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Symmetry in ejaculate volumes of *Centrobolus inscriptus* Attems (Spiroboloidea: Trigoniulidae)

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Abstract
I test for symmetry in ejaculate volumes measured as disintegrations per minute (dpm) deposited by males in ejaculate storage organs of *Centrobolus inscriptus* in single, double and artificially-terminated matings. Combined data showed symmetry in dpm values (left-right: X ± SE = 48.11 ± 126.1828; n = 21). No significant differences between the left and right ejaculate volumes occured (T = 711.5, n = 59, P = 0.19). Symmetry in ejaculate volumes was consistent with the mechanism of sperm displacement *i. e.* mixing-self-sperm displacement by phallopods.

Keywords: asymmetry, fluctuating, symmetry, millipede

1. Introduction
Character symmetry is an effective measure of sexual selection [1-2]. Directional or fluctuating asymmetry of traits is a widespread phenomenon in biological systems [3]. Paired and complex organs are ideal models for studying this symmetry correlated with sexual selection [4]. *Centrobolus* millipedes have paired and complex or elaborate copulatory organs [5]. The male and female reproductive systems are both paired [6]. The objective of this study is to quantify the differences in ejaculate volumes deposited by males in left versus right female sperm storage organs of *C. inscriptus* with the null hypothesis being there is no difference between ejaculate volumes. This should provide insight into the mechanism of sperm displacement.

2. Materials and Methods
Millipedes were collected from indigenous coastal forest at Twin streams farm, South Africa (April 1995). Specimens were identified based gonopod morphology and keys [7]. Live specimens of each sex were transported to the laboratory where conditions were kept constant: 25 °C temperature; 70% relative humidity; 12:12 h light-dark cycle. The experimental protocol was based on radioisotope labelling [8-9]. Animals were placed into glass mating arenas (30 X 22 X 22 mm). They were marked on posterior segments with tipex fluid (perfect A16) prior to mating to allow data from each individual to be integrated. Single, double and artificially-terminated mating with females were allowed. Females of single mating (L) were either dissected immediately (L (0)), or after 24 hours (L (24)). Double mating involved a female copulating in one of two ways, either first with a labelled male, followed by an unlabelled male (L-UL), or *vice versa* (UL-L). Hence four combinations: L-UL (0); L-UL (24); UL-L (0); UL-L (24). Statistical analyses were performed using Statgraphics (version 6.0). Directional asymmetry in spermathecal ejaculate volume was estimated as the signed difference (T) in ejaculate volume of left and right spermathecae.

3. Results
No dpm values were obtained for single unlabelled mating. The mean left and right ejaculate volumes were 235.31dpm and 196.33dpm, respectively. There were no significant differences between the left and right dpm values in any of the mating (Tables 1-2; T = 711.5, n = 59, P = 0.19). The combined data for all mating are shown in Table 3.

Table 1: Means (±l SE) for labelled ejaculate volume present in the spermathecae of female *Centrobolus inscriptus* performing single mating.

Mating experiment	Left ejaculate	Right ejaculate	Total ejaculate	N
L (0)	655 (± 0.00)	1467 (± 0.00)	2121 (± 0. 00)	1
L (24)	172 (± 60. 93)	144 (± 81.75)	316 (± 97. 92)	8
UL	0	0	0	17

Table 2: Means (± l SE) of labelled ejaculate volume (dpm) present in the spermathecae of female *Centrobolus inscriptus* performing double matings.

Mating	Left ejaculate	Right ejaculate	Total ejaculate	N
L-UL (0)	542 (± 0.00)	358 (± 0.00)	901 (± 0.00)	1
UL-L (0)	358 (± 143.83)	339 (± 161.95)	698 (± 221. 09)	7
L-UL (24)	140 (± 98.42)	92 (± 59.36)	232 (± 135.29)	4
UL-L (24)	381 (± 177.23)	300 (± 38.83)	681 (± 214.21)	3

Table 3: Summary statistics of spermathecal ejaculate volume (dpm) and asymmetry in *Centrobolus inscriptus.*

Ejaculate	Mean	Standard Error	N
left	235.31	166.9263	21
right	196.33	137.0948	21
combined	440.74	269.6139	21
left - right	48.11	126.1828	21

4. Discussion

There was symmetry in ejaculate volumes of *C. inscriptus.* This indicates several things. Firstly, it is the result of a sperm mixing strategy employed by males in sperm competition. Differences between the double mating volumes indicate second male sperm precedence determined by the 24 hour interval [9]. This is accompanied by self-sperm displacement [8]. The absence of any differences between left and right dpm values should not imply impediment of flexible use e. g. Arachnida [4]. The dual functional role of the phallopods is sperm transfer and mixing [10]. The result here was not a (false) positive. It is also not seen as a false negative as the sample size is large; millipedes were given a range of polygynandrous matings before dissections and all possible variations except three-male mating sequences were included in the study.

5. Conclusion

Symmetry in ejculate volumes is consistent with the mechanism of sperm displacement *i. e.* mixing-self-sperm displacement by phallopods.

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