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Effects of sex ratio on different biological parameters of *Engytatus varians* (Distant) (Hemiptera: Miridae) adults and their offspring: prey preference for *Bactericera cockerelli* (Sulcer) (Hemiptera: Triozidae)

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Abstract

In the present study, the influence of three sex ratios (1:1, 1:2, and 1:3; female:male) of the mirid *Engytatus varians* (Distant) (Hemiptera) on different biological parameters and on its offspring was evaluated. The prey preference of different developmental stages of this predator for different nymphal instars (N) of *Bactericera cockerelli* (Sulcer) (Hemiptera: Triozidae) was also evaluated. The fertility was significantly higher (24 nymphs/female) in the 1:3 sex ratio than in the 1:1 and 1:2 sex ratios (14 and 16 nymphs/female, respectively). The females in the 1:1 and 1:2 sex ratios lived 1.14 and 1.43 days more (27 and 28 days, respectively) than those in the 1:3 sex ratio (26 days). The nymphs derived from the females of the three sex ratios (first filial generation, F_1) had five instars and a duration of 17 or 18 days. The ratio of the F_1 generation females was not affected by the sex ratio of their parents. In choice tests, independent of whether the preys were placed on a single or multiple tomato (*Solanum lycopersicum* L.) leaflets, the consumption of females and males and N_3, N_4, and N_5 nymphs of *E. varians* on *B. cockerelli*, generally showed the order of $N_2 > N_3 > N_4 > N_5$. In conclusion, the findings revealed in this study can help to improve the rearing methodology for increasing populations of *E. varians*. In addition, they can serve as a guideline for releasing this predator in times when there is an abundance of early instar nymphs of *B. cockerelli*.

Introduction

Engytatus varians (Distant) (Hemiptera: Miridae) is a zoophytophagous species that feeds both on plants and on insects living on them (Martínez *et al*., 2014). This natural enemy, originally described in Guatemala (Hernández and Henry, 2010; Ferreira and Henry, 2011), seems to be already spread from Southern USA to Argentina (Illingworth, 1937; Ferreira *et al*., 2001) and the Caribbean (Castineiras, 1995; Hernández and Henry, 2010). Although there is no available data on the abundance or percentage of natural predation, it has been reported that this predator can feed on different development stages of several phytophagous insects including eggs and larvae of *Manduca sexta* (L.) (Lepidoptera: Sphingidae) (Madden and Chamberlin, 1945) and *Heliothis virescens* F. (Lepidoptera: Noctuidae) (Ayala *et al*., 1982) on tobacco (*Nicotiana tabacum* L.) and on nymphs of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) on horticultural crops (Castineiras, 1995; Wheeler, 2001).

In Brazil and Mexico, *E. varians* was detected for the first time in 2013 and 2014 feeding on eggs and larvae of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tobacco (Bueno *et al*., 2013) and nymphs of *Bactericera cockerelli* (Sulcer) (Hemiptera: Triozidae) on tomato (*Solanum lycopersicum* L.) (Martínez *et al*., 2014), respectively. In both countries, several initiatives have focused on ascertaining the potential of this predator as a biological control agent in order to be included in control programs targeting some of the key pests of crops. In the laboratory, *E. varians* females can consume, per day, 57 eggs and 40 and 34 second- and third-instar, respectively, of *B. cockerelli* (Pineda *et al*., 2020). Thus, this mirid kills on average 13, 20, and 92 eggs/day of *Spodoptera frugiperda* (J. E. Smith), *Spodoptera exigua* (Hübner) (both Lepidoptera: Noctuidae) (Pineda *et al*., 2020), and *T. absoluta* (Bueno *et al*., 2013), respectively. The last authors have also reported that *E. varians* was capable of preying on larvae of *T. absoluta* within leaf mines (Bueno *et al*., 2013). This mirid shows a type III functional response (over a certain range of increasing prey densities, an increasing percentage of prey is killed) to eggs of *T. absoluta* (van Lenteren *et al.*, 2016) and type II (with increasing prey densities, a decreasing percentage of prey is consumed) to nymphs of *B. cockerelli* (Cortés-Piñón, 2017).

