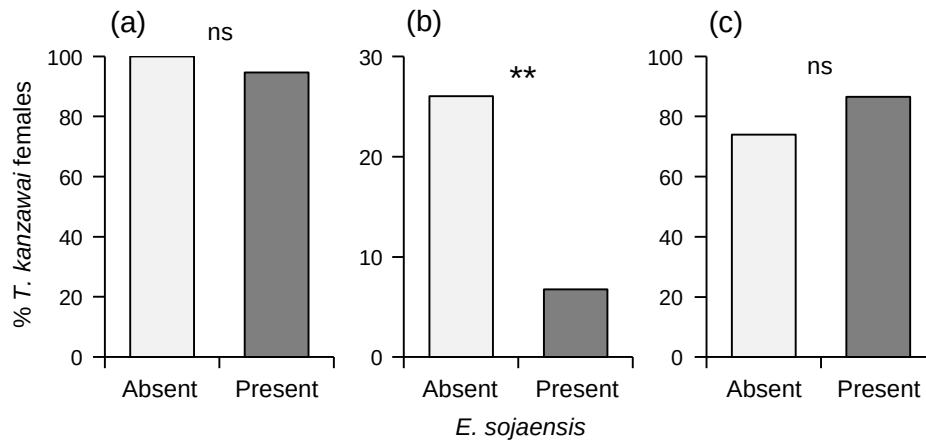


Fig. 6 Effects of the generalist predatory mite *E. sojaensis* on (a) survival, (b) dispersal and (c) remaining rate of *T. kanzawai* females. ** $p < 0.01$ (Fisher's exact test with Holm-Bonferroni correction)

Patch colonization

Nearly all *T. kanzawai* females survived under both treatments, and the survival rate did not differ significantly between the treatments (Fig. 8a). None of the dead females was preyed upon by *N. womersleyi*. In the presence of *N. womersleyi*, significantly more *T. kanzawai* females dispersed to other patches (Fig. 8b), and females colonized significantly more patches in the presence than in the absence of *N. womersleyi* (Fig. 8c).

2.4. Discussion

By using a connected patch system that allowed prey dispersal toward an intact patch, I revealed that generalist predators that cannot suppress spider mites in systems with no opportunity for dispersal effectively prevented spider mite dispersal between patches. *Tetranychus kanzawai* females were directly consumed by the generalist ant *P. punctatus* during dispersal, while they escaped predation by the generalist predatory mite *E. sojaensis* but did fail to access or settle in new patches.

Although *E. sojaensis* did not directly reduce the survival of *T. kanzawai* females (Fig. 6a), the predators would reduce the chance of feeding and oviposition of *T. kanzawai* females that could not either access or settle in intact patches. This may be one of the mechanisms by which generalist predatory mites, which cannot penetrate protective webs produced by spider mites (especially most *Tetranychus* species), do suppress mites in the

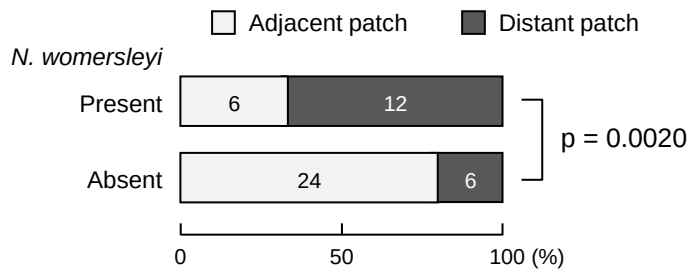


Fig. 7 Effects of the specialist predatory mite *N. womersleyi* on the proportion of *T. kanzawai* females that settled in adjacent (second) or distant (third) patches. The numbers in the bars are the numbers of replication (Fisher's exact test)

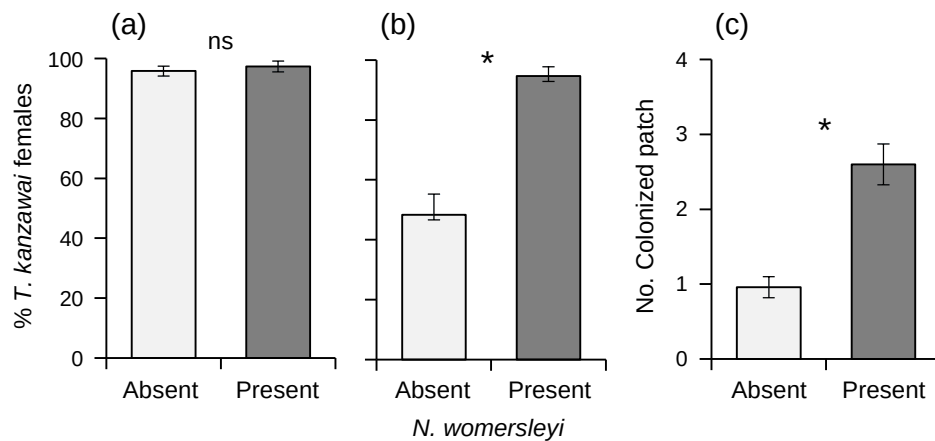


Fig. 8 Effects of the specialist predatory mite *N. womersleyi* on (a) survival, (b) dispersal and (c) patch colonization (mean $\pm$ SE) of *T. kanzawai* females. * $p < 0.05$ (GLM with binomial error distribution adjusted using Holm-Bonferroni correction)

field (McMurtry 1985; Duso 1988, 1989; James 1990; but see Croft & MacRae 1992). Unlike specialist predatory mites, which depend on spider mites, generalist predatory mites subsist on plant-derived alternative foods such as pollen (McMurtry & Johnson 1965; Kennett et al. 1979; Osakabe et al. 1986) and pearl bodies (Ozawa & Yano 2009), and also on mildew infecting plants (Duso et al. 2003), which are relatively stable food resources compared with spider mites. Therefore, 'patrolling' of host plants of spider mites by generalist predatory mites is less correlated with spider mite density (McMurtry 1992).

The non-different survival, the fewer dispersal to the second patches, and the marginally significant tendency to remain on the initial patches in the presence of *E. sojaensis* (Fig. 6) suggest that dispersed *T. kanzawai* females hesitated to settle in intact patches. The females may have been directly threatened by prowling *E. sojaensis* females, and/or been vigilant to chemical cues of the predators (c.f. Grostal & Dicke 1999, 2000; Škaloudová et al. 2007; Bowler et al. 2013) on the bridges and second patches. In contrast, *T. kanzawai* females did not hesitate to disperse in the presence of ants and were

preyed upon. This may be attributed to the ants' higher ability to capture spider mites due to their greater mobility and larger body size compared with predatory mites. Moreover, because ants can approach and attack *T. kanzawai* females from the surrounding wet cotton barrier, which mites could not access, the females might not perceive approaching ants.

I also showed that specialist predatory mites let spider mites escape. In the presence of the specialist predatory mite *N. womersleyi*, more *T. kanzawai* females dispersed (Fig. 8b) than in the absence of *N. womersleyi*, suggesting that spider mite dispersal is dependent on the predators. If specialist predatory mites are absent, dispersal is seemingly triggered by resource deterioration, but if the predators are present, they become important dispersal motivators. This was consistent with results of previous studies reporting higher spider mite departure rates in the presence of specialist predatory mites (Bernstein 1984; Grostal & Dicke 1999; Oku et al. 2004; Škaloudová et al. 2007; Bowler et al. 2013). Although the experiments in this study using setups with more than two patches apparently looks similar to the two-patch system used by Bowler et al. (2013), the two experiments qualitatively differ in that two-patch systems can only compare departure rates of mites from the initial patch, while systems with more than two patches can compare the post-departure processes (transfer and settlement decisions) in the presence or otherwise of the predator. Thus, I further confirmed that specialist predatory mites increased dispersal distance and the number of colonized patches of spider mites.

