

Article

Effect of familiarity and morphological traits on male mating precedence in *Phytoseiulus persimilis* and *Neoseiulus californicus* (Acari: Phytoseiidae)

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Abstract

Male competition to fertilize the eggs of females would result in conflicts between the members of one sex and evolution of secondary behavioral and morphological characters. Here, we have investigated the effect of familiarity and morphological traits on *Phytoseiulus persimilis* Athias- Henriot and *Neoseiulus californicus* (Oudemans) (Acari: Phytoseiidae) mating combat and precedence. Our results showed no significant difference between the number of familiar and unfamiliar *P. persimilis* / *N. californicus* males mated first. The values of ANOVA to compare the mean length of the first leg, Palp, 2nd cheliceral segment, movable digit and spermatodactyl in prevailed familiar, prevailed unfamiliar, defeated familiar, defeated unfamiliar males in male mating precedence experiment was calculated. None of the morphological characters affected the mating precedence in *N. californicus*. The length of the cheliceral segments did not affect the male mating precedence in *P. persimilis*. Tukey HSD post-hoc test for *P. persimilis* showed that both the first leg and palp were significantly longer in prevailed familiar males rather than that in defeated unfamiliar. Also a significant difference was observed between the length of the palp in prevailed familiar and unfamiliar individuals. The spermatodactyl length was significantly more in prevailed familiars in comparison with defeated ones although no significant difference between prevailed familiars and defeated unfamiliar in *P. persimilis*. The relationship between morphological characters, mating precedence and inbreeding depression is discussed.

Key words: Behavior; competition; familiarity; mating precedence; morphology.

Introduction

Male arthropods need to apply different strategies to increase their reproductive success within male-male competition. Male-male conflicts have led to an evolution of mating systems and (behavioral and morphological) adaptations in males for

outcompeting the rivals (Choe and Crespi 1997). Marden and Waage (1990) found that fat reserves of male damselfly, *Calopteryx maculata* (Palisot) affected its ability to win territorial quarrels and increase their reproductive success. Zizzari *et al.* (2013) found that the collembolan *Orchesella cincta* (Linnaeus) (males), a species with dissociated sperm transfer, responded strongly to the presence of a male competitor (by producing more attractive spermatophores), irrespective of the reproductive status of the rival. Blanckenhorn (2005) reported that sexual size dimorphism could cause some important behavioral (mating) consequences.

Mating as a critical process in phytoseiid populations has few studies and is mostly limited to continuous observations over some hours to record the detailed behaviors of male and female. Amano and Chant (1978) proposed two types on mating behavior in phytoseiid mites and called them “*Amblyseius-Typhlodromus* type” in which the males climbed on the female dorsum during the pre-copulatory period and “*Phytoseiulus* type” in which the male moves directly under the female without any climbing. Woyke (1994) studied the mating behavior of *Tropilaelaps clareae* Delfinado and Baker (Acari: Laelapidae) and reported no special courtship behavior prior to mating. Multiple mating has been described in several species i.e. *Amblyseius cucumeris* (Oudemans), *Phytoseiulus macropilis* Banks and *Amblyseius gossipi* Elbadry (Elbadry and Elbenhawy 1968; Castagnoli and Ligouri 1991; Prasad 1967).

Few studies have demonstrated the male-male competition and its effects on mating behavior in phytoseiid mites. Dehghani *et al.* (2015) investigated the effect of mating experience and territoriality on the male mating behavior in two phytoseiid species. They reported that naïve *P. persimilis* males were mostly the ones who were able to mate with the females first, while in *Neoseiulus californicus* (McGregor), previous experience did not have any significant effect on the male behavior. Territoriality also had no significant effect on male mating competition either in *P. persimilis* or *N. californicus*. Strodl and Schausberger (2012) reported that familiarity had a significant effect on foraging traits of *P. persimilis* but they did not address any effect on its mating behavior. Muleta and Schausberger (2013) showed that *P. persimilis* females joined familiar groups to optimize reproduction. Griffiths *et al.* (2004) demonstrated that familiar members of a group had higher efficacy and less needed attention in comparison with unfamiliar.