In tomato and pepper greenhouses, *E. varians* can suppress up to 90% of both nymphal and adult populations of *B. cockerelli*, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) when released at a rate of 1–4 females/plant in spring (Pérez-Aguilar *et al.*, 2019; S. Pineda, Unpublished data). Apart from predation features, other aspects of *E. varians* biology have been elucidated in the past few years: life cycle (Pineda *et al.*, 2016), demographic parameters under artificial or natural supplemented diets (Silva *et al.*, 2016; Palma-Castillo *et al.*, 2019) and susceptibility to some insecticides (Pérez-Aguilar *et al.*, 2018).

To design a biological control program with a natural enemy, it is essential to have an efficient and economic mass-rearing system to make its commercial production possible (Fitz-Earle and Barclay, 1989) but sometimes the mass-rearing produce detrimental sex ratio changes (van Dijken *et al.*, 1993). The influence of *E. varians* sex ratio on its reproduction success and offspring is largely unexplored and to gain knowledge on this aspect is very important for understanding the population growth of sexually reproducing organisms (Foster and Soluk, 2006; Wei, 2008). In general, in insect populations, the adult sex ratio does not deviate from a 1:1 (male:female) because of the segregation of the sex chromosomes in the gametogenesis process (Sheldon and West, 2002; Hoy, 2004). Consequently, they can mate randomly and with an equal resource investment (Hardy, 1994), despite fluctuating response to factors such as mate attractiveness, parental age and condition, and parental investment in offspring (West and Sheldon, 2002). Nevertheless, when the sex ratio is biased toward one sex, the life history traits of the species can be affected and the effect depends on the reproductive physiology and behavior, as reported in some herbivorous insects (Jones *et al.*, 1979; Gou *et al.*, 2019). For example, when comparing 11 sex ratios (male: female) of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae), a higher number of males increases the rate of egg production and mate but decreases female longevity, being optimal the sex ratio 4:6 for assuring sufficient males for mating and a maximum egg hatch (Jones *et al.*, 1979). Similarly, when seven different sex ratios of *Assara inouei* Yamanaka (Lepidoptera: Pyralidae) were evaluated, the fertility was significantly higher at the sex ratio of 3:1 (male:female) (He *et al.*, 2017). Besides, adult sex ratio can affect mate choice and mate competition in crickets (Wehi *et al.*, 2011). Unfortunately, there is scarce information on the influence of sex ratio in natural enemies.

As *E. varians* is a promising candidate for IPM programs targeting different key pests of crops (Martínez *et al.*, 2014; Morales *et al.*, 2018), in this study, our aim was to ascertain the influence of *E. varians* sex ratio on several life parameters in order to optimize the mass rearing. Adult fertility and longevity as well as the duration of the nymphal instars and offspring sex ratio were studied. Besides, the prey preference of *E. varians* nymphs and adults for the different nymphal stages of *B. cockerelli* was also evaluated in order to allow planning an adequate timing when the natural enemy is released in the crops. The pest is a polyphagous phloem feeder that can successfully reproduce in many plants including tomato and potato (*Solanum tuberosum* L.) where it can transmit the zebra chip disease caused by the bacterium '*Candidatus* Liberibacter *psyllaurous*' (Hansen *et al.*, 2008; Liefting *et al.*, 2009).

Materials and methods

Unless different conditions are specifically detailed below, the mass rearing and experiments were conducted under the following laboratory conditions: ~25 °C, 56% relative humidity, and a photoperiod of ~12:12 h (Light:Dark).

Rearing of B. cockerelli *and* E. varians

The *B. cockerelli* and *E. varians* individuals used in this study were obtained from colonies maintained in the Entomology Laboratory of the Instituto de Investigaciones Agropecuarias y Forestales (IIAF) de la Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Tarímbaro, Michoacán. The *B. cockerelli* nymphs and adults were kept in a frame box (80 × 80 × 50 cm) entirely covered by a mesh screen, which contained tomato plants (of the Rio Grande variety) (~30 cm in height with 7–8 fully expanded leaves), which were replaced as necessary. The *B. cockerelli* rearing was maintained in a ventilated greenhouse at 16–30 °C with 60% relative humidity and a photoperiod of ~14:10 h (L:D).

