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ARE A PROMINENT STERNITE, COLEPOD SPINE LENGTH, AND SPINE NUMBER RELATED TO MATING FREQUENCIES 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 gonopod structure. Two sets of linear measurements were made from the SEM micrographs: (1) prominence of the stemite, and (2) colepood spine length; with (3) spine number. Mating frequencies in two species were gaged. Sternite prominence and mating frequencies were positively related (r=0.93, Z score=5.86, n=16, p=0). Spine length and mating frequencies were positively related (r=0.93, Z score=5.86, n=16, p=0). Spine number and mating frequencies were negatively related (r=0.93, Z score=5.86, n=16, p=0).*C. inscriptus* had the highest sternite prominence (0.5 or 50%), highest spine length (10µM), and a low spine number (10) with high mating frequencies, and *C. anulatus* had the lower sternite prominence (0.30 or 30%), lower spine length (5µM), and high spine number (23) with low mating frequencies.

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 [4-9]. *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 [27]. It occurs in all the forests of the coastal belt from the Cape Peninsula to Beira in Mocambique [26]. Spirobolida has two pairs of legs modified into gonopods on the eighth and ninth diplosegments [28]. 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 [1]. The sternites (or stigma-carrying plates [29]) prevent lateral shifting (stabilizer) and stretch the vulva sac in a medial plane [3].

The genital morphology and mechanics of copulation were figured in two *Centrobolus* species [1, 2]. These worm-like millipedes have female-biased SSD [4-9, 12-19, 24]. From the results, correlations between coleopod sternite prominence, spine length, spine number, and mating frequencies were checked for correlations.

II. MATERIALS AND METHODS

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. Three sets of linear measurements were made from the SEM micrographs: (1) prominence of the stemite (%), (2) coleopod spine length (µM), and (3) coleopod spine number. Sternite prominence has been estimated before as a ratio of how far it extends from the basal region up to the top of the coleopod. The collection of SEM micrographs for each species is particularly informative when comparisons are made between congruent views. These results have been published [1]. Sternite prominence, coleopod spine length, and coleopod spine number and mating frequencies were correlated here using Pearson's Correlation Coefficient (https://www.gigacalculator.com/calculators/correlation-coefficient-calculator.php). Sternite prominence, spine length, and spine number were correlated with mating frequencies in two species (*C. anulatus*, *C. inscriptus*) using Pearson's Correlation Coefficient.

III. RESULTS

Sternite prominence and mating frequencies were positively related (Figure 1: r=0.92554221, Z score=5.86394325, n=16, p=0). Coleopod spine length and mating frequencies were positively related (Figure 2: r=0.92554221, Z score=5.86394325, n=16, p=0). Coleopod spine number and mating frequencies were negatively related (Figure 3: r=0.92554221, Z score=5.86394325, n=16, p=0).

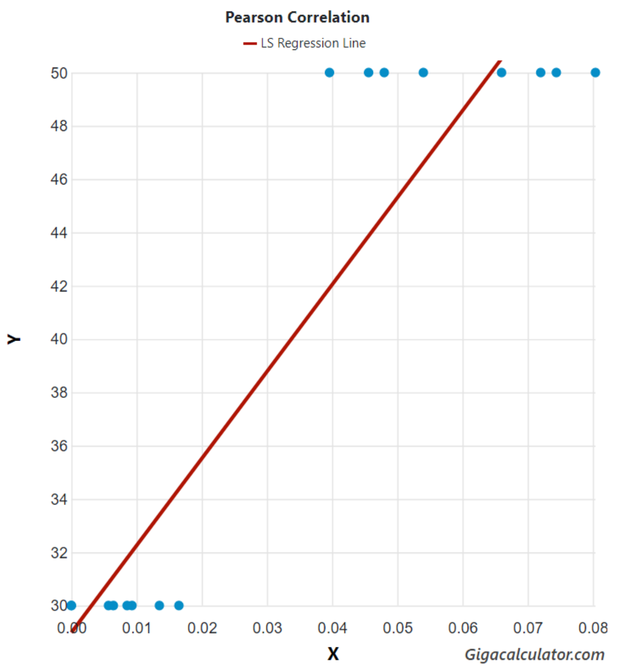

Figure 1. Relationship between sternite prominence (y) and mating frequencies (x) for C. anulatus and C. inscriptus.

