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Biology of Tupiocoris cucurbitaceus (Hemiptera: Miridae), a predator of the greenhouse whitefly Trialeurodes vaporariorum (Hemiptera: Aleyrodidae) in tomato crops in Argentina

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Biology of *Tupiocoris cucurbitaceus* (Hemiptera: Miridae), a predator of the greenhouse whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) in tomato crops in Argentina

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Abstract

The predator Tupiocoris cucurbitaceus is frequently found attacking Trialeurodes *vaporariorum* in greenhouses without pesticide applications in Argentina. The objective of these studies was to evaluate some biological characteristics of this species fed on three types of diet (whitefly nymphs, *Sitotroga cerealella* eggs and a mixed of both) and on two host plants (tomato and tobacco), under controlled experimental conditions. Preimaginal developmental time for female and male bugs was shorter in the presence of whiteflies than with only moth eggs. Females lived longer when they eat only whitefly nymphs compared to a mixed diet or only moth eggs. The amount of adult descendants was greater when bugs could eat whiteflies, regardless of the presence of S. cerealella. Embryonic development time, male longevity and sex proportion were not affected by the diet or the host plant. Prey consumption was evaluated for three T. cucurbitaceus life history stages (4th/5th instar nymphs, female and male adults) on two types of prey (whitefly nymphs and S. cerealella eggs). On tomato, females were more voracious than males and nymphs. On tobacco, adults and nymphs consumed more S. cerealella than T. vaporariorum nymphs, but again, bug females preyed more than males and nymphs. Results demonstrate that T. cucurbitaceus can survive, develop and reproduce normally using both T. vaporariorum and S. cerealella eggs as prey on tobacco or tomato plants. This information can be useful for managing this predator against T. vaporariorum through conservative or augmentative biological control strategies.

Keywords: Dicyphinae, Aleyrodidae, predation, biological control, vegetable crops.

1. Introduction

The use of generalist predators to control whiteflies in vegetable crops has been investigated and practiced in the last twenty five years (Arnó *et al.*, 2005). In Europe, especially in the Mediterranean region, some species of predatory Miridae have been incorporated into management pest programs for field and greenhouse crops. The species *Macrolophus melanotoma* (Costa) (syn. *Macrolophus caliginosus* Wagner) and *Nesidiocoris tenuis* Reuter are commercially available for the biological control of the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) and the cotton whitefly *Bemisia tabaci* Gennadius. Moreover, the use of Integrated Pest Management has promoted the presence and population numbers of *Dicyphus tamanii* Wagnet, another naturally occurring mirid predator (Alomar and Albajes, 1996; Alomar *et al.*, 2002). Although these three species have been mainly used to control whiteflies, they have also been observed to contribute to the control of thrips, leafminers, aphids, spider mites and Lepidoptera species (Perdikis *et al.*, 2008; Urbaneja *et al.*, 2009; Castañé *et al.*, 2011; Perdikis *et al.*, 2011).

In North America several Coleoptera and Hemiptera (e.g. *Delphastus* spp., *Serangium* spp., *Geocoris* spp. and *Orius* spp.) have been studied as predators for *Bemisia tabaci* control (Gerling *et al.*, 2001). Regarding mirid predators, research has been conducted with indigenous species such as *Dicyphus hesperus* Knight and *Deraeocoris nebulosus* (Uhler) (Jones and Snodgrass; 1998, McGregor *et al.*, 1999; Sánchez *et al.*, 2004).

The diversity of whitefly predators in Argentina is poorly known. Some polyphagous coccinellids (*Eriopis connexa* (Germar), *Cycloneda sanguinea* (L.), *Allograpta exotica* (Wiedemann)), syrphids (*Olla abdominalis* (Say)) and chrysopids have been mentioned (Cáceres, 2004). However, the biology and ecology of these species have not been thoroughly studied. Recently, the mirid bug *Tupiocoris cucurbitaceus* (Spinola) has been collected on tomato crops grown in greenhouses (*Solanum lycopersicum* L., var. ceraciforme) infested with *T. vaporariorum* (del Pino *et al.*, 2009).

