

## Article

### **Kin recognition in three samples of *Phytoseiulus persimilis* (Acari: Phytoseiidae)**

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#### **Abstract**

Kin recognition as an important phenomenon which influences species fitness is mediated by mechanisms such as association or familiarity. Here, we have investigated either prior association or relatedness would affect the predatory mite, *Phytoseiulus persimilis* prey choice for cannibalism. Three samples of the predator including UT (University of Tehran), iso-females provided from UT and Turkey population with different degrees of relatedness, were introduced to the prospective cannibal which was selected from the iso-female line. The experimental procedure was consisted of imprinting (on each of the three sample larvae) and choice (between either familiar related and unfamiliar unrelated or familiar unrelated and unfamiliar related larvae) tests. The oviposition tests were designed by introducing each of the samples as the second female to a patch contained eggs related to iso-females as the first female. We found that the cannibal protonymphs preferred unfamiliar larvae for eating regardless of kinship. The number of prey eggs was significantly dependant to the type of relation between the eggs from first and second female. The effect of relatedness on the distances between eggs is discussed.

**Key words:** kinship, familiarity, cannibalism, imprinting, fitness, Phytoseiidae

#### **Introduction**

Kin recognition is the ability to discriminate kin from non-kin that might influence the species fitness considerably and leads to less aggressiveness towards closer relatives (Lizé *et al.* 2012). This behavior has been known in numerous species including cannibals. Species recognition is known as a relevant mechanism in generalist predators that leads to avoid eating kin, but as specialist ones do not show species recognition, a more subtle strategy (i.e. kin recognition) is suggested to be used among them to avoid cannibalism on kin (Schausberger & Croft 2001).

Kin recognition, mostly reported from social arthropods and also with some evidences in non-social ones such as parasitoids (King 1992) and predatory mites (Faraji *et al.* 2000), has been constituted from three phases including labeling, perceiving the label and acting on the cue (Schausberger & Croft 2001). Holmes (2004) has categorized kin recognition to selections according to individual's features and the ones rely on common cues from the individual's common environments. Schausberger (2005) has discussed that the recipients usually imprint on the first individual they meet in their sensitive phase of life such as immediately after hatching (by new emerged larvae) or at the beginning of the oviposition period (by ovipositing female).

Specialist phytoseiids (Acari: Phytoseiidae), the well known predatory mites in biological control programs, are known to put eggs within spider mite patches in clusters so that the offspring emerges aggregated with a high encountering level and competition sufficiency among conspecifics (Sabelis and Janssen 1994). Cannibalism as a common phenomenon among these predatory mites is a sufficient justifier for evolving kin recognition in them (Faraji *et al.* 2000).

Few studies have been focused on phytoseiid predatory mite kin recognition behavior. Faraji *et al.* (2000) have demonstrated that the predatory mite, *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae), not only could distinguish heterospecifics from conspecifics, but also was able to choose the conspecific egg clusters with more or less relatedness for adding new eggs to. They discussed that this behavior would lead to less cannibalism although the adult female had to commute between feeding and ovipositing sites repeatedly. Schausberger (2005) examined whether *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) manipulated imprinting among offspring through egg placement and found out that the protonymphs showed a significant preference to cannibalize unfamiliar larvae rather than familiar ones irrespective of relatedness. This means that *P. persimilis* larvae imprints on the ones encountered first, regardless of being kin or non kin. Schausberger and Croft (2001) studied the kin recognition behavior in different strains of *P. persimilis* and compared it with *P. macropilis*. They reported that both species were able to discriminate even non-descendent related larvae from unrelated ones. Some variations of discriminations among *P. persimilis* strains were recorded.

*Phytoseiulus persimilis*, the specialized predator of *Tetranychus urticae* is the focal species of our study. Its larval stage supposed to be non-feeding but strong in learning the surrounding cues (Schausberger 2007). Here, we have studied the hypothesis whether the adult female *P. persimilis* is able to manipulate imprinting through egg placement. We designed the experiments with three predator samples including University of Tehran population, an iso-female line from University of Tehran population and a sample from Turkey and in two phases (imprinting and choice tests) according to Schausberger (2005). Oviposition experiments were performed to assay how the females from each of the samples above would adjust their oviposition patterns when encountered previous oviposited eggs from iso-female lines.

