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Short Communications

Effect of mating number on longevity and fecundity of the predatory mite *Euseius scutalis* (Acari: Phytoseiidae)

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Abstract

The predatory mite *Euseius scutalis* Athias-henriot is an important natural enemy of tetranychid mites in the southwest of Iran. The effect of number of mating on longevity and fecundity of *E. scutalis* was studied under laboratory conditions (at 25±1 ºC, 60 ± 5 % RH and 16: 8 L:D). Newly emerged adult females (less than 24 h) were held individually in a 9 cm arena. A male (less than 24 h) remained with a female for limited periods of time or continuously. Four treatments were designed: virgin females, single-mated females, multiple-mated females (female had access to a male every five days), multiple-mated females (female had continuous access to a male). Mating was necessary for oviposition. Virgin females lived the longest (25 days). However, no significant differences were found among the longevity of mated females (12- 15 days). Similarly, no significant differences were detected among the fecundity of females with different numbers of mating (18- 22 eggs). Sex ratio was significantly female-biased in progeny of single and multiple mated females with periodic access to males. However, no significant difference was found in the sex ratio of progeny of multiple mated females with continuous access to male. The results of the present study indicated that the Iranian strain of *E. scutalis* has intermediate reproductive behavior compared to two main other reproductive behaviors reported for phytoseiid mites.

Keywords: Number of mating, fecundity, Phytoseiidae, Euseius scutalis

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Introduction

In arthropods such as insects and mites, females typically need one or very limited copulations to maximize their performance, at the same time, males copulate several times and their performance increases with the copulation number (Tornhill & Alcock, 1983). However, in several cases females can copulate many times and this behavior may lead to an increase in egg production. Multiple copulations can have beneficial effects on female performance through the inception of sperm and different substances that are transferred during copulation. On the other hand, multiple copulations may have negative effects due to mating costs, including energy expense, physical harm and transfer of infectious parasites (Radwan & Rysinska, 1999; Arnqvist & Nillson 2000).

Few researchers have studied the effect of multiple copulations in the predacious mites of the family Phytoseiidae. Based on the data available, several phytoseiid mites need multiple mating while a single mating is enough for maximum reproduction activity in some others (Putman, 1962; Prasad, 1967; Elbadry & Elbenhawy, 1968; Laing, 1969; Zaher & Shehata, 1971; Amano & Chant, 1978a; Schulten et al., 1978; Hoy & Smilanick, 1979; Overmeer et al., 1982; Tsunoda & Amano, 2001).

The predatory mite *Euseius scutalis* (Athias-Henriot) (Acari: Phytoseiidae) is a generalist predatory mite (type IV) that can feed on a broad range of diets including various pollens, different insects and mite preys, honeydew and plant exudates (Mcmurtry et al., 2013). *Euseius scutalis* is a common phytoseiid mite in Asia, including Iran, India, Jordan, Lebanon, Turkey and North African countries like Morocco and Algeria (Daneshvar, 1980; Bonfour & McMurtry, 1987; Shishehbor, 1991; Kasap & Sekeroglu, 2004). In Iran, it is found on various plants including Moraceae, Rutaceae, Vitaceae, Convolvulaceae (Daneshvar, 1980), Euphorbiaceae (Shishehbor, 1991) and Malvaceae (Shishehbor et al., 2022). Some laboratory studies reported the life history and life table parameters of *E. scutalis* fed on certain mites (Bonfour & McMurtry, 1987; Shishehbor et al., 2022). The mating behavior of *E. scutalis* was studied by Elbadry & Elbenhawy (1968) and they found significant differences between singlemated and multiplemated females in relation to longevity and fecundity. However, it has been reported that strains (races) of phytoseiid species originating from different areas differ in their biological characteristics (Perring & Lackey 1989; Galazzi & Nicoli 1996). In the current study we examined the effects of multiple and single copulation of females of *E. scutalis* (Iranian strain) on its longevity and fecundity. The results can help us to understand the reproductive behavior of *E. scutalis* to multiple and single copulation. These findings will be useful in the mass production of this useful predatory mite.

Materials and Methods

The *T. turkestani* used in the current study was originally collected from field bindweed *Convolvulus arvensis* L. (Solanales: Convolvulaceae) grown on the vicinity of Shahid Chamran University of Ahvaz, Ahvaz, Iran during February, 2019. A stock colony of *T. turkestani* was kept on cowpea (*Vigna unguiculata* (L.) Walp) seedlings grown in compost in plastic pots (20 cm diameter). Cowpea plants infested with *T. turkestani* were held in rearing cages (120× 60 × 60 cm) covered with nylon mesh (210 µm aperture). The rearing cages containing infested cowpea plants were maintained in a laboratory at 25± 2 °C, 60± 5 RH and a 16: 8 (L: D) h with illumination (4000 lux) provided by fluorescent lamps. New plants were added when needed in order to keep the colony.

