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## RESEARCH ARTICLE OPEN ACCESS

# Females Guarded by Sneaker Males Experience Higher Predation in the Two-Spotted Spider Mite

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## ABSTRACT

Males often employ different reproductive tactics to gain access to females based on their condition and the surrounding environment. Predation risk is expected to have a significant influence on the frequencies of alternative reproductive tactics because these tactics typically differ in activity, which may result in differences in predation rate. In theory, such predation effects can explain the evolution as well as the maintenance of alternative reproductive tactics. Yet, there is little experimental work testing how predation risk affects alternative reproductive tactics. To assess such effects of predation, here we report on experiments with the two-spotted spider mite *Tetranychus urticae*. The two-spotted spider mite is a small arthropod herbivore species, in which males exhibit precopulatory mate guarding by mounting preadult moulting (and hence immobile) females. Two reproductive tactics are observed during mate guarding: The fighting tactic involves attacking other males to drive them away, while the sneaking tactic involves mounting the females and remaining motionless, even when contacted by other males. In this study, we exposed pairs of male and female spider mites to a predatory mite (*Phytoseiulus persimilis*) and observed their survival and male response to a predator when females were guarded by either fighter or sneaker males. We found that predation risk was not significantly different between fighter and sneaker males. However, the immobile females were more often preyed upon when guarded by sneakers than when guarded by fighters. We attribute this indirect effect of predation risk to the sneakers continuing to mount females even when a predator is nearby.

## 1 | Introduction

In general, males compete over access to females for reproduction (Andersson 1994). When the male competition is intense (higher male density, higher male aggression, etc.), they often use alternative reproductive tactics depending on their condition and their surrounding environment (Brockmann 2001;

Gross 1996; Oliveira, Taborsky, and Brockmann 2008). For example, in natterjack toads (*Bufo calamita*), males call to attract females, but some smaller males do not call and parasitise callers to intercept females attracted by callers (satellite tactic). Males frequently adopt the satellite tactic when they are close to larger males with loud calls (Ark 1988). Reproductive activity such as searching, attracting, approaching and guarding

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mates, however, is also vulnerable to predation risk (Lima and Dill 1990; Magnhagen 1991; Zuk and Kolluru 1998). Therefore, predation pressure is likely to have a significant impact on the choice of reproductive tactic. Indeed, there is some empirical evidence for effects of predation risk. For instance, males of the guppy, *Poecilia reticulata*, exhibit sigmoid display to approach females, however, in the presence of predators males refrain from this displaying behaviour, and instead sneak-copulate with females (Godin 1995). Also, males of the tree cricket, *Oecanthus henryi*, commonly call to attract females. When predation risk increases, however, most males stop calling, move towards other callers and display a satellite tactic (Torsekar and Balakrishnan 2020).

These examples notwithstanding, empirical studies into the effects of predation on the evolution and maintenance of alternative reproductive tactics are still quite limited (Torsekar and Balakrishnan 2020). Indeed, alternative reproductive tactics appear primarily functional in conspecific intrasexual competition for mating opportunities (Oliveira, Taborsky, and Brockmann 2008), so it makes sense that the primary focus of the study is on intraspecific interactions. Nevertheless, predation risk is expected to influence the evolution and maintenance of alternative reproductive tactics whenever predators differentially affect the reproductive success of the alternative reproductive tactics (Torsekar and Balakrishnan 2020). Here, we study how predation risk affects alternative reproductive tactics in an arthropod herbivore: the two-spotted spider mite *Tetranychus urticae*. Mites of this species live in groups under complicated silk webs constructed on the surface of host plants. In these groups, male competition over access to females is very intense, especially because males need to mate with unmated females to reproduce. Females of this mite mate with multiple males. Unless the first mating is interrupted, however, females use sperm only from the first mating to fertilise the eggs they lay throughout their life (Helle 1967; Morita et al. 2020; Potter and Wrensch 1978; Satoh, Yano, and Takafuji 2001). Therefore, the male mites often engage in precopulatory mate guarding by mounting preadult moulting females (teleiochrysalis stage), and fight with rival males for the females during the mate guarding (Potter, Wrensch, and Johnston 1976a, 1976b).