The results showing that most *T. kanzawai* females settled in adjacent patches in the absence of predators (Fig. 7) support published theoretical research (Poethke et al. 2011). These authors predicted that dispersing individuals should settle in adjacent patches unless the cost of between-patch dispersal is extremely low because the costs of resource competition should sufficiently decrease after one dispersal step. Although dispersal costs were not simulated in my experiment testing dispersal distance, the average costs of between-patch dispersal that spider mites should incur in the wild may be considerable, because dispersing mites are exposed to harmful abiotic conditions such as rain, wind, and ultraviolet rays (Das 1959; Jeppson et al. 1975; Sakai & Osakabe 2010), as well as generalist predators (Ozawa & Yano 2009; Yano 2012; Otsuki & Yano 2014; also see Figs. 5 & 6). The results in this study imply that ignoring the first encountered patch is disadvantageous for spider mites when the predation risk by specialist predatory mites is low. Conversely, the majority of *T. kanzawai* females dispersing in the presence of the specialist predatory mite *N. womersleyi* passed through adjacent patches without settling, which implies that the benefits of greater dispersal distances under predation risk may outweigh the average costs of dispersal. Because specialist predatory mites prefer spider mite eggs laid in the webs to adult females (Takafuji & Chant 1976; Fernando & Hassell

1980; Sabelis 1990; Blackwood et al. 2001; Furuichi et al. 2005), it is not surprising that all *T. kanzawai* females escaped predation (2.3.3), and hence just one dispersal step may be enough for the females to avoid predation on themselves. Rather, moving farther away from the predator-intruded patches would reduce future predation to spider mite offspring, eggs and immatures that cannot readily disperse from predators. Nonetheless, some *T. kanzawai* females settled in adjacent patches even in the presence of the specialist predatory mite *N. womersleyi* (Fig. 7). This result and the one that *T. kanzawai* females escaping from *N. womersleyi* scattered (Fig. 8c) suggest individual variation in dispersal strategy; or possible benefits of not aggregating when escaping from specialist predators.

Most theoretical and empirical studies on dispersal ecology have focused only on departure (Bowler & Benton 2005). While departure rate is informative, dispersal distances that are determined by both transfer and settlement decision makings post-departure are necessary to predict the full consequences of dispersal (Travis et al. 2013). An increasing number of theoretical studies have investigated the influences of population density and/or kin competition on the evolution of dispersal distance (e.g. Rousset & Gandon 2002; Poethke et al. 2011). Furthermore, empirical studies have demonstrated that these factors induce plasticity for dispersal distance in actively dispersing organisms such as small mammals (Ims & Andreassen 2005) and also spider mites (Bitume et al. 2013). However, only a few studies have considered the effects of predators on prey dispersal distances (Tamaki et al. 1970; Dixon & Agarwala 1999; Weisser et al. 1999; Meng et al. 2012). Tamaki et al. (1970) compared spatial distributions of apterous aphid populations in the presence or absence of parasitoids, and inferred that those aphids dispersed a greater distance if they encountered parasitoids. Similarly, predatory ladybirds induce production of winged offspring in aphids (Dixon & Agarwala 1999; Weisser et al. 1999), suggesting predator-induced long-distance dispersal. However, Meng et al. (2012) did not detect increased dispersal distances in adult whiteflies in the presence of predators. The scarcity of empirical studies examining dispersal distances may be due largely to the difficulty in tracking flying organisms that disperse great distances. From this viewpoint, the system of tiny, wingless spider mites and their predators is a good model for experimental studies of dispersal. This is the first experimental demonstration of predators increasing dispersal distances in prey organisms. It remains to be examined how greater dispersal distances in response to predation risk contribute to the stability of prey populations and how this, in turn, influences the effectiveness of predators in suppressing prey populations.

Chapter 3.

Within-Patch Oviposition Site Shifts by Spider Mites to Reduce Offspring Predation

3.1. Introduction

Oviparous animals lacking post-ovipositional parental care have to ensure offspring survival with pre-ovipositional care. Eggs and juveniles are generally more vulnerable to predators compared to adults. Because loss of offspring reduces parents' inclusive fitness (Hamilton 1964), parental attention to avoiding offspring predation is particularly important, even when predators never attack the parents (Kats & Sih 1992; Gross 1993; Faraji et al. 2001).

Females can reduce present and/or future predation risk for offspring by ovipositing in suitable sites for offspring survival. Many studies have shown that ovipositing females avoid habitat patches with predators of their offspring (Chesson 1984; Resetarits & Wilbur 1989; Spieler & Linsenmair 1997; Kats & Dill 1998; Magalhães et al. 2002; Kiflawi et al. 2003; Nomikou et al. 2003; Blaustein et al. 2004). Oviposition site qualities that determine offspring survival vary both between habitat patches and on a smaller scale within patches. When predator-free patches are unavailable or movement between patches is costly for the ovipositing female, within-patch microhabitat use for oviposition (hereafter “micro-oviposition selection”) against predators is favored (Gall et al. 2012; Meng et al. 2012). Predator-induced micro-oviposition selection that reduces offspring predation has been known in newts (Gall et al. 2012), predatory midges (Lucas & Brodeur 1999), and water striders (Amano et al. 2008; Hirayama & Kasuya 2009). Compared to between-patch oviposition site selection (see Chapter 2 and references therein), however, micro-oviposition selection against predators has been poorly studied, in part because its potential benefits can be overlooked or underestimated in typical laboratory conditions where predators do not have the opportunity to disperse from prey patches due to the experimental design. In such environments, all of the prey will eventually be consumed. Because predators leave prey patches partly based on foraging efficiency within patches (Charnov 1976), prey micro-oviposition selection against predators should decrease predation within patches before predator patch leaving (“decrease predator patch exploitation”), and its benefits should be better examined in systems where predator dispersal from the patch is allowed.

Specialist predatory mites that intrude into webs prefer spider mite eggs to adult females (Takafuji & Chant 1976; Fernando & Hassell 1980; Sabelis 1990; Blackwood et al. 2001; Furuichi et al. 2005). Oku & Yano (2007) and Lemos et al. (2010) reported that spider mites shift oviposition sites onto their webs within patches with specialist predatory mites or cues of their presence. Although the eggs laid on webs were less predated than those on leaf surfaces (Lemos et al. 2010), I consider the reported micro-oviposition shift observed in artificially closed patches needs further investigation for the following reasons: (i) spider mite females may have laid eggs onto webs while seeking an escape route, such that the reported micro-oviposition shifts may have been artifacts; and (ii) the survival of eggs on webs may have been underestimated because predatory mites without dispersal opportunities eventually consume all of the eggs on both leaf surfaces and webs in the closed patches (Otsuki, unpublished).

To confirm the benefits of micro-oviposition shifts to spider mites, I used spider mite females that have previously experienced predators. Because predator-experienced females oviposit onto webs even in predator-free patches from which the females do not have to disperse (Murase et al. 2017), the learned micro-oviposition shift onto webs is less likely an artifact. I tested egg survival using an experimental design in which predatory mites could voluntarily disperse from prey patches.

Using *T. kanzawai* and its native specialist predatory mite *N. womersleyi*, I examined the following four questions:

- i. Whether micro-oviposition shift by predator-experienced females an artifact or not? (3.2.2)
- ii. Does the learned micro-oviposition shift decrease predator patch exploitation, thereby increase offspring survival? (3.2.3)
- iii. Does the micro-oviposition shift incur costs in predator-experienced females? (3.2.4)
- iv. Do previously experienced predation risks affect between-patch oviposition site selection by females? (3.2.5)

3.2. Materials and methods

3.2.1. Animals

I collected *T. kanzawai* study population from kudzu vines in 2014 in Kyoto, and reared them on leaf discs, which were free from predators. I collected *N. womersleyi* population from bushkiller in 2014 in Kyoto. The population was reared on leaf discs infested heavily with *T. urticae* as prey.

3.2.2. Preparation of predator-experienced and -naive females

Based on the method of Murase et al. (2017), I prepared predator-experienced *T. kanzawai* females as follows. I introduced 20–40 randomly selected quiescent female deutonymphs of *T. kanzawai* onto uninfested leaf discs. Immediately after synchronized adult emergence (see Ikegami et al. 2000 for detailed methods), I introduced the same number of adult males to allow mating. After 1 h, I transferred the females singly onto bean leaf squares (10 × 10 mm) which were artificially bent 90° (“bent squares”; Fig. 9) to standardize the patch structure for building three-dimensional webs and to prevent further leaf deformation by *T. kanzawai* females (Oku & Yano 2007), as well as to prevent dispersal of predators that are not accustomed to the new conditions. The bent squares had previously been inhabited by three preceding *T. kanzawai* females for 24 h and harbored their eggs and webs. Then, I introduced one starved mated female *N. womersleyi* onto each square to create predator-experienced *T. kanzawai* females (Fig. 9a). Meanwhile, *T. kanzawai* females on predator-free squares served as the predator-naive group (Fig. 9b). After 19 h of predator introduction, when newly emerged mated females began oviposition, the *T. kanzawai* females were transferred to the following experiments. No *T. kanzawai* females were killed during the treatment because *N. womersleyi* females preferentially feed on spider mite eggs (Furuichi et al. 2005) and the *T. kanzawai* females took refuge against predators by staying on webs (Oku et al. 2004) built by the preceding females.