The original population of *E. scutalis* was collected from hollyhock (*Althea officinalis* L.) leaves grown in the premises of Shahid Chamran University of Ahvaz, Ahvaz, Iran, and infested with *T. turkestani* in April 2021. The *E. scutalis* colony was maintained in rearing units consisting of a plastic green sheet (5× 5 × 0.1 cm) placed on the top of a sponge mat with similar size in a

round plastic Petri dish (9 cm diameter and 1 cm height) that was half-filled with water. The sheet edges were covered with moist tissue paper to serve as a water supply source and as a barrier to prevent the predators from escaping. A few cotton threads were placed on plastic green sheet to serve as shelter and oviposition sites. Cowpea leaves infested with *T. turkestani* were added to the rearing units every three days as food supply.

Experimental setup

The experimental units were similar to the stuck culture rearing unit of *E. scutalis*. fresh eggs (less than 24 hours) of *E. scutalis* were collected from the stock colony and placed in experimental units, one egg per unit, using a fine hair brush (000). The experimental units were kept in a growth chamber set at 25 ±1 °C, 60 ± 5 % R. H. and a 16 L: 8 D photoperiod. After emergence of the larvae, the development and survival of immature stages were monitored daily. A piece of cowpea leaves infested with different stages of *T. turkestani* were added to each experimental unit every two days and the old leaves were removed.

Copulation number experiment

The newly emerged virgin adult male and female (less than 24 h old) of *E. scutalis* were used for all experiments. There were four treatments:

1- Virgin females. Females were kept singly without male throughout their life.

2- Single-copulated females. Newly emerged female with a young male were transferred to an experimental unit. The couple were observed every 5 minutes using a stereomicroscope (Olympus, 25X) with cool light. After the first contact, the couple showed sexual behavior which resulted to venter-to-venter copulation position and stayed at that stage for approximately 15 minutes. According to the previous results, this copulation time was enough to complete mating (Zergani, 2023). After the separation of couple, males were removed and females were kept individually without male during the rest of their life.

3- Multiple-copulated females with access to males at five days intervals. In this treatment a couple of newly emerged female and male was transferred to an experimental unit similar to those described above, to copulate. After copulation the male was removed and the number of eggs laid by female was recorded. Five days after the removal of first male another young male was introduced to experimental unit in order to copulate with the female. After mating, the second male was also removed and the number of eggs oviposited by female was documented. This process was repeated two more times during the life span of females. At the end of the experiment the longevity and fecundity of female was recorded. It should be noted that our preliminary experiment showed that *E. scutalis* females tend to lay eggs approximately five days after copulation and during this period they are not interested in new mating. However, after around five days the female *E. scutalis* was ready for a new mating (Zergani, 2023).

4- Multiple-copulated females with continuous access to males. In this treatment a newly emerged female and male were confined in an experimental unit to copulate. Every day the couple was observed and the number of eggs laid by the female was recorded. In the case the male died or was stuck in water around plastic sheet, a new young male was added to the experimental unit. When the female mite died the experiment was ended and the number of eggs laid was documented.

In all four treatments mentioned above 30 females were studied. Females that were stuck in water barricade surrounding the leaf discs or died because of inappropriate handling were excluded from data analysis. In addition, since age and mating history of males may affect its fertility (Amano & Chant, 1977) one young and less than 24 hours old males were used in all treatments of this experiment. As mentioned above, these males were also reared individually in the same way as females and used in related experiments.

In all treatments a small piece of cowpea leaves (2 × 2 cm) infested with different stages of *T. turkestani* was added to each experimental unit every two days and the old leaves were removed. The survival and fecundity of females studied in all treatments were recorded every day until the death of females.

In order to study the progeny sex ratio, in all treatments tested eggs were collected daily and transferred to an experimental unit described above and kept in a growth chamber at 25 ±1 °C, 60 ± 5 % R. H. and a 16 L: 8 D photoperiod. A piece of cowpea leaf infested with different developmental stages of *T. turkestani* was added to the plastic sheet as food for immature mites. After the immature mites reached adult stage, their sex was recorded.

