ARE MATING FREQUENCIES RELATED TO MALE AND FEMALE SIZE 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. Three sets of measurements were made from data: (1) male size (mm), (2) female size (mm), and (3) mating frequencies. Mating frequencies and male and/or female size were correlated (r=0.93, Z score=5.86, n=16, p=0). Female mating frequencies were related to female size (r=0.93, Z score=3.63, n=8, p<0.01). Male mating frequencies were related to male size (r=0.93, Z score=3.73, n=8, p<0.01). Male lengths were negatively related to mating frequencies (r=-0.93, Z score=-3.73, n=8, p<0.01). Female lengths were negatively related to mating frequencies (r=-0.93, Z score=-3.63, n=8, p<0.01). Lengths were negatively related to mating frequencies (r=-0.73, Z score=-3.34, n=16, p<0.01). Male width was positively related to mating frequencies (r=0.93, Z score=3.73, n=8, p<0.01). Female width was positively related to mating frequencies (r=0.93, Z score=3.63, n=8, p<0.01). Width was positively related to mating frequencies (r=0.60, Z score=2.48, n=16, p<0.01).Size and mating frequencies were discussed.**

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 [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 [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 male and female sizes 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. Three sets of measurements were made (1) female size, (2) male size, and the third set included field data for (3) mating frequencies. Additionally, size was broken down into lengths (Appendix 2) and widths (Appendix 3) for each sex. Male size, female size, (and their components), and mating frequencies were correlated using Pearson's Correlation Coefficient (https://www.gigacalculator.com/calculators/correlation-coefficient-calculator.php).

III. RESULTS

Male and female size and mating frequencies were given [1, 19]. Male and female sizes and mating frequencies were positively related (r=0.92554221, Z score=5.86394325, n=16, p=0). Female mating frequencies were related to female size (Figure 1: r=0.92532821, Z score=3.63333017, n=8, p=0.00013993). Male mating frequencies were related to male size (Figure 2: r=0.93139991, Z score=3.73166936, n=8, p=0.00009514). Male lengths were negatively related to mating frequencies (Figure 3: r=-0.93139991, Z score=-3.73166936, n=8, p=0.00009514). Female lengths were negatively related to mating frequencies (Figure 4: r=-0.92532821, Z score=-3.63333017, n=8, p=0.00013993). Lengths were negatively related to mating frequencies (Figure 5: r=-0.72862970, Z score=-3.33801930, n=16, p=0.00042195). Male width was positively related to mating frequencies (Figure 6: r=0.93139991, Z score=3.73166936, n=8, p=0.00009514). Female width was positively related to mating frequencies (Figure 7: r=0.92532821, Z score=3.63333017, n=8, p=0.00013993). Width was positively related to mating frequencies (Figure 8: r=0.59705867, Z score=2.48265267, n=16, p=0.00652042).

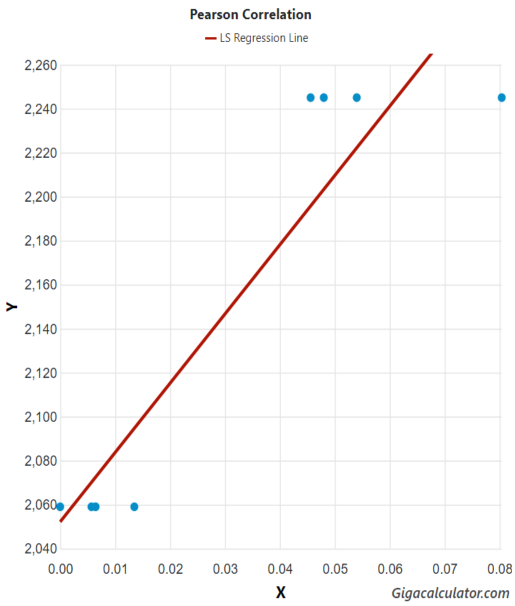

Figure 1. Relationship between female mating frequencies and female size in *Centrobolus*.

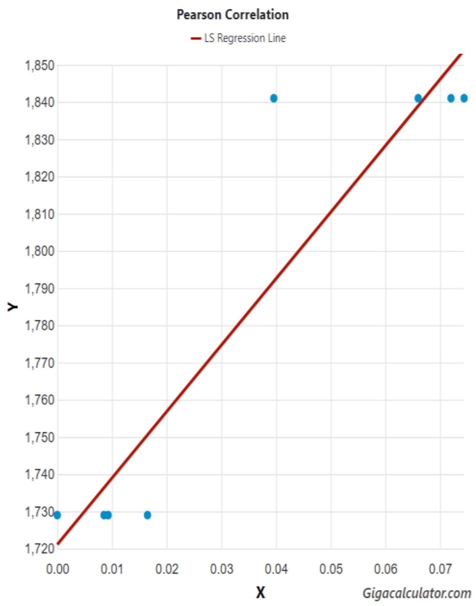

Figure 2. Relationship between male mating frequencies and male size in *Centrobolus*.

