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Competition affected by re-mating interval in a myriapod

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Abstract

Copulation duration, re-mating interval, and ejaculate volume (disintegrations per minute of H^{3+}) was analyzed in the millipede *Chersastus inscriptus*. Re-mating interval negatively related to second duration ($y = 36 + 0.01x$) and relative ejaculate volume increased from 21% to 77% in favor of second males after 24h intervals.

Keywords: arthropod, copulation, diplopod, invertebrate, pachybolid, spirobolid

1. Introduction

In millipedes, copulation duration is known from some African ^[1-7], Australian ^[8] and American species ^[9], however few studies investigate sperm or ejaculate precedence ^[1]. Second male sperm precedence correlates with prolonged second copulation duration and can be analyzed from sperm volumes ^[1, 7]. Ejaculate volume precedence of males was analyzed in double mating experiments with 24 hour (h) re-mating intervals in *Chersastus inscriptus*.

2. Materials and methods

Chersastus inscriptus were collected from Twin Streams (28°55'S, 31 °45'E) (1995 - 1998). Live specimens of each sex were taken to a laboratory, kept at 25°C; 70% relative humidity; 12:12 h light-dark. Vegetables were provided *ad lib*. Unisex groups were kept in plastic containers lined by moist vermiculite (\pm 5cm deep). Ejaculate volume was measured as disintegrations per minute (dpm) tritium in a 1600 scintillation counter (low count reject = 0; dpm multiplier = 1). Millipedes were marked on posterior segments with colored tipex and placed in glass aquaria (30 X 22 X 22 mm). Pairs were isolated in plastic beakers (13cm diameter) and mating timed. Double mating sequences were performed to test for order or interval effect. Mating order was controlled by mating females with labelled and un-labelled males in reciprocity. Females were given the opportunity for second mating immediately (0-h delay) or later (24-h delay). As controls, females that had single mating with labelled males were dissected immediately or 24 h later. Females that had single mating with unlabeled males were dissected to control for background radiation. Data were analyzed with Pearson's correlation (r), t-tests (t), Spearman's rank order correlation coefficients (r), two-tailed Mann-Whitney U-tests (Z), and Wilcoxon tests (T). A Kruskal Wallis 1-way ANOVA (H) was used to test for differences in dpm data prior to Mann-Whitney U-tests.

3. Results

Copulation duration in *C. inscriptus* lasted 173 ± 41 minutes. First duration (174 ± 35 min.) and second duration (173 ± 47 min.) were the same ($T = 507$, $n = 46$, $P = 0.91$) and were not correlated ($r = -0.06$, $n = 46$, $P = 0.69$). The interval was not related to first duration ($r = 0.07$, $n = 46$, $P = 0.64$) but was negatively related to second duration ($r = -0.31$, $n = 46$, $P = 0.04$); $y = 36 + 0.01x$. Differences in successive copulations were present ($r = 0.33$, $n = 46$, $P < 0.05$). No differences between duration of isotope-labelled and unlabeled males ($t = 0.38$, d. f. = 28, $P = 0.70$), or between left and right vulvae of females ($T = 711.5$, $n = 59$, $P = 0.19$) occurred. Overall differences were found in total dpm ($H = 30.67$, d. f. = 5, $P = 0.00001$). Immediate impact of the second male on the first male was absent ($Z = 0.84$, $n = 6, 12$, $P = 0.40$). The dpm of the first male was higher than the second male ($Z = 3.37$, $n = 6, 12$, $P = 0.0007$) but with a delay of up to 24 h precedence reversed and second male's was higher ($Z = -2.59$, $n = 8, 14$, $P = 0.01$). There was no difference in dpm between single and double mating with

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controlled intervals ($Z=0.58$, $n = 7.8$, $P = 0.56$). There was a drop in dpm post-mating ($Z = 3.00$, $n = 6, 7$, $P = 0.003$). Ejaculate displacement negatively related to copulation durations after 24 h ($r = -0.88$, $P = 0.004$, $n = 8$) but not when females mated immediately ($r = -0.10$, $P = 0.75$, $n = 12$).

4. Discussion

This study successfully uses a hypothetico-deductive approach to unravel three major factors (re-mating interval, mating duration, ejaculate volume) important to mate competition in a spirobolid millipede. Ejaculate volume (dpm) in *C. inscriptus* is a function of re-mating, rising from 21% to 77%, in favor of second males after 24 h. This is only the second case of female control of ejaculate in millipedes^[1]. The ejaculate may equate with the female storage capacity. An untested assumption is ejaculate precedence equates with second male sperm precedence at the molecular level. Future research should investigate the effect of third males on mate guarding, and natural re-mating interval.

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