Data analysis

The longevity and fecundity data were analyzed using ANOVA (SAS Institute 2002). Means were separated using Tukey's test (P< 0.05). Pearson's chi-square test was used to compare the sex ratio of progeny during oviposition period.

Results

Effect of mating number on longevity and fecundity

The longevity of virgin females was significantly higher than the mated females (F= 25.5; d. f. = 3, 57; P <0.0001) (Table 1) whereas no significant difference was detected among the longevity of females with different numbers of mating (Table 1).

The virgin female did not lay eggs. The females with different histories of mating numbers laid different numbers of eggs, however, the differences were not significant (F= 25.5; d. f. = 3, 57; P <0.0001) (Table 1).

Sex ratio of progeny

The sex ratio of progeny of single mated females was significantly female-biased (X^2 = 9.54; $X^2_{0.01;\,1}$ = 6.63) (Figure 1). Similarly, the sex ratio of progeny of females that copulated every five days was significantly female-biased (X^2 = 14.09; $X^2_{0.01;\,1}$ = 6.63) (Figure 1). However, the sex ratio of progeny of multiple-mated females with continuous access to males was not significantly different (X^2 = 3.03; $X^2_{0.01;\,1}$ = 3.84) (Figure 1).

Discussion

The results of our experiment indicated that virgin females of *E. scutalis* were could not produce eggs. Similarly, Sabelis (1985) reported that mating is necessary for oviposition in all phytoseiid species. It should be noted that the necessity of mating for oviposition is a rare phenomenon and is also reported in mites belonging to the family Dermanyssidae (Mesostigmata) in the subclass Acari (Weeks et al., 2001).

The results of the current study indicated that the presence of male and resultant mating significantly reduced female longevity. The reduction in female longevity under the presence of male may be due to the struggle and discomfort of females to avoid male sexual attack or to food competition between females and males (Pappas et al., 2007), in addition to extra search for suitable sites to lay eggs.

Table 1 Effect of mating number on mean (±SE) longevity and fecundity of females of *E. scutalis*

Treatments	N	Longevity (days)	Fecundity (number of egg/female)
Virgin females	25	25.44 ± 1.28a	00.00 ± 0.00b
Single mated females	25	15.14 ± 1.43b	18.62 ± 0.04a
Multiple-mated females with periodic presence of males (3-4 times during their adult life)	25	15.02 ± 1.29b	22.15 ± 0.08a
Multiple-mated females with continuous presence of males throughout their adult life	25	12.20 ± 1.00b	19.29 ± 0.08a
	F	25.5	25.5
	P	<0.0001	<0.0001

Values in each column followed by the same letter are not significantly different, using the Tukey HSD test at 5% significance level. N = number of individuals tested.