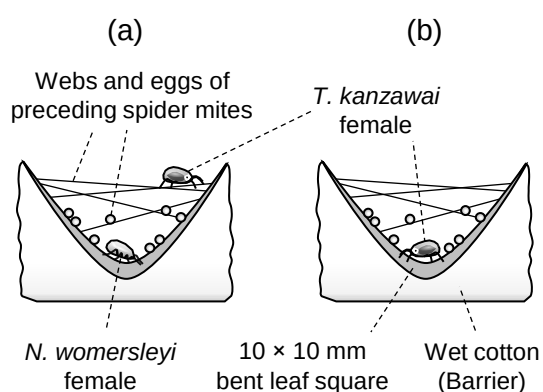


Fig. 9 Experimental setup to prepare (a) predator-experienced and (b) -naive *T. kanzawai* females

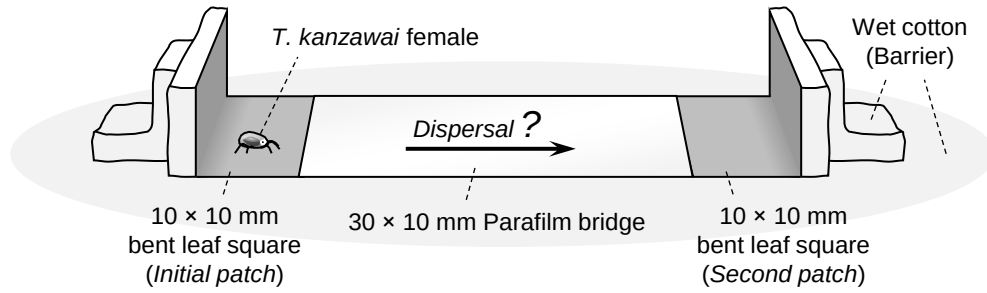


Fig. 10 Experimental setup to investigate whether predator-experienced *T. kansawai* females disperse from predator-free patches

To preliminarily confirm that micro-oviposition shifts by predator-experienced spider mites are not artifacts, I examined whether experienced females disperse from predator-free patches, in which they oviposit onto webs (Murase et al. 2017). If they settle in predator-free patches and lay eggs onto webs, the shift is not an artifact. Otherwise, i.e. if they disperse from the patches without laying eggs on webs, the shift observed in closed setups may have been artifacts; the females may have been on webs seeking an escape route. I introduced experienced *T. kansawai* females singly onto uninfested bent squares (10 × 10 mm; “initial patches”). After 30 min of acclimation, each square was connected to another uninfested bent square (10 × 10 mm; “second patches”) with a Parafilm bridge (10 × 30 mm; Fig. 10). I preliminarily confirmed that the females were not attracted to leaf squares at a distance of 30 mm (see subsection 2.2.2 for detailed methods), such that the adjacent patches did not affect female dispersal and settlement decisions. Both patches were free from predators. The second patches were also bent to facilitate the settlement of dispersed females and thereby prevent their return to the initial patches. As a result, all *T. kansawai* females remained on the initial patches ($n = 29$), and $49.1 \pm 7.4\%$ (mean $\pm$ SE) of the eggs were laid on webs in the initial patches. Therefore, I can reasonably conclude that the learned micro-oviposition shift onto webs is not an artifact, but rather, is a good model for examining the maternal care by spider mites.

3.2.3. Effects of micro-oviposition shift on predator patch exploitation

I introduced two experienced or two naive *T. kansawai* females onto uninfested bent square without predators (10 × 10 mm; “initial patches”), allowing them to build webs and oviposit. After 2 days, I removed the females and counted the number of eggs laid both on the leaf surface and the web. To adjust the total number of eggs to 24, I carefully removed excess eggs from both leaf surfaces and webs to approximately maintain the

proportion of eggs on webs in each patch before and after manipulation. I excluded patches with fewer than 24 eggs. Then, I introduced one starved mated female *N. womersleyi* onto each patch. After 30 min of acclimation, I connected each initial patch to another bent leaf square (15 × 15 mm; “second patches”) with a Parafilm bridge (10 × 30 mm; Fig. 11). The second patches served as traps for dispersed predators; the traps harbored ample (ca. 50) spider mite eggs and webs, and I preliminarily confirmed that predators dispersed from the initial patches to the second patches within 2 days of introduction and did not return to the initial patches within that period. Another preliminary test showed that *N. womersleyi* females were not attracted to the infested bent leaf squares harboring ample prey eggs and webs at a distance of 30 mm (see subsection 2.2.2 for detailed methods). That is, the second patches did not affect predator patch exploitation in the initial patches. After 2 days, when all predators had dispersed to the second patches, I counted the surviving eggs on the leaf surfaces and webs in the initial patches.

I first compared the proportion of eggs on webs (/24 eggs) between experienced and naive groups using Mann-Whitney *U*-test. I used linear regression to determine the relationship between the proportion of surviving eggs and the proportion of eggs on webs, and used the Wilcoxon signed-rank test to analyze the effects of egg position (web or leaf surface) on egg survival. All of the statistical analyses in this chapter were performed using R v.3.2.2 (R Core Team 2015). The numbers of replications were 36 (experienced) and 41 (naive), respectively.

3.2.4. Cost of micro-oviposition shift onto webs

To examine whether micro-oviposition shift onto webs by predator-experienced *T. kanzawai* females incurs fitness costs, I compared egg numbers and longevity of predator-experienced and -naive *T. kanzawai* females. I introduced experienced or naive

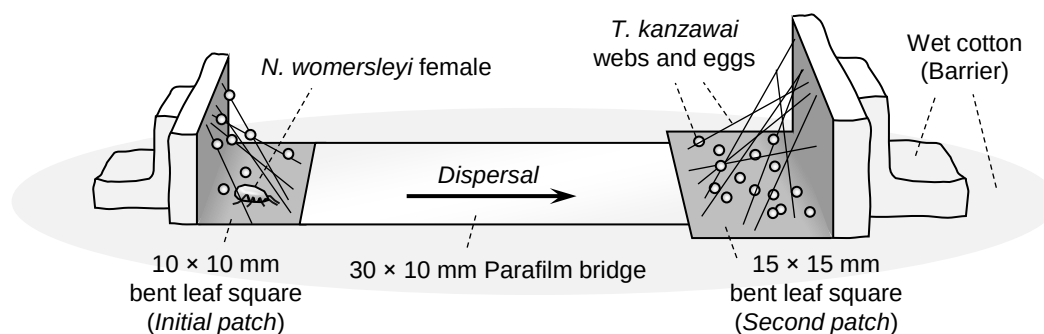


Fig. 11 Experimental setup to investigate the effects of micro-oviposition shift of *T. kanzawai* on egg predation. Initial patches harbored webs and 24 eggs of either experienced or naive *T. kanzawai* females

females singly onto uninfested bent squares (10×10 mm) without predators. I transferred the females onto new squares every 3 days, to minimize the possible effects of leaf deterioration.

I recorded females' survival every 24 h. I also counted the number of eggs laid, on the leaf surfaces and webs, on the first and every third day, until day 12 at which time more than half of the females remained alive. I used the every-third-day egg data, because egg numbers and/or micro-oviposition selection are influenced by the degree of leaf deterioration.

The numbers of replications were 33 (experienced) and 47 (naive), respectively. I compared the proportion of eggs on webs between experienced and naive groups using Mann-Whitney *U*-test, where I excluded a few females that had laid fewer than two eggs. To compare longevity, I estimated survival rates using the Kaplan-Meier method with censored samples due to accidental death during the transfer process, and analyzed differences in survival curves between experienced and naive females using the log-rank test. I compared the number of eggs between groups using Student's *t*-test. The Holm-Bonferroni correction was used to adjust *p*-values for multiple comparisons.

3.2.5. Between-patch dispersal of predator-experienced and -naive females

Spider mite females ensure offspring survival by dispersing from patches with predator intrusions to those that are free of predators, settling in farther patches compared to when they disperse in response to food deterioration (2.3.3, Figs. 7 & 8). To determine whether previously experienced predation risks, which induce micro-oviposition shifts in *T. kanzawai* females, affect future between-patch oviposition site selection against predators, I compared dispersal timing and distance of predator-experienced and -naive *T. kanzawai* females from predator-intruded patches.

I created setups with three patches similar to that in Fig. 3. I introduced experienced or naive *T. kanzawai* females singly onto bent squares (10×10 mm; "initial patches") harboring spider mite eggs and webs. After 24 h, I introduced one starved mated female *N. womersleyi* onto each patch. After 30 min of acclimation, I connected each initial patch to two consecutive flat uninfested leaf squares (10×10 mm each; "second/third patches") with Parafilm bridges (10×30 mm).

I recorded the location and state of *T. kanzawai* females every 24 h for 3 days, after which *T. kanzawai* offspring in the initial patches hatched. Almost all of the experienced (95.5%) and naive (96.0%) females dispersed within the period. I identified which patch each female had first settled in; I considered a patch to be settled if it contained webs, injury scars, feces, and eggs, regardless of female presence.