The *E. varians* individuals were reared on tomato plants in a frame box (45 × 65 × 45 cm) covered by a mesh screen. Every 3 days, plants from the *B. cockerelli* rearing cages infested with third, fourth, and fifth *B. cockerelli* instars were used to sustain the predator colony. Eggs of the grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) (Bio-bich, Uruapan, Michoacán, Mexico), deposited on tomato leaves were also supplied to the adults and nymphs of *E. varians*. The tomato plants were used as an oviposition substrate and a water source for the adults and nymphs of this predator.

Biological parameters of E. varians

To determine whether the sex ratio affects the fertility and longevity of the *E. varians* females as well as the development time and the sex ratio of their offspring, the following three sex ratios (i.e., treatments) were evaluated: (i) 1:1, (ii) 1:2, and (iii) 1:3 (female: male). Prior to the test, freshly emerged unmated females and males (parental generation, F_0) were individualized for 5 days in Petri dishes (9 cm diameter × 1.5 cm height) containing a tomato leaflet infested with a mixture of 15 second and third instar (N_2–N_3) *B. cockerelli*. As complimentary food, 10 mg of *S. cerealella* eggs were dispersed on the tomato leaflet.

For the test, tomato plants (~10–15 cm tall with four fully expanded leaves) were individually transplanted into Dart (Dart de México, Atlacomulco, Estado de México, Mexico) styrofoam cups (1 liter capacity). The two upper leaves were infested with a mixture of 15 N_2–N_3 *B. cockerelli* nymphs (7 or 8 nymphs on each leaf). In addition, 10 mg of *S. cerealella* eggs was added to these tomato leaves. The tomato plants were covered with cylindrical plastic tubes (15 cm height × 12 cm diameter), which were open at both ends. The top of the cylinder was covered with a fine mesh screen to permit air circulation and to prevent the escape of the insects. Afterwards, naïve *E. varians* adults (5 days old) were introduced, in accordance with the previously described treatments. The adult predators were released through a side opening (0.5 mm diameter), which was made in the middle section of the plastic tube and covered with a piece of cotton. The tomato plants, with a mixture of N_2–N_3 *B. cockerelli* and *S. cerealella* eggs, were replaced every 4 days when the *E. varians* adults were 9, 13, 17, 21, 25 days old. Seven replicates were used for each treatment.

To evaluate fertility, the tomato plants on which the *E. varians* females had oviposited endophytically (Pineda *et al.*, 2016) were maintained under laboratory conditions mentioned above until the emergence of the nymphs (first filial generation, F_1). For each *E. varians* female age, the number of nymphs emerged in each plant and treatment was recorded every 8 h. In addition, the total fertility was also determined as the cumulative number of nymphs per female during its lifespan. To determine their longevity, the females of each treatment were observed every 24 h until their death. The record of the males' longevity was interrupted when the last female died.

After emergence, each nymph of the F_1 generation derived from each treatment was placed into an individual Petri dish, which contained an excised tomato leaflet with a mixture of 15 N_2–N_3 *B. cockerelli* and 10 mg of *S. cerealella* eggs. Each nymph was maintained under these conditions until nymphal development was completed. The food was replaced every 48 h. The petiole of each leaflet was enveloped with a piece of moist cotton to delay dehydration. Each Petri dish was examined at 24 h intervals to determine when the nymphs had moulted, and the shed exuviae if present, were removed. From the number and timing of the moults, the number and duration of the nymphal instars as well as the total duration of nymphal development (duration from the first to last instar) were determined. After emergence, the adults were sexed, and the sex ratio was calculated as the percentage of females [females/(females + males) × 100].