Tupiocoris cucurbitaceus has been recorded in many South American countries (Carvalho, 1947; Carvalho and Ferreira, 1972; Carvalho and Afonso, 1977). In Argentina this mirid has been found in a wide area on a variety of host plants including

wild and cultivated species (industrial, ornamental and horticultural crops) (Carpintero and Carvalho, 1993; Carpintero, 1998; Carpintero, 2004). This species shows a marked preference for developing on hairy plants, mainly Solanaceae and Cucurbitaceae, since their surface provides more protection and enough water availability to the eggs that are inserted into the plant tissue (Agustí and Gabarra, 2009; Ingegno *et al.*, 2011). As for the other Dicyphinae, *T. cucurbitaceus* is primarily a predaceous bug although its phytophagy has been recorded (Carpintero, 1998; Ohashi and Urdampilleta, 2003). Although *T. cucurbitaceus* has been frequently found associated with whiteflies in the field, its actual prey range is still unknown.

The objective of this research was to examine some basic biological characteristics (development time, fecundity, longevity, sex ratio and prey consumption) of *T. cucurbitaceus* in order to improve our knowledge about this species, which is being considered as a potential biological control agent against *T. vaporariorum* in tomato crops. The studies were carried out on tomato and on tobacco plants, and we supplied the bugs with three different diets so that the biological information obtained would be useful for improving our rearing system.

2. Materials and methods

Sources of insects. Adults and nymphs of *T. cucurbitaceus* used in the experiments were obtained from the colony reared in the Insectario de Investigaciones para Lucha Biológica (IILB), Instituto de Microbiología y Zoología Agrícola (IMYZA), Instituto Nacional de Tecnología Agropecuaria (INTA), Castelar. They were originally collected in February, 2009 from tomato crops grown in greenhouses located in La Plata, Buenos Aires, in 2009. The species was identified by Diego Carpintero of the Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina, and voucher specimens were preserved in the collection of the IILB. On the basis of previous experience on mirid rearing (Agustí and Gabarra, 2009), the bugs were maintained on tobacco plants (*Nicotiana tabacum* L.) and fed on *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) eggs, a moth available in our laboratory.

T. vaporariorum nymphs and *S. cerealella* eggs were also obtained from IILB laboratory rearings.

Preimaginal developmental time. Through a completely randomized design, 30 bug adults (20 females, 10 males) from the rearing were isolated during one day in an acetate cage (21 cm in diameter, 40 cm high, top covered with muslin) with a host plant (25-30 cm high, 3-4 leaves) for egg-laying. Thirteen plants (replicates) of each host plant (tobacco and tomato) were used. Following exposure, the bugs were removed and the cages with the plant were held at 25°C±2°C, RH: 50-80% and 14L:10D photoperiod in an environmental controlled chamber. Plants (stems and leaves) were carefully inspected every 24 h and the time for the eggs to hatch was recorded until all nymphs emerged. The mean embryonic developmental time was estimated for each replicate. Nymphs emerged from the eggs in each host plant were placed individually into a Petri dish (5.5 cm diameter and 1.5 cm high) whose bottom was covered by a moistened filter paper, under the environmental conditions above mentioned. Nymphs were fed on one of the following diets during their development: 1) *S. cerealella* eggs, 2) *T. vaporariorum* nymphs and 3) a mixed diet consisting of *S. cerealella* eggs and *T. vaporariorum* nymphs.

Diets were offered to the nymphs according to the following procedures: 1) *S. cerealella* eggs. Three times a week, about 100 fresh eggs were sprinkled on the surface of a piece of leaf (aprox. 3 x 2 cm) placed upside down in the Petri dish. Eggs were previously sterilized (36W UV-lamp; 1 h exposure). Each time the eggs were added, the piece of leaf was also replaced and an appropriate amount of water was added to the filter paper to maintain the leaf in good condition; 2) *T. vaporariorum* nymphs. In order to obtain pieces of host plant leaves (aprox. 3 x 2 cm) with >100 3^{rd} -4th instar whitefly nymphs, tomato and tobacco plants were introduced into a cage and infested during a week with > 100 adult whiteflies 2 weeks before the experiment. One of these pieces of leaf with whitefly nymphs was placed upside down in the Petri dish containing the mirid nymph. The piece of leaf was replaced three times a week and the filter paper was moistened to maintain the leaf in good condition; 3) mixed diet of *S. cerealella* eggs and *T. vaporariorum* nymphs. Both procedures described above were jointly followed. In this

case, the two pieces of leaf in each Petri dish were also renewed three times a week together with an appropriate amount of water to maintain the leaves in good condition. Out of the thirteen plants of each host plant, nymphs coming from four plants were fed on diets 1) and 3), and nymphs coming from the other five plants on diet 2).

