Mating Preferences and Consequences of Choosing Sibling Or Non-Sibling Mates by Females of the Predator Podisusnigrispinus (Heteroptera: Pentatomidae)

Authors: Poderoso, Júlio Césae Melo, Zanuncio, José Cola, Puker, Anderson, Ramalho, Francisco De Souza, Wilcken, Carlos Frederico, et al.

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MATING PREFERENCES AND CONSEQUENCES OF CHOOSING SIBLING OR NON-SIBLING MATES BY FEMALES OF THE PREDATOR *PODISUS NIGRISPINUS* (HETEROPTERA: PENTATOMIDAE)

Júlio César Melo Poderoso[1], José Cola Zanuncio[1,*] Anderson Puker[1], Francisco de Souza Ramalho[2], Carlos Frederico Wilcken[3] and Eraldo Lima[1]

[1]Departamento de Entomologia, Universidade Federal de Viçosa, 36570-000 Viçosa-Minas Gerais, Brazil

[2]Unidade de Controle Biológico, EMBRAPA Algodão, 174, 58107-720, Campina Grande, Paraíba, Brazil

[3]Departamento de Produção Vegetal, Faculdade de Ciências Agronômicas, Universidade Estadual Paulista, 18603-970, Botucatu, São Paulo, Brazil

*Corresponding author: E-mail: zanuncio@ufv.br

Abstract

The sex with the greater investment in offspring is more careful in selecting sexual partners. Mating preferences and consequences of choosing sibling or non- sibling males by *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) females were investigated. Females of this predator were placed with sibling, non- sibling or both types of males in 500 mL plastic containers. The numbers of matings and egg masses per female were registered after 12 h. Eggs were transferred to Petri dishes (9.5 × 1.5 cm) and the number of nymphs that hatched were recorded daily until 10 days after each oviposition. Female longevity with sibling, non-sibling or both types of males was registered and adults were weighed and measured after their deaths. Females of *P. nigrispinus* did not discriminate between sibling or non- sibling males, which can lead to inbreeding. However, the greater number of eggs laid and the higher egg viability of *P. nigrispinus* females that mated with non- sibling males indicate that this predator can avoid inbreeding depression including reduced fitness by mating with unrelated males.

Key Words: biological control, choice mate, sexual selection, stinkbug

Resumo

O sexo que mais investe na prole é mais seletivo na escolha dos parceiros sexuais. Preferências e consequências da escolha de parentes (irmãos) ou não-irmãos por fêmeas de *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) foram estudadas. Fêmeas desse predador foram colocadas com machos irmãos ou não-irmãos em recipientes plásticos (500 ml). O número de acasalamentos e o de massas de ovos foram registrados por fêmea em intervalos de 12 horas. Os ovos foram transferidos para placas de Petri (9,5 × 1,5 cm) e o número de ninfas eclodidas observado, diariamente, até 10 dias após cada oviposição. A longevidade de fêmeas copuladas com machos irmãos, não-irmãos ou ambos foi registrada e após a morte, os adultos foram pesados e medidos. Fêmeas de *P. nigrispinus* não discriminam entre machos irmãos ou não-irmãos, indicando que este predador pode evitar a endogamia incluindo redução da aptidão por meio de cruzamentos com machos não relacionados.

Palavras Chave: controle biológico, escolha de cópula, percevejo, seleção sexual

Pentatomid predators (Hemiptera: Pentatomidae) have been evaluated for the augmentative biological control of insect pests (Tipping et al. 1999), which necessitates the study of their biology to develop mass rearing methodologies (Zanuncio et al. 2001). The predator *Podisus nigrispinus* (Dallas, 1851) (Heteroptera: Pentatomidae) is common in the Americas and in several agroecosystems in Brazil where it is an important biological control agent of defoliating caterpillars and other insects (Molina-Rugama et al. 1997; Medeiros et al. 2003; Torres & Zanuncio 2001).