The numbers of replications were 22 (experienced) and 25 (naive), respectively. I compared the dispersal timing (days after predator introduction within 3 days) of experienced and naive females using the Kaplan-Meier method and the log-rank test. I compared the dispersal distance (the proportion of female settlement in second to third patches) between groups using the Fisher's exact test, where I excluded a few females that had dispersed but not settled in either consecutive patch within the period.

3.3. Results

3.3.1. Effects of micro-oviposition shift on predator patch exploitation

The proportion (mean $\pm$ SE) of eggs on webs (/24 eggs) was 38.4 ± 4.0 and $2.4 \pm 0.8\%$ in the initial patches founded by predator-experienced and -naive females, respectively. The proportions differed significantly (Mann-Whitney *U*-test, $p < 0.0001$), confirming the micro-oviposition shift of females in predator-free environments. Egg survival was significantly higher in the patches founded by experienced females than those founded by naive females (Fig. 12a). In the patches founded by experienced females, egg survival was positively correlated with the proportion of eggs on webs (Fig. 12b), and egg survival on webs was significantly higher than on leaf surfaces (Fig. 12c).

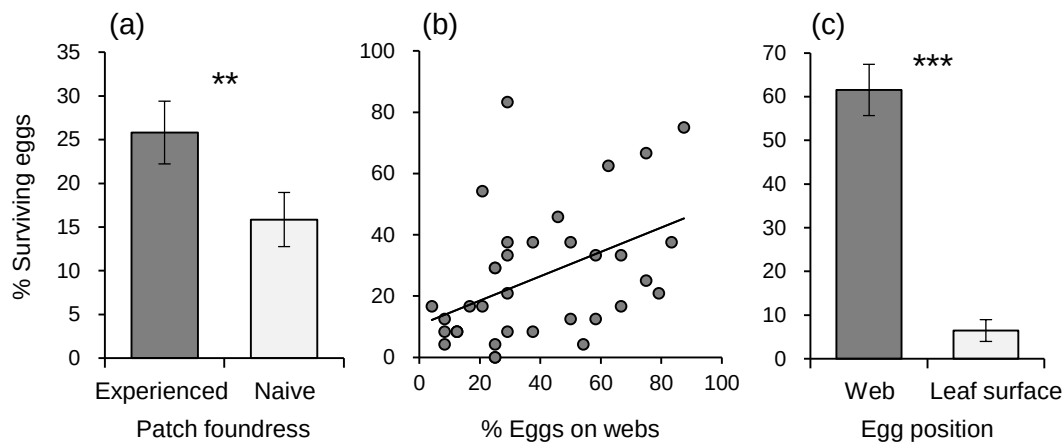


Fig. 12 Increased offspring survival by micro-oviposition shift onto webs in predator-experienced *T. kanzawai* females. (a) Egg survival (mean $\pm$ SE) of experienced and naive females. ** $p < 0.01$ (Mann-Whitney *U*-test). (b) The proportion of eggs laid on webs in relation to egg survival of experienced females (linear regression analysis, $R^2 = 0.2025$, $p < 0.01$). (c) Egg survival (mean $\pm$ SE) of experienced females with respect to the position of laid eggs. *** $p < 0.0001$ (Wilcoxon signed-rank test)

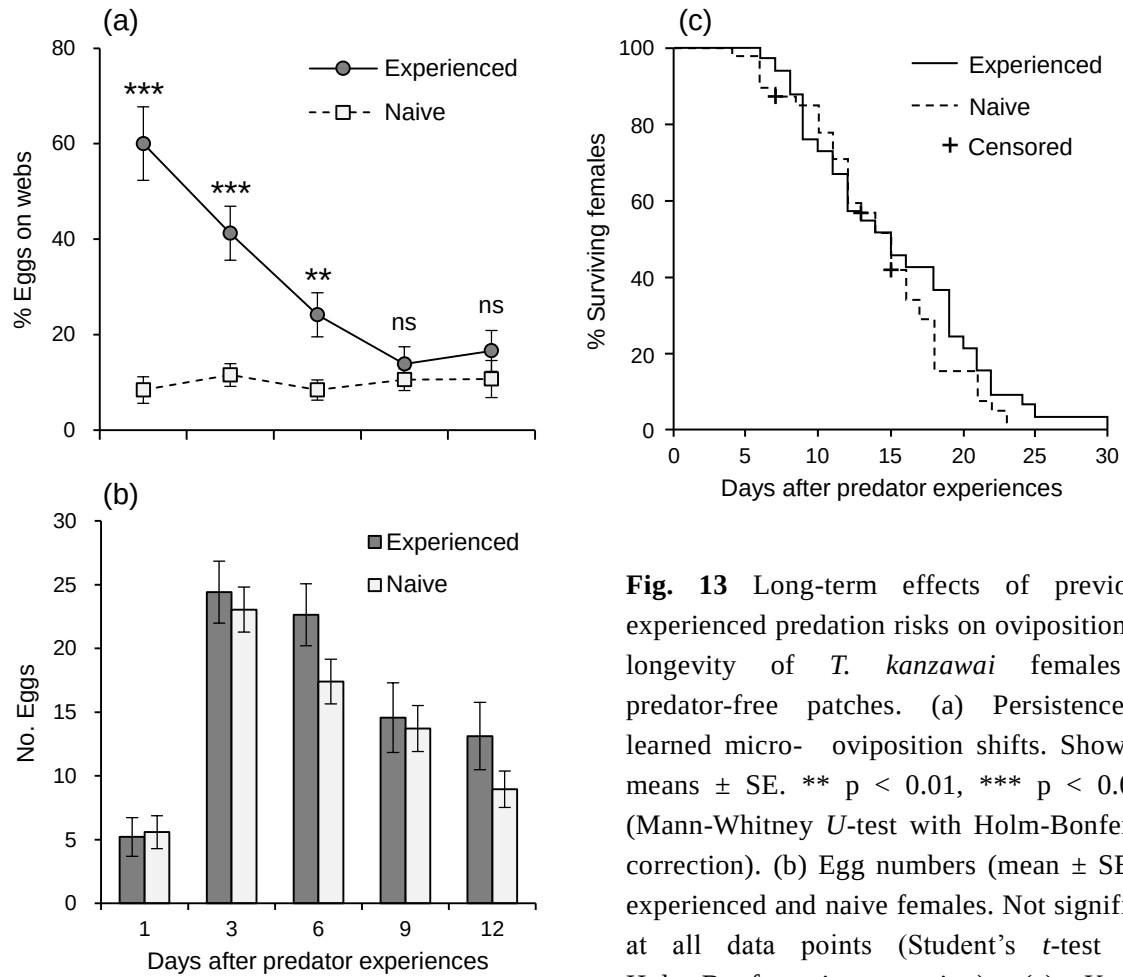


Fig. 13 Long-term effects of previously experienced predation risks on oviposition and longevity of *T. kanzawai* females in predator-free patches. (a) Persistence of learned micro-oviposition shifts. Shown as means $\pm$ SE. *** $p < 0.0001$ (Mann-Whitney U -test with Holm-Bonferroni correction). (b) Egg numbers (mean $\pm$ SE) of experienced and naive females. Not significant at all data points (Student's t -test with Holm-Bonferroni correction). (c) Kaplan-Meier survival curves of experienced and naive females

3.3.2. Cost of micro-oviposition shift onto webs

Differences in the proportion of eggs on webs between experienced and naive females, were largest on day 1 and persisted for a maximum of 6 days after predator experience (Fig. 13a). However, the numbers of eggs laid did not differ between experienced and naive females (Fig. 13b). Moreover, the survival rate was not significantly different between experienced and naive females (log rank $\chi^2 = 1.2396$, $df = 1$, $p = 0.27$; Fig. 13c).

3.3.3. Between-patch dispersal of predator-experienced and -naive females

Experienced and naive females dispersed from the initial patches after (mean $\pm$ SE) 1.6 ± 0.15 and 1.9 ± 0.15 days of predator introduction, respectively, and dispersal timing did not differ significantly between groups (log rank $\chi^2 = 0.8091$, $df = 1$, $p = 0.37$). No *T. kanzawai* female was preyed upon. The proportion of females that had settled in the third

patches did not differ significantly between groups (experienced: 63.2% [n = 19]; naive: 61.9% [n = 21]; Fisher's exact test, $p > 0.99$).