Time from nymph to adult emergence and the sex of each individual were daily recorded. In order to avoid pseudoreplication, each plant was considered as a replicate and the mean nymphal developmental time was estimated for each replicate from the developmental time of all nymphs emerging on it.

Differences due to the host plant 0n the mean embryonic developmental time were analyzed by a Kruskal-Wallis test since data did not meet the ANOVA assumptions (Statsoft, 2000). The effect of host plant and type of diet on the mean nymphal developmental time of females and males was analyzed through a two-way ANOVA. Means were separated using the Tukey test at the 0.05 level (Statsoft, 2000).

Longevity, fertility and sex ratio. Pairs of T. cucurbitaceus adults (< 24 h-old) from the rearing were individually isolated in an acetate cage with a host plant (25-30 cm high, 3-4 leaves). Twenty-six plants of tobacco and twenty-eight plants of tomato were used. The pairs (replicates) were fed on one of the three following diets: 1) S. cerealella eggs, 2) T. vaporariorum nymphs and 3) a mixed diet consisting of S. cerealella eggs and T. vaporariorum nymphs. Diets were offered to the adults according to the following procedures: 1) S. cerealella eggs. About 1000 sterilized fresh eggs were sprinkled on the leaves surface twice a week; 2) T. vaporariorum nymphs. In order to obtain 3rd-4th instar whitefly nymphs, tomato and tobacco plants were introduced into a cage and infested during a week with > 100 adult whiteflies 2 weeks before the experiment. These procedures guaranteed the presence of >300 nymphs at different instars per leaf (> 1000 nymphs per plant). When these nymphs were consumed or the plant became deteriorated, a new plant infested with whitefly nymphs was introduced into the cage so that the developing bug nymphs had new prey to consume; 3) mixed diet of S. cerealella eggs and T. vaporariorum nymphs. Both procedures described above were jointly followed. Out of the twenty six pairs isolated on a tobacco plant, eleven pairs were fed on diet 1), five (5) on diet 2) and ten (10) on diet 3). Out of the

twenty-eight pairs individually placed on a tomato plant, twelve were fed on diet 1), five on diet 2) and eleven in diet 3).

Each pair was checked daily in order to record adult longevity and their offspring were left to develop on the same plant until adulthood. Plants were inspected every day and emerged adult offspring counted, sexed and removed. The experiment was carried out in an environmental controlled chamber at 25°C±2°C, RH: 50-80% and 14L:10D photoperiod.

Differences in female and male longevity due to the host plant and the type of diet were evaluated through the Gehan's generalized Wilcoxon test for multiple-sample comparison and the Cox's F test for comparing two samples. Sex ratio (females/(females + males)) of offspring was analyzed by a logistic regression with host plant and diet as independent variables and model fit was checked by Deviance (Agresti, 1996). The effect of host plant and diet on the fertility (number of progeny / bug female) was analyzed using an analysis of covariance with the longevity of the female as covariable. Means were separated using the Tukey test at the 0.05 level. All the statistical analyses were performed using Statistica for Windows (StatSoft Inc., 2000).

Prey consumption. Prey consumption was evaluated separately for three mirid lifehistory stages $(4^{th}/5^{th} \text{ instar nymphs}, \text{ female and male adults})$ on two types of prey (whitefly nymphs or *S. cerealella* eggs). Studies were carried out on tobacco and tomato in separate experiments.