Predation of E. varians*: choice tests*

The preference of nymphal instars, males and females of *E. varians* for the different nymphal instars of *B. cockrelli* was tested in the laboratory. Each experiment consisted of seven replicates per predator life stage and nymphal instar of the prey. The individuals representing the life stages of *E. varians* were starved for 6 h before the bioassay to induce a higher feeding rate. After 24 h of exposure, the number of consumed *B. cockerelli* nymphs for each developmental stage of the predator was recorded using a 50× stereoscopic microscope. *Bactericera cockerelli* nymphs that had been preyed upon were distinguishable because no more hemolymph was left in the body and because of the presence of a little brown spot on their dorsum, indicating the place where the predator inserted its stylet for feeding.

Bactericera cockerelli *nymphs on different leaflets*

For this bioassay, tomato leaves with four leaflets were used. These leaflets were consecutively numbered from the bottom to the top and from the right to the left as leaflet number 1, 2, 3, and 4. On the adaxial surface of these leaflets, five N_2, N_3, N_4, or N_5 *B. cockerelli* nymphs (24 h old) were placed using a small brush, in total 20 *B. cockerelli* nymphs. To avoid dehydration, the petiole of the tomato leaf was placed in a plastic cup (3.8 cm diameter × 3 cm height) containing approximately 28 ml of a 15% nutritive solution described by Hoagland and Arnon (1950). Afterwards, this tomato leaf was enclosed in a cylindrical plastic tube (15 cm high × 12 cm diameter), which was open at both ends. Finally, N_3, N_4, or N_5 nymphs (all 6 h old) or females or males (both 6 h old) of *E. varians* were individually released into the cylinder. The top of the cylinder was covered with a fine mesh screen to permit air circulation and to prevent the escape of the insects.

Bactericera cockerelli *nymphs on the same leaflet*

This test was performed using the same procedure described for the first assay, but randomly placing the five nymphs of each instar (N_2, N_3, N_4, and N_5) of *B. cockerelli* (i.e., the prey) on a single tomato leaflet.

Data analysis

A generalized linear model procedure (PROC GLM), with the LSMEANS test ($P < 0.05$) to separate means, was used for all analyses, except for evaluating the sex ratio of F_1 generation where a binomial distribution model was used. All the analyses were performed without transforming data because they met the assumption of normality (PROC UNIVARIATE) and homoscedasticity (PROC GLM).

Fertility of *E. varians* females in F_0 generation and the duration of nymphal instars of F_1 generation was studied with a 3 × 5 factorial design with treatments as a common factor (1:1, 2:1, 3:1 sex ratios) and different female ages (9, 13, 17, 21, 25-day-old) or nymphal instars (N_1, N_2, N_3, N_4, N_5), respectively. Predation of *E. varians* on *B. cockerelli* in the two different choice tests was analyzed independently. In both cases, the experiment consisted of a 5 × 4 factorial design with different *E. varians* developmental stages (female, male, N_5, N_4, and N_3) and different *B. cockerelli* preys consumed (N_2, N_3, N_4, and N_5). Analyses were performed using the fixed-effects model. All statistical tests were performed using SAS/STAT (version 8.1; SAS Institute, Cary, NC, USA) and all data are expressed as the mean ± SE.

Results

Fertility and longevity of E. varians

The sex ratio affected the fertility of *E. varians* females (Table 1). In the 1:3 sex ratio, the 9, 13, and 17-day-old females produced significantly more nymphs/female (6 or 7) than those of the same ages in the 1:1 and 1:2 sex ratios (between 3 and 5 nymphs/female). In contrast, irrespective of the treatment, when the *E. varians* females were 21 and 25 days old, they produced 2–3 and 1 nymph/female, respectively, and no significant differences were observed. In addition, throughout their lifespan, females in the 1:3 sex ratio significantly produced 1.8 and 1.6 more nymphs than females in the 1:1 and 1:2 sex ratios, respectively (Table 1).

Regarding longevity, *E. varians* females in the 1:1 and 1:2 sex ratios lived 1.14 and 1.43 days, respectively (27.28 ± 0.36 and 27.57 ± 0.48 days, respectively), more than the females in the 1:3 sex ratio (26.14 ± 0.34 days). Although minimal, this difference was statistically significant ($F = 3.60$; df = 2, 18; $P = 0.04$).