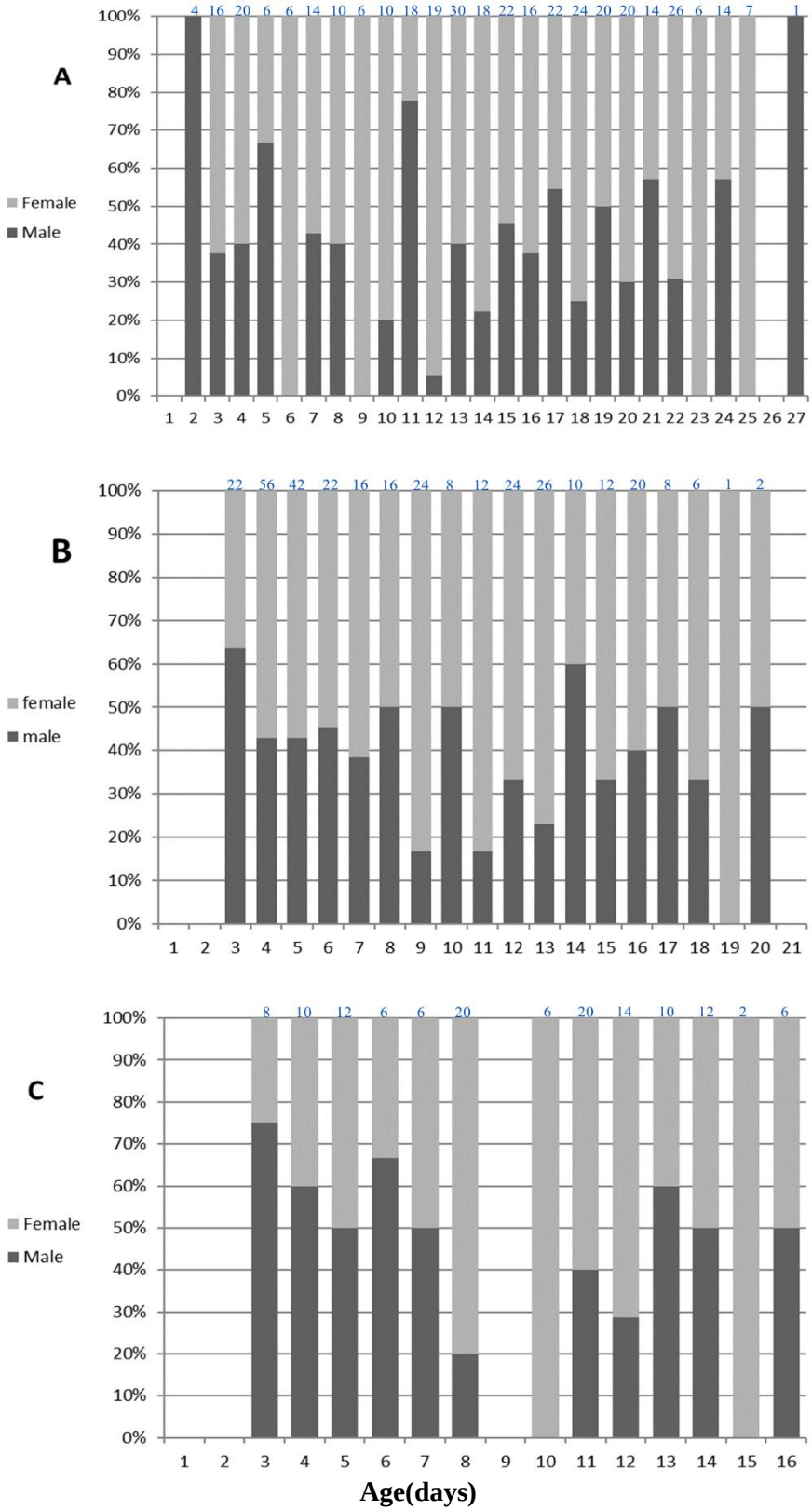

Figure 1. Sex ratio of the progeny of single-mated (A) and multiple-mated females with periodic (B) or continuous (C) access to males of *E. scutalis*. The mites were maintained at 25°C and a 16:8 LD. Bars indicate the proportion of male and female progeny at each different day of the oviposition period.

The results of the current study showed that copulation number did not affect the fecundity of *E. scutalis* females. In contrast to our results, increased fecundity as a result of multiple copulations has been reported for *E. scutalis* (Elbadry & Elbenhawy, 1968; Bonfour & McMurtry, 1987). Differences in the reproductive behavior of *E. scutalis* may be due to differences in strains (populations) of this predatory mite studied as proposed by Perring & Lackey (1989) and Galazzi & Nicoli (1996). They mentioned that phytoseiid strains from different areas differ in their biological attributes.

In general, there are two types of phytoseiid species with regard to mating frequency and its effect on egg production: increased fecundity due to multiple mating has been reported for *Euseius brazilli* (El Benhawy) (El Benhawy, 1975), *Amblyseius swirskii* Athias-Henriot (Momen & El-Saway, 1993), *Neoseulus bibens* (Blommer)(Schulten et al., 1978), *Galendromus occidentalis* (Nesbitt) (Hoy & Smilanick, 1979), *Typhlodromus pyri* Schueten (Overmeer et al., 1982), *Galendromus halveolus* (Chant) (Caceres & Childers, 1991) and *Kampimodromus aberrans* Oudemans (Pappas et al., 2007). However, in other phytoseiid species such as *Phytoseius persimilis* Athias- Henriot (Amano & Chant 1978a; Schulten et al. 1978) and *Amblyseius colimensis* Aponte and McMurtry (Aponte & McMurtry, 1992) a single copulation resulted in maximum egg laying. Therefore, we could consider the Iranian strain of *E. scutalis* as an intermediate type that although females tend to do multiple copulations; however, no distinct increase in egg production occurs in comparison to singlemated females.

The findings of the current study also showed that most of the eggs laid during the first three to four days of the oviposition period gave rise to male offspring. This trend has also been reported for *E. scutalis* (Bonfour & McMurtry, 1987) and other phytosiid species such as *P. persimilis, A. andersoni* (Amano & Chant, 1978b; Schulten et al., 1978) and *K. aberrans* (Pappas et al., 2007). It has been reported that a male-biased sex ratio in phytoseiid mites at the early oviposition period after copulation could result in early fertilization of females (Pappas et al. 2007). Then these fertilized females disperse to find suitable prey. Therefore, an early male-biased sex ratio could enhance the survival of this predatory mite.

