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Do copulation durations of sympatric red millipedes vary seasonally with mating frequencies?

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Abstract: **Mating frequencies and copulation duration were assessed in sympatric red millipedes of the genus *Centrobolus* across the ground and tree substrata early and late in a season. There was a correlation between male and female mating frequencies of *C. anulatus* and *C. inscriptus* and copulation durations (r=0.72, Z-score=2.02, n=8, p=0.02). There was a correlation between male and female mating frequencies and copulation durations recorded late (r=0.83, Z score=2.64, n=8, p<0.01), on the ground late (r=0.93, Z score=3.74, n=8, p<0.01), and in the trees late (r=0.89, Z score=3.13, n=8, p<0.01). This suggested copulation duration is seasonal when mating frequencies were controlled.**

Keywords: **duration; frequency; ratio, size; species.**

1. INTRODUCTION

Millipedes mate multiply and store sperm which are the prerequisites for the process of sperm competition [3, 5]. The millipede genus *Centrobolus* is found in the temperate South African subregion, its northern limits on the east coast of southern Africa being about -17° latitude South (S) and its southern limits being about -35° latitude S. It consists of taxonomically important species with 12 species considered threatened and includes nine vulnerable and three endangered species [26]. It occurs in all the forests of the coastal belt from the Cape Peninsula to Beira in Mocambique [25]. Common with worm-like millipedes is the mating frequency which is known to differ in several populations of the genus [6]. Mating frequencies are seasonal and probably covary with the copulation durations for each species at any one time [18-20].

Mating frequencies are compared during the breeding season in two sympatric species in the pachybolid millipede genus *Centrobolus* Cook, 1897 [4, 21, 25]. The aim is to test the null hypothesis that there are no correlations between mating frequencies and copulation duration across species at different times in the breeding season.

2. MATERIALS AND METHODS

Two species (*C. anulatus* and *C. inscriptus*) were identified as belonging to the genus *Centrobolus* Cook, 1897 [4]. The mating frequencies on the ground and in the trees during the breeding season were obtained for *C. anulatus* (0.00, 0.03) and *C. inscriptus* (0.0372, 0.0828) [6] (Table 1). This included the data for early and late in the season. The Pearsons Correlation Coefficient Calculator was used to test the null hypothesis (https://www.gigacalculator.com/calculators/correlation-coefficient-calculator.php). Correlation coefficients were compared at https://www.medcalc.org/calc/comparison_of_correlations.php. Mating frequencies were controlled.

3. RESULTS

There was no overall correlation between mating frequencies and copulation durations (r=-0.23409041, Z score=-0.85996965, n=16, p=0.19490284). There were correlations between male and female mating frequencies of *C. anulatus* and *C. inscriptus* and copulation durations on the ground and in the trees (Fig. 1: r=0.71760070, Z-score=2.01845566, n=8, p=0.02177184), recorded late (Fig. 2: r=0.82697968, Z score=2.63521771, n=8, p=0.00420421), recorded on the ground late (Fig. 3: r=0.93163748, Z score=3.73568551, n=8, p=0.00009363) and recorded in the trees late (Fig. 4: r=0.88540411, Z score=3.13103805, n=8, p=0.00087102). There were no correlations between male and female mating

frequencies of *C. anulatus* and *C. inscriptus* and copulation durations recorded early (r=-0.36839071, Z score=-0.86437406, n=8, p=0.19369117), recorded early and late pooled (r=-0.36266190, Z score=-0.84958849, n=8, p=0.19777690), recorded on the ground early (r=-0.36103538, Z score=-0.84540354, n=8, p=0.19894273), or recorded in the trees early (r=-0.36430370, Z score=-0.85381855, n=8, p=0.19660272). There was no difference between the correlation coefficients of mating frequencies and copulation duration recorded late on the ground compared to late in the trees (z statistic=0.4276, n=8, 8, p=0.6690). Mating frequencies at the beginning of the season were normal (D=0.1441, K=0.2882, n=4, p=0.9937). Mating frequencies at the end of the season were normal (D=0.2454, K=0.4907, n=4, p=0.4907). Mating frequencies on the ground were normal (D=0.3044, K=0.6088, n=4, p=0.2122) Mating frequencies in the trees were normal (D=0.2505, K=0.5009, n=4, p=0.5039). There was no difference between mating frequencies recorded early versus late (t=0.000, d. f. =6, p=1) (Standard Error=2.353).