Environmental stressors might cause phenotypic plasticities in body size. Male body size, influenced by natural and sexual selection, affects its biological and ecological behaviors during lifetime (Walzer and Schausberger 2014). Walzer and Schausberger (2014) assessed the mating and reproductive traits of small and standard-sized males of *P. persimilis* and *N. californicus*. They reported that small body size did not affect the number of fertilized females but decreased the lifetime reproductive success (LRS) in *P. persimilis*.

Here, we have questioned whether the mating precedence of the predatory mites, *Phytoseiulus persimilis* and *Neoseiulus californicus* would be affected by the male familiarity [the ability to recognize conspecific individuals following prior association, Strodl and Schausberger (2012)] with the mating female. Also, we investigated whether male morphological characteristics make some males introduced to the mating females sooner than the others. The morphological characteristics we considered (Walter and Proctor 1999; Sato *et al.* 2013) were: the length of the first leg, palpi, the second cheliceral segment, and the movable digit and spermatodactyl.

Material and Methods

Host plants and pest mites

Common bean plants [*Phaseolus vulgaris* L. (Fabaceae)] were grown in plastic pots containing perlite. Plants were irrigated every day by tap water and fertilizer solution of NPK (20×20×20) and were kept in controlled conditions (23±2 °C, complete photoperiod, 60–70% RH) in growth chambers at the Mite Behavior Laboratory, Department of Plant Protection, Faculty of Agriculture, University of Tehran, Karaj, Iran. Spider mites (*Tetranychus urticae* Koch) were reared on bean plants in a separate greenhouse (24±2 °C, complete photoperiod, 65±5% RH). New fresh bean plants were added into the rearing system regularly. The spider mite colony originated from the experimental greenhouse of the Acarology Laboratory in Jalal Afshar Zoological Museum.

Predatory mites

The predatory mites (*P. persimilis*/ *N. californicus*) were reared on detached bean leaves infested with *T. urticae* in the germinator (25±2 °C, 16L: 8D hour photoperiod and 70 ± 5% RH). They were kept on several detached bean leaves, placed upside down on a plastic sheet that was on a water saturated sponge. The plastic sheet was surrounded by napkin tapes, which were put into the water from another side so that the predatory mites could drink water. The predator colonies originated from the experimental greenhouse of the Acarology Laboratory in Jalal Afshar Zoological Museum.

General experimental conditions

We prepared the predatory mites for the experiments by randomly selecting a mated female from the main predator colony (for each of the predator species). The mated females started to reproduce for several generations, so that iso-female lines (mother-son mating) were prepared. As we needed same-aged females for each experiment, several adult females were transferred to another leaf disc for oviposition. After 24 hours the females were removed and the eggs were monitored until the mites reached the deutonymphal stage (about 4 days). The same-aged male or female deutonymphs were placed to separate Petri dishes singly to avoid mating. All experiments were performed under controlled conditions (25±2 °C, 16L: 8D hour photoperiod and 70±5% RH).

The effect of familiarity on mating precedence

Two iso-female lines (A and B) were prepared for each of the predator species. Experimental arenas were consisted of spider mite infested bean leaves (1.7×1.7 cm²) put upside down on water saturated sponge in a Petri dish (9 cm in diameter). Same-aged newly matured *P. persimilis*/*N. californicus* females (both related to the line A) were placed on the leaf disc singly. Immediately, two same-aged newly matured males (one related to line A and the other related to line B, signed with watercolor) were added to the disc. Males had no previous mating experience. Leaf discs were monitored continuously until the first successful mating happened. We recorded which line the mating male belonged to. Each experiment was replicated 30 times. Data was analyzed through non-parametric binomial-exact test of statistical significance of deviations from expected distribution of observations in two categories-test in SPSS 19.

The effect of morphological character variation on mating precedence

Two iso-female lines (A & B) were prepared and kept for 6 months for each of the predator species. Experimental arenas were prepared as above. Same-aged newly matured *P. persimilis*/*N. californicus* females (both related to the line A) were placed on the leaf disc singly. Immediately, two same-aged newly matured males (one related to line A and the other related to line B, signed with watercolor) were added to the leaf disc. Males had no previous mating experience. Leaf discs were monitored continuously until the first successful mating happened. We recorded which line the mating male belonged to. Microscopic permanent slides were prepared from the male specimens [mites cleared in Nesbitt's fluid and mounted in Faure medium, Walter and Krantz (2009)]. The morphological characters including the length of the first leg, palpi, the second cheliceral segment, the movable digit and spermatodactyl were measured using Zeiss Standard 20 microscope. All measurements are given in micrometers (μm). Each experiment was replicated 30 times. Data was analyzed using AVOVA with means separated using Tukey HSD if any significant differences were found. Variances were compared using Leven's test.