## Material and Methods

### *Host plants and mites*

Common bean plants (*Phaseolus vulgaris* L. (Fabaceae) var. Red Alamouti) were grown in plastic pots containing mixture of perlite and soil. Plants were irrigated everyday by tap water and fertilizer solution of NPK (20×20×20) and were kept in controlled condition (23±1°C, 16L: 8D hours photoperiod, 65±5% RH) in growth chambers at the Department of Plant Protection, Faculty of Agriculture, University of Tehran, Karaj, Iran. Spider mites were reared on bean pots in a separate greenhouse (24±2 °C, complete photoperiod, 65±5% RH). New fresh bean plants were added to the rearing system regularly.

The predatory mite, *Phytoseiulus persimilis* was selected from three cultures, one so called University of Tehran population (with the rearing time length of 6 Years) in the mite behavior laboratory of University of Tehran (UT), another so called iso-female line, prepared from a female separated from UT culture and produced a dependent culture in a separate experimental unit. The other so called Turkey population, imported from Hatay,

Turkey and reared at laboratory condition for one year in Karaj. The predators were reared on detached bean leaves infested with *T. urticae* in a separate growth chamber under the conditions specified above.

#### *General experimental conditions*

##### *Oviposition test: egg placement and the probability of egg aggregation*

We hypothesized that the type of relation between ovipositing female predators would affect the distances between second female eggs and also the distances between second and first female eggs. Experimental units were contained of 9 cm agar filled Petri dishes with an upside down bean leaf on. Twenty spider mites (contained all of life stages) were transferred into the leaf disc using a thin brush. A female predator separated from the iso-female line was introduced to each disc for a 24 hours oviposition period. Removing the female predator, we started counting the eggs under stereomicroscope. The egg sites were marked by watercolor so that we could measure their distances after hatching. Second females were chosen either from related or unrelated ones and inserted into arenas. Number and the distances between eggs were analyzed by Multiple Univariate Anova through SPSS 17.

##### *Imprinting and kin recognition*

We hypothesized that the protonymph prey preference would depend on familiarity, relatedness and even the interaction between familiarity and relatedness. Taking ovipositing females from each culture randomly, putting each on a separate bean leaf disc, we let them continue ovipositing. Eggs collected once every 24 hours were kept in 7°C temperature to prevent hatching so that we could provide a sufficient number of eggs for the experiment.

For the imprinting phase, a single egg related to the prospective cannibal (from the iso-female line) was put on a leaf disc. Two other eggs (same aged) related to prospective prey were added to the same disc 12 hours later which could be either related or unrelated to the prospective cannibal. Leaf discs were put in growth chamber (23±1°C, 16L: 8D hours photoperiod, 65±5% RH) and monitored in close time intervals not to miss the exact time of hatching. As the prey eggs were younger than cannibal's, a retarded hatching was expected so that the cannibal larva was able to meet the prey larvae for a short time. Only those replicates which the cannibal and prey had an overlapped larval stage, were chosen for the next experimental phase. Those prey which the cannibal protonymph met them on the leaf disc, were considered as familiar regardless of genetic relatedness.

##### *Choice tests*

For the choice tests, the cannibal protonymphs were put on new leaf discs individually. Two prey larvae from each of the three cultures (familiar or unfamiliar) were added to the discs. Each protonymph should have made a choice between familiar related and unfamiliar unrelated larvae or familiar unrelated and unfamiliar related larvae for cannibalism. The prey larvae were marked by a small watercolor dot for being recognized till the end of the experiment. Leaf discs were kept in growth chamber under controlled conditions (23±1°C, 16:8 h photoperiod, 65±5% RH). The discs were checked in close time intervals until one of the larvae was eaten. The status of the eaten larva was recorded. The replicates in which each of the prey larvae were molted to protonymph were excluded. Thirty to 32 replicates were considered for each treatment. The proportion of familiar and unfamiliar larvae eaten by protonymphs were compared by non-parametric

binomial test in SPSS 17. The influence of relatedness and familiarity on the larva choice tested by logit-loglinear model in SPSS 17.