3.4. Discussion

Females can reduce offspring predation by ovipositing in relatively protected microhabitats within patches (e.g. Lucas & Brodeur 1999; Amano et al. 2008; Gall et al. 2012). I demonstrated that precautionary micro-oviposition shifts (i.e. oviposition site shift within patches) of spider mites in predator-free patches, based on previously experienced predation risks, could decrease predator patch exploitation. I observed that the specialist predatory mite *N. womersleyi* preferentially fed on *T. kanzawai* eggs on leaf surfaces and abandoned the patch with more eggs unpredated when a higher proportion of eggs were laid on webs (Fig. 12). This may be because the predator had not noticed the eggs on the webs or because handling the prey eggs on webs may have been too costly. Although many studies have examined the effects of current predation risks on oviposition site selection, there are very few studies on the effects of previously experienced predation risks, at either between-patch (Nomikou et al. 2003) or within-patch (Hirayama & Kasuya 2009) scales. Nomikou et al. (2003) showed that whitefly females that had experienced predatory mites learned to avoid host plants with predators more strongly than did naive females. Moreover, Hirayama & Kasuya (2009) found that predator-experienced water striders oviposit at deeper positions underwater not to avoid current risks, but rather, to avoid future egg parasitism (i.e. precautionary anti-predator behavior). The findings in this study confirmed spider mites' potential for adaptive learning (e.g. Egas & Sabelis 2001; Agrawal et al. 2002; Hackl & Schausberger 2014), and suggest that the potential influences of predators on parental care for offspring in many other organisms may have been underestimated and require further study.

In the absence of predation risk, spider mite females lay almost all their eggs on leaf surfaces (Oku & Yano 2007; Lemos et al. 2010; also see Results), which suggests that there are costs associated with micro-oviposition shifts. In water striders, oviposition at sites deeper under water reduces egg parasitism, but also imposes costs of increased drowning and decreased egg numbers on ovipositing females (Hirayama & Kasuya 2014). I examined whether the possible cost of spider mite micro-oviposition shift is reflected in egg numbers and female longevity. Despite persistent micro-oviposition shifts, there was no significant difference between predator-experienced and -naive *T. kanzawai* females. The similarity in egg numbers may have resulted from equivalent feeding times of

experienced and naive females, at least 9 h after predator experience (Otsuki, unpublished), since spider mite egg numbers depend on time allocation to feeding (Agrawal et al. 2002; Oku et al. 2004). Previous work by Lemos et al. (2010) reported reduced egg numbers in patches with predation risks (cues), where spider mites exhibited a micro-oviposition shift. However, because egg numbers were measured in artificially closed patches with predation risks, the numbers may have reflected not only the costs of the micro-oviposition shift itself, but also the costs associated with other anti-predator behaviors of females such as dispersal attempts from predator-intruded patches, as reported in other works (Bernstein 1984; Grostal & Dicke 1999; Oku et al. 2004; Škaloudová et al. 2007; Bowler et al. 2013; 2.3.3, Figs. 7 & 8). Since I examined the egg numbers of predator-experienced females that exhibited micro-oviposition shifts in predator-free patches, from which the females do not disperse (3.2.2), the potential costs associated with female anti-predator behaviors are precluded. Therefore, the results in this study suggest that micro-oviposition shifts onto webs do not incur costs in terms of egg reduction and longevity. In the wild, however, eggs laid on webs may incur costs caused by harmful abiotic stressors that are not examined in this study. For example, eggs laid on webs are more likely to be washed away with rainwater compared to those on leaf surfaces (Yano, personal communication). Further investigations are needed to determine in which fitness indices the costs of micro-oviposition shift in spider mites are reflected.

In general, micro-oviposition selection is advantageous when predation risk is ubiquitous in most patches (Gall et al. 2012), the ability of females to disperse between patches or detect adjacent patches is limited (Meng et al. 2012), and lethal and non-lethal costs of dispersal (Bonte et al. 2012) are high for females. In the case of *T. kanzawai*, predatory mites are not ubiquitously distributed, but spatially associated with spider mites on host plants (Nachappa et al. 2011). Moreover, *T. kanzawai* is a polyphagous herbivore that feeds on hundreds of host plant species (Jeppson et al. 1975), and herbivores are generally present in low densities in the wild (Hairston et al. 1960). Therefore, uninfested predator-free patches should always be available for *T. kanzawai* females and patch availability should not be a constraint of between-patch oviposition site selection. Conversely, during dispersal, spider mites suffer costs caused by both biotic (generalist predators; Ozawa & Yano 2009; Yano 2012; Otsuki & Yano 2014; also see Chapter 2, Figs. 5 & 6) and abiotic stressors (e.g. rain; Das 1959). These expected costs in the wild might have made *T. kanzawai* females reluctant to disperse, resulting in the similar between-patch dispersal patterns of predator-experienced and -naive females in this study (3.3.3). I predict that spider mite females can minimize costly between-patch oviposition site selection by micro-oviposition shifts, thereby maximizing their inclusive fitness. Although the adaptive significance has not been sufficiently

explored, micro-oviposition selection to reduce predation risk, induced by current or learned predation risks, may be commonly observed in animals using patchy resources surrounded by hostile environments.

Broad species in the spider mite family (Tetranychidae), consisting of the genus *Tetranychus*, are reported to be social animals (Saito 1995). *Tetranychus* species, such as *T. urticae* and *T. kanzawai*, have slightly advanced subsociality for two reasons: (i) the adult females lay eggs in their nest webs, which leads to temporary cohabitation of mother and offspring (Saito 1995) and to offspring protection from biotic and abiotic stressors outside the webs, as mentioned in the previous paragraph; and (ii) they benefit from living in small groups (Le Goff et al. 2010; Yano 2012). The findings in this study on maternal care by predator-experienced *T. kanzawai* females, which reduces offspring predation, clearly support subsociality in this species.

Chapter 4.

The Stealthiness of Predatory Mites as Spider Mite Biological Control Agents

4.1. Introduction

Predation becomes difficult when the threatened prey adopts defensive behaviors or traits before the attack (Tollrian & Harvell 1999). Therefore, predators have developed various ways to reduce the probability of being detected by their prey, such as creeping and aggressive mimicry (Wickler 1968). From this perspective, stealthiness could be one such key to predation success.

Adult female spider mites change behaviors when they are aware of specialist predatory mites. Females leave the patches containing predators (Bernstein 1984; Grostal & Dicke 1999; Oku et al. 2004; Škaloudová et al. 2007; Bowler et al. 2013) and disperse farther and scatter more than those dispersing in response to resource deterioration (2.3.3, Figs. 7 & 8). Moreover, females that are aware of predators can reduce offspring predation by shifting their oviposition site onto the webs (micro-oviposition shift) (Oku & Yano 2007; Lemos et al. 2010; Murase et al. 2017) where eggs are predated less than those on leaf surfaces (see Chapter 3, Fig. 12). Therefore, whether predatory mites are detected by spider mites should largely determine the success of biological control of spider mites using predatory mites. The utility of predatory mites as spider mite biological control agents has conventionally been evaluated using indices such as the predation rate and intrinsic rate of natural increase (e.g. Janssen & Sabelis 1992; Gotoh et al. 2006). These indices are, however, measured in artificially closed setups where neither spider mites nor predatory mites have opportunities for dispersal. Because the predatory mites eventually consume all of the spider mites under such conditions even if the prey is aware of the predators and tries to avoid predation, the conventional indices may fail to reflect possible differences in predator stealthiness to spider mites.

To quantify predator stealthiness to spider mites, I examined two types of anti-predator behaviors tested in the second and third chapters:

- i. How do the departure rates of spider mite females from predator-intruded patches differ among predator species? (4.2.2)

- ii. How do micro-oviposition shifts by spider mite females in response to predation eggs differ among predator species? (4.2.3)

I examined two commercialized biological control agents used against tetranychid mites, *P. persimilis* (Hussey & Bravenboer 1971) and *N. californicus* (Copping 2001), and *N. womersleyi*, a native predator of *T. kanzawai* in Japan (Hamamura 1986). *Phytoseiulus persimilis* is mainly associated with tetranychid mite such as *T. urticae* and strongly aggregates in prey patches (Zhang & Sanderson 1993). *Neoseiulus californicus* and *N. womersleyi* feed on tetranychid mites as well as mites in other genera producing various types of nest webs (McMurtry & Croft 1997). Although *N. womersleyi* has not been commercialized as a biological control agent, it can control *T. kanzawai* populations under certain conditions (Kondo 2004). To examine whether predator stealthiness is affected by the species and populations of spider mite used, I also used European and Japanese populations of *T. urticae*, a related spider mite species to *T. kanzawai* (4.2.3). Based on these results, I discuss whether the degree of stealthiness is consistent across species and populations of prey mites.

4.2. Materials and methods

4.2.1. Animals

I used *T. kanzawai* study population collected from kudzu vines in 2014 in Kyoto. The study populations of *T. urticae* were collected from rose in 2008–2009 in the northern Netherlands (European population; Khajehali et al. 2011) and from florists' daisy (*Chrysanthemum × morifolium*; Asteraceae) in 1998 in Nara (Japanese population). Each spider mite population was reared on leaf discs.