Results

The effect of familiarity on mating precedence

No significant difference was observed between the number of first *P. persimilis* males (19 individuals vs. 10 individuals, familiar and unfamiliar respectively) that started mating with the females ($P > 0.05$) (Table 1, a). Neither was observed for *N. californicus* (18 individuals vs. 11 individuals, familiar and unfamiliar respectively) ($P > 0.05$) (Table 1, b).

Table 1. The values of binomial test to compare the familiar/unfamiliar male mating precedence in *P. persimilis* (a) and *N. californicus* (b).

Mating precedence	category	N		Observed Prop.		Test Prop.	Asymp. Sig. (2-tailed) ^a	
		a	b	a	b		a	b
familiar	1.00	19	18	0.66	0.62	50%	0.136	0.265
unfamiliar	0.00	10	11	0.34	0.38			
Total		29	29	1.00	1.00			

^a Based on Z Approximation

The effect of morphological character variation on mating precedence in P. persimilis

A significant difference was estimated between the mean length of the first leg related to the prevailed familiar, defeated unfamiliar, prevailed unfamiliar and defeated familiar males ($P < 0.05$) (Table 2, a). A Tukey post-hoc test (Table 3) revealed that the mean length of the first leg was significantly different between prevailed familiar (longer) and defeated unfamiliar (shorter) individuals ($P < 0.05$). Other pairwise comparisons showed no significant difference.

A significant difference was estimated between the mean length of the palp related to the prevailed familiar, defeated unfamiliar, prevailed unfamiliar and defeated familiar males ($P < 0.05$) (Table 2, b). The mean length of palp was significantly longer not only in prevailed familiars in comparison with defeated unfamiliars, but also in prevailed familiars rather than prevailed unfamiliars ($P < 0.05$) (Table 3). Other pairwise comparisons showed no significant difference.

No significant difference was estimated between the mean length of the second cheliceral segment related to the prevailed familiar, defeated unfamiliar, prevailed unfamiliar and defeated familiar males ($P < 0.05$) (Table 2, c) so that no Tukey HSD post-hoc multiple comparison was performed.

Table 2. The values of ANOVA to compare the mean length of first leg, Palp, 2nd cheliceral segment, movable digit and spermatodactyl in prevailed familiar, prevailed unfamiliar, defeated familiar, defeated unfamiliar in *P. persimilis* (dark bars)/*N.californicus* (light bars) males in male mating precedence experiment.

Source of variation (μm)	Prevailed familiar Mean \pm SE	Defeated unfamiliar Mean \pm SE	Prevailed unfamiliar Mean \pm SE	Defeated familiar Mean \pm SE	Mean square	F	d_f	Sig.
(a) First leg	387.33 \pm 4.46	368.50 \pm 7.77	384.85 \pm 2.33	373.21 \pm 3.51	1131.03	3.9	3	0.013*
	239.75 \pm 1.68	241.85 \pm 1.92	241.59 \pm 2.17	238.44 \pm 1.36	42.035	0.81	3	0.49
(b) Palp	114.83 \pm 1.36	108.00 \pm 1.58	108.20 \pm 1.75	110.31 \pm 1.33	129.07	3.4	3	0.02*
	86.31 \pm 0.92	85.67 \pm 0.87	84.44 \pm 0.88	84.55 \pm 1.05	13.064	0.91	3	0.44
(c) 2nd cheliceral segment	66.25 \pm 0.62	65.17 \pm 0.61	64.41 \pm 0.56	64.68 \pm 0.55	9.09	1.7	3	0.17
	61.56 \pm 0.44	60.08 \pm 0.60	60.94 \pm 0.55	59.72 \pm 0.69	11.39	2.06	3	0.11
(d) Movable digit	21.58 \pm 0.26	20.67 \pm 0.19	21.61 \pm 0.43	21.11 \pm 0.24	2.71	1.7	3	0.17
	20.44 \pm 0.27	19.69 \pm 0.43	20.71 \pm 0.39	20.22 \pm 0.44	2.67	1.08	3	0.36
(e) Spermatodactyl	38.50 \pm 0.47	36.67 \pm 0.38	37.11 \pm 0.47	36.58 \pm 0.38	10.29	3.1	3	0.03*
	30.87 \pm 0.24	30.46 \pm 0.24	30.71 \pm 0.36	30.78 \pm 0.19	0.439	0.37	3	0.76

