

ARE MATING FREQUENCIES RELATED TO SEX RATIO 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 measurements were made from field data: (1) sex ratios, and (2) mating frequencies. Mating frequencies and sex ratios were negatively correlated early and late in the season on the ground and in the trees ($r=-0.51401217$, $Z \text{ score}=-2.04855684$, $n=16$, $p=0.02025267$). Mating frequencies and sex ratios were negatively correlated in the trees and on the ground early and late in the season in *C. anulatus* ($r=-0.84$, $Z \text{ score}=-2.75$, $n=8$, $p<0.01$) and not in *C. inscriptus* ($r=0$, $Z \text{ score}=0$, $n=8$, $p=0.50$). In *C. anulatus* mating frequencies and sex ratios on the ground and the trees early were correlated ($r=-0.94$, $Z \text{ score}=-3.03$, $n=6$, $p<0.01$), and on the ground and in the trees late were correlated ($r=-0.87$, $Z \text{ score}=-2.32$, $n=6$, $p=0.01$).

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 [3-8]. *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 [23]. It occurs in all the forests of the coastal belt from the Cape Peninsula to Beira in Mocambique [22]. Spirobolida has two pairs of legs modified into gonopods on the eighth and ninth diplosegments [25]. 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 [26]) prevent lateral shifting (stabilizer) and stretch the vulva sac in a medial plane [3].

These worm-like millipedes have female-biased SSD [3-8, 11-18, 20]. From the results, correlations between sex ratios and mating frequencies were checked for correlations.

II. MATERIALS AND METHODS

Millipedes were hand collected in coastal forest habitat at Mtunzini ($28^\circ 55' S$; $31^\circ 45' E$) during the summer season (1995-1996). Individual millipedes were identified to 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 ($>30\text{cm}$ but $<3\text{m}$ 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. Sex ratio was calculated as ($[\text{number of males}] / [\text{males} + \text{females}]$). 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. Two sets of measurements were made from the field data (1) sex ratios, and (2) mating frequencies. Sex ratios and mating frequencies were correlated here using

Pearson's Correlation Coefficient
<https://www.gigacalculator.com/calculators/correlation-coefficient-calculator.php>.

III. RESULTS

Mating frequencies and sex ratios were negatively correlated early and late in the season on the ground and in the trees (Figure 1: $r=-0.51401217$, Z score= -2.04855684 , $n=16$, $p=0.02025267$). Mating frequencies and sex ratios were negatively correlated in the trees and on the ground early and late in the season in *C. anulatus* (Figure 2: $r=-0.84202618$, Z score= -2.74610628 , $n=8$, $p=0.00301542$) and not in *C. inscriptus* ($r=0$, Z score= 0 , $n=8$, $p=0.50$). In *C. anulatus* mating frequencies and sex ratios on the ground and the trees early were correlated (Figure 3: $r=-0.94132296$, Z score= -3.03028909 , $n=6$, $p=0.00122167$) and on the ground and in the trees late were correlated (Figure 4: $r=-0.87212311$, Z score= -2.32420473 , $n=6$, $p=0.01005724$).

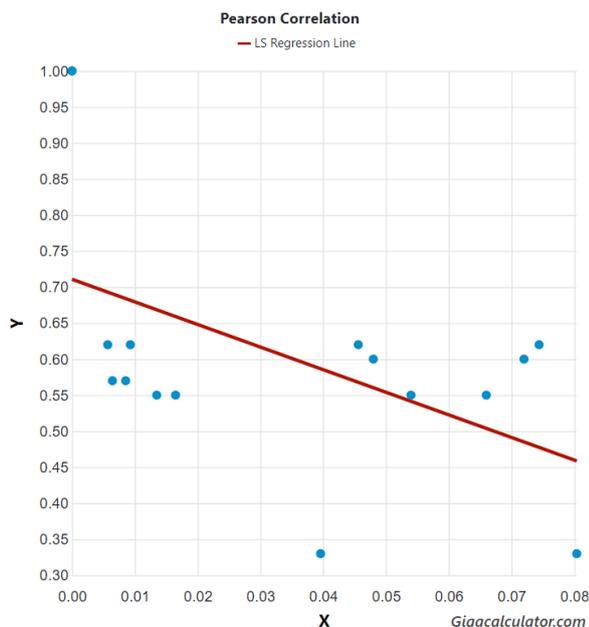


Figure 1. Relationship between sex ratios (y) and mating frequencies (x) for *C. anulatus* and *C. inscriptus* late and early in the season on the ground and in the trees.