Our results indicated that maximum fecundity of female *E. scutalis* could occur with single or multiple copulations. In multiple copulations (continuous presence of male) females laid a mean of 19.29 eggs. In contrast to our results, Bonfour & McMurtry (1987) reported an unusually higher fecundity of 64.8 eggs for *E. scutalis* (Morocco strain) fed on the pollen of *Melaphora crocea* Jacq. with continuous male presence at a similar temperature (25 ºC), which may be due to different food types and/or experimental conditions. In our experiment the singlemated female laid a mean number of 18.62 eggs. In a similar study Momen & Abdel-Khalek (2008) reported the mean total fecundity of 37.6, 26.08, 24.14, 21.81 and 20.50 eggs for singlemated females of *E. scutalis* (Egyptian strain) when fed on castor bean pollen, *Aceria ficus* (Cotte), *Rhyncaphytoptus ficifoliae* (Keifer, *Eutetranychus orientalis* (Klein) nymphs, *Icerya aegyptica* (Douglas), respectively, at 25 ºC. Furthermore, Kasap & Sekeroglu (2004) reported a mean total of 39.7 eggs for single-mated females of *E. scutalis* (Turkish strain) fed on *Panonychus citri* (McGregor) at 25 ºC. Differences between our and Momen & Abdel-Khalek (2008) and Kasap & Sekeroglu (2004) results may be due to differences in the strain of predator, food type and experimental method.

The results of the present study indicated that number of mating had no significant effect on total fecundity of *E. scutalis* females. The positive effect of this rare case is that the female *E. scutalis* can lay maximum fecundity by single copulation in case it does not encounter a male for the rest of her life span. The information concerning the reproductive strategy of *E. scutalis* can help us recognize the population dynamics of this useful natural enemy under field conditions.

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گزارش کوتاه انگلیسی

اثر تعداد جفت گیری بر طول عمر و زادآوری کنه شکارگر *Euseius scutalis* (Acari: Phytoseiidae)

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چکیده

اثر تعداد جفت گیری بر طول عمر و زادآوری کنه شکارگر *Euseius scutalis* Athias-Henriot در شرایط آزمایشگاهی (دمای ۱ ± ۲۵ درجه سلسیوس، رطوبت نسبی ۵ ± ۶۰ درصد و دوره روشنایی: تاریکی ۱۶: ۸ ساعت) مورد مطالعه قرار گرفت. کنه های ماده تازه پوست اندازی کرده (با عمر کمتر از ۲٤ ساعت) به صورت تکی در درون یک واحد آزمایشی با ۹ سانتی متر قطر گذاشته شدند. یک کنه نر (با عمر کمتر از ۲٤ ساعت) برای یک دوره محدود زمانی یا به صورت دائمی در کنار کنه ماده در واحد آزمایشی گذاشته شد. چهار تیمار تعیین شد: تیمار ماده های باکره، ماده‌های یک بار جفت گیری کرده، ماده‌های چند بار جفت گیری کرده (هر پنج روز کنه نر برای یک بار جفت گیری کنار کنه ماده گذاشته شد)، ماده های چند بار جفت گیری کرده (کنه نر به صورت مستمر در کنار کنه ماده قرار داشت). کنه های ماده باکره هیچ تخمی نگذاشتند بنابراین جهت تخمگذاری لازم است جفت گیری انجام شود. ماده های باکره طولانی ترین میانگین طول عمر را داشتند (۲۵ روز). با این حال، اختلاف معنی داری بین طول عمر کنه های ماده با تعداد متفاوت جفت گیری دیده نشد (۱۲- ۱۵ روز). به همین ترتیب، اختلاف معنی داری بین زادآوری کنه های ماده با تعداد متفاوت جفت گیری دیده نشد (۱۸- ۲۲ تخم). نسبت جنسی نوزادان در کنه های ماده یک بار جفت گیری کرده و کنه های ماده ای که هر پنج روز جفت گیری کردند به صورت معنی داری متمایل به ماده بود. با این حال، نسبت جنسی نوزادان در کنه های ماده ای که نر به صورت مستمر در کنارشان بود معنی دار نبود.

کلیدواژه‌ها: تعداد جفت گیری، زادآوری، Phytoseiidae ، Euseius scutalis

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