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

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Abstract
I test an assumption of the lock-and-key hypothesis that paired and complex copulatory organs may impede flexibility. I found no differences in ejaculate volumes (dpm) deposited by males in left versus right female storage organs of *Centrobolus inscriptus* involved in single, double and artificially-terminated matings. When the data for all matings were combined there was symmetry in spermathecal dpm values (left-right: X±SE = 48.11±126.1828; n=21). No significant differences between the left and right ejaculate volumes occurred (T=711.5, n =59, P=0.19). My findings partially support the species isolating lock-and-key hypothesis for explaining divergence in millipede genital morphology.

Keywords: asymmetry, complex, fluctuating, lock-and-key, symmetry, millipede

1. Introduction
Paired and complex copulatory organs may impede flexible use as maintained by the lock-and-key hypothesis for the evolution of complex genitalia [1-2]. *Centrobolus* millipedes have paired and complex or elaborate copulatory organs [3]. Directional or fluctuating asymmetry has been reviewed for biological systems with exception of millipedes [4]. The female reproductive system in the spiroboloidan millipede *C. inscriptus* is paired [5]. Here I test the hypothesis that paired and complex copulatory organs impede flexible use by testing for differences in ejaculate volumes deposited by males in left versus right female sperm storage organs of *C. inscriptus* after single, double and artificially-terminated matings.

2. Materials and methods
Millipedes were collected from indigenous coastal forest at Twin streams farm, South Africa (April 1995). Live specimens of each sex were transported to the laboratory where conditions were kept constant: 25 °C temperature; 70% relative humidity; 12:12 hrs light-dark cycle. The experimental protocol was based on radioisotope labelling [6-7]. Animals were placed into glass mating arenas (30 X 22 X 22 em). 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 matings with females were allowed. Females of single matings (L) were either dissected immediately (L(0)), or after 24 hours (L(24)). Double matings 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 and Discussion
No dpm values were obtained for single unlabelled matings. 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 matings (Tables 1-2; T = 711.5, n =59, P = 0.19).

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

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

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

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

The combined data for all matings are shown in Table 3. Hence there was symmetry in spermathecal dpm values, which means the lock-and-key hypothesis for the evolution of paired and complex genitalia by impairment cannot be completely rejected for paired and complex genitalia of *Centrobolus*. The finding of a female *C. inscriptus* with torn off gonopods also supports the lock-and-key in these millipedes. The lock-and-

key and cryptic female choice hypotheses are not mutually exclusive in millipedes with complex and paired genitalia.

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

Factor	Mean	SE	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. References

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