## Results

### *Egg placement and the probability of egg aggregation*

Our results showed that the second females which transferred into leaf discs containing unrelated eggs, put their eggs closer in comparison with the ones transferred into leaf discs with related eggs ( $P= 0.004$ ) (Table 1). The distances between second female eggs from the first female ones, was not depended to the relation between the eggs of first and second female ( $P= 0.850$ ). The number of eggs from second female was significantly depended to the type of relation between the eggs from first and second female ( $P= 0.005$ ). The distances between first female eggs and the effect of first female egg number did not affect the distances between second female eggs, the distances between second female eggs and first female eggs and also the second female egg number ( $P= 0.689, 0.854$  and  $0.7$ , respectively) (Table 1).

**Table 1.** Multiple univariate ANOVA for the number of and distances between eggs of related and unrelated female predators

Source of variation	Dependent variable	<i>df</i>	Type III sum of squares	F	<i>P</i>
Relatedness between eggs	Dist. eggs female 2	2	589	5.6	0.004**
Relatedness between eggs	Dist. eggs female 1 & 2	2	24.78	0.16	0.850
Relatedness between eggs	Number of eggs female 2	2	19.88	23.7	0.005**
Distance between eggs female 1	Dist. eggs of female 2	1	684	6.5	0.11
Distance between eggs female 1	Dist. eggs of female 1 & 2	1	183.12	1.2	0.27
Distance between eggs female 1	Number of eggs of female 2	17	2.15	1.82	0.054
Number of eggs of female 1	Dist. eggs of female 2	1	6.86	0.07	0.7
Number of eggs of female 1	Dist. eggs female 1 & 2	1	5.69	0.34	0.85
Number of eggs female 1	Number of eggs female 2	4	0.838	0.564	0.69

The distances between second female eggs were compared with the distances between second female eggs and first female eggs in the treatments of iso-female line and iso-female line (less:  $P < 0.01$ ), iso-female line and UT population (no significant

difference:  $P= 0.02$ ) and also iso-female line and Turkey sample (less:  $P< 0.01$ ) as first and second females respectively (Table 2).

**Table 2.** Number and distance between eggs of related and unrelated female predator (mean  $\pm$  SE)

First and second female	N	No. eggs female 1	No. eggs female 2	Dist. egg female 1	Dist. egg female 2	Dist. egg female1 & 2
related	22	4.72 $\pm$ 0.19	4.72 $\pm$ 0.26	8.14 $\pm$ 1.16	7.28 $\pm$ 0.84	14.51 $\pm$ 1.17
Unrelated (with Pop. Univ. Tehran)	20	4.9 $\pm$ 0.143	3.05 $\pm$ 0.15	6.59 $\pm$ 1.18	13.01 $\pm$ 1.80	15.64 $\pm$ 1.76
Unrelated (with Turkey sample)	22	4 $\pm$ 0.25	4.77 $\pm$ 0.15	11.27 $\pm$ 1.21	8.49 $\pm$ 0.92	15.16 $\pm$ 1.16

### *Imprinting and kin recognition*

In kin recognition experiments which the cannibal protonymph was selected from iso-female line, there were 17 replicates included familiar-related and unfamiliar-unrelated larvae and 16 replicates included familiar-unrelated and unfamiliar-related larvae. From the first 17 replicates, five familiar related (29%) and 12 unfamiliar unrelated (71%) larvae and from the second 16 replicates, 12 unfamiliar related (25%) and four familiar-unrelated (75%) were chosen for cannibalism. The proportions of familiar and unfamiliar eaten larvae were significantly different in both of the replicate series ( $P=0.01$  and  $0.04$  respectively).

**Table 3.** Logit-loglinear analysis for the effect of relatedness and familiarity on the predator choice for eaten larvae when the second females were chosen from the samples, iso-female line, UT and Turkey populations.