Podisus nigrispinus is polyandrous and its females perform up to 13 copulations with 3 to 4 males over a 1-2 wk period, which is sufficient to fertilize its eggs and to produce nymphs for over 80% of its lifetime (Torres & Zanuncio 2001). Costs and benefits of reproduction are asymmetric for males and females, resulting in sexual conflict (Watson et al. 1998). Mate choice can affect reproductive success and females may have multiple copulations to maximize their offspring (Ridley 1990). This behavior may increase female gains in genetic and nutritional material (Reynolds 1996) resulting in higher fertility and offspring heterogeneity (Koshiyama et al. 1996). Disadvantages to multiple mating include: 1) energy expenditure, 2) increased risk of predation (Crudgington & Siva-Jothy 2000); 3) contamination by pathogens (Rolff & Siva-Jothy 2002) and 4) toxic effects of substances transferred during ejaculation (Chapman et al. 1995).

Females of *P. nigrispinus* with several long copulations are more fecund (Torres & Zanuncio 2001; Sousa-Souto et al. 2006; Rodrigues et al. 2009), but consequences of multiple mating remain unclear. The objective of this research was to elucidate female preference for mates and to determine the consequences of these preferences.

Materials and Methods

The experiment was conducted under laboratory conditions at the Laboratory of Biological Control of the Department of Animal Biology of the Federal University of Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil in a climate chamber at 28 ± 1 °C, 70 ± 10% R.H. and 12:12 h L:D.

Insect Culture

Eggs of *P. nigrispinus* were obtained from different cages from rearing facilities of the Laboratory of Biological Control of the UFV and monitored separately. Hatched nymphs were kept in Petri dishes (13.0 × 1.5 cm) with moistened cotton wool and fed with *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) pupae until the nymphs transformed into the adult stage. Water was supplied in 5 mL anesthetic tubes plugged with moistened cotton wool. Egg masses deposited in the cages were collected with cotton wool and transferred to Petri dishes (9.0 × 1.5 cm) with moistened cotton wool. Nymphs were maintained in these dishes and fed *T. molitor* pupae.

Choice of Sexual Partner

Newly emerged *P. nigrispinus* females were transferred to plastic containers (500 mL) and fed every 2 days with 2 *T. molitor* pupae. Treatments were: 1) one virgin female with one virgin sibling male, 2) one virgin female with one virgin non sibling male and 3) one virgin female simultaneously with 2 virgin males (a sibling and a non-sibling male). Sibling males were marked on the back (scutellum) with water-based ink. Females of the no-choice tests were kept in containers either with a single sibling or non- sibling male. In the choice test, each female was kept with one sibling and one non-sibling male. Ten replications were used per treatment (Oliver & Cordero 2009). The weight of the females used ranged from 70-80 mg and that of males from 43-50 mg (Torres & Zanuncio 2001).

Data Collection

Number of matings, eggs and egg masses were observed every 12 h from the time adults were placed in the containers. Eggs laid were removed from the plastic containers with cotton tips and transferred to Petri dishes (9.5 × 1.5 cm) containing moistened cotton wool to prevent dehydration. Egg viability was evaluated during the first 10 days after each oviposition. Longevities of females with sibling, non-sibling or both males were recorded.

Statistical Analysis

The number of matings of females without a choice of male was subjected to analysis of variance (ANOVA) ($P < 0.05$). A chi-square test was applied to the data from females with a choice of partners. The numbers of eggs, eggs/egg mass, egg masses, nymphs and duration of oviposition period and female longevity were analyzed with the non-parametric Kruskal-Wallis test.

Results

In the no-choice tests, the numbers of matings were similar for females mated with siblings or non-siblings males (Fig. 1A) (3.3 ± 1.36, and 1.7 ± 0.73 times, respectively; $\chi^2 = 2.0$; $P > 0.05$).

