DOES EJACULATE VOLUME, MASS, AND COLEOPOD SPINE LENGTH AND NUMBER VARY WITH MOMENTS OF INERTIA AND SEX RATIO IN CENTROBOLUSCOOK, 1897?

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***Abstract*- Ejaculate volume, moments of inertia and sex ratios were checked for correlations in the red millipede genus Centrobolus.There was a significant relationship between ejaculate volumes and sex ratio (Kendall's τ: r=-0.27854301, Z score=-120000, n=8, p=0). Moments of inertia were checked for a correlation against ejaculate volume. Moments of inertia were correlated against ejaculate volume (r=-0.95306049, Z score=-3.22879652, n=6, p=0.00062162). Moments of inertia were checked for a correlation against mass. Moments of inertia were correlated against mass (r=0.81667541, Z score=3.44025764, n=12, p=0.00029063); in males (r=0.87842566, Z score=2.37088604, n=6, p=0.00887274) and marginally in females (r=0.72642819, Z score=1.59543164, n=6, p=0.05530787). Coleopod spine length (n=10, 23) and moments of inertia (n=4, 2) were positively related to spine length (Kendall's τ: r=0.54772256, Z score=60000 ,n=6, p=0). Spines counted were negatively related to moments of inertia (Kendall's τ: r=-0.54772256, Z score=-60000, n=6, p=0). C. inscriptus had the highest spine length (10µM) and the highest moments of inertia while C. ruber males had the lowest spine length (2.5µM) and the lowest moments of inertia. This supports the function of the spine length as an allometric device in sperm competition or stimulation of cryptic female choice. The above suggests the pattern of mate guarding was positively associated with ejaculate volume, mass and the intensity of intra-male competition.**

I. INTRODUCTION

The millipede genus *Centrobolus* Cook, 1897 is found in the temperate South African subregion, its northern limits on the east coast of southern Africa being about -17° latitude South (S) and its southern limits being about -35° latitude S. It consists of taxonomically important species with 12 species considered threatened and includes nine vulnerable and three endangered species. It occurs in all the forests of the coastal belt from the Cape Peninsulato Beira in Mocambique. Common with worm like millipedes is the absolute abundance known to differ in several populations of the genus. These worm-like millipedes show female-biased Sexual Size Dimorphism (SSD) [57]. Absolute abundance is seasonal and triggered byprecipitation and determines the ejaculate volume which correlates with the copulation durations. Ejaculate volumes are tested for a correlation with sex ratio during the breeding season in the pachybolid millipede genus Centrobolus. The aim is to determine if there is a correlation between ejaculate volume, mass, moments of inertia and sex ratio. Correlations between coleopod spine length,number, and moments of inertia were checked.

II. MATERIALS AND METHODS

Two species belonging to the genus *Centrobolus* Cook, 1897 were identified. The ejaculate volume during the breeding season was obtained for C. anulatus and C. inscriptus. The number of individualmillipedes was hand collected, counted, and sexed in situ from the Mick's Park Conservation area inTwin Streams farm (Mtunzini) over a period of up to 3 days early and late in a season. The ejaculate volume was calculated as the disintegrations per minute. Ejaculte volume and sex ratio during early and late in the breeding season were checked for correlations usingthe Pearson Correlation Coefficient calculator(https://www.gigacalculator.com/calculators/correlationcoefficient-calculator.php) (Appendix 1). Tests for normalitywere conducted. Moments of inertia and mass (g) were calculated. Moments of inertia (and mass) and ejaculate volumes were correlated using a Pearson Correlation Coefficient(https://www.gigacalculator.com/calculators/correlation-coefficient-calculator.php) (Appendix 2-3). One set of linear measurements was made from the SEMmicrographs: (1) spine length (µM); and number. The collection of SEM micrographs for each species is particularly informative when comparisons are made between congruent views. These results have been published [1]. Moments of inertia were calculated. Spine length,number, and moments of inertia were correlated using a Pearson Correlation Coefficient (https://www.gigacalculator.com/calculators/correlation-coefficient-calculator.php). Spine length and numbers were correlated with moments of inertia in two species (C. inscriptus, C. ruber, C. fulgidus) (Appendix 4-5) using Pearson's Correlation Coefficient.