A piece of host plant (aprox. $3 \ge 2$ cm) holding roughly 100 3^{rd} -4th whitefly nymphs or *S. cerealella* eggs was placed on the bottom of a Petri dish (9 cm diameter and 1.5 cm high) that had been lined with a filter paper. One 4th-5th instar or adult female or male was placed in the arena for 24 h, and thereafter the number of fully consumed prey was recorded using a stereo microscope. Preyed nymphs and eggs can be easily distinguished because predation leaves an empty whitefly or egg cuticle. In order to obtain the experimental specimens of the predator, mirid nymphs (4th/5th instar) were collected randomly from the rearing and provided with only water through a moistened cotton for a period of 24 h before the experiment. In addition, late 5th instar nymphs

were also collected from the rearing and isolated individually in a glass tube until adult emergence. Then, they were provided with water during 24 h before the experiment. There were ten replicates per prey type and predator life-history stage. Experiments were carried out in a controlled chamber at 25°C±2°C, RH: 50-80% and 14L:10D photoperiod.

The influence of mirid life-history stage on the number of prey consumed was analyzed by a one way ANOVA for each prey type. Means were compared by the Tukey test at the 0.05 level (Statsoft, 2000).

3. Results

Preimaginal developmental time. *Tupiocoris cucurbitaceus* eggs hatched 9.6 ± 0.1 days after oviposition. There were no significant differences in the mean embryonic developmental time between host plants (Kruskal-Wallis statistic=0.01, P=0.93).

Both females and males developed more quickly when they fed upon whitefly nymphs than on only *S. cerealella* eggs. Host plant did not affect nymphal developmental time. There was no interaction between factors (Females: $F_{host plant x diet}=0.65$, df=2,20, P=0.53; $F_{host plant}=3.73$, df=1,20, P=0.068; $F_{diet}=6.09$, df=2,20, P=0.0086; Males: $F_{host plant x diet}=1.02$, df=2,20, P=0.38; $F_{host plant}=2.36$, df=1,20, P=0.14; $F_{diet}=5.46$, df=2,20, P=0.013; Tukey test, P<0.05) (Table 1).

Longevity, fertility and sex ratio Female longevity was significantly modified by the diet (Gehan's generalized Wilcoxon test: $Chi^2=17.09$; df=6; p=0.0043; Cox's *F* tests: $F_{tobacco\ vs\ tomato}=1.23$, df=52,54, P=0.23; $F_{mixed\ diet\ vs\ Tv\ nymphs}=2.48$, df=42,20, P=0.016; $F_{mixed\ diet\ vs\ Sc\ eggs}=1.22$, df=42,44, P=0.25; $F_{Tv\ nymphs\ vs\ Sc\ eggs}=2.36$, df=44,20, P=0.020).

Fertility was also significantly affected by the diet ($F_{host plant x diet}=1.1$, df=2,47, P=0.36; $F_{host plant}=2.45$, df=1,47, P=0.12; $F_{diet}=4.86$, df=2,47, P=0.012; Tukey test, P<0.05). Regardless of the host plant, females lived longer when they ate only whitefly nymphs than when they consumed a mixed diet or only moth eggs. The number of adult descendants was greater when parental and filial generation could eat whiteflies, regardless of the presence of *S. cerealella* (Table 1).

Male longevity was not significantly modified by host plant and diet (Gehan's generalized Wilcoxon test: $\text{Chi}^2=3.20$; df=6; P=0.67). Also, sex ratio (proportion of females) of the progeny was not affected neither by the host plant ($\text{Chi}^2_{(1)} = 1.90$, P=0.17) nor by the type of diet ($\text{Chi}^2_{(2)} = 5.13$, P=0.081) (Table 1).

Prey consumption. Consumption on tomato plants differed significantly depending on the different bug life-history stage (Figure 1). For both kind of prey, female adults were more voracious than male adults and nymphs, without significant differences between these two last stages (Whitefly nymphs: F= 23.50, df=2,27, P=0.000; *S. cerealella* eggs: F=3.63, df=2,27, P=0.04).

The same pattern was observed on tobacco, with females of *T. cucurbitaceus* consuming more preys than males and nymphs (Whitefly nymphs: F= 25.57, df=2,26, P=0.000; *S. cerealella* eggs: F=16.42, df=2,27, P=0.000) (Figure 2).

4. Discussion

Egg development period of *T. cucurbitaceus* was within the range of values (8.6 - 12 d) recorded for other mirid predators (*M. caliginosus, M. pygmaeus, D. tamanii* and *N. tenuis*) at 24-25°C on different host plants (Fauvel *et al.*, 1987; Perdikis and Lykourissis, 2002; Agustí and Gabarra, 2009; Mohd Rasdi *et al.*, 2009; Sánchez *et al.*, 2009). The embryonic developmental time of *T. cucurbitaceus* was not influenced by the host plant suggesting that eggs either do not require nutrients from the plant or are provided with equal resources by both host plants.