Nymphal development and sex ratio of the F_1 generation of E. varians

The nymphs of the F_1 generation derived from *E. varians* females of the three sex ratios tested had five instars. Irrespective of the sex ratio, the duration of the N_1 and N_5 instars was around 4 days, and that of N_2, N_3, and N_4 instars around 3 days (Table 2). Some slightest differences in duration were however registered in some cases. The duration of N_2 and N_3 instars derived from females in the 1:3 sex ratio was significantly higher than in the others and that of N_4 and N_5 instars derived from females in the 1:2 sex ratio significantly shorter compared to the 1:1 sex ratio.

Table 1. Fertility (number of nymphs/female ± SE) of *Engytatus varians* females of different ages from three sex ratios

	Age of *Engytatus varians* females (days)^{a}					
Sex ratio of *Engytatus varians* (female:male)	9	13	17	21	25	Total fertility^{b}
1:1	4.00 ± 0.69a	4.57 ± 0.43a	2.57 ± 0.37a	1.86 ± 0.26a	0.71 ± 0.18a	13.71 ± 1.11a
1:2	4.43 ± 0.48a	5.00 ± 0.87a	3.43 ± 0.65a	1.71 ± 0.36a	1.0 ± 0.0a	15.57 ± 1.87a
1:3	6.86 ± 0.88b	7.28 ± 1.04b	5.86 ± 0.40b	3.0 ± 0.31a	1.43 ± 0.20a	24.43 ± 2.06b

Means followed by the same letter in the same column are not significantly different ($P < 0.05$; GLM, LSMEANS test).

$^{a}F = 14.16$; df = 14, 90; $P < 0.0001$.

$^{b}F = 10.96$; df = 2, 18; $P < 0.0008$.

Table 2. Duration (days ± SE) of the nymphal instars (N) of the F_1 generation of *Engytatus varians* derived from females from three sex ratios

		Engytatus varians nymphal instars (days)^{a}					
Sex ratio of *Engytatus varians* (female:male)	*n*	N_1	N_2	N_3	N_4	N_5	Total nymphal stage^{b}
1:1	96	4.04 ± 0.08a	2.77 ± 0.08a	3.14 ± 0.09a	3.20 ± 0.08b	3.94 ± 0.1b	17.08 ± 0.16ab
1:2	109	4.20 ± 0.10a	2.85 ± 0.08a	3.32 ± 0.08a	2.89 ± 0.08a	3.71 ± 0.12a	16.93 ± 0.15a
1:3	171	4.01 ± 0.06a	3.06 ± 0.06b	3.55 ± 0.07b	3.08 ± 0.07ab	3.76 ± 0.07ab	17.45 ± 0.12b

Means followed by the same letter in the same column are not significantly different ($P < 0.05$; GLM, LSMEANS test). *n,* represents the number of specimens studied.

$^{a}F = 41.76$; df = 14, 1830; $P < 0.0001$.

$^{b}F = 3.94$; df = 2, 363; $P < 0.02$.

The total duration of nymphal development of the individuals derived from the females in the 1:1, 1:2, and 1:3 sex ratios was between 16.93 and 17.45 days (Table 2) and, although minimal, significant differences were only observed between the last two sex ratios.

The sex ratio of the parents did not influence the sex ratio of their offspring ($F = 1.29$; df = 2, 366; $P = 0.27$). The percentage of females of the F_1 generation derived from the females in the 1:1, 1:2, and 1:3 sex ratios was 58 ± 5, 58 ± 4, and 50 ± 5%, respectively.

Predation of E. varians

When *B. cockerelli* nymphs were exposed to *E. varians* either on different or on the same tomato leaflet, the predation depended on the developmental stage of the predator (the nymphal instar and sex of the adults), as well as on the nymphal instar of the prey (Table 3). During 24 h, the females and males as well as the N_3, N_4, and N_5 nymphs of *E. varians* preyed, in general, significantly more on the N_2 nymphs of *B. cockerelli* than the other three nymphal stages in both types of choice tests bioassayed.