While engaged in precopulatory mate guarding, males show two different reproductive tactics: the fighting tactic, in which the male attacks other males to drive them away, and the sneaking tactic, in which the male continues to mount the female and remains motionless even when contacted by other males, resulting in avoidance of attacks from other males (Sato et al. 2013). The reproductive tactics are flexible; males often exhibit the fighting tactic but change to the sneaking tactic depending on their condition and surrounding environments. For example, young males tend to be sneakers, whereas older males are often fighters (Sato, Sabelis, and Egas 2014). This age-dependent reproductive tactic makes sense from a life-history perspective: Young males may avoid fighting for future reproduction as fighting incurs a survival cost (Sato et al. 2016). Young fighters, who are surrounded by old, aggressive fighters during the precopulatory mate guarding, often change their tactic to sneaking. The change of tactic to sneaking increases the success rate of keeping the mounting

position against older fighters (Sato, Sabelis, and Egas 2014). In addition, it is known that the proportion of sneakers changes depending on male density (Sato, Sabelis, and Egas 2014). Body length and width were measured in fighters and sneakers, but no significant difference was detected between them (Sato et al. 2013). Which tactic a male is taking can be determined by an artificial disturbance test; when we make the legs of a male placed on a wet brush tip touch a mounting male, it can be judged a fighter if the mounting male shows a fighting posture, or a sneaker if it does not show any response and continues to be mounting (Sato et al. 2013).

Predation pressure is an important factor in the evolution of herbivores, affecting them in diverse ways, including defence mechanisms, adaptation to environments and changes in reproductive strategies. Indeed, one of the features of *T. urticae* is the construction of complicated webs on the leaf surface of host plants function, and the webs function as shelters against predators (McMurtry, Moraes, and Sourassou 2013; McMurtry and Croft 1997). However, the webs are not effective against all predators. A few predatory mites, such as *Phytoseiulus persimilis*, are able to invade the webs (McMurtry, Moraes, and Sourassou 2013; McMurtry and Croft 1997). All life stages of *P. persimilis* prefer to not only prey on the eggs but also prey on the juveniles and adults. In particular, the females guarded by males are more likely to be preyed upon by *P. persimilis* than the solitary females (Oku et al. 2018). Phytoseiid mites do not have eyes and rely on chemical cues for searching prey (De Boer and Dicke 2006; Sabelis and Van de Baan 1983). Differences in odour have been found between a teleiochrysalis female with guarding male and a solitary teleiochrysalis female (Oku et al. 2015), and the difference was considered to cause the difference in predation risk between guarded and solitary females (Oku et al. 2018). Therefore, not only in the presence of *P. persimilis* but also in the presence of chemical cues of predation, *T. urticae* often reduces or delays its activities including reproduction (Choh, Uefune, and Takabayashi 2010; Fernández Ferrari and Schausberger 2013; Grostal and Dicke 1999; Pallini, Janssen, and Sabelis 1998, 1999; Rocha et al. 2020; Škaloudová, Zemek, and Křivan 2007). However, those males that continue reproductive activities are expected to display the reproductive tactics with lower predation risk. To date, however, it is unknown which tactic is more vulnerable to predators. In order to investigate the influence of predation risk on male reproductive tactics, it is first necessary to determine which tactic yields greater predation risk.

Therefore, in this study, we investigated which reproductive tactic, fighting or sneaking, makes either male or female *T. urticae* mites more vulnerable to predation by *P. persimilis*. We introduced a starved, gravid female predator into an arena where a fighter or sneaker male was guarding a female. Then, we observed and compared the probability of being preyed upon between fighters and sneakers and between females guarded by fighters and sneakers. Additionally, we observed the male response to the predator and compared the timing of when they stopped guarding and retreated from the predator. We predicted that sneaker couples would be at higher risk of predation than fighter couples, as sneaker males do not respond to stimuli and would, therefore, continue to guard in the presence of predators.