The average oviposition period of a female mated both with a sibling + a non-sibling male was shorter (6-7 days) than the oviposition periods following a mating with either a sibling or a non- sibling male (16-16.5 days) (Fig. 1B). Number of eggs per female was the lowest when the female had mated with a sibling + a non-sibling male (choice) (Fig. 2A). However, numbers of eggs/egg mass and of egg masses did not differ between females mated with siblings, non- siblings or with both types of males (Fig. 2B, C).

The average number of nymphs per female that had mated with a non-sibling male was the highest, the number of nymphs per female that had mated with a sibling were intermediate and the number of nymphs per female that had mated with both kinds of males produced the fewest nymphs (Fig. 3A). The type of male with which the female mated did not affect female longevity (Fig. 3B).

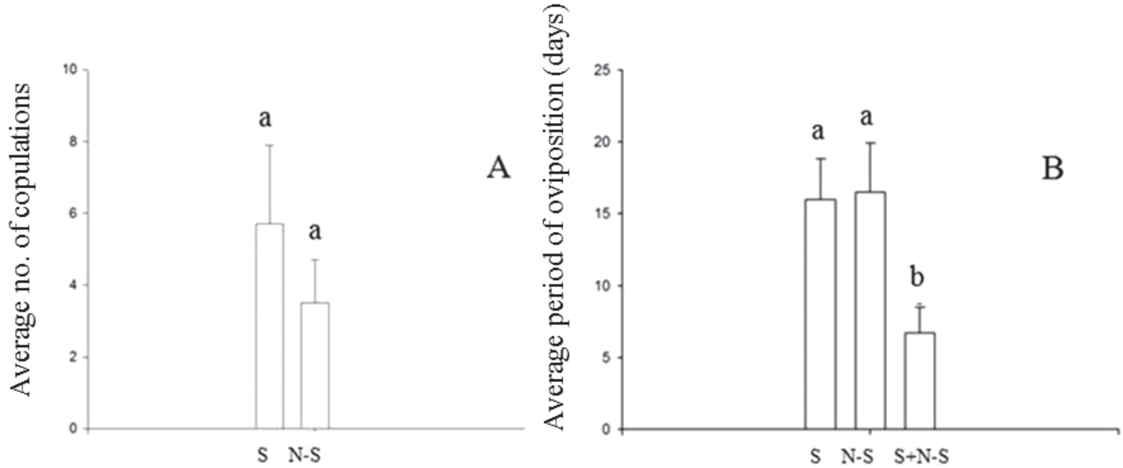

Fig. 1. Number of matings (A) (ANOVA: $F_{1;\,18} = 0.7458$; $P > 0.05$) and oviposition periods (days) (B) (Kruskal-Wallis test: KW = 8.2518; $P < 0.05$) of *Podisus nigrispinus* females each held either with a sibling, a non-sibling or both kinds of males in the laboratory at 28 ± 1 °C, 70 ± 10% RH and 12:12 h L:D. Bars on the columns represent standard errors. Abbreviations: S - sibling; N-S - non-sibling; and S + N-S - sibling plus non-sibling.