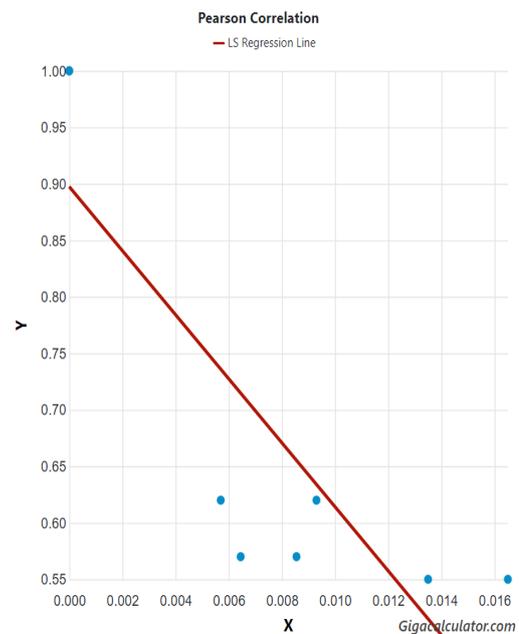


Figure 2. Relationship between sex ratios (y) and mating frequencies (x) for *C. anulatus* late and early in the season on the ground and in the trees.

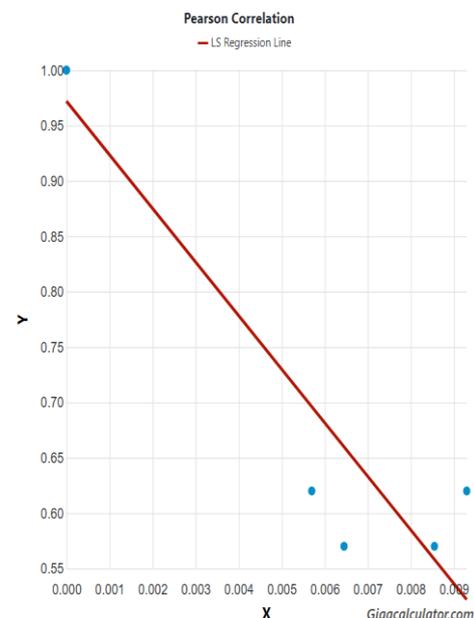


Figure 3. Relationship between sex ratios (y) and mating frequencies (x) for *C. anulatus* early in the season on the ground and in the trees.

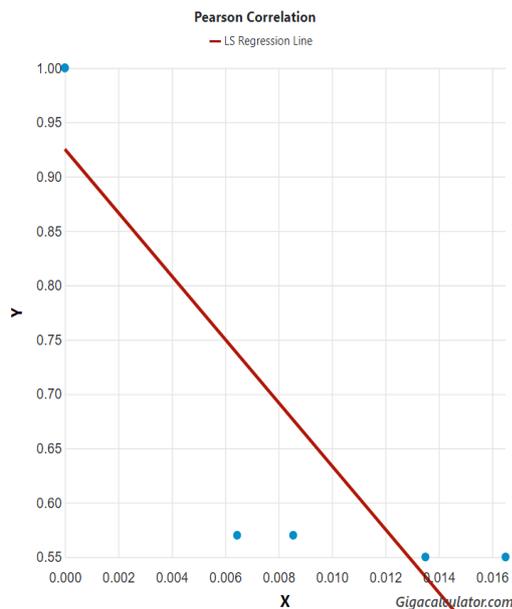


Figure 4. Relationship between sex ratios (y) and mating frequencies (x) for *C. anulatus* late in the season on the ground and in the trees.

IV. DISCUSSION

The sex ratios and mating frequencies were estimated in two *Centrobolus* species. A direct relationship between two factors (absolute abundance and mating frequencies) in the millipedes is compared which certainly supports the relationship in two ways, i. e. early, late, on the ground, and in the trees across species and one species. A relationship between these behavioral factors is present across one species suggesting adaptation to lifetime reproductive success. *C. inscriptus* had the mating frequencies independent of sex ratios while *C. anulatus* had lower mating frequencies and a negative relationship with sex ratio. In *C. anulatus* mating frequencies and sex ratios on the ground and the trees early were correlated while the same two factors on the ground and in the trees late were correlated. The *C. anulatus* - *C. inscriptus* species pair represents an example of "homotypy", "nondeceitful homotypy" and "arithmetic homotypy" [24].

V. CONCLUSION

New relationships between sex ratios and mating frequencies among the *Centrobolus* millipedes

support the function of this behavior as adaptive toward mate competition and assuring paternity among increased mating frequencies with sex ratios. Frequency-dependent and frequency-independent selection were demonstrated in two species.

APPENDIX.

Male and female mating frequencies (early, and late in a season, on the ground, and in the trees), in two species of *Centrobolus* are followed by sex ratios.

- 0, 1 (*C. anulatus*).
- 0, 1 (*C. anulatus*).
- 0.0165, 0.55 (*C. anulatus*).
- 0.0135, 0.55 (*C. anulatus*).
- 0.0093, 0.62 (*C. anulatus*).
- 0.0057, 0.62 (*C. anulatus*).
- 0.00855, 0.57 (*C. anulatus*).
- 0.00645, 0.57 (*C. anulatus*).
- 0.066, 0.55 (*C. inscriptus*).
- 0.054, 0.55 (*C. inscriptus*).
- 0.0744, 0.62 (*C. inscriptus*).
- 0.0456, 0.62 (*C. inscriptus*).
- 0.072, 0.60 (*C. inscriptus*).
- 0.048, 0.60 (*C. inscriptus*).
- 0.0396, 0.33 (*C. inscriptus*).
- 0.0804, 0.33 (*C. inscriptus*).

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