III. RESULTS

There was a significant relationship between ejaculate volumes and sex ratio (Fig. 1: Kendall's τ: r=-0.27854301, Z score=-120000, n=8, p=0).

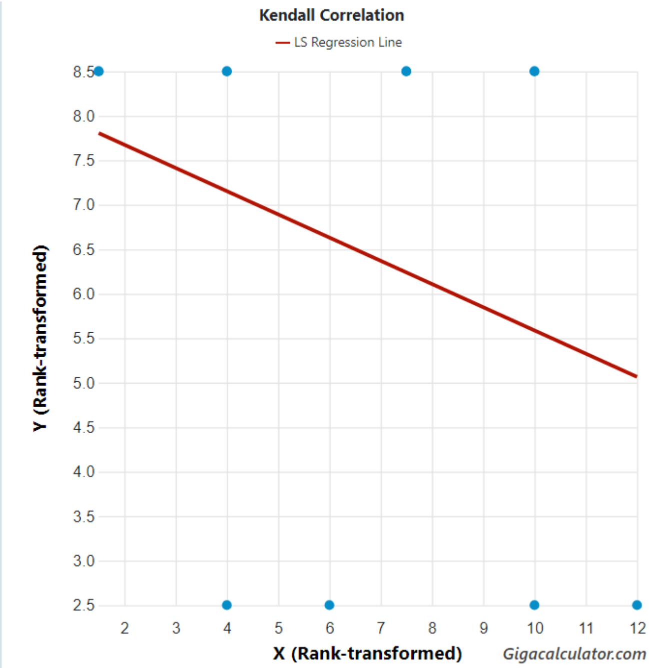

Figure 1. Correlation between sex ratio and ejaculate volume in *Centrobolus.*

Moments of inertia were correlated against ejaculate volume (Figure 2: r=-0.95306049, Z score=-3.22879652, n=6, p=0.00062162).

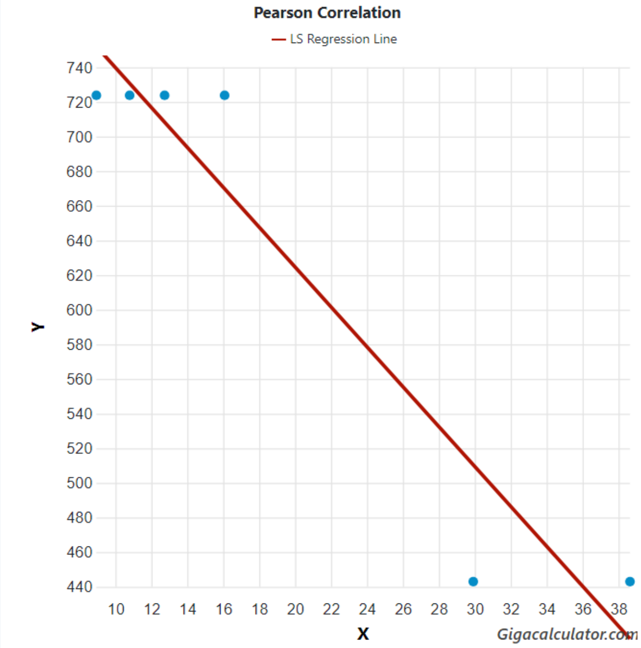

Figure 2. Correlation between ejaculate volume and moments of inertia in *Centrobolus*.

Moments of inertia were correlated against mass (Figure 3: r=0.81667541, Z score=3.44025764, n=12, p=0.00029063); in males (r=0.87842566, Z score=2.37088604, n=6, p=0.00887274) and marginally in females (r=0.72642819, Z score=1.59543164, n=6, p=0.05530787).