Second female \ effect	Relatedness	Familiarity	Relatedness $\times$ Familiarity
Iso-female	$Z= -0.267$	$Z= 2.453$	$Z= 0.267$
UT	$Z= 0.781$	$Z= 2.394$	$Z= -0.346$
Turkey	$Z= -0.238$	$Z= 1.99$	$Z= -0.238$

The influence of relatedness and familiarity on the choice of cannibal was analyzed by logit-loglinear model and the results showed that the cannibal protonymphs from iso-female line, preferred unfamiliar larvae for eating regardless of being related or unrelated ( $P= 0.014$ ,  $Z= 2.453$ ) and kinship did not affect the cannibal tendency towards prey ( $P= 0.789$ ,  $Z= -0.267$ ). The interaction between kinship and familiarity did not affect the predator choice ( $P= 0.789$ ,  $Z= 0.267$ ) (Table 3).

When the cannibal protonymphs were selected from UT population, 15 replicates included familiar-related and unfamiliar-unrelated larvae and 15 replicates included

familiar-unrelated and unfamiliar-related larvae. From the first 15 replicates, three familiar related (20%) and 12 unfamiliar unrelated (80%) and from second 15 replicates, five familiar unrelated (33%) and 10 unfamiliar unrelated (67%) were chosen for cannibalism. The proportions of familiar and unfamiliar eaten larvae were compared in both of the replicate series ( $P= 0.05$  and  $0.3$ , respectively).

The cannibal protonymphs from UT population, preferred unfamiliar larvae for eating regardless of being related or unrelated ( $P= 0.017$ ,  $Z= 2.394$ ) and kinship did not affect the cannibal tendency towards prey ( $P= 0.435$ ,  $Z= 0.781$ ). The interaction between kinship and familiarity did not affect the predator choice ( $P= 0.729$ ,  $Z= 0.346$ ) (Table 3).

When the cannibal protonymphs were selected from Turkey population, 15 replicates included familiar-related and unfamiliar-unrelated larvae and 15 replicates included familiar-unrelated and unfamiliar-related larvae. From the first 15 replicates, five familiar related (33%) and 10 unfamiliar unrelated (67%) and from second 17 replicates, five familiar unrelated (29%) and 12 unfamiliar unrelated (71%) were chosen for cannibalism. The proportions of familiar and unfamiliar eaten larvae were compared in both of the replicate series ( $P= 0.3$  and  $0.2$ , respectively).

The protonymphs from UT population, preferred unfamiliar larvae for eating regardless of being related or unrelated ( $P= 0.046$ ,  $Z= 1.99$ ) and kinship did not affect the cannibal tendency towards prey ( $P = 0.812$ ,  $Z= -0.238$ ). The interaction between kinship and familiarity did not affect the predator choice ( $P= 0.812$ ,  $Z= -0.238$ ) (Table 3).

## Discussion

The principal of a successful imprinting is to recognize the related and unrelated eggs in order to put the new eggs close to the more related ones. Our experiments showed that the relation between eggs (kinship) could affect the distances between the second female eggs in UT population and Turkey samples. In the other word, the more the relatedness between first and second female decreases, the closer the second female eggs will be put to each other. This was in coincident with Schausberger (2005) that reported the effect of kinship on oviposition behavior. He discussed that the predatory mites use this strategy to increase the probability of imprinting on kins, so that the total fitness in the next generation would increase. We expected that the predators chosen from iso-female line, would oviposit more scattered regarding their closest relation with the first females, but they decreased the distance between eggs much more in comparison with two other treatments. This needs to be more investigated. A probable environmental stress should have made the second females decide to gather the eggs.

An interesting observation when the second female was selected from iso-female line and UT population, was the effect of kinship on the number of eggs. By decreasing the relatedness between the first and second female, not only the second female eggs were put closer, but also the total number of eggs was decreased. Ovipositing less eggs might be due to either egg resorption or egg retention which was not the subject of our research. Neither the populations tested by Schausberger (2005) nor our Turkey population showed reduction in oviposition. It seems that the priority of using each of the strategies discussed above could differ depending to the population genetic system.