Table 1: Copulation duration in *C. anulatus* (39.4 min) and *C. inscriptus* (170 min) and mating frequencies for males and females on the ground, in the trees, early, and late in a season.

Spatio-temporal position	Copulation duration	Mating frequency
On the ground	39.4	0
On the ground	39.4	0
In the trees	39.4	0.0165
In the trees	39.4	0.0135
On the ground	170	0.066
On the ground	170	0.054
In the trees	170	0.0744
In the trees	170	0.0456
Early in the season	39.4	0.0093
Early in the season	39.4	0.0057
Early in the season	170	0.072
Early in the season	170	0.048
Late in the season	39.4	0.00855
Late in the season	39.4	0.00645
Late in the season	170	0.0396
Late in the season	170	0.0804

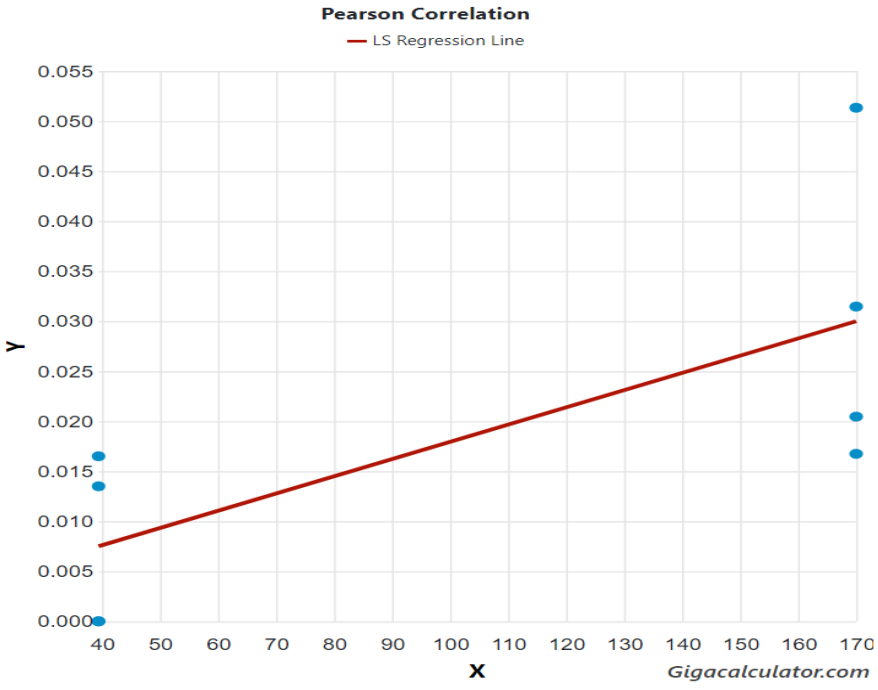

Figure 1: Correlation between copulation duration (x) and mating frequencies (y) in *Centrobolus anulatus* and *C. inscriptus* on the ground and in the trees.