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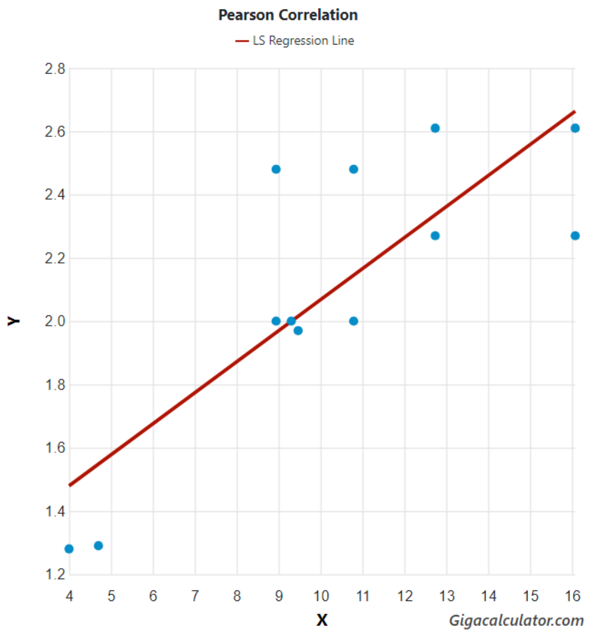

Figure 3. Correlation between mass and moments of inertia in *Centrobolus*.

Coleopod spine length (n=10, 23) and moments of inertia (n=4, 2) were positively related to spine length (Figure 4: Kendall's τ: r=0.54772256, Z score=60000 ,n=6, p=0). Spines counted were negatively related to moments of inertia (Figure 5: Kendall's τ: r=-0.54772256, Z score=-60000, n=6, p=0). C. inscriptus had the highest spine length (10µM) and the highest moments of inertia while C. ruber males had the lowest spine length (2.5µM) and the lowest moments of inertia.

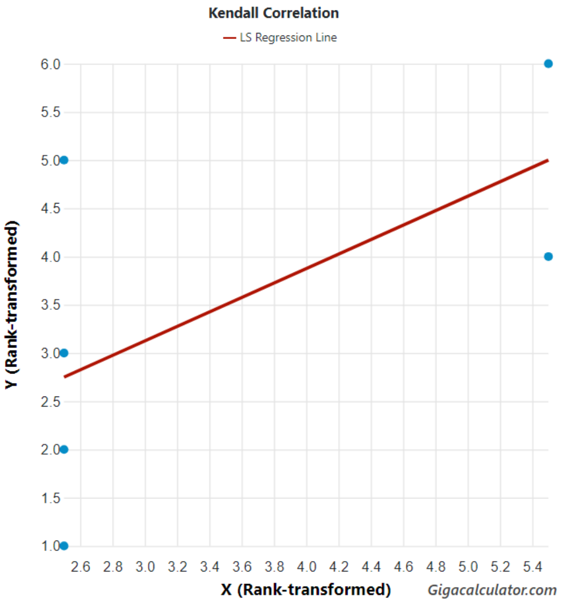

Figure 4. Correlation between coleopod spine length and moments of inertia in Centrobolus.

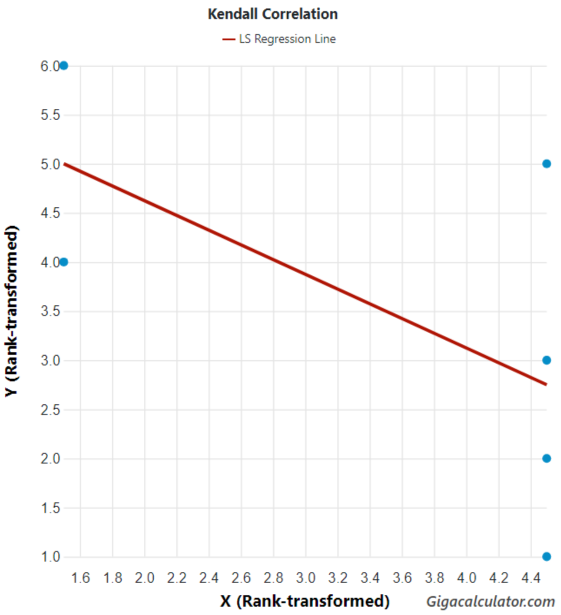

Figure 5. Correlation between coleopod spine number and moments of inertia in Centrobolus.