In general, no significant differences were observed on the voraciousness of the different development stages of *E. varians* when fed on N_2, N_3, N_4, or N_5 nymphs of *B. cockerelli*, regardless of the prey was exposed on different or on the same tomato leaflet ($P \geq 0.074$ in all cases; Table 4). However, N_3 nymphs of the predator consumed significantly more N_2 ($P = 0.007$) and less N_4 ($P = 0.001$) nymphs of the prey when they were placed on the same tomato leaflet.

Discussion

Biological control – the use of natural enemies to decrease the density of key pest organisms – is one of the most environmentally safe and effective IPM management tactics in many crops worldwide (Williams *et al.*, 2013; Nafiu *et al.*, 2014). To release natural enemies, the implementation of optimal rearing methods is required to obtain a large quantity of good quality individuals (van Lenteren, 2012). Therefore, it is important to know the biological life parameters of natural enemies as well as those of their offspring. In this study, both the fertility and longevity of *E. varians* females from three different sex ratios were evaluated.

It has been reported that the pre-oviposition period of *E. varians* females was 3.5 or 3.6 days (S. Pineda, Unpublished data; Silva *et al.*, 2016) and that the type of diet influences the fertility. The fertility of *E. varians* was high (107 nymphs/female) when fed with a mixture of eggs and first instars larvae of *T. absoluta* (Silva *et al.*, 2016). Similarly, females of *Tupiocoris cucurbitaceus* (Spinola) (Hemiptera: Miridae) fed with *T. vaporariorum* Westwood (Homoptera: Aleyrodidae) nymphs produced significantly more nymphs throughout their lifespan (62 nymphs/female) than those fed with *S. cerealella* eggs (36 nymphs/female) (López *et al.*, 2012). In our study, the sex ratio had an influence on fertility, which was significantly higher with the sex ratio 1:3 than with 1:2 and 1:1. The fertility values recorded in the present study are much lower (14–24 of nymphs/female for the three sex ratios bioassayed) than those reported by López *et al.* (2012) and Silva *et al.* (2016). In these studies, the total fertility of *T. cucurbitaceus* and *E. varians* is also estimated, but using ≤1-day-old instead of 5-day-old females as in our study. Therefore, the females used were non-naïve in mating experience as ours, which could have wasted some time accepting males and mating and consequently, the fertility potential could have been underestimated. Besides, tests of Silva *et al.* (2016) were done under different climatic conditions [24 ± 1 °C, 70 ± 10% RH and 12:12 h (L:D)] than ours [~25 °C, ~56% RH, and ~12:12 h (L:D)], which could have also influenced results. Our findings cannot explain the greatest *E. varians* offspring when the females were placed with a higher number of males. Nevertheless, considering that in

Table 3. Predation of *Engytatus varians* adults and nymphs (N) on different nymphal instars (N) of *Bactericera cockerelli* placed on one or multiple tomato leaflets

Engytatus varians developmental stage	*Bactericera cockerelli* nymphs N_2	N_3	N_4	N_5
Nymphal instars on different leaflets[a]				
Female	4.71 ± 0.18d	3.43 ± 0.43c	1.86 ± 0.26b	0.71 ± 0.18a
Male	4.71 ± 0.18b	0.43 ± 0.20a	0.00 ± 0.0a	0.00 ± 0.0a
N_5	3.43 ± 0.20c	2.43 ± 0.22b	0.71 ± 0.18a	0.28 ± 0.18a
N_4	4.14 ± 0.34c	3.57 ± 0.20c	1.42 ± 0.20b	0.14 ± 0.14a
N_3	3.28 ± 0.36c	2.86 ± 0.26c	1.43 ± 0.20b	0.00 ± 0.0a
Nymphal instars on the same leaflet[b]				
Female	4.71 ± 0.18d	3.85 ± 0.46c	1.86 ± 0.34b	0.57 ± 0.20a
Male	4.28 ± 0.28b	0.57 ± 0.20a	0.00 ± 0.0a	0.00 ± 0.0a
N_5	3.86 ± 0.26c	1.86 ± 0.26b	0.57 ± 0.20a	0.43 ± 0.20a
N_4	4.43 ± 0.20d	3.71 ± 0.18c	1.00 ± 0.0b	0.00 ± 0.0a
N_3	4.14 ± 0.34c	3.28 ± 0.18b	0.28 ± 0.18a	0.28 ± 0.18a

Means followed by the same letter in the same row are not significantly different ($P < 0.05$; GLM, LSMEANS test).

