

Short communication

Effect of starvation on the mating behavior of an aphidophagous ladybird beetle, *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae) in laboratory conditions

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اثر گرسنگی بر رفتار جفت‌گیری کفشدوزک شسته‌خوار *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae) در شرایط آزمایشگاهی

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چکیده

شرایط اکولوژیک مثل دسترسی به غذای کافی و مناسب از عواملی است که می‌تواند روی رفتار جانوران تاثیرگذار باشد. در این بین، حشرات مختلفی در زمان گرسنگی سعی می‌کنند تا رفتارهای آمیزشی و حتی طول مدت آمیزش را کاهش دهند. هدف از این مطالعه، بررسی اثر گرسنگی بر عملکرد آمیزشی کفشدوزک *Hippodamia variegata* بود. در این آزمایش، از نرها و ماده‌های ۱۰ تا ۱۵ روزه و باکره استفاده شد. در هر تکرار، یک نر و یک ماده به مدت ۳ ساعت (از ساعت ۱۲ الی ۱۵) برای آمیزش در کنار هم قرار داده شدند و مدت زمان تاخیر در آمیزش و مدت زمان آمیزش ثبت شد. آزمایش‌ها در شرایط دمای 25 ± 1 درجه سلسیوس و رطوبت 50 ± 5 درصد انجام شد. نتایج نشان داد که طول مدت جفت‌گیری و مدت زمان تاخیر در آمیزش در گروه گرسنه و سیر تفاوت معنی‌داری ندارد، لذا این گونه قابلیت سازگاری با شرایط گرسنگی در محیط‌هایی با مقدار غذای متفاوت را دارد.

واژه‌های کلیدی: تاخیر در آمیزش، جفت‌گیری، طول مدت آمیزش، Coccinellidae، گرسنگی

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Ecological conditions such as access to adequate and appropriate nutrition is one of the factors that affect animal behavior (Ortigosa & Rowe, 2002) as well as their growth, development and fitness (Singh *et al.*, 2020). Various studies have investigated the role of adequate or inadequate nutrition on mating behavior and quality of offspring in different taxa of vertebrates and invertebrates (Duquette & Millar, 1995; Rossiter, 1996; Cheung & Lam, 1999). Various insects try to avoid copulation and even decrease the duration of copulation under starvation (Scharf, 2016). For example, the behavior of the *Propylea dissecta* (Mulsant,

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1850) ladybird suggested if adult insects are properly fed, they start copulating much faster and increase the duration of it (Singh *et al.*, 2020). This study aimed to investigate the effect of starvation on the mating performance of *Hippodamia variegata* (Goeze) in which latency to copulation and copulation duration were also recorded. This aphidophagous ladybird is native to Asia and is an effective biological control agent (Tan *et al.*, 2014). Little is known about the sexual behavior of this ladybird beetle (Pervez & Navodita, 2011; Pervez & Singh, 2013) and most of the studies have focused on the aspects of its predatory behavior (Farhadi *et al.*, 2010).

For this study, adult ladybirds of *H. variegata* were collected from the College of Agriculture and Natural Resources field, University of Tehran in Karaj in May 2019 and then were identified by valid identification keys (Bienkowski, 2018). After transferring ladybirds to the laboratory, they were maintained in the laboratory conditions of $25 \pm 1^\circ\text{C}$, $55 \pm 5\%$ RH and under a 16 L: 8 D photoperiod. The ladybirds were daily fed on an *ad libitum* supply of *Aphis fabae* Scopoli which were reared on *Vicia faba* L.

In each container, crumpled papers and leaves of fava bean plants were placed for females to lay eggs on. Afterward, eggs that were laid on the paper or leaves would be transferred to the plastic containers ($18.0 \times 15.0 \times 5.0$ cm). The lids of these plastic containers were cut (a hole of 4.5 cm in diameter) and covered by lace and eggs would be kept in them until the time of hatching (Fig. 1).



Fig.1. Plastic container used for rearing *Hippodamia variegata*

To avoid cannibalism after hatching, all larvae were transferred to separate containers and fed on daily an *ad libitum* supply of *A. fabae*. All larvae were kept in the same abiotic conditions until the emergence from the pupae to adults. In this experiment, young (10–15 days old) and virgin males and females of *H. variegata* were used and copulation latency (i.e., latency in the establishment of genital contact) and copulation duration (i.e., insertion

of the male's aedeagus into the female's genitalia till the natural dislodging) was recorded. In the laboratory conditions of $25 \pm 1^\circ\text{C}$ temperature and $50 \pm 5\%$ humidity, in each container (specifications as mentioned above) one male and one female were placed for 3 hours (from 12:00 to 15:00 p.m.) for mating, after which they were separated. The pairs which did not mate within 3h or mated for less than 1 min were removed from the experiment. There were 13 replicates of this experiment with pairs not fed for 6 hours and 13 replicates with pairs properly fed with 18 wingless aphids in a day.

All analyses were performed using IBM SPSS Statistics V25.0 software. Since the data distribution of this experiment was normal, the Independent Samples T-Test was used to compare the means of copulation latency and mating duration. Results showed no significant difference in the copulation duration for fed versus hungry groups (t-test $t = 0.251$, $df = 24$, $p = 0.804$) and the duration of copulation for fed and hungry group was ($M = 115.15$, $SD = 14.32$) and ($M = 113.62$, $SD = 16.88$), respectively. In addition, the results for copulation latency does not show any significant difference at 5% level (t-test $t = -0.720$ $df = 24$, $p = 0.478$). Data for copulation latency for fed and hungry groups were ($M = 7.38$, $SD = 5.22$) and ($M = 9.00$, $SD = 6.17$), respectively (Table 1).

Table 1. The results of the mean (\pm SE) copulation latency and duration of *Hippodamia variegata*

Individuals	Replicates	Mean (\pm SE) copulation latency (min.)	Mean (\pm SE) copulation duration (min.)
Fed	13	7.38 ± 1.448	115.15 ± 3.972
Starved	13	9.00 ± 1.713	113.62 ± 4.682
Test-statistics	Independent Samples T-Test	$t = -0.720$	$t = 0.251$

For the survival of a species, reproduction is a necessity (Pervez, 2002) and sexual reproduction is the most common way of animal reproduction in the wild (Alcock, 2009; Matthews & Matthews, 2009) which could be affected by various ecological factors such as nutritional stress (Ortigosa & Rowe, 2002).

We hypothesized that according to the results of (Singh *et al.*, 2020) conducted on *P. dissecta* (Mulsant, 1850), our experimental species would no longer be interested in copulating in the starvation context or at least would increase copulation latency and decrease copulation duration; however, our results indicated that starvation context does not have any meaningful effect on the mating behavior of *H. variegata* and results are not statistically significant.

Due to the fact that starvation context does not affect the mating behavior of this species, it probably does not have any significant effect on their fecundity either and this ladybird can be adapted to environments with different nutrition quantities; these results are in accordance with the findings of (Hatt & Osawa, 2021) on *Harmonia axyridis* (Pallas, 1773).

Since studying the biology and mating behavior of ladybirds can help us in their mass production as effective biological control agents (Sahu & Kumar, 2013), we highly recommend studying the effect of starvation context on fertility and fecundity of this species

for further studies, so we could have a wider perspective on the effect of starvation on the reproductive patterns of *H. variegata* ladybird.

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