IV. DISCUSSION

A significant relationship was found between ejaculate volumes and sex ratio in sympatric *Centrobolus* was found. *Centrobolus* has sex ratios which correlate with ejaculate volume. This study found sex ratio recorded in Centrobolus was negatively related to ejaculating volumes. So the sex ratios probably determined the opportunity for selection,degree of polygynandry, and ejaculate volume in these species. This study supports using sex ratio as a correlate of ejaculate volume across Centrobolus. Examples of ejaculate volume varying sex ratio are unknown. Ejaculate volume variation with the sex ratio occurs during seasonal activity patterns in species such as millipedes. Sex ratio can bias the ejaculate volume and covary with many other factors depending on the time and place in the season. Spatial changes in habitat preference areknown in C. fulgidus and C. richardii [29]. These differences are linked to the effects of SSD differences (65%) between the latter two species. Similarly, ejaculate volumes may be usefully investigated and compared with this study. Copulation duration was positively related to absolute abundances across Centrobolus. Low ejaculate volumes (in C. anulatus) were associatedwith male biased sex ratio and large ejaculatevolumes (in C. inscriptus) were associated with female biased sex ratios. This suggests the pattern of mate guarding is positively associated with sex ratio and the intensity of intra-male competition. This implies the probability of a female remating is a function of male density.

The genital morphology and mechanics of copulation were figured in two *Centrobolus* species. A direct relationship between ejaculate volume and the moments of inertia of the millipedes is compared. *C. inscriptus* had the highest ejaculate volume and the lowest moments of inertia while *C. anulatus* had thelowest ejaulcate volume and the highest moments of inertia. *C. inscriptus* had the highest mass and the highest moments of inertia while C. ruber males had thelowest mass and the lowest moments of inertia.

The genital morphology and mechanics of copulation were figured in two Centrobolus species.

A direct relationship between an ultrastructural feature (spine length and number) and the moments of inertia of the millipedes is compared which certainly supports the function of the spine as a device adapted for sperm competition. A relationship between this structural feature is present across two species suggesting adaptation to insemination. C. inscriptus had the most extended spine length (10µM) and the highest moments of inertia while C. ruber males had thelowest spine length (2.5µM) and the lowest moments of inertia. Although there was neither a relationship between male nor female moments of inertia and spine length when moments of inertia are combined from both sexes a relationship exists. It can be challenging to understand the functionality and where there is no functional significance this could have been overlooked. However, the spine lengths and their numbers in Centrobolus millipede coleopods predictably function in assuring paternity.

V. CONCLUSION

Ejaculate volumes varied systematically with the sex ratio in two Centrobolus species.Higher ejaculate volumes occurs with female-biased sex ratios. New relationships between ejaculate volume and moments of inertia of the *Centrobolus* millipedes are supported. New relationships between mass and moments of inertia of the *Centrobolus* millipedes are supported.

New relationships between ultrastructural features of the morphology (spine length and number) and moments of inertia of the Centrobolus millipedes support the function of the spines as allometric devices adapted toward reducing sperm competition and assuring paternity. Spine length and number are related across species.

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APPENDIX 1.Sex ratio followed by ejaculate volumes (DPM) in two species of Centrobolus.
0.33, 723.79
0.60, 723.79
0.55, 723.79
0.62, 723.79
1.00, 443
1.00, 443
0.55, 443
0.55, 443
0.62, 443
0.62, 443
0.57, 443
0.57, 443

APPENDIX 2.Male and female moments of ineria and ejaculate volume (dpm) in two species of *Centrobolus*.
10.7911, 723.79
8.9401, 723.79
12.7375375, 723.79
16.0777305, 723.79
29.91585, 443
38.6391, 443

APPENDIX 3.Male and female moments of ineria and mass (g) in three species of *Centrobolus*.
10.7911, 2.48
10.7911, 2.00
4.70205, 1.29
4, 1.28
8.9401, 2.48
8.9401, 2.00
12.7375375, 2.27
12.7375375, 2.61
9.46585, 1.97
9.3025, 2.00
16.0777305, 2.27
16.0777305, 2.61

APPENDIX 4.Male and female moments of ineria and spine length (μM) in two species of Centrobolus
with the first species (C. inscriptus) having two measurement sets.
10, 10.7911
10, 12.737537
10, 8.9401
10, 16.077730
2.5, 4
2.5, 9.3025

APPENDIX 5.Male and female moments of ineria and spine number in two species of Centrobolus with
the first species (C. inscriptus) having two measurement sets.
10, 10.7911
10, 12.737537
10, 8.9401
10, 16.077730
23, 4
23, 9.3025