[a] $F = 56.08$; df = 19, 120; $P < 0.0001$.

[b] $F = 63.20$; df = 19, 120; $P < 0.0001$.

Table 4. Influence of *Engytatus varians* adults and nymphs (N) on the predation rate of *Bactericera cockerelli* (second, third, fourth, and fifth instars; [N]) placed on one or multiple tomato leaflets

Engytatus varians developmental stage	*Bactericera cokcerelli* nymphs N_2	N_3	N_4	N_5
Female vs. female	1.000	0.183	1.000	0.654
Male vs. male	0.180	0.654	1.000	1.000
N_5 vs. N_5	0.180	0.074	0.654	0.654
N_4 vs. N_4	0.371	0.654	0.180	0.654
N_3 vs. N_3	0.007	0.180	0.001	0.371

$F = 58.17$; df = 39, 240; $P < 0.0001$ ($P < 0.05$; GLM, LSMEANS test).

insects with sexual reproduction, sperm transference from males during mating has important effects on the reproductive parameters and longevity of females (Simmons, 2001; Wedell *et al.*, 2002), this increase in fertility could be indicative of the adaptive phenomenon known as polyandry. In some insect species, females mate with different males to ensure fertilization of their eggs, which increases the genetic diversity and maintain the mean level of offspring fitness (Fedorka and Mousseau, 2002). As a consequence of this phenomenon, a greater offspring production has been observed in several insect species, e.g., *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae; Abdel-Azim *et al.*, 2012), *Pieris napi* L. (Lepidoptera: Pieridae; Wiklund *et al.*, 1993), and *Euborellia plebeja* Dohrn (Dermaptera: Anisolabididae; Kamimura, 2003). In the females of mirid species, however, information related to the occurrence of polyandry is very limited. The only available study (Franco *et al.*, 2011) shows that *N. tenuis* females are polyandrous and mate regularly to maintain a good sperm supply; nonetheless, it is unknown whether the fertility of this predator can increase as a consequence of this phenomenon. Therefore, more studies must be performed to confirm whether polyandry could be responsible for the increase in fertility found in *E. varians* females in the present study.

In the 1:3 sex ratio, the longevity of *E. varians* females was ~0.95 times shorter and the fertility was 1.6 and 1.8 times higher than those recorded in the other two sex ratios tested (1:2 and 1:1, respectively). Our findings are in agreement with Arnqvist and Nilsson (2000) who argue that insect females gain from multiple matings an increase in lifetime offspring production despite a negative effect on longevity. Therefore, we hypothesize that in the presence of various males, *E. varians* females could have had multiple matings, which is very costly in terms of energy and this could have originated the decrease in the lifespan. Besides, the higher fertility, the higher the energy demand for embryonic development (Arnqvist and Nilsson, 2000), which could have also played a role in the decrease. The studies of Silva *et al.* (2012) seem to support our hypothesis because of the longevity of *Edessa meditabunda* (F.) (Hemiptera: Pentatomidae) females, at a 1:1 sex ratio (female:male), also decreased 0.9-fold when they had two or more matings compared with the longevity of females that did not mate.

There is no information available regarding the transgenerational effects caused by the sex ratios in zoophytophagous mirids. In the present study, it was observed that the number of *E. varians* instars of the F_1 generation derived from the females in the three sex ratios tested was the same (five instars) as those recorded previously by Pineda *et al.* (2016) for this same species as well as for other mirid predators (e.g., *N. tenuis*; Kim *et al.*, 2016, and *Macrolophus pygmaeus* Rambur; Perdikis and Lykouressis, 2002). Also, in general, the total nymphal development duration recorded in the three sex ratios studied was similar (~17 days) to that reported previously (15–17 days) in the species (Pineda *et al.*, 2016) or in *M. pygmaeus* (Mollá *et al.*, 2014). It is very important to point out that all these authors determined either one or another of these biological parameters using cohorts of individuals from laboratory-rearing cultures instead of offspring derived from different sex ratios, as was done in the present study. Only the N_2 and N_3 stages of the nymphs derived from the females in the 1:3 sex ratio had durations 1.1 times longer than those recorded for the nymphs of the same instars derived from the females of two other sex ratios (1:1 and 1:2; female:male). An increase in the number of an insect's larval or nymphal instars, or in the length of the developmental period, could be indicative of inadequate nutrition (Slansky and Rodriguez, 1987). In the present work, however, the parental females of the three sex ratios as well as their offspring received the same high-quality diet (a mixture of N_2–N_3 *B. cockerelli* nymphs and *S. cerealella* eggs). The mechanism by which these effects might be exerted is poorly understood; therefore, they merit further study.