## 2 | Materials and Methods

### 2.1 | Mites

We used the strain 'Houten-1' of *T. urticae*, in which alternative reproductive tactics for males have been first described (Sato et al. 2013) and therefore used in the previous studies associated with the tactics, obtained from the Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, in 2021. We reared the mites at the University of Tsukuba on detached leaves of common bean, *Phaseolus vulgaris* var. Naga-Uzura, placed on wet cotton wool in styrofoam and plastic trays. Some of the rearing mites were used for experiments and some for rearing *P. persimilis*. We used *P. persimilis* obtained from Arysta Life Science (Japan) in 2020. We reared *P. persimilis* with *T. urticae* on detached leaves of common beans at the University of Tsukuba. The mite rearings were carried out under about 25°C and L15:D9 h photoperiod. In the experiment, *T. urticae*, which had never been in contact with *P. persimilis*, and *P. persimilis*, which had grown by feeding on *T. urticae*, were used.

### 2.2 | Experiment

To obtain unmated 0-day-old adult males of spider mites, we collected teleiochrysalis males from the *T. urticae* colony. We placed five teleiochrysalis males on separate bean leaf discs (1.5 cm diameter), which were placed on wet cotton wool in insect breeding dishes (5 cm diameter, 1.5 cm high; SPL Life Sciences, Gyeonggi-do, Korea) (hereafter called mating arena). We checked their emergence and used the males, which emerged within 24 h (0-day-old males) in the experiment. When some of the five males did not emerge or were dead, we replaced them with 0-day-old males, which were prepared in the same way. To prepare starved gravid females of *P. persimilis*, we collected gravid females from the *P. persimilis* culture and placed them into 1.5-mm microcentrifuge tubes individually. We placed a piece of bean leaf into the tube together as a source of water for the mites. We kept them for more than 48 h to starve them so that they would be interested in and attack prey, and then used them in the experiment.

We placed a teleiochrysalis female collected from the *T. urticae* colony on a mating arena where five unmated 0-day-old males were present. For males, it takes time to find and mount teleiochrysalis females, so every hour for four hours after the female introduction, we checked whether one of the five males mounted the teleiochrysalis female.

When we detected the presence of a mounting male, we checked the reproductive tactic of each mounting male (fighter or sneaker) using the artificial disturbance test (Sato et al. 2013). We removed the other four males from the mating arena, and then introduced a starved gravid predator. We observed their behaviour for 30 min and recorded predator-prey contact, whether contact was made with a male or female, predation on (killed and eaten) female/male, continued/stopped male mounting, male retreat and those timings. If the predatory mite preyed on both the male and female spider mites within 30 min, we stopped observation at that point. We

carried out these manipulations under the same conditions as those used for mite rearing. The number of replicates was 30 each for fighters and sneakers.

### 2.3 | Statistical Analysis

For the statistical analyses, we used the software R v.4.0.2 (R Core Team 2024). We categorised the timing with which males stopped mounting to retreat from a predator into three: before contact with a predator, after contact with a predator and not at all (males remained mounted even after contact with a predator). Because a male appears as part of the female he mounts, we regarded predator contact with a female as the same as contact with a male. To determine whether the probability of being preyed on differed between fighters and sneakers and between females guarded by fighters and by sneakers, we analysed the probability of being preyed with male reproductive tactics (fighter and sneaker), male retreat timing (before contact, after contact or no retreat) and the interaction using a generalised linear model with binomial error distribution (binomial GLM) in each sex. We tested the effects of explanatory variables in the GLMs using a likelihood ratio test (LRT). When the effect of interaction was not significant, the interaction was removed from the model and re-analysed. We confirmed there was no overdispersion problem in the binomial GLMs. To determine whether the response to a predator differed between fighters and sneakers, we analysed the male retreat timing (before contact, after contact and no retreat) with male reproductive tactics by a multinomial log-linear model. We tested the effect of explanatory variable in the model using an LRT. For the multinomial model, the package *nnet* was used (Venables and Ripley 2002). We used the odds ratio (OR) by dividing the odds (preyed on/survived) of sneaker males or of sneaker-guarded females by the odds of fighter males or fighter-guarded females to show the results of comparison between tactics in each sex clearly.