Nymphs of the mirid bug developed faster to adult stage (both females and males) when they were fed whitefly nymphs, either alone or together with moth eggs, and on both host plants evaluated. Orozco and López (2010) observed that the performance of *T*. *cucurbitaceus* nymphs and females dropped drastically in absence of prey supply indicating that this bug is essentially zoophagous. The results herein suggest not only that the presence but also the kind of prey is important for the development of *T*. *cucurbitaceus* nymphs since the individuals that fed on *S. cerealella* eggs had a delay in their development. The influence of different items such as diet and host plant on the immature development time of mirid predators has been recorded by other authors (Perdikis and Lykouressis, 2000; Agustí and Gabarra, 2008; Calvo and Urbaneja, 2004). *T. vaporariorum* nymphs seem to be a more suitable prey than *S. cerealella* eggs for the development of *T. cucurbitaceus* nymphs, probably due to a higher nutritional value. In addition, young nymphs (1^{st} to 3^{rd} instars) could have certain difficulty in eating *S. cerealella* eggs compared to *T. vaporariorum* nymphs due to chorion resistance and difficulty in manipulating them, delaying their development. The preference for whitefly nymphs instead of lepidopteran or whitefly eggs due to the greater hardness of the chorion has been recorded for some other predaceous mirids (Bonato *et al.*, 2006; Izquierdo *et al.*, 1994).

All *T. cucurbitaceus* females successfully oviposited except one fed on *T. vaporariorum-S. cerealella* on tobacco and two reared on *S. cerealella* on tomato, although these three bugs died in the first week of their life. Presumably they were in their preoviposition period since mirid predators need about 7 days at 25°C before they can lay eggs (Perdikis and Lykouressis, 2002).

Like nymphs, females of *T. cucurbitaceus* were also influenced by the prey offered. Several authors have found that polyphagous predators show differences in fecundity and longevity according the suitability of the prey they fed upon. Fauvel *et al.* (1987) and Alomar *et al.* (2006) found that the fecundity of *M. caliginosus* was higher when they preyed on *E. kuehniella* eggs than with *T. vaporariroum* and *B. tabaci* nymphs. Agustí and Gabarra (2008) observed a benefit for the fecundity of *D. tamanii* fed on *E. kuehniella* alone or mixed with *T. vaporariorum* compared to the whitefly alone. These results may be explained by the high egg quality of *E. kueniella* for this and other Dicyphinae mirids. However, our study indicates that *T. vaporariorum* nymphs are a more suitable food than *S. cerealella* eggs for *T. cucurbitaceus* adults, because they live more and lay more offspring when they prey on whiteflies. Moreover, the combined supply of both prey did not provide any advantage to adult performance. Further studies are warranted to evaluate the nutritional value of each prey in order to understand its possible influence on the biology of *T. cucurbitaceus*.

Despite the lower longevity and fertility displayed by *T. cucurbitaceus* when fed upon *S. cerealella* eggs, this food resource allowed for bug reproduction and development.

This is an important result for the mass rearing of *T. cucurbitaceus* because it means that this mirid species can be produced in an adequate and cheaper way using the same food (*S. cerealella* eggs) suitable for rearing other insects (e.g. *Trichogramma* spp., *Orius* sp., *Chrysopa* sp.). Regarding predator management, in European greenhouse crops, moth eggs are added on the plants to improve the establishment of the predator, particularly when prey availability is low (Perdikis *et al.*, 2008). Since *S. cerealella* eggs are a suitable prey for *T. cucurbitaceus*, a similar scheme could be tested with *T. cucurbitaceus* in greenhouse crops.

The progeny produced by a female mirid can be influenced not only by the prey but also by the characteristics of the host plant. The hardness and thickness of the leaves, petioles and midribs, and possibly the moisture content of the host plant influence the number of eggs laid by a female and the survival of offspring (Constant *et al.*, 1996). Nutrients also influence insect development. Tobacco and tomato, both solanaceus species, appear to be equally good host plants for *T. cucurbitaeeus* reproduction, sharing structural characteristics such as leaf hardness, stickmess and hairiness. The fact that tobacco has been used as the rearing host for several generations of *T. cucurbitaeeus* did not give it an advantage over the tomato in the adult performance (longevity and progeny production). Male longevity and the sex proportion of the filial generation were not influenced neither by the prey nor by the host plant.

