



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2019; 7(4): 183-186

© 2019 JEZS

Received: 23-05-2019

Accepted: 27-06-2019

Mark CooperSchool of Animal, Plant &
Environmental Sciences,
University of the Witwatersrand,
Johannesburg, South Africa

When is the change in sperm precedence in the millipede *Centrobolus inscriptus* (Attems, 1928) (Diplopoda, Pachybolidae)?

Mark Cooper**Abstract**

There were three scenarios of sperm precedence; first-male, last-male and no order effect. I replicated the H^{3+} radiolabel technique and showed sperm precedence patterns in the sexually dimorphic millipede *Centrobolus inscriptus* (Attems, 1928). Disintegration rates (D.P.M.) from single labelled mating were significantly lower than those of unlabelled-labelled mating ($U=-2.6$, $n=13$, $p=0.005$) but not to labelled-unlabelled double mating ($U=0.45$, $n=14$, $p=0.65$). In the initial experiment without the interval $V_2=43.66\%$ and with the interval $V_2=74.59\%$. D.P.M. from the immediate dissections of twice mated females were higher ($Z = 3.81$, $n = 12$, 12 , $P = 0.0001$) than females with a remating interval of up to 24 hours. When the experiment was replicated significant differences ($H = 30.67$, d.f. = 5, $P = 0.00001$) between total D.P.M values were again found. D.P.M. values were higher when second mating followed immediately after the first ($V_2= 21\%$). D.P.M. from labelled-unlabelled mating were significantly lower than the D.P.M. of unlabelled-labelled mating after 10 hours (600 minutes) resulting in a reduction of up to 57% in favour of the second male when second-male ejaculate volume precedence arises to 77%.

Keywords: Interval, millipede, precedence, remating, sperm**Introduction**

Recent evidence for mechanisms of sperm competition in arthropods includes the millipede *Centrobolus inscriptus* ^[10, 17], the fly *Drosophila melanogaster* ^[19], the moth *Ephesia kuehniella* ^[23], the squid *Euprymna tasmanica* ^[20] and the ladybird *Menochilus sexmaculatus* ^[3]. In the fly *Drosophila melanogaster* female remating rate influences the relative share of paternity ^[19]. In the polyandrous moth the intermating duration is positively correlated with the number of the first male's sperm moving to the spermatheca ^[23]. In the squid *Euprymna tasmanica* significant variation among females in patterns of sperm precedence result in the second mating male siring up to 75% of offspring at the beginning of the laying period ^[20]. In the ladybird males which mated last were found to sire up to 72% indicating that prolonged matings by first males are essentially examples of post-copulatory mate guarding to prevent last male sperm precedence ^[3]. In the polygynandrous millipede *Centrobolus inscriptus* with sperm dumping and sperm storage, remating interval negatively relates to second copulation duration ^[10, 11, 13]. Three scenarios exist for sperm precedence, namely, first-male precedence, last-male precedence, and no order effect, although this may change with additional mating ^[10]. In all these cases sperm precedence patterns vary with remating interval which gives the female a chance to select between different male ejaculates ^[2]. Sperm competition can be selected in the remating interval ^[1].

When the radio-isotope method is used to quantify sperm precedence, rather than the sterile male method, genetic marking or some forms of DNA fingerprinting, the females' influence on the storage and use of sperm may be detected ^[2]. Here the H^{3+} radiolabel technique was implemented in order to account for the female role, and replicated to show sperm precedence patterns in the diplopod *C. inscriptus*. *C. inscriptus* belongs to the forest genus of millipedes in the Order Spirobolida found along the eastern coast of southern Africa ^[4]. It has evolved sperm competition strategies through either conflict of interest or cooperation ^[12, 24]. It was questioned when there is sperm competition during the remating interval, does sperm precedence become one male favoured?

Correspondence**Mark Cooper**School of Animal, Plant &
Environmental Sciences,
University of the Witwatersrand,
Johannesburg, South Africa

2. Materials and Methods

2.1 Observations

Centrobolus inscriptus (Attems, 1928) was collected from Zululand (Twin streams farm, Mtunzini), where they inhabited indigenous coastal forest in the ecologically significant Siyaya coastal catchment area (28°59'13.4"S, 31°43'41.3"E). Live specimens of each sex were transported to the laboratory where conditions were kept under a constant 25°C temperature regime; 70% relative humidity; 12:12 hrs light-dark cycle. Food was provided in the form of fresh vegetables *ad libitum*. Individuals had unknown mating histories and unisex groups were housed in plastic containers containing moist vermiculite (± 5 cm deep) for 10 days before commencing the first mating experiments.