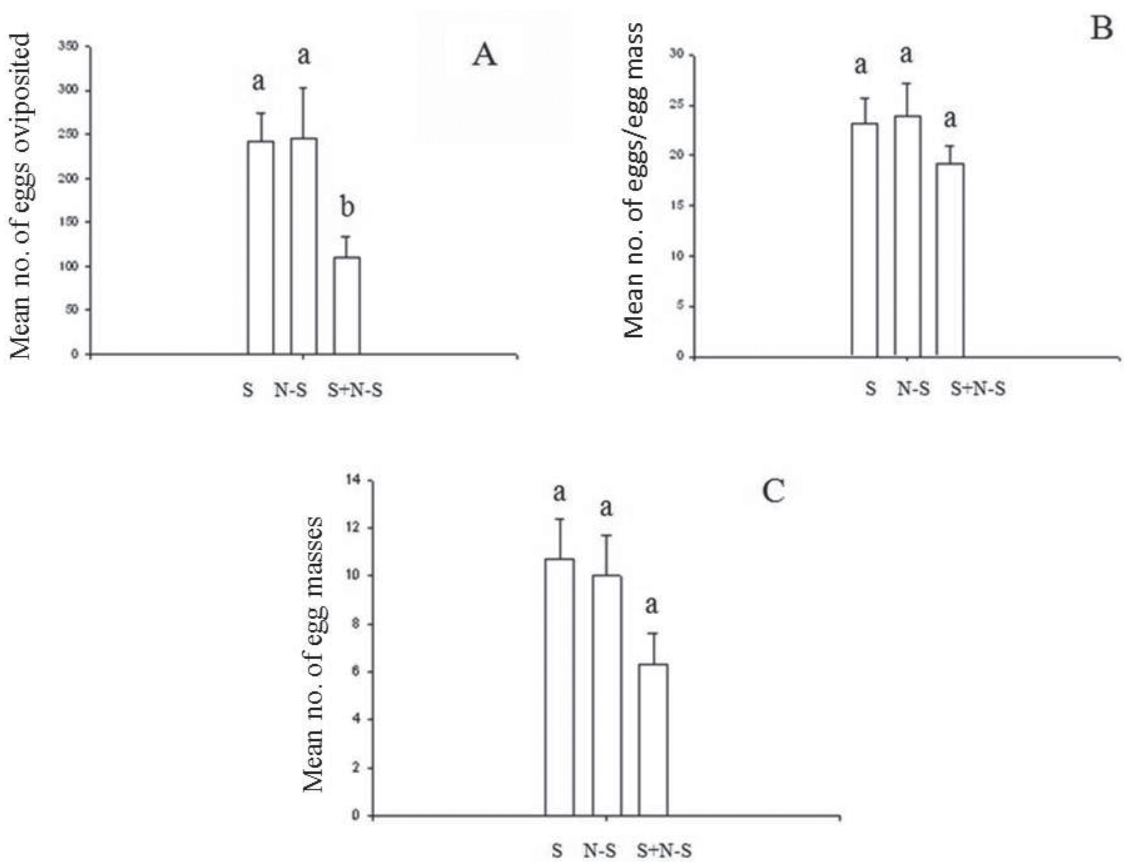

Fig. 2. Number of eggs oviposited (A) (Kruskal-Wallis test: KW = 6.0351; $P < 0.05$); number of eggs/egg mass (B) (Kruskall-Wallis test: KW = 3.2535; $P > 0.05$); and number of egg masses (C) (Kruskal-Wallis test: KW = 1.9766; $P > 0.05$) per *Podisus nigrispinus* female held either with sibling, non- sibling or both kinds of males in the laboratory at 28 ± 1 °C, 70 ± 10% RH and 12:12 h L:D. Bars on the columns represent standard errors. Abbreviations: S - sibling; N-S - non-sibling; and S + N-S - sibling plus non-sibling.