No significant difference was estimated between the mean length of the movable digit related to the prevailed familiar, defeated unfamiliar, prevailed unfamiliar and defeated familiar males ($P < 0.05$) (Table 2, d) so that no Tukey HSD post-hoc multiple comparison was performed.

Table 3. The values of multiple comparisons (Tukey HSD post-hoc test, Mean difference (Md), Standard Error (SE)) to determine which group(s) among the sample in specific level have significant difference in *P. persimilis*. Comparisons are presented pairwise among prevailed familiar (PF), prevailed unfamiliar (PU), defeated familiar (DF) and defeated unfamiliar (DU).

Source of variation	PF-PU	PF- DF	PF-DU	PU-DF	PU-DU	DF-DU
First leg	Md: 2.48	14.12	18.83	11.63	16.34	4.71
	SE: 6.3	6.2	6.9	5.59	6.33	6.2
	Sig: 0.97	0.12	0.042*	0.25	0.058	0.87
palp	Md: 6.62	4.51	6.83	2.11	0.2	2.31
	SE: 2.27	2.24	2.48	2.00	2.27	2.24
	Sig: 0.025*	0.19	0.03*	0.72	1.00	0.73
spermatodactyl	Md:1.38	1.92	1.83	0.53	0.44	0.8
	SE:0.67	0.66	0.74	0.59	0.67	0.66
	Sig:0.18	0.02*	0.07	0.8	0.91	0.9

A significant difference was estimated between the mean length of the spermatodactyl related to the prevailed familiar, defeated unfamiliar, prevailed unfamiliar and defeated familiar males ($P < 0.05$) (Table 2, e). A Tukey post-hoc test (Table 3) revealed that the mean length of the spermatodactyl was significantly longer in prevailed familiars in comparison with defeated familiars ($P < 0.05$). Other pairwise comparisons showed no significant difference.

The effect of morphological character variation on mating precedence in N. californicus

No significant difference was estimated between the mean length of the first leg, palp, second cheliceral segment, movable digit and spermatodactyl related to the prevailed familiar, defeated unfamiliar, prevailed unfamiliar and defeated familiar males in *N. californicus* ($P < 0.05$)(Table 2, a-e, dark bars) so that no Tukey HSD post-hoc multiple comparison was performed.

Discussion

The effect of familiarity on mating precedence

Many animals have evolved the ability to recognize familiar and unfamiliar conspecifics based on their previous association or contact and use the advantage of associate with familiars (Strodl and Schausberger 2012). Limited attention theory has predicted that associating with familiars is preferential for individuals both from physiological and behavioral points of view (Dukas 2002; Strodl and Schausberger 2012). Strodl and Schausberger also studied the social familiarity and its effect on foraging behavior of juvenile *P. persimilis* and showed that familiar mites grouped together and foraged more efficiently. To our knowledge, no publication has yet addressed the effect of familiarity on mating precedence of predatory mites. Simmons (1988) demonstrated that the field cricket, *Gryllus bimaculatus* (de Geer) evaded from courting full siblings more than unrelated males. Yoshioka and Yano (2014) also showed that *Tetranychus urticae* males preferentially guarded unfamiliar quiescent females. They suggested that this behavior was due to inbreeding avoidance based on prior associations. Here, although the number of *P. persimilis/N. californicus* familiar males that mated first, was higher than unfamiliar ones (unlike our expectation according to literature review), no significant difference was estimated between them. As the rival males were the same aged, with no previous experience and owned no territoriality (Dehghani *et al.* 2015), this could be either because of the equal competition ability between the males or narrow genetic difference between the lines. Our results contrast with Hebets (2003) who reported that the wolf spider, *Schizocosa uetzi* Stratton (Araneae: Lycosidae) preferred males of a familiar phenotype to mate with. They stressed the importance of learning and memory on mate choice behavior in arthropods. Yoshioka and Yano (2014) demonstrated that spider mite male preference for mating with unfamiliar females was not only based on memory of their webs but also on chemical compounds of female body. We do not know whether the short-time direct contact of individuals (familiarization time) within the colony was sufficient for *P. persimilis* to recognize the familiar or unfamiliar individuals of its group, so further investigations are needed to explore the least mean time needed for familiarization and substances by which this species could detect the relatedness degree of surrounding individuals.