2.2 Data analysis

Three measurements were taken for all individuals once copula pairs had disengaged; body mass (accurate to 0.01 g), body length (mm), and dorsal tergite width (mm). Morphometric data were tested for normality before comparing males and females using a Student's t-test.

2.3 Experimental layout

Animals were placed into glass mating arenas (30 X 22 X 22 cm) for single, double, and artificially-terminated mating with females. Four combinations of double mating were possible: L-UL (0); L-UL (24); UL-L (0); UL-L (24). In *C. inscriptus* an experimental protocol was based on the Tritiated [methyl-3H] thymidine radioisotope labelling technique [1, 24]. The radioactivity of ejaculates inseminated into a female spermatheca, from labelled males, were quantified as the volume of labelled (L) ejaculate present in the female spermatheca. V_2 was calculated as a surrogate variable for second-male sperm precedence (P_2).

2.4 Statistical analysis

Statistical analyses were performed using Statgraphics (version 6.0). Morphometric data were tested for normality

before comparing males and females using a Student's t-test. Two-tailed Mann-Whitney u-tests were employed as non-parametric analogues to the t-tests. Pearson's correlation coefficients were used to analyse any other relationships.

2.5 Inter-mating interval

Although the interval between successive matings was planned at 24 hours there was natural variation which was monitored in the laboratory. The remating interval essentially refers to the duration between successive matings. The remating interval was plotted against the disintegrations per minute (D.P.M.) inseminated from the first and second males to mate with a twice-mated female (Figure 1).

3. Results

From figure 1 it may be seen the disintegration rates (D.P.M.) from immediate dissections of once-mated females were higher than from females dissected after approximately 24 hours (L (0) » L (24)). There was a significant difference between mean D.P.M. values of double mating (L-UL vs. UL-L: Two-tailed Mann-Whitney U-test (unpaired), $U=2.4$, $n=15$, $p=0.001$). D.P.M. values were also higher when second mating followed immediately after the first (UL-L (0) > UL-L (24) and L-UL (0) » L-UL (24): $Z = 3.81$, $n = 12, 12$, $P = 0.0001$). D.P.M. rates from L mating were significantly lower than those of UL-L mating ($U=-2.6$, $n=13$, $p=0.005$) but not to L-UL double mating ($U=0.45$, $n=14$, $p=0.65$). Without the interval $V_2=43.66$ and with the interval $V_2=74.59$.

C. inscriptus is clearly sexually dimorphic and shows length- and width-based variability. Male body mass explains a significant percentage of body length ($R^2=0.65$, d. f. = 54, $p<0.005$) and dorsal tergite width ($R^2=0.09$, d.f.=54, $p<0.005$). The same is true for female body length ($R^2=0.79$, d.f.=39, $p<0.005$) and dorsal tergite width ($R^2=0.58$, d.f.=39, $p<0.005$). Coefficients of variation for mass differed between the sexes ($F=0.47345$, d.f.=55, 40, $P = 0.01025$) indicating directional sexual selection.