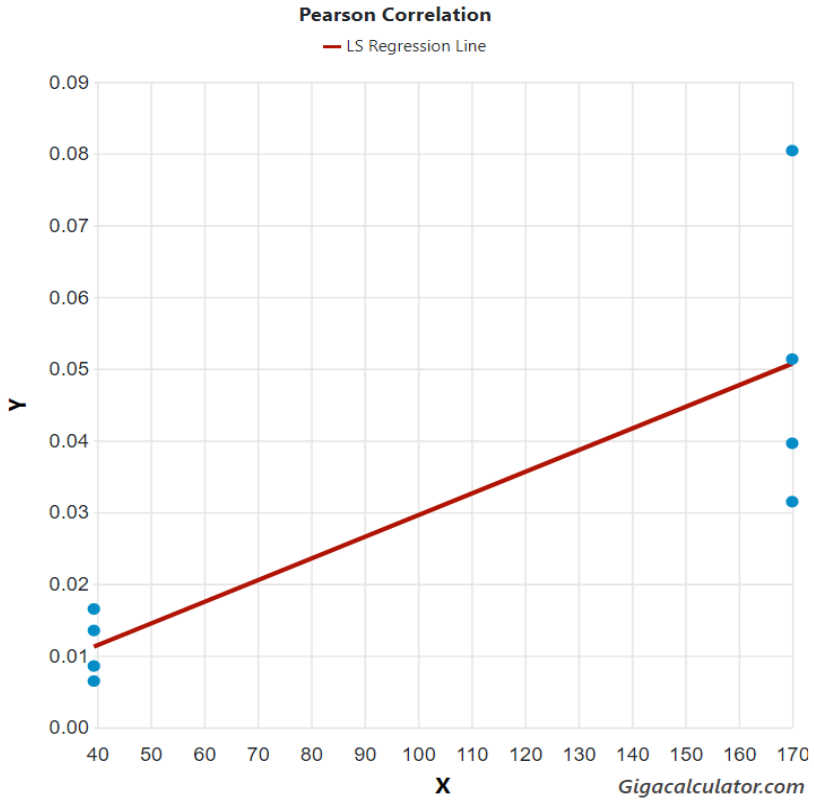

Figure 2: Correlation between copulation duration (x) and mating frequencies (y) in *Centrobolus anulatus* and *C. inscriptus* late in the season.

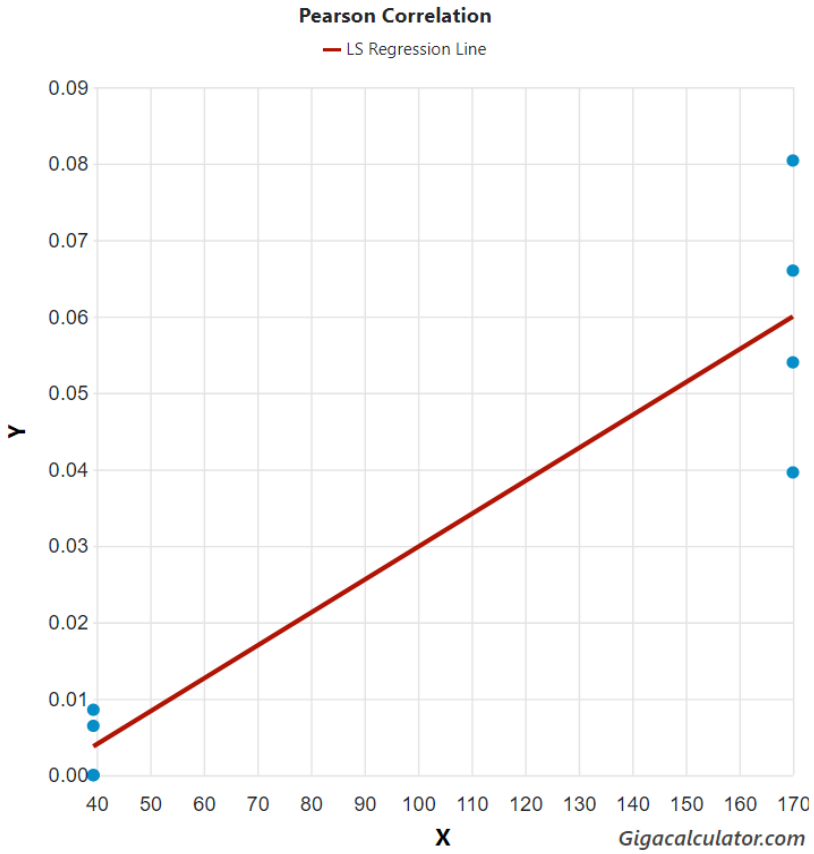

Figure 3: Correlation between copulation duration (x) and mating frequencies (y) in *Centrobolus anulatus* and *C. inscriptus* was recorded on the ground late in the season.