I obtained a population of *P. persimilis* from a commercial source in Europe (Spidex; Koppert Biological Systems, Berkel en Rodenrijs, the Netherlands), and maintained the population on leaf discs heavily infested with *T. urticae* (Japanese population) as prey. Therefore, the population should not have a coevolutionary history with *T. kanzawai*, which occurs only in Asia (Ehara 1999). The *N. californicus* study population was collected from Nashi pear (*Pyrus pyrifolia* var. *culta*; Rosaceae) in 2000 in Nagano, Japan, and that of *N. womersleyi* from bushkiller in 2014 in Kyoto. These populations were reared on bean leaf discs in the same manner.

4.2.2. Comparing anti-predator dispersal of spider mites among predator species

To quantify the stealthiness of predatory mites, I first examined the predator-induced dispersal within- and between-patch dispersal of *T. kanzawai* females. I introduced three *T. kanzawai* females onto uninfested bent leaf squares (10 × 10 mm) without predators. After 24 h, when the leaf harbored ample prey eggs and three-dimensional webs, two of the three females were removed. Then, I introduced a starved mated female of one of the three predatory mite species onto each bent square. The bent leaf and ample eggs and webs thereon were intended to prevent dispersal of the predators. Ample prey eggs were also intended to delay predation on *T. kanzawai* females because predators seldom attack prey females as long as prey eggs are available. For comparison, I also prepared bent leaves without predators, which served as controls. During 30 min of acclimation, no *T. kanzawai* female was preyed upon by the predator that settled under the spider mite webs. I then placed an uninfested flat leaf square (10 × 10 mm) close to the original bent leaf square so as to form an initial patch with a bent square (Fig. 14). I connected each uninfested leaf square to another uninfested flat leaf square (10 × 10 mm; “second patches”) with a Parafilm bridge (10 × 30 mm; Fig. 14).

I recorded the location and survival status of both *T. kanzawai* females and predatory mites at 10:00, 13:00, 16:00, 19:00, and 22:00 for 3 days, after which predator offspring began to hatch. I stopped the observations when *T. kanzawai* females had dispersed either within or between patches before 3 days had elapsed. I identified which patch each spider mite first settled; I considered a *T. kanzawai* female to have dispersed within the patch if an uninfested area of the initial patch contained webs, injury scars, feces, and eggs, regardless of female presence, whereas I considered the female to have dispersed between patches if the second patch contained these signs.

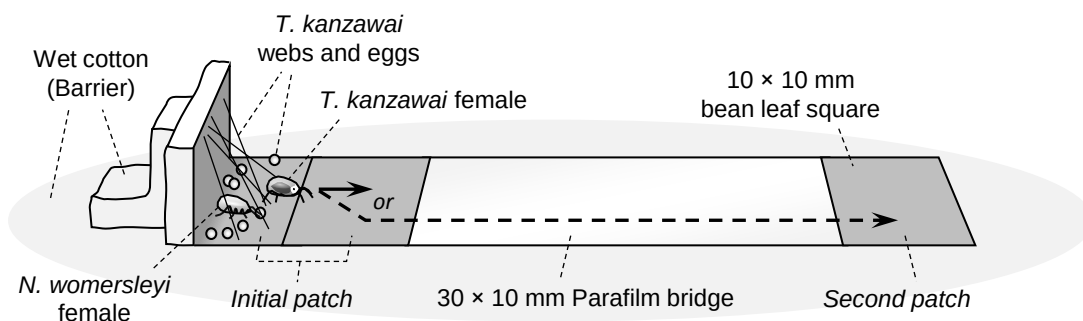


Fig. 14 Experimental setup to investigate the stealthiness of predatory mites: within- (solid arrow) and between-patch (dashed arrow) dispersals of *T. kanzawai* females in response to predator intrusion

The numbers of replications were 39 (without predators), 43 (with *P. persimilis*), 45 (with *N. californicus*), and 45 (with *N. womersleyi*), respectively. The proportion of *T. kanzawai* females that had dispersed from the bent leaf squares (both within- and between-patch) in response to each predatory mite species was compared with the control without a predator using Fisher's exact test. The proportion of *T. kanzawai* females preyed upon during the experiment was compared among predatory mite species using Fisher's exact test. The Holm-Bonferroni correction was used to adjust p-values for multiple comparisons. All of the statistical analyses in this chapter were performed using R v.3.2.2 (R Core Team 2015).

4.2.3. Micro-oviposition shift by spider mites in response to predator eggs

Comparison among predator species

As another stealthiness index of predatory mites, I examined the proportions of spider mite eggs laid on webs in response to predator eggs. I did not use adult predators in this experiment because they consume spider mite eggs, making it impossible to calculate the proportion of eggs on the webs.

I prepared uninfested bent leaf squares (10 × 10 mm). I obtained eggs of the three predatory mite species within 24 h of oviposition, and transferred three eggs of one species onto the bottom of the bent leaf surfaces. I then introduced *T. kanzawai* females singly onto each bent square.

After 24 h, I counted the number of eggs laid on the leaf surface and the web. The proportions of *T. kanzawai* eggs laid on webs were compared among the predatory mite species using the Steel-Dwass test. The numbers of replications were 42 (with *P. persimilis* eggs), 38 (with *N. californicus* eggs), and 41 (with *N. womersleyi* eggs), respectively.

Concealing vs. detectable materials

To examine whether concealing or detectable surface materials were responsible for the different degrees of stealthiness, I compared the proportion of spider mite eggs laid on webs in response to untreated predator eggs and to those washed using a detergent. I hypothesized that removing the surface materials would make the washed *P. persimilis* eggs (the most stealthy, see subsection 4.3.2) more detectable (resulting in more oviposition on webs) if the materials served as concealment; whereas detectable surface materials should make the washed *N. womersleyi* eggs (the least stealthy, see subsection 4.3.2) less detectable (resulting in less oviposition on webs).

I dipped eggs of *P. persimilis* and *N. womersleyi* in kitchen detergent (Kyukyutto; Kao, Tokyo, Japan) diluted 10-fold with water. After 10 s, the eggs were rinsed with a fine

brush in ample distilled water for 10 s to remove the detergent from the eggs. Since the detergent is for dishwashing, it should be completely removed by rinsing in water. I preliminarily confirmed that the treatment did not have harmful effects on predatory mite eggs.

The proportions of *T. kanzawai* eggs laid on webs in response to three untreated or washed eggs of the respective predator species were measured in the manner described above. The proportions were compared between untreated and washed eggs using Mann-Whitney *U*-test. The numbers of replications were 39 (with untreated *P. persimilis* eggs), 37 (with washed *P. persimilis* eggs), 38 (with untreated *N. womersleyi* eggs), and 43 (with washed *N. womersleyi* eggs), respectively.

Comparison among origins of mite population

To examine whether the stealthiness index is consistent across prey mites or is affected by the species and origins of the prey populations used, I also examined the oviposition site pattern using a related spider mite, *T. urticae*. Because the *P. persimilis* used originated from Europe, while *N. californicus* and *N. womersleyi* are from Japan, I used European and Japanese populations of *T. urticae* to examine this question.

The proportions of eggs laid on webs were examined in the manner described above and compared among predatory mite species for the various *T. urticae* populations using the Steel-Dwass test. The numbers of replications were 36 (with *P. persimilis* eggs), 33 (with *N. californicus* eggs), and 34 (with *N. womersleyi* eggs) for the European *T. urticae* population, as well as 31, 34, and 35, respectively, for the Japanese *T. urticae* population.

4.3. Results

4.3.1. Comparing anti-predator dispersal of spider mites among predator species

The within-patch dispersal of *T. kanzawai* females in response to *P. persimilis* and *N. womersleyi* did not significantly differ from that of the control without predators. Only within-patch dispersal in response to *N. californicus* was significantly lower than that of the control (Fig. 15a). Between-patch dispersal of *T. kanzawai* females in response to *P. persimilis* and *N. californicus* did not significantly differ from that of the control, whereas between-patch dispersal in response to *N. womersleyi* was significantly higher than that of the control (Fig. 15b). In addition, the proportion of *T. kanzawai* females preyed upon by *P. persimilis* was the highest, while that by *N. womersleyi* was the lowest. The differences were significant between all pairs of predatory mite species (Fig. 16).