## 3 | Results

For both males and females, the predation probability was not significantly affected by the interaction between male reproductive tactics and male retreat timing (Table 1a,c). Therefore, the predation probability was re-analysed without the interaction. In the 30-min observation, either the male, the female or both male and female were preyed on in 76.7% of fighter couples and in 96.7% of sneaker couples (Figure 1a). In males, the predation probability was not significantly different between fighters and sneakers (odds ratio: 0.585; Table 1b; Figure 1b). However, the predation probability was significantly higher for females guarded by sneakers than for those guarded by fighters (odds ratio: 4.571; Table 1d; Figure 1b).

The male retreat timing seemed to be different between fighters and sneakers, although not statistically significant (multinomial log-linear model:  $df = 2$ ,  $LRT = 4.541$ ,  $p = 0.103$ ). In fighters, 20% of males (6 out of 30 males) stopped mounting and ran away before contact with a predator, but in sneakers, only 3% of males (1 out of 30 males) stopped mounting and ran away before contact with a predator (Figure 2a). Most

males retreated from a predator after contact with a predator regardless of the male tactic (Figure 2a). The male retreat timing significantly affected the predation probability in males (Table 1b): nonretreating males were more preyed on than retreating males (Figure 2a,b). In females, no clear trend was

detected between the predation probability and male retreat timing (Table 1d; Figure 2a,b).

#### 4 | Discussion

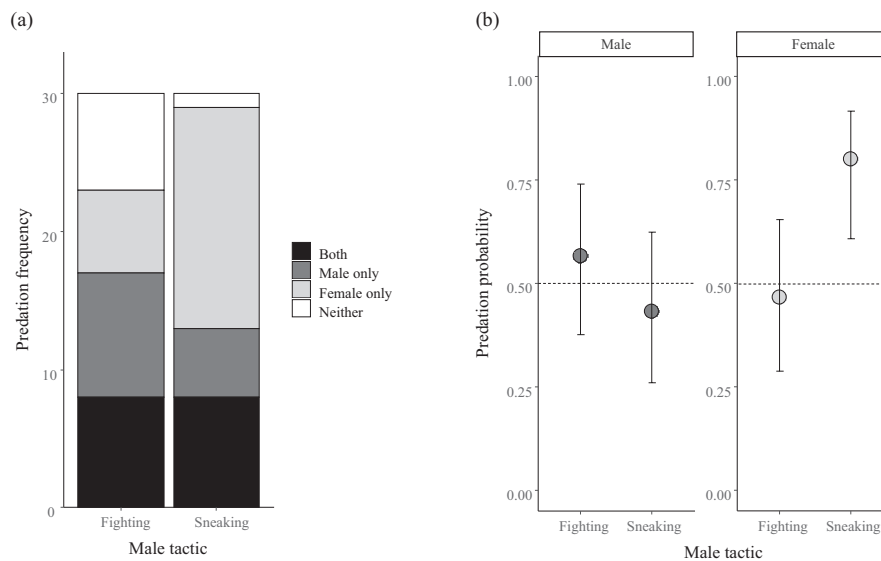
In this study, we investigated the predation effects on fighter and sneaker males, and the females they were guarding, of the two-spotted spider mite under attack by the predatory mite, *P. persimilis*. The predation probability during precopulatory mate guarding was not significantly different between fighter and sneaker males. However, females guarded by sneaker males were more preyed upon than those guarded by fighter males. Precopulatory pairs often attract more attention from predators than solitary individuals (Cothran 2004; Fairbairn 1993). This is also the case in the system of *T. urticae* and *P. persimilis*; the females guarded by males are more likely to be preyed upon by predators than the solitary females (Oku et al. 2018). In addition, females are much more vulnerable to predation than males in precopulatory pairs (Oku et al. 2018) because males can run away, but females cannot due to being in the quiescent stage of moulting. In our observation, sneakers tended to stay in the mounting position even when a predator was nearby. Although the effect of male retreat timing on the predation probability was not significant in females but in males, delayed timing of retreat from a predator by sneaker males may have increased the predation probability for the females they guarded.

There are two possible explanations for why the sneakers did not stop mounting females even when a predator was nearby. One possible explanation is that fighters, who are always vigilant for approaching rival males, may be aware of predator invasion quickly, while sneakers, who continue to mount the female and remain motionless even when contacted by other males,

**TABLE 1** | Results of likelihood ratio test (LRT) in generalised linear models with binomial error distribution in which explanatory variables are male reproductive tactics ('Tactics'), male retreat timing ('Retreat timing') and the interaction as the saturated model for male survival (a), the model without interaction for male survival (b), the saturated model for female survival (c), and the model without interaction for female survival (d).