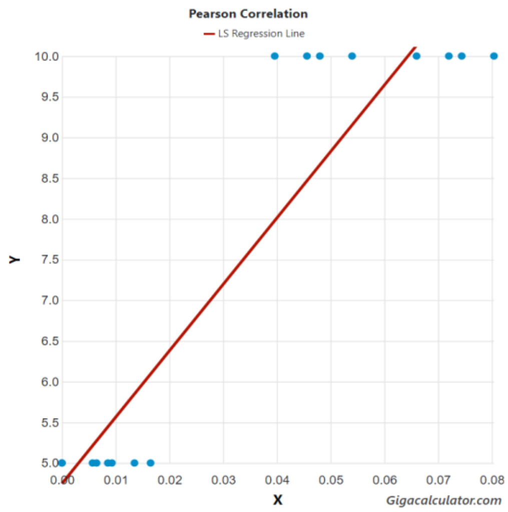

Figure 2. Relationship between coleopod spine length (μM) (y) and mating frequencies (x) for C. anulatus (left) and C. inscriptus (right).

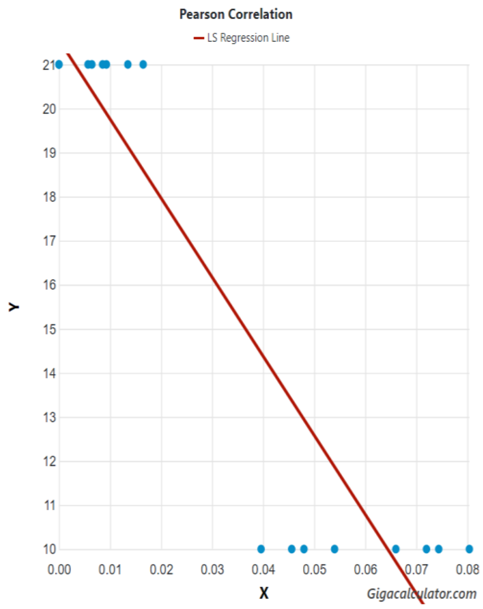

Figure 3. Relationship between coleopod spine number (y) and mating frequencies (x) for *C. anulatus* (left) and *C. inscriptus* (right).

IV. DISCUSSION

The genital morphology and mechanics of copulation were figured in two *Centrobolus*species [1, 2]. A direct relationship between three ultrastructural features (sternite prominence, spine length, and spine number) and mating frequencies in the millipedes is compared which certainly supports the function of the sternite and the colepood spines as devices adapted to mating [10, 30]. A relationship between these structural features is present across two species suggesting adaptation to insemination. *C. inscriptus*had the highest sternite prominence (0.5 or 50%), longest coleopod spines (10μM), least number of colepod spines (10), and highest mating frequencies. *C. anulatus* had the lowest sternite prominence (0.30 or 30%), lowest colepod spine length (5μM), highest number of coleopod spines, and lower mating frequencies. It can be challenging to understand the functionality and where there is no functional significance this could have been misjudged [25]. However, the sternites and accessory spines in *Centrobolus*millipedes predict a functional relevance in assuring paternity and mating or copulation. This was explained with a mechanical fit and stimulatory one-size-fits-all arguments [22, 23].

V. CONCLUSION

New relationships between ultrastructural features of the morphology (sternite prominence, spine length, spine number) and mating frequencies among the *Centrobolus* millipedes support the function of the sternite and its accessory structures as hypoallometric devices adapted toward reducing sperm competition and assuring paternity among various mating frequencies. Aprominent sternite with longer fewer spines is adapted to function in higher mating frequencies.

APPENDIX.

Male and female mating frequencies (on the ground, in the trees, early, and late in a season) and sternite prominence (%), spine length (μM), and spine number in two species of *Centrobolus.*

0, 30, 5 (23) (*C. anulatus).*
0, 30, 5 (23) (*C. anulatus).*
0.0165, 30, 10 (10) (*C. anulatus).*
0.0135, 30 5 (23) (*C. anulatus).*
0.066, 50, 10 (10) (*C. inscriptus).*
0.054, 50, 10 (10) (*C. inscriptus).*
0.0744, 50, 10 (10) (*C. inscriptus).*
0.0456, 50, 10 (10) (*C. inscriptus).*
0.0093, 30, 5 (23) (*C. anulatus).*
0.0057, 30, 5 (23) (*C. anulatus).*
0.072, 50, 10 (10) (*C. inscriptus).*
0.048, 50, 10 (10) (*C. inscriptus).*
0.00855, 30, 5 (23) (*C. anulatus).*
0.00645, 30, 5 (23) (*C. anulatus).*
0.0396, 50, 10 (10) (*C. inscriptus).*
0.0804, 50, 10 (10) (*C. inscriptus).*

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