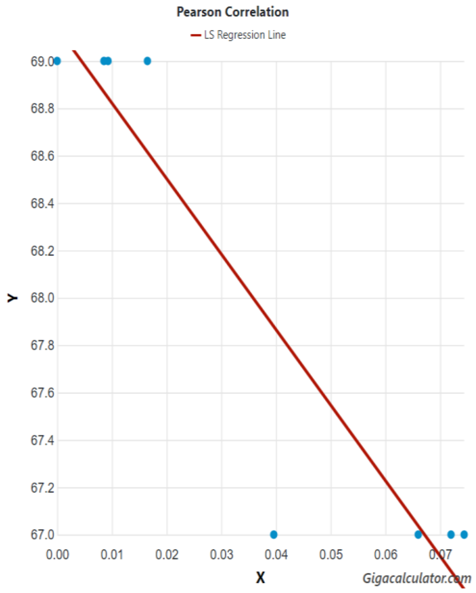

Figure 3. Relationship between male mating frequencies and male length across *Centrobolus*.

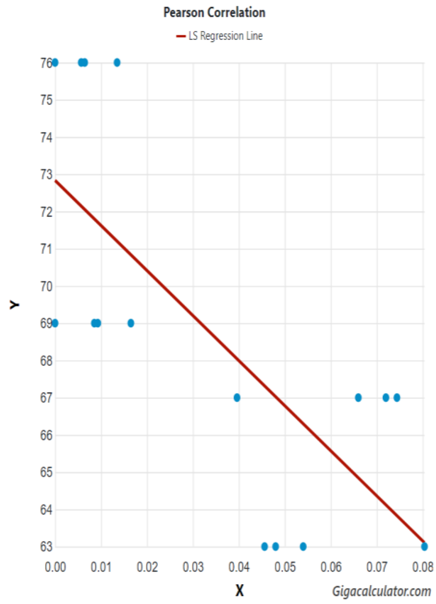

Figure 5. Relationship between mating frequencies and length across *Centrobolus*.

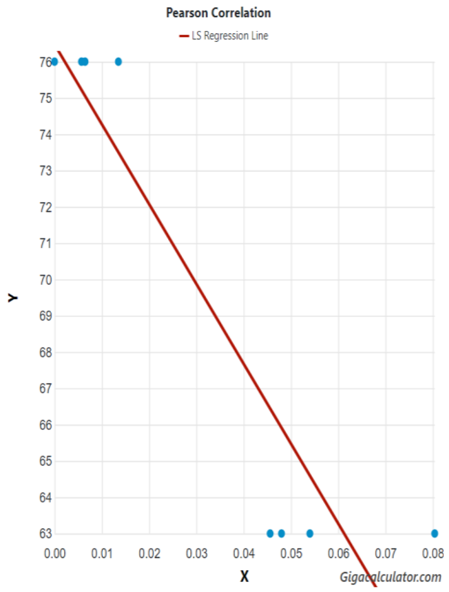

Figure 4. Relationship between female mating frequencies and female length across *Centrobolus*.

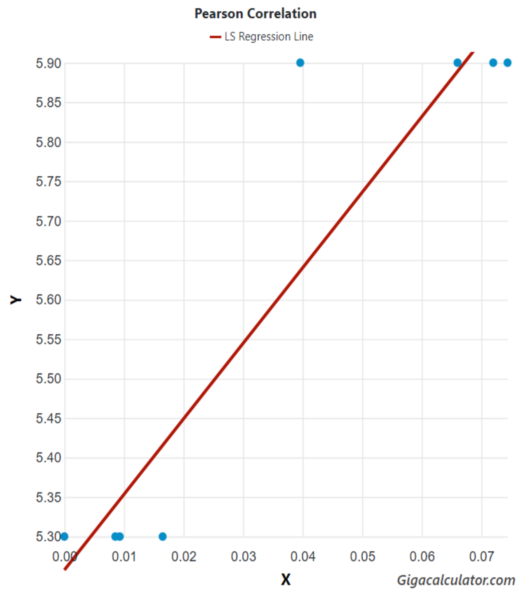

Figure 6. Relationship between male mating frequencies and male width across *Centrobolus*.

