ARE MATING FREQUENCIES RELATED TO TARSAL PAD LENGTH 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 and tarsal pads. Three sets of measurements were made from data: (1) tarsal pad length (μm), (2) tarsal pad to tarsus length ratios, and (3) mating frequencies. Mating frequencies and tarsal pad lengths were correlated (r=0.93, Z score=5.86, n=16, p=0). Tarsal pad lengths to tarsus ratios and mating frequencies were positively related (r=0.93, Z score=5.86, n=16, p=0).

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 [25]. It occurs in all the forests of the coastal belt from the Cape Peninsula to Beira in Mocambique [24]. Spirobolida has two pairs of legs modified into gonopods on the eighth and ninth diplosegments [27]. 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 [26]) prevent lateral shifting (stabilizer) and stretch the vulva sac in a medial plane [5].

These worm-like millipedes have female-biased SSD [5-10, 13-20, 22]. 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 tarsi and 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. Tarsal pads and 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. Two sets of measurements were made from the micrographs (1) tarsal pad length, (2) ratio of tarsal pad length to total tarsus length and the third set included field data for (3) mating frequencies. Tarsal pad lengths, tarsal pad to tarsus ratios, and mating frequencies were correlated using Pearson's Correlation Coefficient (https://www.gigacalculator.com/calculators/correlation-coefficient-calculator.php).

III. RESULTS

Tarsal pad lengths in *C. anulatus* (540µm, n=8) and *C. inscriptus* (1018µm, n=10), tarsal pad length to tarsus ratios in *C. anulatus* (0.83), and *C. inscriptus* (1.04), and mating frequencies were given and taken [1, 3]. Tarsal pad lengths and mating frequencies were positively related (Figure 1: r=0.92554221, Z score=5.86394325, n=16, p=0). Tarsal pad lengths to tarsus ratios and mating frequencies were positively related (Figure 2: r=0.92554221, Z score=5.86394325, n=16, p=0).

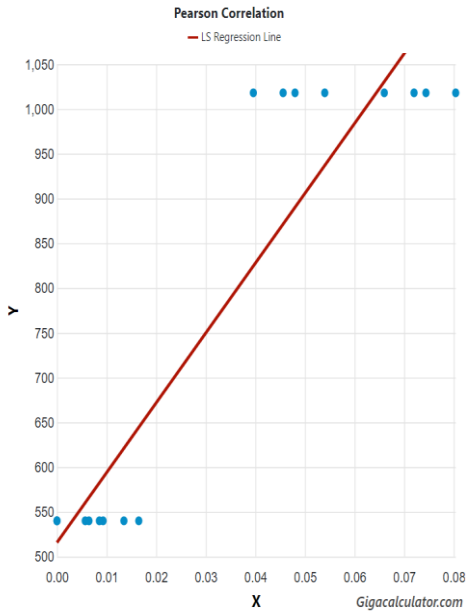

Figure 1. Relationship between tarsus pad length (Y) and mating frequencies (x) for *C. anulatus* and *C. inscriptus*.

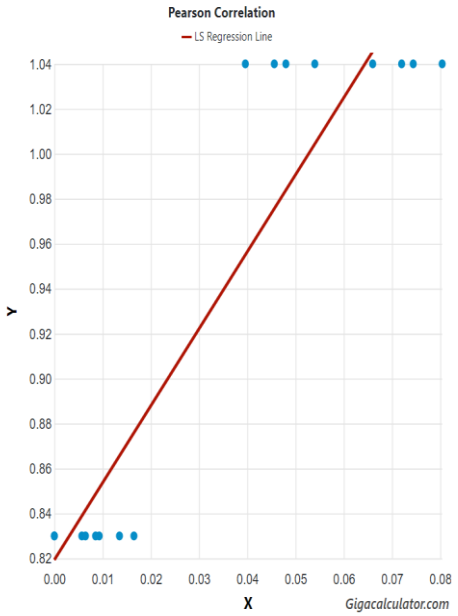

Figure 2. Relationship between tarsus pad length to tarsus ratio (Y) and mating frequencies (x) for *C. anulatus* and *C. inscriptus*.

IV. DISCUSSION

The tarsal pads are important taxonomic and secondary sexual characters of *Centrobolus* [24]. The tarsal pad length, tarsal pad-to-tarsus ratios, and mating frequencies were estimated in two *Centrobolus* species [3]. A direct relationship between three factors (tarsal pad length, tarsal pad to tarsus ratios, 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 longer tarsal pads while *C. anulatus* had lower mating frequencies and a shorter tarsal pad length and tarsal pad to tarsus ratio. In other words, there were two positive relationships between tarsal pad length, tarsal pad length to tarsus ratio, and mating frequencies.

V. CONCLUSION

New relationships between tarsal pad length, tarsal pad to tarsus ratios, and mating frequencies among the *Centrobolus* millipedes support the function of this character as adaptive toward mate competition

and acquiring mates among increased mating frequencies.

APPENDIX.

Male and female mating frequencies (early, and late in a season, on the ground, and in the trees), in two species of *Centrobolus* followed by tarsal pad lengths (μm) and tarsal pad length to tarsus ratio.
0, 540, 0.83 (*C. anulatus*).
0, 540, 0.83 (*C. anulatus*).
0.0165, 540, 0.83 (*C. anulatus*).
0.0135, 540, 0.83 (*C. anulatus*).
0.066, 1018, 1.04 (*C. inscriptus*).
0.054, 1018, 1.04 (*C. inscriptus*).
0.0744, 1018, 1.04 (*C. inscriptus*).
0.0456, 1018, 1.04 (*C. inscriptus*).
0.0093, 540, 0.83 (*C. anulatus*).
0.0057, 540, 0.83 (*C. anulatus*).
0.072, 1018, 1.04 (*C. inscriptus*).
0.048, 1018, 1.04 (*C. inscriptus*).
0.00855, 540, 0.83 (*C. anulatus*).
0.00645, 540, 0.83 (*C. anulatus*).
0.0396, 1018, 1.04 (*C. inscriptus*).
0.0804, 1018, 1.04 (*C. inscriptus*).

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