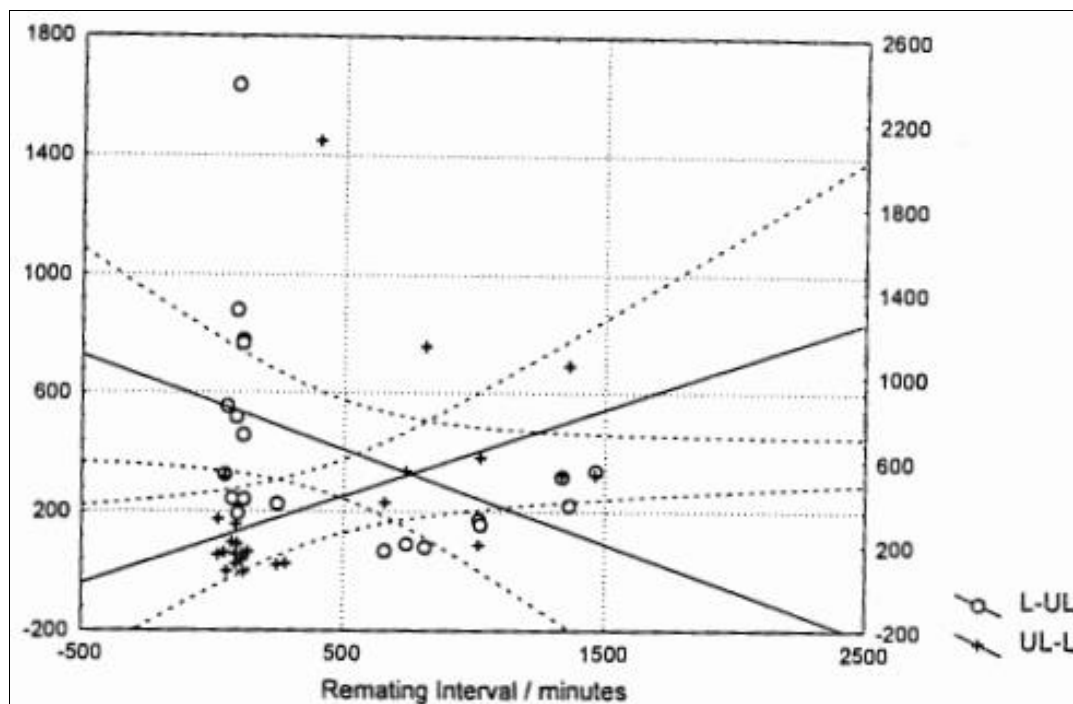


Fig 1: The change in ejaculate volumes (disintegrations H^{3+} per minute) originally deposited by the first male (L-UL) and those deposited by the second male (UL-L) after different remating intervals are indicative of volumetric ejaculate priorities.

When the experiment was replicated significant differences between total D.P.M values were again found ($H = 30.67$, D.F. = 5, $P = 0.00001$). In effect there was first male precedence without a remating interval ($V_2 = 0.21$). When there was a delay between mating of 24 hours between successive mating, precedence reversed (UL-L (24) > L-UL (24): $z = -2.59$, $n = 8, 14$, $P = 0.01$) to $V^2 = 0.77$. These findings are illustrated in figure 1 which successfully depicts the remating interval on the x-axis and the D.P.M on the y-axis. There is clearly a cross-over and change in the precedence from initial first-male to later second-male sperm precedence. This makes sense considering the longest copulation is *c. a.* 440 minutes in this species and take-overs are rare as a low percentage of triplets were found in the field.

4. Discussion

C. inscriptus is sexually dimorphic showing length- and width-based variability, factors common to members of the worm-like millipedes which were correlated with mass [4-7, 14, 15]. There was a reduction of up to 57% in favour of the second male after 10 hours within the 24 hour remating interval which changed with the number of mating an individual had experienced. Size-assortative mating was found in the laboratory and morphometric trends suggest males may be adapted for increased mobility and ability to locate females because males that mate last prolonged copulations in accordance with their body size [17].

We know there are three scenarios for sperm precedence which were tested *i. e.* first-male precedence, last-male precedence, and no order effect. We also know sperm-precedence may be female-mediated in millipedes [2]. In *C. inscriptus* second-male precedence was evident from the lower labelled ejaculate volumes present when a 24-h delay was imposed between mating. This begins at about 10 hours and equates to a decline in V_2 over time seen in arthropods where second-male precedence is female mediated from 10-hours [10, 17, 18]. This also demonstrates that successive mating produces opposite patterns on ejaculate volume and selection on female remating interval which is influenced by male sperm competition strategies as was shown in an ancient arthropod model [1, 21]. Body size and reproductive performance may be related in *C. inscriptus* because second-male sperm precedence is achieved by those individuals which prolong mate-guarding relative to body size based on mass [13].

Extensive telopodite retraction-release cycles are associated with prolonged copulations which is a behaviour which will only provide benefits in the presence of rival ejaculates *i.e.* when males copulate with a previously mated female. Last-male precedence may be more effective in assuring paternity when a female has already mated with two males for some reason [21]. Last-male precedence is affected when sex ratios are male-biased [9], perhaps because of the increased probability a female has already mated which may be associated with competition.

In millipedes (*Antichiropus variabilis*) genital morphology may influence paternity success *e. g.* spinescent hairs occurring on the distal regions of the gonopod coxites which are thought to displace sperm [20]. The spines on the gonopod telopodite plates in *C. inscriptus* may have other functions including female choice, sperm displacement, and male 'anchoring' [17]. Spinescent hairs in *C. inscriptus* are suggested to maybe function in female choice as the probability that a female will mate promiscuously is positively correlated to

genital complexity and copulation behaviour [22]. The less complex telopodites of *C. inscriptus* may necessitate a longer retraction-release cycle in order to achieve the equivalent effect of the more complex telopodites in other millipedes such as *D. uncinatus* [17].

Prolonged copulation durations in *C. inscriptus* is probably a consequence of simple gonopods being less effective than complex gonopods in sperm mixing and 'repositioners' having relatively short copulations (seconds) compared with these 'movers' (minutes-hours). The operational sex ratio and copulation duration are plastic and change through the mating season which is the result of competition and in turn results in sperm competition [9]. When the operational sex ratio becomes male-biased competition intensifies and the remating interval contracts. Here I measured the remating interval and sperm precedence at one point in evolutionary time to show the point at which precedence changes over from first to second male sperm precedence due to female mediation control [2].

