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Zoomorphic variation with copulation duration in *Centrobolus*

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Abstract

Centrobolus typically has prolonged copulation as a form of syn-copula mateguarding. Variations in the copulation duration were calculated and analysed in four species of the millipede genus *Centrobolus*. Mean copulation durations differed between all four species but only two species were different intra-specifically. *C. inscriptus* was different from *C. anulatus* in copulation duration coefficient of variation (CV) (F=0.41490, d.f.=114, 7, p=0.04892) and C. *fulgidus* and *C. anulatus* were different in copulation duration CV (F=0.38912, d.f.=50, 7, p=0.04836). Copulation duration was variable intra-specifically but tends to be intermediate and determining evolutionarily (interspecifically). Copulation duration was significantly correlated (Spearman's Rho Calculator) with male and female volumes (r=1, p=0, n=4, 4; 4, 4). When I controlled for sex, I found copulation duration was significantly correlated with size (volumes) (r=0.6655, r^2=0.4429, p=0.004897, n=8, 8). Larger male and female body size correlate with copulation duration both intra-specifically and inter-specifically in millipedes, and perhaps in animals.

Keywords arthropoda; copulated; mated time; variance; variation.

1 Introduction

Copulation duration is a response to sperm competition (Kelly and Jennions, 2016). It determines insemination, fertilization, egg number/ production and paternity but not necessarily nuptial gifts (Micholitsch et al., 2000; Zhong and Hua, 2013; Cooper, 2015; Ullah et al., 2019). Even heat stress in males can negatively affect copulation duration (Zhang et al., 2016). There are many determining factors of copulation duration across arthropods, such as the timing of insemination varies to with spermatophore size and male genital titillation when there is indirect sperm transfer (Vahed et al., 2011; Cooper, 2016). It was "propose[d] that prolonged copulations gain meaning in multiple mating situations and should play a role in sperm competition or other forms of sexual selection" (Szira´nyi et al., 2005).

Optimal copulation duration is dependent on male and female size (Charnov and Parker, 1995; Parker and Simmons, 1994; Parker et al., 1999). Size-dependent copulation duration and mate guarding occurs in the fly *Drosophila melanogaster* (LaFranc and Bundgaard, 2004), the scorpionfly (*Panorpa cognata*) (Engqvist, 2003), spiders (Elgar, 1995), the skeleton shrimp *Caprella penantis* (Takeshita and Henmi, 2010), the carrion beetle *Necrophila americana* (Knox and Scott, 2006), orb-web spiders (Prenter, 2003), millipede *Centrobolus inscriptus* (Cooper, 2017) and yellow dungflies (Parker, 1974; Grafen and Ridley, 1984; Alcock, 1994; Arnqvist and Danielsson, 1999). This is not the case in the millipede *Nyssodesmus pythos* (Adolph and Geber, 1995).

Here I investigate intraspecific and interspecific variance in size with copulation duration in the Arthropod case where there is mate-guarding in *Centrobolus* (Cooper, 2016, 2017, 2019). In these millipedes, copulation duration determines what sperm precedence is and when sperm precedence changes relative to the interval between mating (Cooper, 2019). In order to calculate optimal copulation duration I first calculate intraspecific and interspecific variance and coefficients of variation in copulation duration of four species of millipedes and perform interspecific comparisons to test the null hypothesis copulation is size-dependent and look for statistical differences in between species.

2 Materials and Methods
2.1 Observations
Centrobolus was collected from KwaZulu-Natal where they inhabited the indigenous coastal forest. Live specimens of each sex were transported to the laboratory where conditions were kept under a constant 25°C temperature regime; 70% relative humidity; 12:12 hrs light-dark cycle. Food was provided in the form of fresh vegetable ad libitum. Individuals had unknown mating histories and unisex groups were housed in plastic containers containing moist vermiculite (±5cm deep) for ten days before commencing the first mating experiments.

2.2 Data analysis
Three calculations were made for all individuals once copula pairs had disengaged; copulation duration, standard deviation, and coefficient of variation (CV). CV data were compared using a coefficient of variation comparison calculator MEDCALC®. Mean copulation duration was simulated (to within 10 minutes of the mean) with 5 occurrences for each conspecific species and compared with hetero-specific species using a T-test for 2 independent means. Initially differences between copulations were investigated using Free Statistics Calculator version 4.0 One-Way ANOVA on summary data available from https://www.danielsoper.com/statcalc/calculator.aspx?id=43.