Tupiocoris cucurbitaceus females were more voracious irrespective of the type of prey and the host plant. In general, predatory activity is intensified in the adult stage. Differences in consumption rates among predator stages/sexes can be explained by differences in the nutritional needs of each group, i.e. growth for immature, fecundity for females, maintenance for all of them. It is clear that females need a higher nutritional input related to oogenesis and oviposition requirements.

Consumption rates obtained for *T. cucurbitaceus* were higher than that of *D. tamaninii*, *M. caliginosus*, *Deraecoris* sp. and *Campylomma nicolasi* Reuter tested on *B. tabaci* and *T. vaporariorum* by Barnadas *et al.* (1998), Kapadia and Puri (1991) and Mohd Rasdi *et al.* (2009), and agreed with those recorded for *D. tamaninii*, *M. caliginosus* and

D. hesperus by McGregor *et al.* (1999), Bonato *et al.* (2006) and Castañé *et al.* (2002), who used a similar starvation period, aged classes and environmental conditions.
Although *T. cucurbitaceus* consumption rate was evaluated in a small and confined area

and could be lower in field settings, our study indicates that this bug may be useful for biological control of *T. vaporariorum*. Therefore, it would be interesting to conduct a complementary study under greenhouse conditions with different whitefly stages as prey and with other potential prey in tomato crops such as *B. tabaci* and the tomato moth *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae).

In conclusion, our results demonstrate that *T. cucurbitaceus* can survive, develop and reproduce normally using both *T. vaporariorum* immature and *S. cerealella* eggs as prey and that both tobacco and tomato plants are good hosts for this predator. The prey consumption falls within the range of values recorded for other predaceous mirids, being the females more voracious than males and nymphs. Our study suggests that *T. cucurbitaceus* can be an appropriate biological control agent to be used against *T. vaporariorum*.

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Figure 1. Prey consumption by *Tupiocoris cucurbitaceus* on tomato plants (mean + standard error). Prey: *Trialeurodes vaporariorum* nymphs or *Sitotroga cerealella* eggs. Different letters within a particular type of prey indicate significant differences among predator life-history stages (ANOVA, P<0.05).



Figure 2. Prey consumption by *Tupiocoris cucurbitaceus* on tobacco plants (mean ± standard error). Prey: *Trialeurodes vaporariorum* nymphs or *Sitotroga cerealella* eggs. Different letters within a particular type of prey indicate significant differences among predator life-history stages (ANOVA, P<0.05).

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Table 1: Biological attributes of *Tupiocoris cucurbitaceus* (mean \pm standard error). Tv: *Trialeurodes vaporariorum*; Sc: *Sitotroga cerealella*. Mean fertility values were computed by ANCOVA with the covariate at its means (Female longevity: 16.15 d). Means followed by the same letter within rows are not significantly different (P> 0.05). Numbers in brackets show the number of replicates used for mean calculations.

	Diet		
Biological attribute	Tv nymphs + Sc eggs	Tv nymphs	Sc eggs
Development time of female nymphs (d)	14.1 ± 0.6 (8) b	14.9 ±0.4 (10) ab	16.4 ± 0.4 (8) a
Development time of male nymphs (d)	13.6 ± 0.7 (8) b	14.5 ± 0.4 (10) ab	16.1 ± 0.5 (8) a
Female longevity (d)	14.0 ± 2.3 (21) b	25.0 ± 1.8 (10) a	14.3 ± 1.8 (23) b
Fertility (adults/female)	47.83 ± 4.61 (21) ab	61.77 ± 7.26 (10) a	35.90 ± 4.40 (23) b
Male longevity (d)	20.7 ± 2.9 (15) a	22.6 ± 2.6 (10) a	17.8 ± 2.6 (16) a
Sex ratio (females/(females + males))	0.58 ± 0.035 (20) a	0.53 ± 0.019 (10) a	0.56 ± 0.039 (21) a