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ARE MATING FREQUENCIES RELATED TO EJACULATE VOLUMES IN *CENTROBOLUS* COOK, 1897?

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***Abstract*- Two species of *Centrobolus* were identified (*C. anulatus, C. inscriptus*) based on morphology and confirmed using Scanning Electron Microscopy (SEM) of gonopods. Two sets of measurements were made: (1) mating frequencies, and (2) mean ejaculate volume. Mating frequencies and ejaculate volume were correlated (r=0.93, Z score=5.86, n=16, p=0). A new relationship between mating frequencies and mean ejaculate volume among the *Centrobolus* millipedes support the higher ejaculate volumes as adaptive toward higher mating success or choice.**

I. INTRODUCTION

The red millipede genus *Centrobolus* is well known for studies on sexual size dimorphism (SSD) and displays prolonged copulation durations for pairs of individuals of the species [5-10]. *Centrobolus* is distributed in temperate southern Africa with northern limits on the east coast of southern Africa at -17° latitude South (S) and southern limits at -35° latitude S. It consists of taxonomically important species with 12 species considered threatened and includes nine vulnerable and three endangered species [31]. It occurs in all the forests of the coastal belt from the Cape Peninsula to Beira in Mocambique [30]. Spirobolida has two pairs of legs modified into gonopods on the eighth and ninth diplosegments [33]. In *Centrobolus* the coleopods are the anterior gonopods of leg-pair eight. They can be classed as paragonopods or peltogonopods because they are fused into a single plate-like structure and play a subsidiary role as inseminating devices. In contrast, leg-pair nine is sperm-transferring [2]. The sternites (or stigma-carrying plates [32]) prevent lateral shifting (stabilizer) and stretch the vulva sac in a medial plane [5]. Like other millipedes, these worm-like millipedes have female-biased SSD [5-10, 13-26, 28]. From the results, correlations between tarsal pad lengths and mating frequencies were checked for correlations.

II. MATERIALS AND METHODS

Millipedes were hand collected in coastal forest habitat at Mtunzini (28° 55' S; 31° 45' E) during the summer season (1995-1996). Individual millipedes were identified as species and sexed based on the presence of gonopods in males and their absence in females. Individuals were counted as either on or above ground (>30cm but <3m above ground surface). The number of mating pairs was recorded. The total number of adults was used to estimate the relative abundance. Intercalary males were excluded from the counts. Two species of *Centrobolus* were identified based on morphology and confirmed using Scanning Electron Microscopy (SEM) of gonopod structure (*C. anulatus*, *C. inscriptus*). The gonopods were dissected from males of these two species and prepared for SEM. Specimens were fixed, first in 2.5% glutaraldehyde (pH 7.4 phosphate-buffered saline) at 4 °C for 24 hours, then in osmium tetroxide (2%). Dehydration through a graded alcohol series (50%, 60%, 70%, 80%, 90% to 100% ethanol) and critical point drying followed. Specimens were mounted on stubs and sputter coated with gold palladium. Gonopods were viewed under a Cambridge S200 SEM. SEM micrographs were examined and the individual components of the gonopods were identified according to the available species descriptions. The ejaculate volume was calculated as the disintegrations per minute [3]. Ejaculate volume and mating frequencies during early and late breeding seasons were checked for correlations. Mean ejaculate volumes and mating frequencies were correlated using Pearson's Correlation Coefficient (https://www.gigacalculator.com/calculators/correlation-coefficient-calculator.php).

III. RESULTS

Mating frequencies were related to mean ejaculate volumes (Figure 1: r=0.92554221, Z score=5.86394325, n=16, p=0). Mating frequencies early in the season were related to mean ejaculate volume (r=0.93633673, Z score=5.12241798, n=12, p=0.00000015). Mating frequencies late in the season were related to mean ejaculate volume

(r=0.90668502, Z score=4.52571110, n=12, p=0.00000301). Mating frequencies on the ground early and late in the season were related to mean ejaculate volumes (r=0.94397135, Z score=5.31993758, n=12, p=0.00000005). Mating frequencies in the trees early and late in the season were related to mating frequencies (r=0.91764638, Z score=4.72174712, n=12, p=0.00000117).

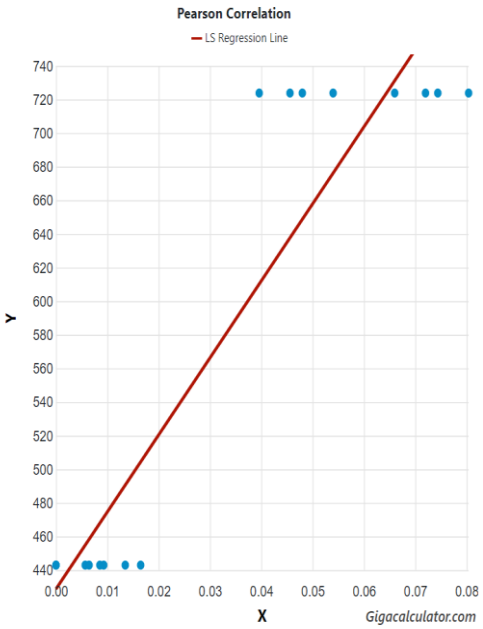

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Figure 1. Relationship between mating frequencies (x-axis) and mean ejaculate volumes (y-axis) across two species of *Centrobolus.*

IV. DISCUSSION

The mating frequencies and mean ejaculate volumes were estimated in two *Centrobolus* species [3]. A direct relationship between two factors (mean ejaculate volumes and mating frequencies) in the millipedes is compared which certainly supports the relationship. A relationship between these behavioral and morphological factors is present across the two species suggesting an adaptive character. *C. inscriptus* had higher mating frequencies and higher mean ejaculate volumes while *C. anulatus* had lower mating frequencies and a lower mean ejaculate volume. In other words, there was one positive relationship between mating frequencies and mean ejaculate volume. At the breakdown, this relationship remained on the ground and in the trees, early and late in a season.

V. CONCLUSION

A new relationship between mating frequencies and mean ejaculate volume among the *Centrobolus* millipedes support the function of this behavior as adaptive toward mating success.

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