In general, the first nymphal instars of zoophytophagous mirids feed on their host plants (Lucas and Alomar, 2001); nevertheless, this dietary habit can change according to the abundance or availability of their preys (Goula and Alomar, 1994; Urbaneja *et al.*, 2005; Dalin *et al.*, 2011). In the choice tests performed in the present study, regardless of whether the prey was offered on different or on the same leaflet, the consumption by all the *E. varians* life stages tested (females and males and the N_3, N_4, and N_5 nymphs) on *B. cockerelli* was generally $N_2>N_3>N_4>N_5$. In agreement with our results, other zoophytophagous mirids showed similar trends in the consumption of their preys either in choice- or no-choice tests. In choice tests, *Dicyphus hesperus* (Knight) (Hemiptera: Miridae) females consumed more N_2 and N_3 nymphs than N_4 nymphs of the prey *B. cockerelli* (Ramírez-Ahuja *et al.*, 2017), while N_5 nymphs of *M. pygmaeus* consumed more N_1 and N_2 nymphs than N_3 and N_4 nymphs of the prey *M. persicae* (Sulzer) (Hemiptera: Aphididae) (Fantinou *et al.*, 2009). Also, in no-choice tests, *E. varians* females and males consumed more N_2 nymphs than N_3 nymphs of *B. cockerelli* (Pineda *et al.*, 2020), while the unsexed adults of *N. tenuis* consumed more N_1–N_2 nymphs than N_3–N_4 nymphs of the prey *T. vaporariorum* (Valderrama *et al.*, 2007). Size and mobility are two factors that can explain the dietary preference of all these mirid predators for the earlier instars of their prey (Fauvel *et al.*, 1987). Bigger prey offers greater nutritional content, but they are more difficult for predators to handle (Fantinou *et al.*, 2009). Similarly, younger instars of some preys have a very limited mobility (e.g. *T. vaporariorum)* and predatory mirids such as *N. tenuis* preferred them because of the easier manipulation (Valderrama *et al.*, 2007).

In our study, in general, each developmental stage of *E. varians* consumed a similar amount of the different *B. cockerelli* instars regardless if they were exposed on different or on the same tomato leaflet. Even though we do not have a clear explanation for these results yet, our hypothesis is that the optimal foraging theory can help explaining the results. According to this theory, a predator makes a decision on whether to attack an available prey or to move to a new patch of a more preferable prey type (Pyke, 1984). In addition, a predator is able to rank prey types according to their suitability and prey selection by preference is based on a mechanism of prey discrimination (Chesson, 1983). More studies should be conducted in *E. varians* to fully understand its prey foraging.

In conclusion, this is the first report about the influence of the sex ratio on some biological parameters of the adults of the predator *E. varians* and on their offspring. One of the main findings was that females had greater offspring when they could mate with more males in spite of the decrease in longevity. Besides, some aspects of the dietary behavior have been revealed: the natural enemy preferred to attack younger nymphal stages when different developmental stages were available. This allows using together other ecologically acceptable options to complement the control of *B. cockerelli* if needed, such as the ectoparasitoid *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae), which prefers parasitizing more mature nymphs (Morales *et al.*, 2013). Both findings could contribute to improve the rearing method of this predator in laboratories, with a view to obtaining a larger number of individuals and to improve the timing of field releases, since other natural enemies preferentially attacking older nymphal instars.

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