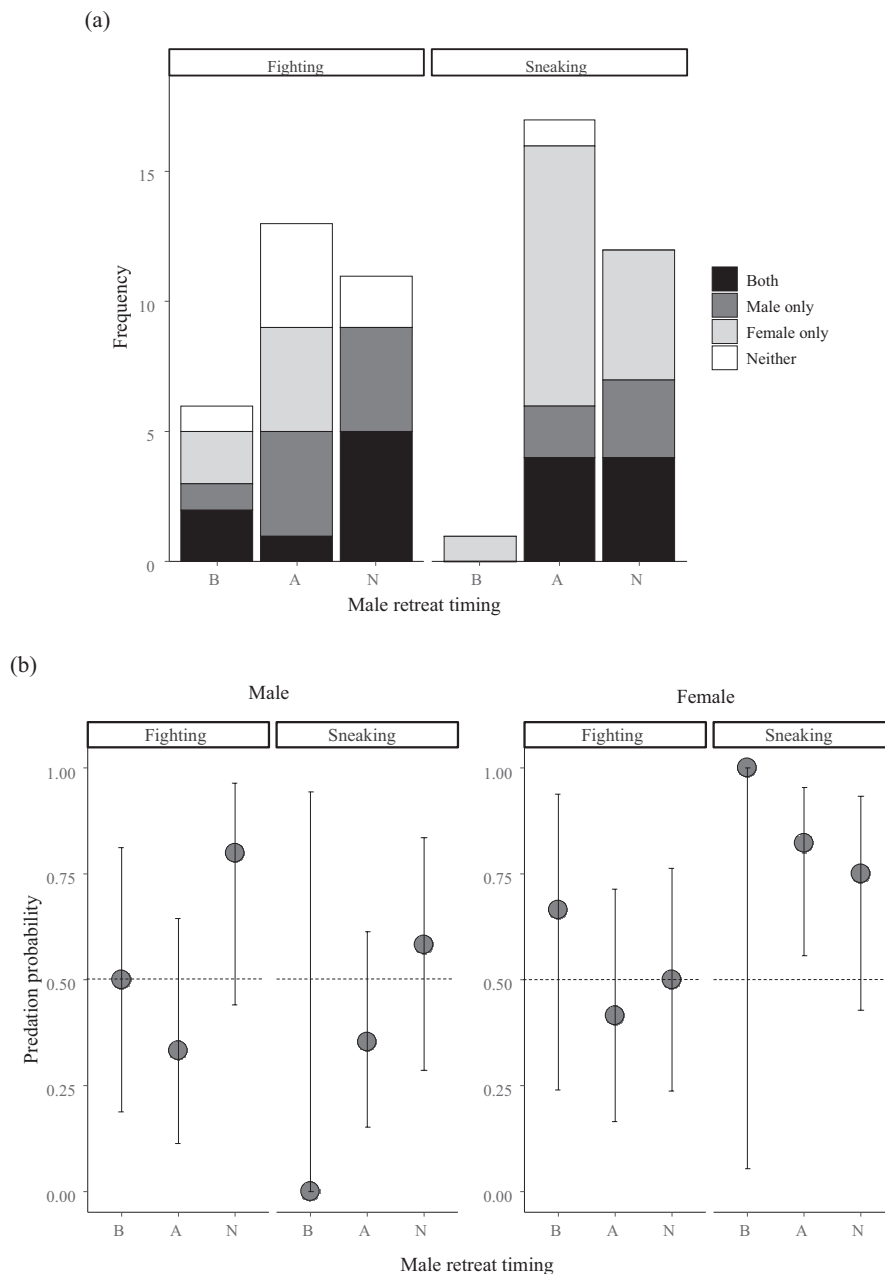
Explanatory variable	df	LRT	p
(a) Saturated model for male survival			
Tactics × Retreat timing	2	1.516	0.469
(b) Model for male survival without interaction between tactics and retreat timing			
Tactics	1	1.294	0.255
Retreat timing	2	6.145	0.046*
(c) Saturated model for female survival			
Tactics × Retreat timing	2	0.516	0.773
(d) Model for female survival without interaction between tactics and retreat timing			
Tactics	1	8619	0.003**
Retreat timing	2	1.504	0.471

Note: \* $p < 0.05$ , \*\* $p < 0.01$ .