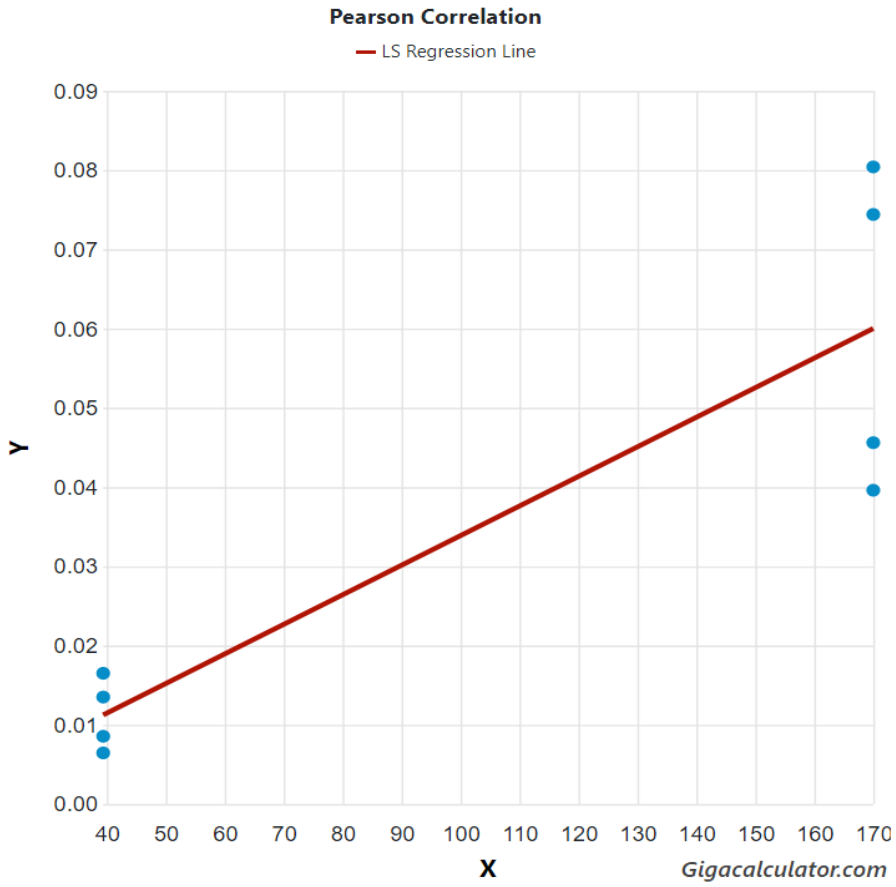

Figure 4: Correlation between copulation duration (x) and mating frequencies (y) in *Centrobolus anulatus* and *C. inscriptus was* recorded

4. DISCUSSION

Correlations were found between mating frequencies and copulation durations across sympatric *Centrobolus*. *C. anulatus* has the lower mating frequencies (0.00; 0.03) and shorter copulation durations while *C. inscriptus* has higher mating frequencies (0.0372; 0.0828) and longer copulation durations. This study found mating frequencies recorded in the trees and on the ground in *C. anulatus* and *C. inscriptus* late in the season were correlated with copulation duration. This study supports using mating frequency as a component of sexual selection in *Centrobolus* because there were correlations with copulation duration across substrata. Mating frequencies are positively related to copulation duration late in the season.

This is another example of covariance with mating frequencies between sympatric millipede species. When mating frequencies were controlled the copulation duration varied during seasonal activity patterns in species [1, 18, 19, 23-24]; and probably also during daily activity patterns [2, 22, 27]. Copulation durations vary with mating frequencies depending on the time in the season. Spatial changes in habitat preference are known in *C. fulgidus* and *C. richardii* [20]. These differences are likely due to the effects of differences in mating frequencies and copulation durations between the sympatric species. Similarly, temporal differences in mating frequencies and copulation duration may be usefully investigated and compared with this study.

Mating frequency is an indicator of mating success and also paternity success in the water strider *Aquarius remigis* [28]. Temporal changes in copulation duration and mating frequency are probably suitable indicators of paternity success in polygynandrous mating systems such as millipedes and paternity can be determined from copulation duration, and mating frequency, and sperm precedence.

5. CONCLUSION

Copulation duration varied systematically with mating frequencies in two *Centrobolus* species. Variance in the mating frequencies occurs with higher frequencies of both species having more prolonged copulations late in the season.

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