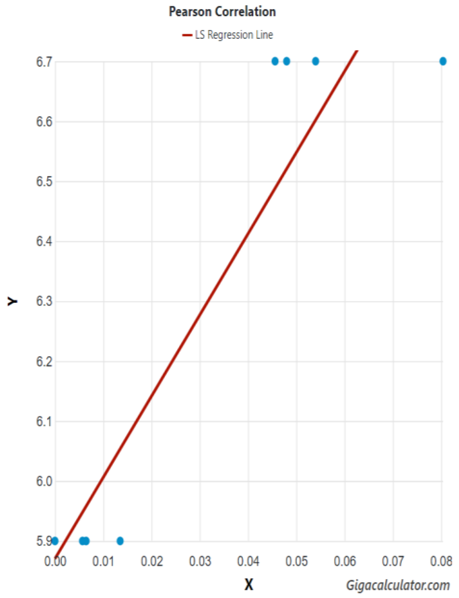

Figure 7. Relationship between female mating frequencies and female width across *Centrobolus*.

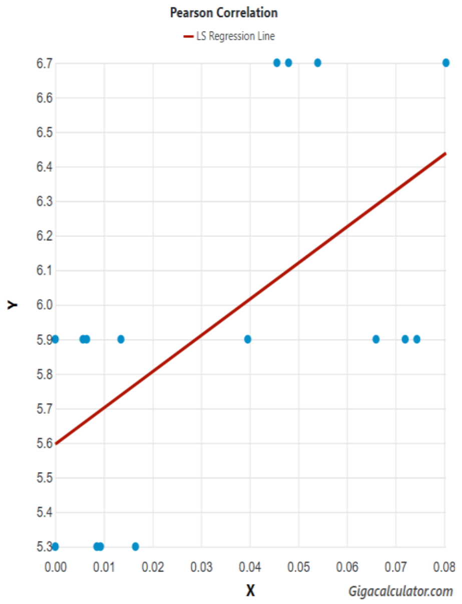

Figure 8. Relationship between mating frequencies and width across *Centrobolus*.

IV. DISCUSSION

The male and female sizes are important taxonomic and secondary sexual characters of *Centrobolus* [24]. The male and female sizes and mating frequencies were estimated in two *Centrobolus* species [3]. A direct relationship between three factors (male size, female size, and mating frequencies) in the millipedes is compared which certainly supports two relationships. Furthermore, lengths are negatively related to mating frequencies while widths are positively related to mating frequencies. A relationship between these behavioral and morphological factors is present across the two species suggesting width as an adaptive character and length as a maladaptive character. *C. inscriptus* had higher mating frequencies and larger sizes while *C. anulatus* had lower mating frequencies and smaller sizes. There were two positive relationships between male and female size and their mating frequencies. These can be attributed to the relationship between larger widths and higher mating frequencies. These results suggest that size itself contributes to mating success in these millipedes which would be due to the effects of width, not length. A similar result was found in the brachypterous grasshopper *Podisma sapporensis* [27].

V. CONCLUSION

New relationships between male and female sizes and mating frequencies among the *Centrobolus* millipedes support larger size as adaptive toward mating success 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 male and female size (mm^3).
0, 1729, 2059 (*C. anulatus*).
0, 1729, 2059 (*C. anulatus*).
0.0165, 1729, 2059 (*C. anulatus*).
0.0135, 1729, 2059 (*C. anulatus*).
0.066, 1841, 2245 (*C. inscriptus*).
0.054, 1841, 2245 (*C. inscriptus*).
0.0744, 1841, 2245 (*C. inscriptus*).
0.0456, 1841, 2245 (*C. inscriptus*).
0.0093, 1729, 2059 (*C. anulatus*).
0.0057, 1729, 2059 (*C. anulatus*).
0.072, 1841, 2245 (*C. inscriptus*).
0.048, 1841, 2245 (*C. inscriptus*).
0.00855, 1729, 2059 (*C. anulatus*).
0.00645, 1729, 2059 (*C. anulatus*).

0.0396, 1841, 2245 (*C. inscriptus*).
0.0804, 1841, 2245 (*C. inscriptus*).

APPENDIX 2.

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 male and female length (mm).
0, 69
0, 76
0.0165, 69
0.0135, 76
0.066, 67
0.054, 63
0.0744, 67
0.0456, 63
0.0093, 69
0.0057, 76
0.072, 67
0.048, 63
0.00855, 69
0.00645, 76
0.0396, 67
0.0804, 63

APPENDIX 3**.**

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 male and female width (mm).
0, 5.3
0, 5.9
0.0165, 5.3
0.0135, 5.9
0.066, 5.9
0.054, 6.7
0.0744, 5.9
0.0456, 6.7
0.0093, 5.3
0.0057, 5.9
0.072, 5.9
0.048, 6.7
0.00855, 5.3
0.00645, 5.9
0.0396, 5.9
0.0804, 6.7

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