**FIGURE 1** | Predation outcomes for male–female pairs of spider mites (*Tetranychus urticae*) exposed to a predatory mite (*Phytoseiulus persimilis*) when males use precopulatory mate guarding tactics. (a) The frequency of predation on males, females or both, when males used either tactic. (b) The probability of predation on males (including trials where both males and females were preyed upon) and females (including trials where both males and females were preyed upon), when males used either tactic. The number of replicates is 30 for both fighters and sneakers. Error bars in (b) show the 95% confidence intervals obtained by *prop.test* (Newcombe 1998a, 1998b; Wilson 1927) in the package *stats*, the software R v.4.0.2 (R Core Team 2024).





**FIGURE 2** | Predation outcomes for male–female pairs of spider mites (*Tetranychus urticae*) exposed to a predatory mite (*Phytoseiulus persimilis*) when males using either tactic had different timing of retreat from predators (A: after contact with a predator, B: before contact with a predator, N: no retreat even after contact with a predator). (a) The frequency of predation (males, females or both). (b) The probability of predation on males (including trials where both males and females were preyed upon) and females (including trials where both males and females were preyed upon). Error bars in (b) show the 95% confidence intervals obtained by *prop.test* (Newcombe 1998a, 1998b; Wilson 1927) in the package *stats*, the software R v.4.0.2 (R Core Team 2024).

may pay less attention to the surroundings and therefore notice predator invasions later. This explanation is very difficult to test because that requires to determine whether they are aware of the approaching predator but choose not to escape or whether they are unaware and therefore do not escape.

The other possible explanation is that stopping the current mounting is more costly to the sneakers than to the fighters, so they might have continued to mount and only gave up at the last instant. When a male is approached by a suspected predator object during precopulatory mate guarding, immediate retreat may

ensure their survival. However, if it turns out not to be a predator, retreat may not be necessary and could lead to a loss of mating opportunity (Ydenberg and Dill 1986). In addition, delayed retreat may allow them to gather information about the approaching object and avoid unnecessary retreat. Therefore, the timing of retreat may change depending on the trade-off between the amount of effort expended on current precopulatory mate guarding and future mate opportunities (Ydenberg and Dill 1986). For example, in the triplefin blenny, *Enneapterygius etheostoma*, there are two different male reproductive tactics; territorials in which males hold a small territory as a spawning site with territories,

and sneaking in which males slowly approach the spawning sites and get as close to the females in the spawning site as possible to obtain a better ejaculation position to fertilise eggs (Ota 2019). In the presence of its predator, the marbled rockfish, *Sebastes marmoratus*, sneakers that are closer to females have been found to have slower timing to escape (Ota 2020). This is thought to be due to the fact that the closer the distance to the female, the more reproduction opportunities are lost through escape (Ota 2016). In the tree lizard, *Urosaurus ornatus*, there are territorial and non-territorial males, and territorial males escape later and allow predators to get closer (Thaker, Lima, and Hews 2009). This is thought to be because territorial males avoid the loss of reproduction opportunities due to conspecific males' invasion into their territory, rather than the cost of predation. In this spider mite, male reproductive success is achieved by mounting females that are close to emerging into adulthood. A sneaker male does not fight, making it difficult to mount a female if a fighter male is already there. On the other hand, a fighter can drive away a mounted male by fighting. Given these options, it would make sense that sneaker males would start mounting females earlier than fighter males, that is, sneakers spend more time for mounting until copulation with females. Therefore, it is predicted that the loss of mating opportunities due to retreating from the female may be greater for sneakers than for fighters, and this may be the reason why sneakers escape from predators later, as in sneakers of the triplefin blenny and territorial males of the tree lizard (Thaker, Lima, and Hews 2009). Both of these two potential explanations are likely, and they are not mutually exclusive.