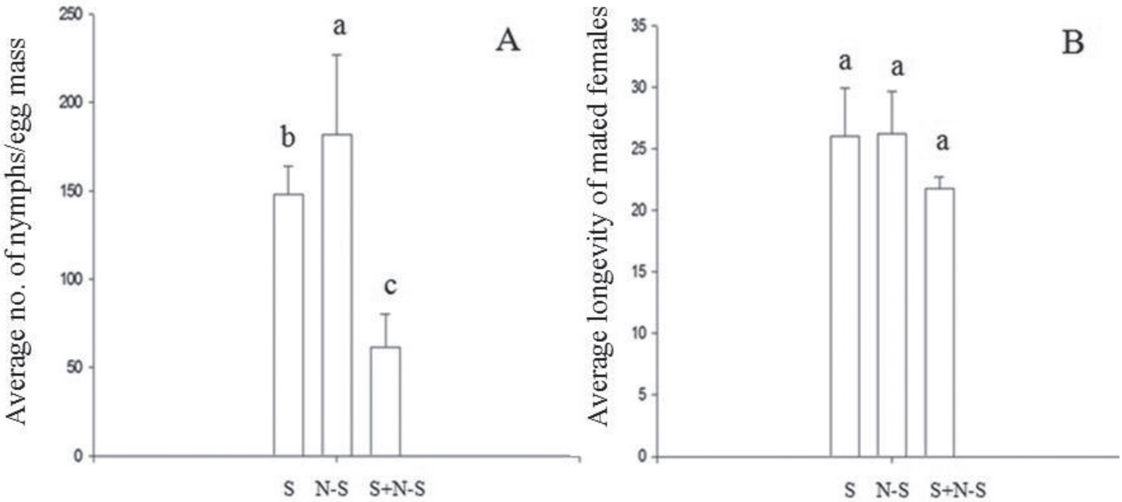

Fig. 3. Mean number of nymphs per egg mass (A) (Kruskal-Wallis test: KW = 6.1170; $P < 0.05$) and mean longevities (B) (Kruskal-Wallis test: KW = 1.0721; $P > 0.05$) of *Podisus nigrispinus* females each held either with a sibling, a non- sibling or both kinds males in the laboratory at 28 ± 1 °C, 70 ± 10% RH and 12:12 h L:D. Bars on columns represent standard errors. Abbreviations: S - sibling; N-S - non-sibling; and S + N-S - sibling plus non-sibling.

Discussion

Females of *P. nigrispinus* did not discriminate between sibling and non- sibling males in the laboratory. This may lead to decline of fitness due to inbreeding as observed for many insect species (Henter 2003; Clutton-Brock 2007; Shuker 2010)

The shorter oviposition period of females in the choice-test may reflect conflict between males, because the number of copulations should have been adequate to fertilize most eggs produced (Torres & Zanuncio 2001). Quantity of sperm transferred during mating can be low and sperm competition high in polygamous insects, which can decrease oviposition periods (Parker et al. 1997; Arnqvist & Nilsson 2000; Elgar et al. 2003; Crudgington et al. 2010; Rodrigues et al. 2008).

The greater number of eggs laid and the higher egg viability of *P. nigrispinus* females mated with non-sibling males indicate that this predator can reduce inbreeding by mating with unrelated males. Inbreeding could be reduced or avoided in laboratory rearing facilities by keeping *P. nigrispinus* females separate from sibling males. Sexual conflict between males and females after mating is ubiquitous and increases competition between the first ones to fertilize eggs (Stockley 1997).

The similar longevities of females in the various treatments may indicate no differences in quantities of sperm and accessory substances transferred by *P. nigrispinus* males. The nutritional content of sperm of this stinkbug has been poorly studied, but males of other insects provide sperm rich in nutrients as a nuptial gift (Pivnick & McNeil 1987; Koshiyama et al. 1996; Smedley & Eisner 1996). Accessory substances are used in the processes of cellular respiration and vitellogenesis (Koshiyama et al. 1996), oviposition (Herndon & Wolfner 1995), sperm activation (Osanai & Chen 1993) and to maintain sperm viability during storage in the spermatheca (Tram & Wolfner 1999), all of which tend to increase female longevity.

The increased oviposition and egg viability rates for females that had mated with non-sibling males confirm the need of multiple mating for the reproductive success of this species (Torres & Zanuncio 2001). *Podisus nigrispinus* nymphs aggregate from the time of hatching until transformation into adults (Torres & Zanuncio 2001), which increases the chances of mating with siblings and inbreeding.

Conclusions

Females of *P. nigrispinus* did not discriminate between sibling or non- sibling males for mating, which can lead to genetic inbreeding. However, the greater number of eggs laid and the higher egg viability of *P. nigrispinus* females mated with non-sibling males indicate that this predator can reduce genetic inbreeding by mating with genetically unrelated males.

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