Although we expected an increasing procedure in distances between unrelated eggs by decreasing the relatedness between ovipositing females, the distance of second female eggs from the first ones was not affected by kinship. This might be a protective strategy taken by second female: when the first female eggs hatch, they will imprint on both first and second female eggs, so that the cannibalism rate on second female eggs will decrease.

Schausberger (2005) has noted the important role of age gap between the first and second female eggs. If the eggs are the same aged, there will be no sufficient time for imprinting and an increased bilateral cannibalism effect will be expected. So the age gap should be considered as a variable affects the oviposition pattern significantly.

In the present study, relatedness did not affect the cannibalism rate of protonymphs on conspecific larvae. In another word, the iso-female predators did not make a preference when encountered larvae from the same line, conspecifics from out of the line and even the larvae from another sample or population. The protonymphs ate numerous unfamiliar larvae regardless of kinship. It means that familiarity had a significant effect on the proportion of eaten larvae. This is consistent with Schausberger (2005) reported that imprinting happens on the first met larva after hatching and contrasts with Schausberger (2004) noted that imprinting always happens on related familiar larvae. There is a similar report from another phytoseiid predator, *Iphiseius degenerans* which preferred to cannibal on non-kin individuals and its clustering oviposition behavior considered as a strategy for avoiding kin to be eaten (Faraji *et al.* 2000). Stordl and Schausberger (2012) have discussed that life stage could affect the associating behavior in *P. persimilis* and larvae would be closer to each other than protonymphs.

Very few studies have been focused on predatory mite imprinting and kin recognition behavior. Schausberger (2005, 2007) and Stordi and Schausberger (2012) as the founders of this research point of view have reported several aspects of this behavior and its effect on foraging behavior in *P. persimilis*. Our samples in mite behavior laboratory of University of Tehran have represented different behaviors in comparison with the samples discussed above. Further investigations are needed to define other aspects of imprinting, familiarization and their effects on the predator life parameters.

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### *Phytoseiulus persimilis* شکارگر از کنه‌های شکارگر (Acari: Phytoseiidae)

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#### چکیده

خویشاوندشناسی، پدیده مهمی است که شایستگی گونه‌ها را تحت تاثیر قرار می‌دهد و به واسطه سازوکارهای مختلفی از جمله قرابت و آشنایی گونه‌ها با هم رخ می‌دهد. در این پژوهش، نقش قرابت و آشنایی پیشین در نحوه انتخاب شکار توسط کنه شکارگر *Phytoseiulus persimilis* و تاثیر آن بر میزان هم‌خواری در این شکارگر بررسی شده است. سه نمونه از کنه شکارگر شامل جمعیت دانشگاه تهران، ماده‌های هم‌سان حاصل از جمعیت دانشگاه تهران و ماده‌های هم‌سان حاصل از نمونه‌های وارداتی از کشور ترکیه، در اختیار کنه شکارگر هم‌خواری قرار گرفتند که از نمونه ماده‌های هم‌سان انتخاب شده بود. مراحل آزمون، از نشان‌گذاری (روی لاروهای هر کدام از سه نمونه) و انتخاب (بین خویشاوند آشنا و غیرخویشاوند غیر آشنا و یا غیرخویشاوند آشنا و خویشاوند غیر آشنا) تشکیل شد. آزمون تخم‌گذاری با وارد کردن فرد ماده‌ای از هر یک از نمونه‌ها به عنوان ماده دوم به لکه‌ای انجام شد که حاوی تخم‌های ماده‌های هم‌سان به عنوان ماده‌های نخست بود. مشاهده شد که پوره‌های سن یکم هم‌خوار، لاروهای غیر آشنا را صرف‌نظر از خویشاوندی، برای خوردن ترجیح می‌دهند. تعداد تخم‌های

شکار، به طور معنی داری به نوع ارتباط و قرابت تخم‌های ماده‌های اول و دوم بستگی داشت. در مورد تاثیر ارتباط کنه‌ها بر فاصله تخم‌های گذاشته شده توسط ماده‌ها بحث می‌شود.

**واژگان کلیدی:** خویشاوندشناسی، آشنایی، هم‌خواری، نشان‌گذاری، شایستگی، *Phytoseiidae*

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