So far, we have discussed the reasons why females guarded by sneaker males are more likely to be preyed upon by predators than females guarded by fighter males, from the viewpoint of male behaviour. However, it is also necessary to consider the effects of differences in the females themselves. Guarded females are more attractive to males (attract additional males) than unguarded females (Potter, Wrench, and Johnston 1976b) and more likely to be preyed upon by predators (Oku et al. 2018). Odour differences were also detected between guarded and unguarded females (Oku et al. 2015). It is possible that odour may differ between females guarded by sneaker males and females guarded by fighter males. Indeed, Schausberger and Sato (2019) observed synchrony in male reproductive tactics at a higher rate than expected by chance when different males guarded the same female in succession. We cannot exclude the possibility that odour differences are an underlying factor that affects the tactic displayed by guarding males as well as the predation risk of females. Therefore, differences in female odour will also need to be investigated in future.

This study does not provide a clear answer to the question of which tactic is more vulnerable to predators. This is because fighters and sneakers did not exhibit a significant difference in the predation probability. However, behavioural observations showed that sneakers tended to stay in the mounting position even when a predator was nearby. This suggests that the results could be different if experimental designs and environments were different. For example, in this experiment, we used a small bean leaf disc (1.5 cm diameter) without any structures for precopulatory guarding and predation arena. However, *T. urticae* usually construct complicated webs on the leaf surface. Although specialist predators such as *P. persimilis* can intrude the webs, spider mites constructing

complicated webs change their oviposition and quiescent sites from leaf surfaces to webs to hide from the predators (Oku, Yano, and Takafuji 2003; Oku and Yano 2007; Otsuki and Yano 2017). If we had used a larger bean leaf disc with a structure, which allows the males to hide, fighters who retreat quickly likely avoid predation more. In addition, if the reason why retreat timing was slower in sneakers is due to greater losses in reproductive opportunities, as discussed above, males should not take a sneaking tactic under predation pressure, that is, in an environment that forces frequent interruptions to the mounting behaviour. Above all, sneaking tactics lead to a significant loss of available females, that is, mating opportunities, as females guarded by sneakers were more preyed on than those by fighters. These results suggest that males should not take sneaking tactics under predation pressure. It would be very interesting, then, to see whether males avoid sneaking tactics in response to cues of predation. Previous studies have shown that not only the condition and environments that the males experienced but also the condition and environment that their parents experienced affect the reproductive tactics and the associated behaviour of the males (Sato, Egas, and Schausberger 2023; Schausberger, Gotoh, and Sato 2019; Schausberger and Sato 2019). For example, sneaker-mated mothers and mothers who experienced a female-biased operational sex ratio produce sneakers that initiate mate guarding earlier. In both cases, it is considered that the mothers predict intense male competition in their son's generation based on sex ratio and mating partners, and alter their sneaker sons' mating behaviour to give their sons an advantage in intense male competition (Sato, Egas, and Schausberger 2023; Schausberger and Sato 2019). Therefore, it would be worth to see whether males avoid sneaking tactics when their parents experienced predation risk, also.

In conclusion, predation risk did not differ significantly between fighter and sneaker males. However, sneaker males continued to mount females even when a predator was nearby. It is likely that differences could have been observed if the experimental design had not been as simple as it was in this study. On the other hand, immobile females were predated more frequently when guarded by sneaker males than when guarded by fighter males. This suggests that sneaking tactics lead to a significant loss of available females. These results suggest that males should not display sneaking tactics under predation pressure. As a maternal effect on reproductive tactics was previously reported, it is important to test for maternal effects on reproductive tactics of males in the presence of predators as well.

#### Author Contributions

**Taito Sano:** conceptualization, investigation, writing – original draft. **Tanvi Gurjar:** investigation. **Martijn Egas:** conceptualization, writing – review and editing. **Yukie Sato:** conceptualization, writing – original draft, writing – review and editing.

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## Ethics Statement

The authors have nothing to report.

## Consent

The authors have nothing to report.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

The data sets generated during and/or analysed during the current study are available from figshare (<https://doi.org/10.6084/m9.figshare.26969029.v1>).

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