3 Results
No differences appeared between species copulation duration (ANOVA: F=1.101, d.f.=3, P=0.350). Mean copulation durations differed between all four species: *C. inscriptus* and *C. fulgidus* (t=32.7612, p<0.00001, n=5, 5), differed between *C. inscriptus* and *C. ruber* (t=41.17286, p<0.00001, n=5, 5), differed between *C. inscriptus* and *C. annulatus* (t=41.29935, p<0.00001, n=5, 5), differed between *C. fulgidus* and *C. annulatus* (t=853.81497, p<0.00001, n=5, 5) and *C. annulatus* and *C. ruber* (t=12.64911, p<0.00001, n=5, 5). *C. inscriptus* variation in copulation duration was not different to *C. fulgidus* (F=1.06626, d.f.=114, 50, p=0.81484) or *C. ruber* (F=0.78374, d.f.=114, 31, p=0.35692) but was different from *C. anulatus* (F=0.41490, d.f.=114, 7, p=0.04892). *C. ruber* and *C. anulatus* were not different in copulation duration CV (F=0.52938, d.f.=31, 7, p=0.21086). *C. fulgidus* and *C. anulatus* were different in copulation duration CV (F=0.38912, d.f.=50, 7, p=0.04836). *C. fulgidus* and *C. ruber* were not different in copulation duration CV

(F=0.73504, d.f.=51, 30, p=0.32648). Copulation duration was significantly correlated (Spearman's Rho Calculator) with male and female volumes (r=1, p=0, n=4, 4; 4, 4). Copulation duration was not related to male surface area (r=0.2, p=0.8, n=4, 4) or female surface area (R=-0.4, p=0.6, n=4, 4). Copulation duration was significantly correlated with male surface area to volume ratio (R=-1, p=0, n-=4, 4) and female surface area to volume ratio (r=-1, p=0, n=4, 4). Coefficient's of variation in copulation duration were not correlated with male or female volume (R=-0.8, p=0.2, n=4, 4; 4, 4). When I controlled for sex, I found copulation duration was significantly correlated with size (volumes) (r=0.6655, r2=0.4429, p=0.004897, n=8, 8).

Table 1 Copulation durations (C), variance (Var.), coefficients of variation (CV), Volumes (V), Surface Areas (SA) and Surface Area to Volume (SA: V) for male (M) and female (F) *Centrobolus* spp.; sample sizes (N).

Spp.	C (minutes)	Var. (min.)	N	CV (%)	MV (mm^3)	FV (mm^3)	MSA (mm^2)	FSA (mm^2)	MSA: V (mm^{-1})	FSA: V (mm^{-1})
anulatus	39.4±18.6	432.6	8	47.3	2058	1729	2462,874	3026,009	0,000486	0,000578
fulgidus	66.4±418.6	124044.84	51	28.0	4587	7554	1934,216	2512, 269	0,000218	0,000132
inscriptus	170±49	14641	115	29.0	7327	8885	2717,289	2934, 185	0,000136	0,000113
ruber	39.8±13.2	707.56	32	33.1	4555	7248	1972,92	2621, 596	0,00022	0,000138

4 Discussion

Here I found species-specific mean copulation durations in *Centrobolus* and variation in copulation duration which was different between species. This highlights there is a species-specific difference in variation of copulation duration. Copulation durations in millipedes extend beyond the time necessary to transfer sperm (insemination) (Assis and Foellmer, 2019). When there is variation at the intraspecific level, "[M]ating durations exceeding female optima serve males as a form of 'extended mate guarding': by inducing mating refractoriness in the female, a male extends the time over which its sperm is exclusively used to sire progeny and reduces the likelihood of the female being inseminated by a competitor" (Mazzi et al., 2009).

Significant correlations between copulation duration and volume were found giving an inverse relationship between surface area to volume ratios and copulation duration. Copulation duration was directly correlated and increased with body size across species. I found no difference between the relationships between copulation duration with male versus female sizes which was found in some studies of *Drosophila melanogaster* (LaFranc and Bundgaard, 2004). Millipedes were similar to spiders where the duration of copulation correlates with intra-specific size variation, also found in a scorpionfly (*Panorpa cognata*) where males in good condition copulate longer (Elgar, 1995; Engqvist, 2003). However, this study showed there was also a trend interspecifically for copulation duration to correlate with body size in and across millipedes. This emphasises the importance of body size on mate guarding as was the case in the skeleton shrimp *Caprella penantis* where male body size was the most important factor affecting competition for a receptive female (Takeshita and Henmi, 2010). The success of guarding males in millipedes is similar to the carrion beetle (*Necrophila americana*) which depends on size relative to other males and the operational sex ratio (Knox and Scott, 2006; Cooper, 2016). Results from removal experiments in orb-web spiders show that larger males have a clear advantage in monopolizing females (Prenter et al., 2003). The relative size of each species is thought to be significant in determining a copulation duration (Cooper, 2017). Together, copulation duration and size dimorphism contribute to calculating optimal copulation durations independent to the operational sex ratio of each population (Charnov and Parker, 1995; Parker and Simmons, 1994; Parker et al., 1999). "[P]atterns need

to determine whether sperm selection is applied differently, or consistently, on given males by different females in the same population" (Ball and Parker, 2003).

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