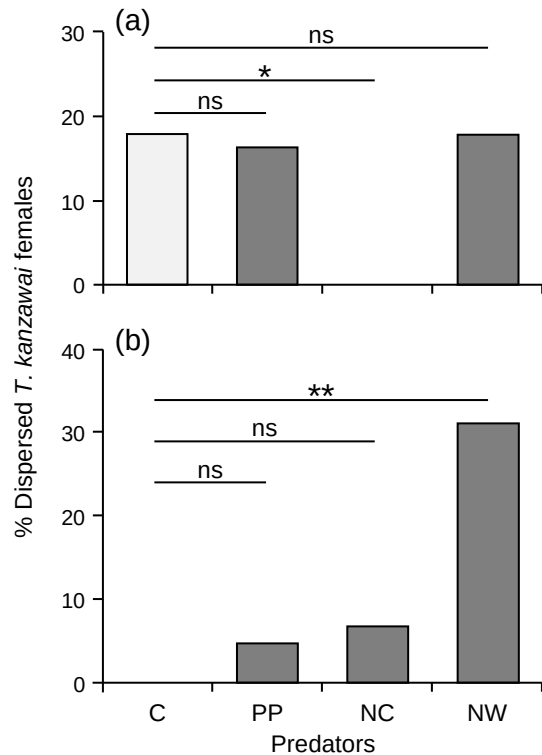


Fig. 15 The proportion of *T. kanzawai* females that dispersed (a) within and (b) between patches. C, PP, NC, and NW indicate the control, *P. persimilis*, *N. californicus*, and *N. womersleyi* treatments, respectively. * $p < 0.05$, ** $p < 0.001$ (Fisher's exact test with Holm-Bonferroni correction)

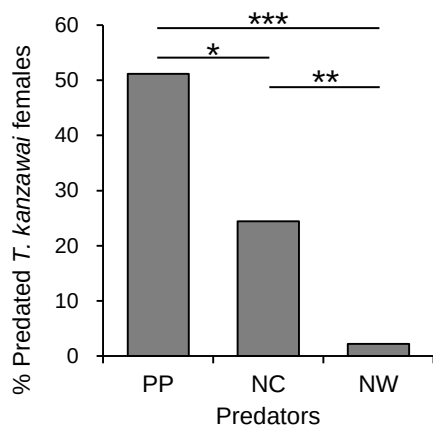


Fig. 16 The proportion of *T. kanzawai* females preyed upon by each predatory mite species. PP, NC, and NW indicate the *P. persimilis*, *N. californicus*, and *N. womersleyi* treatments, respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$ (Fisher's exact test with Holm-Bonferroni correction)

4.3.2. Micro-oviposition shift by spider mites in response to predator eggs

Comparison among predator species

The proportions of *T. kanzawai* eggs laid on webs in response to *N. womersleyi* was the highest (the least stealthy), while that in response to *P. persimilis* was the lowest (the greatest stealthiness). Significant differences were found between all pairs of predatory mite species (Fig. 17).

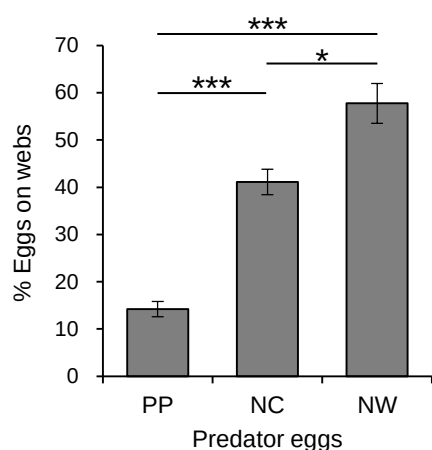


Fig. 17 The proportions (mean $\pm$ SE) of *T. kanzawai* eggs laid on webs in response to eggs of each predatory mite species. PP, NC, and NW indicate the *P. persimilis*, *N. californicus*, and *N. womersleyi* treatments, respectively. * $p < 0.05$, *** $p < 0.0001$ (Steel-Dwass test)

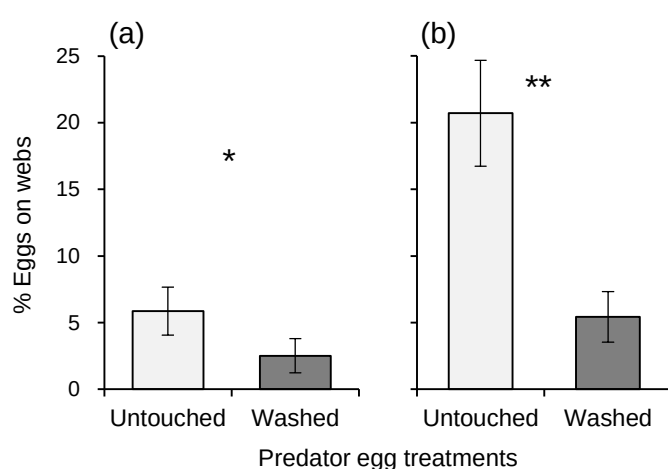


Fig. 18 The proportions (mean $\pm$ SE) of *T. kanzawai* eggs laid on webs in response to untreated and washed predator eggs of (a) *P. persimilis* and (b) *N. womersleyi*. * $p < 0.05$, ** $p < 0.001$ (Mann-Whitney *U*-test)

Concealing vs. detectable materials

The proportions of *T. kanzawai* eggs laid on webs in response to washed *P. persimilis* eggs were slightly lower than that in response to untreated eggs (Fig. 18a), whereas those in response to washed *N. womersleyi* eggs were considerably lower than that in response to untreated *N. womersleyi* eggs (Fig. 18b). These results suggest the existence of detectable, rather than concealing, surface materials on *N. womersleyi* eggs.

Comparison among origins of mite population

The proportions of eggs laid on webs by *T. urticae* females of European and Japanese populations in response to predatory mite eggs showed the same pattern seen using *T. kanzawai* females. Significant differences were found between all pairs, except for the *N. californicus* and *N. womersleyi* pair examined with the European *T. urticae* population and the *P. persimilis* and *N. californicus* pair examined with the Japanese *T. urticae* population (Fig. 19).

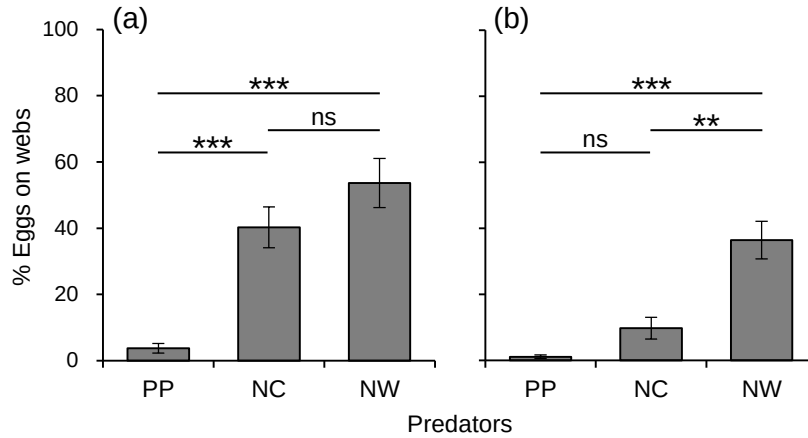


Fig. 19 The proportions (mean $\pm$ SE) of eggs laid on webs by females of (a) European and (b) Japanese *T. urticae* populations in response to eggs of each predatory mite species. PP, NC, and NW indicate the *P. persimilis*, *N. californicus*, and *N. womersleyi* treatments, respectively. ** $p < 0.001$, *** $p < 0.0001$ (Steel-Dwass test)

4.4. Discussion

I systematically measured stealthiness indices of predatory mites, the proportion of *T. kanzawai* females dispersed from their patches in response to predator intrusion, and the proportions of *T. kanzawai* eggs laid on webs in response to predator eggs. I found that both indices resulted in the same ranking across prey species and prey populations, with *P. persimilis* ranking highest and *N. womersleyi* lowest.

Tetranychus kanzawai females dispersed from patches intruded by *N. womersleyi* more often than from patches intruded by *P. persimilis* or *N. californicus* (Fig. 15b). If this result was only due to the relatively low predation rate by *N. womersleyi*, compared to the other two predator species (Fig. 16), then the proportion of dispersed *T. kanzawai* females in the absence of predation (i.e. control) should have been the highest. In contrast, the proportion of dispersed females from control patches did not differ from those in response to *P. persimilis* or *N. californicus* (Fig. 15b). Therefore, I may reasonably conclude that *T. kanzawai* females were less aware of *P. persimilis* and *N. californicus* than of *N. womersleyi*, and hence more females were preyed upon by the former two predator species.

Another stealthiness index of predatory mites, the proportions of spider mite eggs laid on webs in response to predator eggs, showed the same ranking, the highest stealthiness in *P. persimilis* and lowest in *N. womersleyi*. Because all of the predatory mites oviposited during the experiments examining spider mite dispersal, the stealthiness index

described above should in part reflect the stealthiness of the predatory mite eggs. Moreover, the ranking of the stealthiness index was also consistent with both European and Japanese populations of *T. urticae*, indicating that the stealthiness of predatory mites is not affected by the origin of the spider mite or by the predatory mite populations tested. These results suggest that the relative levels of stealthiness are consistent across prey mite species, although their absolute values may depend on the specific combination of prey and predatory mite populations. Therefore, predator stealthiness could be used as a novel index to evaluate the utility of predatory mite populations as biological control agents.