5. Conclusion

Radiolabelling of millipede ejaculates successfully shows female-mediated second-male sperm precedence changes while male-specialized genital structures function in sperm redistribution. Second males benefit from mate-guarding prolonged copulation in accordance with body sizes to counter sperm competition only after 10 hours.

6. References

1. Alonzo SH, Pizzari T. Selection on female remating interval is influenced by male sperm competition strategies and ejaculate characteristics. Philosophical Transactions of the Royal Society B: Biological Sciences. 2013; 368(1613):20120044.
2. Barnett M, Telford SR, Tibbles BJ. Female mediation of sperm competition in the millipede *Alloporus uncinatus* (Diplopoda: Spirostreptidae). Behavioural Ecology and Sociobiology. 1995; 36(6):413-419.
3. Chaudhary DD, Mishra Omkar G. Last male wins the egg fertilization fight: A case study in ladybird, *Menochilus sexmaculatus*. Behavioural Processes. 2016; 131:1-8.
4. Cooper M. A review on studies of behavioural ecology of *Centrobolus* (Diplopoda, Spirobolida, Pachybolidae) in southern Africa. Arthropods. 2019; 8:38-44.
5. Cooper M. *Centrobolus titanophilus* size dimorphism shows width-based variability. Arthropods. 2019; 8(2):80-86.
6. Cooper M. Non-significant intersexual differences in millipede mass. Journal of Entomology and Zoology Studies. 2019; 7(3):763-765.
7. Cooper M. Size dimorphism and directional selection in forest millipedes. Arthropods, 2019, 8(4).
8. Cooper M. Size dimorphism in six juliform millipedes. Arthropods, 2019, 8(3).
9. Cooper MI. Sex ratios, mating frequencies and relative abundance in *Chersastus* millipedes (Diplopoda: Pachybolidae). Arthropods. 2014; 3(4):176-178.
10. Cooper MI. Competition affected by re-mating interval in a myriapod. Journal of Entomology and Zoology Studies. 2015; 3(4):77-78.
11. Cooper MI. Sperm storage in *Centrobolus inscriptus* (Attems). Journal of Entomology and Zoology Studies. 2016; 4(4):392-393.
12. Cooper MI. Sexual conflict over the duration of

- copulation in *Centrobolus inscriptus* (Attems). Journal of Entomology and Zoology Studies. 2016; 4(6):852-854.
13. Cooper MI. Sperm dumping in *Centrobolus inscriptus* (Attems). Journal of Entomology and Zoology Studies. 2016; 4(4):394-395.
14. Cooper MI. Copulation and sexual size dimorphism in worm-like millipedes. Journal of Entomology and Zoology Studies. 2017; 5(3):1264-1266.
15. Cooper MI. Lawrence's red millipede *Centrobolus lawrencei* shows length-based variability and size dimorphism. Journal of Entomology and Zoology Studies. 2019; 7(2):1037-1039.
16. Cooper MI. Quasi-experimental determination of a mass standard in the forest millipede *Centrobolus inscriptus*. Journal of Entomology and Zoology Studies. 2019; 7(3):772-774.
17. Cooper MI. Underlying sperm precedence pattern in the millipede *Centrobolus inscriptus* (Attems, 1928) (Diplopoda, Pachybolidae). Journal of Entomology and Zoology Studies. 2019; 7(3):1066-1069.
18. Hook KA. Female remating decisions and a shorter inter-mating interval diminish last-male sperm precedence. Behavioural Ecology and Sociobiology. 2017; 71:121.
19. Laturney M, Van Eick R, Billeter JC. Last male sperm precedence is modulated by female remating rate in *Drosophila melanogaster*. Evolution. 2018; 2(3):180-189.
20. Squires ZE, Wong BBM, Norman MD, Stuart-fox D. Last male sperm precedence in a polygamous squid. Biological Journal of the Linnean Society. 2015; 116(2):277-287.
21. Vrech DE, Oviedo-Diego MA, Olivero PA, Peretti AV. Successive matings produce opposite patterns on ejaculate volume and spermatozoa number in an ancient arthropod model with indirect sperm transfer. Canadian Journal of Zoology. 2019; 579-587.
22. Wojcieszek J, Simmons LW. Male genital morphology influences paternity success in the millipede *Antichiropus variabilis*. Behavioural Ecology and Sociobiology. 2011; 65:1843.
23. Xu J, Wang Q. Mechanisms of last male precedence in a moth: sperm displacement at ejaculation and storage sites. Behavioral Ecology. 2010; 21(4):714-721.
24. Yang JN. Cooperation and the evolution of anisogamy. Journal of Theoretical Biology. 2010; 264(1):24-36.