The effect of morphological character variation on mating precedence

First leg

The only significant difference was observed between the mean length of the first leg in prevailed familiar (longer) and defeated unfamiliar (shorter) individuals ($P < 0.05$). We interpret these two observations [longer first legs in familiars (Table 3) and no significant mating precedence between familiar and unfamiliar individuals (Table 1)] as follows: Many studies have demonstrated the role of the first leg in mating behavior.

Woyke (1994) reported that male arthropods use their first legs to clasp the female. Cone (1985) reported that male spider mites used their first legs during male-male combat. Olomski (2012) showed that the male *Brachypoda versicolor* (Müller) used its first legs to crawl on the female. Therefore, possessing longer first leg in the prevailed familiar males in comparison with defeated unfamiliar should be due to a higher mating ability in the first group. On the other hand, many studies have demonstrated that male mites avoid mating with familiar individuals (Tien 2010; Tien *et al.* 2011; Yoshioka and Yano 2014). Our data could be a correlation among these findings: familiar individuals possessed longer first legs than unfamiliar, but they did not show a significant higher mating precedence despite more (19 vs. 10, Table 1) familiar individuals mated first. This could be because of male avoidance of inbreeding.

Palp

Palpal damage during mating has been reported in many species of *Latrodectus* spiders (Araneae: Theridiidae) (Berendonck and Greven 2002). Jackson and Hallas (1986) studied the general trends of salticid spiders during intrasexual interactions and reported the palp engagements during copulation and sperm induction. Here we showed that, not only the mean length of the palp in prevailed familiar males, was significantly more in comparison with that in defeated unfamiliar ($P < 0.05$) but also prevailed unfamiliar possessed significantly shorter palps ($P < 0.05$) (Table 3). Therefore, it seems that the length of the palp is a less practical phenotypic character in comparison with the first legs as 34% of individuals (unfamiliar: 10 out of 29) (Table 1) with shorter palp length were constituted the winner population of the mating precedence experiment (not observed for the first leg).

Spermatodactyl

There is lack of information about the evolutionary forces responsible for structural complexity of mating organs in animals. Richmond (2014) studied the role of aedeagus size in *Drosophila mojavensis* male-female interactions. Eberhard (2004) proposed that dipteran males with average sized aedeagi had a fitness advantage over those with large or small one. Hosken and Stockley (2004) noted that some insects with large genital structures exhibited a higher fertilization efficiency. Alberti *et al.* (2010) believed that *Veigaia* sp. had a well-developed spermatodactyl with developed spiral organ. To our knowledge this is the first research about the correlation between the length of the spermatodactyl and mating precedence in mites. Despite the records above (length of the first leg and palp) with significant difference between prevailed familiar and defeated unfamiliar, here this difference has just been recorded between prevailed and defeated familiar individuals. Since the females had not experienced any male previous to the experiment, this could not be due to their female mate choice (Hebets 2003). Our suggestion is that some other traits rather than length should be responsible for mating combat between males.

According to the descriptive and ANOVA table (Table 2) no significant difference was observed among the mentioned characters in familiar/unfamiliar individuals of prevailed/defeated *N. californicus* females. This could be a simpler explanation for the non-significant difference between the male precedence in familiar and unfamiliar individuals in this species (Table 1). Haghani *et al.* (2015) have addressed the higher aggressiveness level of *N. californicus* rather than *P. persimilis* towards their heterospecific larva in intraguild predation and related interactions but they have not

mentioned whether this is also true in mating males towards females of the same species.

In conclusion, this study suggests that morphological traits could exert a strong influence on the predatory mite mating precedence. The results of this study demonstrates a basis for regarding these traits more delicately and their effects on other courtship and mating behaviors and strategies. Here, familiarity does not seem to affect mating precedence, but for a precise advice, preparing different lines with varied degrees of kinship is necessary. Further experiments are needed to expand this scope on which traits the mating competition could be manipulated.

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