The stealthiness indices of the two commercialized biological control agents, *P. persimilis* and *N. californicus*, were higher than those of *N. womersleyi*. This might not be coincidental if the two predatory mites had been adopted as biological control agents based on their empirical utility in crop fields, which should in part reflect their stealthiness to spider mites. In comparison, *N. womersleyi* seems relatively unsuitable as a biological control agent against spider mites because the predator failed to predate spider mites before dispersal, probably due to its low level of stealthiness (but see Kondo 2004 for a successful case in a greenhouse).

Considering the higher stealthiness of the other two species, *N. womersleyi* might have the potential for improved stealthiness. The low stealthiness (high detectability) of *N. womersleyi* eggs seemed to be due to the existence of detectable surface materials rather than to the lack of special concealing materials (Fig. 18b). Although *N. womersleyi* has much lower stealthiness than *P. persimilis* and *N. californicus*, the mite thrives under natural conditions involving predation of spider mites (McMurtry & Croft 1997). Therefore, the inability of *N. womersleyi* to reduce such maladaptive materials suggests that the materials have unknown adaptive functions, which remain to be addressed in future investigations.

Chapter 5.

General Discussion

I have studied interactions between spider mites and their predators in systems with dispersal opportunities which I developed. We must remember that rearing experimental animals under laboratory conditions usually deprives them of dispersal opportunities and we may observe artifacts under such conditions, i.e. behaviors of target experimental animals that would otherwise have dispersed from the patch. I would like to emphasize that all the results could be adequately obtained in systems where between-patch dispersal of both spider mites and their predators was possible.

The main findings on the questions given in Chapter 1 are as follows:

Answer to Question 1. How do spider mites disperse between habitat patches against specialist or generalist predators? (Chapter 2):

Spider mite dispersal was effectively hindered by generalist predators, which cannot suppress spider mites in closed systems without dispersal opportunities. Spider mite females failed to settle in new patches when they were preyed upon by the ant *P. punctatus* during dispersal, and also when the females refrained from dispersal in the presence of the generalist predatory mite *E. sojaensis*. In contrast, spider mite females could easily escape from patches intruded by the specialist predatory mite *N. womersleyi*, which can suppress spider mites in closed systems. The females dispersed farther and colonized more patches than those dispersing in response to resource deterioration.

Answer to Question 2. Do within-patch defensive behaviors against specialist predatory mites benefit spider mites? (Chapter 3):

Spider mites benefited from oviposition site shifts within patches based on previously experienced predation risks. When higher proportions of spider mite eggs were laid on webs, the specialist predatory mite *N. womersleyi* left the patches with more eggs unpredated. The egg survival on webs was higher than on leaf surfaces.

Answer to Question 3. Is predatory mite stealthiness consistent across spider mite species? (Chapter 4):

Among the three tested specialist predatory mite species (*P. persimilis*, *N. californicus*, and *N. womersleyi*), the stealthiness of *P. persimilis* against spider mites (*T. kanzawai* and *T. urticae*) was consistently the highest and that of *N. womersleyi* was the lowest.

Phytoseiulus persimilis successfully stalked spider mite females and consumed them, whereas *N. womersleyi* induced between-patch dispersal and within-patch oviposition site shift by spider mite females. The stealthiness of predator eggs showed the same ranking, and the lowest stealthiness of *N. womersleyi* eggs seemed to be due to the existence of detectable surface materials.

Because anti-predator defenses incur fitness costs, conditional defensive strategies in response to predation risk would be more advantageous than fixed strategies (Tollrian & Harvell 1999). The key to the success of risk-sensitive defense is an ability to perceive predation risk that varies among/within habitat patches. Spider mites can perceive intruding specialist predators (e.g. Bernstein 1984; Grostal & Dicke 1999; Oku et al. 2003a). Although stealthiness to spider mites differed among predator species, *N. womersleyi* was most detectable to *T. kanzawai* (Chapter 4). Its low stealthiness should have caused the observed predator-prey interactions between *N. womersleyi* and *T. kanzawai* as described above.

Spider mite females selected oviposition sites in response to specialist predatory mites on two scales, between and within patches. Because individuals dispersing from an intruded patch must avoid being tracked by predators (Lima & Dill 1990), dispersing farther away from specialist predators seems advantageous to spider mites. However, extra between-patch dispersal would be costly in the wild, because spider mite females are exposed to harmful abiotic conditions (Das 1959; Jeppson et al. 1975; Sakai & Osakabe 2010) and generalist predators during dispersal, which they avoid by remaining inside their webs on the underside of leaves. Moreover, the probability of being tracked by specialist predators substantially decreases with every between-patch dispersal event where multiple dispersal directions are available. Therefore, the costs of between-patch dispersal will outweigh the benefits of predator avoidance as dispersal steps increase. Therefore, spider mites that minimize dispersal distance and shift oviposition sites within patches after settlement may be favored under some conditions. This may be responsible for the results that some spider mite females that had dispersed from specialist predators settled in adjacent patches (in the second and third chapters). Hinomoto & Takafuji (1995) also showed that spider mites dispersing in response to food deterioration in the absence of predators mostly settle in adjacent patches. In conclusion, the benefits and costs of between-patch dispersal, as well as the benefits and possible costs of oviposition on webs, would have formed spider mite oviposition site use among and within patches.

Contrary to conventional understanding, the results in this study suggest that generalist predators can be more effective agents for conservation biological control against *Tetranychus* species than can specialist predatory mites at least under some

conditions. Unlike specialist predatory mites, generalist predatory mites, which subsist on alternative food resources, would ‘patrol’ host plants of spider mites and hinder the colonization of spider mites particularly under low spider mite density where most patches are left intact. Successful dispersal and subsequent establishment of local populations are crucial for spider mite metapopulation persistence (Levins 1969) because local spider mite populations are often in danger of extinction due to voracious predatory insects (Janssen et al. 1998), coincidental intraguild predation by larger herbivores (Shiotsuka & Yano 2012), and natural or human-induced disturbance (e.g. Das 1959). From this viewpoint, generalist predators would impose long-term costs on spider mites by inhibiting the establishment of local populations and by imposing short-term costs on dispersing females as discussed above. Therefore, conservation of native generalist predators might help maintain spider mites at low endemic densities (Funayama et al. 2015). Outbreaks of spider mites may partly be caused by the absence of those generalist predators due to chemical spraying (Kondo & Hiramatsu 1999) and undergrowth removal (Funayama 2016).

On the other hand, specialist predatory mites with low stealthiness, such as *N. womersleyi*, would scatter spider mite females, increasing the number of infested patches in the area, particularly under low spider mite density. Although specialist predatory mites would impose short-term fitness costs on spider mites by consuming eggs (e.g. Takafuji & Chant 1976), the predatory mites may reduce the extinction rate of spider mite metapopulations in the long term by increasing the number of local populations. Moreover, specialist predators, in general, do not remain on a reward-less patch (Charnov 1976; Symondson et al. 2002). Thus, specialist predatory mites can suppress spider mite populations only under high spider mite density (e.g. Burnett 1979; Janssen et al. 1997; Schausberger & Walzer 2001).

Nonetheless, specialist predatory mites with low stealthiness may not always be useless for controlling spider mites. By making spider mites move out of their protective webs and disperse between patches, the specialist predators would enhance the lethal/non-lethal effects of generalist predators that cannot consume spider mites inside the webs (Otsuki & Yano 2014). Although predator-induced prey dispersal seems maladaptive for predator individuals in the short term, it may, in turn, contribute to a stable prey supply for future offspring habitats. In other words, specialist predatory mites with low stealthiness may ‘pasture’ female prey mites, as long as prey eggs are abundant within the intruded patch. Therefore, it is necessary to discuss whether high/low stealthiness is advantageous to specialist predatory mites in light of the metapopulation dynamics of both prey and predatory mites.

In summary, biological control of spider mites would have still room to consider the importance of generalist predators, and to consider adopting new indices for evaluating conventionally used specialist predatory mites. Generalist predators should be useful under low spider mite density. In contrast, under high spider mite density, where most habitat patches are covered by defensive webs of spider mites and hence generalist predators have little chance to attack spider mites, specialist predatory mites with high stealthiness would be effective. This study will contribute to the efficient use of biological control agents for successful sustainable management of spider mites, and suggests a need for further experimental studies, which should, of course, be conducted in systems with dispersal opportunities.

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List of Publications

Chapter 2

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The final publication is available at Springer via
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Chapter 3

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Chapter 4

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