SEXUAL DIMORPHISM IN FORELIMB LENGTHS OF INTERTIDAL JUMPING SPIDERS (*Terralonus californicus*) AND IMPLICATIONS FOR CURRENT THEORY ON ALLOMETRIC PATTERNS AND TRAIT FUNCTION

by

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Abstract

Sex-specific selection theory suggests that secondary sexual characteristics can vary between sexes in their mean, their variance, and their allometric scaling due to differences in optimal resource investment and condition. The intertidal jumping spider, *Terralonus californicus*, is an unstudied species with the potential to exhibit a wide range of possible sexual dimorphisms due to its high degree of intrasexual agonistic encounters and female mate choice. From qualitative behavioural examinations, I observed that males appeared to use forelimbs as both a 'weapon' and an 'ornament.' Therefore, I predicted that forelimb length (FL) would exhibit dimorphism between males and females via differences in mean, variance, and allometric scaling both between sexes and between traits. I measured the cephalothorax width, forelimb length, and hindlimb length of mature males and females using images taken under a dissection microscope along with a calibrator of known distance to tabulate measurements in millimeters. I found that FL differed significantly between males and females ($T = 4.17$, $p < 0.001$) and that the variance was significantly higher in male FL than in females ($F = 1.81$, $p = 0.0485$). A one-way ANCOVA found that only cephalothorax width had a significant effect on forelimb length ($F = 90.1$, $p < 0.001$), while comparisons of regression slopes found no difference between male FL and HL ($b = 0.91 \pm 0.15$ for FL and 0.84 ± 0.19 for HL). These results confirmed my first two predictions but did not provide support my latter two predictions for a positive allometric scaling in male forelimb length, contradicting theory on patterns of allometry and function. This study provided empirical evidence for sexual dimorphism in the forelimbs of a unique species that had received no attention in the current body of literature, warranting future research into mechanisms of both sexual selection and intersexual ecological niche differentiation that may act on the forelimbs of this unstudied local species.

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I would like to thank my roommates for tolerating all of the spiders that I stored in our house throughout the semester, Lastly, I thank all of my friends and my family for their endless support and assistance in getting me through this year. I could not have made it this far without them. Thank you!

Introduction

Sexual Dimorphism and Sex-Specific Selection

Many gonochoristic species exhibit sexual dimorphism, defined as the variation in non-reproductive traits such as secondary sexual characteristics or conspicuous behaviours between males and females (Darwin, 1871). Sexual dimorphism arises due to a difference in the optimal investment into a trait for each sex (Darwin, 1871; Bonduriansky and Rowe, 2003). There are different evolutionary mechanisms through which sexual can occur; for example, intersexual niche differentiation in which each sex evolves separate characters as an adaptation to their respective ecological niches (Przybylo and Merilk, 2000; Sobczyk *et al.*, 2020; Wasiljew *et al.*, 2021). Sexual selection is another explanatory evolutionary mechanism for the evolution of sexual dimorphism, as it posits that evolution will favour the exaggeration of traits in a given sex as means of sexual signalling (Darwin, 1871). Intrasexual selection (*i.e.*, the selection of traits favourable in same-sex interactions) and intersexual selection (*i.e.*, the selection of traits favourable in male-female courtship) are two longstanding proposed mechanisms that explain how sexual selection acts to establish these sexually dimorphic traits in a given species (Berglund *et al.*, 1996).

Dimorphism in Trait Means

Differences in the mean of a single character between sexes due to differences in optimal investment resulting from both intrasexual selection and intersexual selection was first described by Charles Darwin and has become a widely recognized and well-documented phenomenon (Darwin, 1871; Kirkpatrick, 1982; Arnold and Wade, 1984). However, sexual selection can also create other patterns in character morphology as well, which have not yet garnered the same degree of study as differences in a single mean.

Dimorphism in Trait Variance

Theory postulates that the variance of a trait under sexual selection, in addition to the mean, may also differ between males and females (Rowe and Houle, 1996; Punzalan, Cooray, *et al.*, 2008). Theory as to why this occurs can be split into two main arguments. The first argument poses that variance itself is selected for since directional selection favours extremes among a population (Pomiankowski and Møller. 1995). The second argument hypothesizes that variance is only a by-product of selection due to the dependence of sexually selected traits on condition (*i.e.*, the total sum of resources available to an individual) to allocate resources towards the growth or maintenance of the trait (Rowe and Houle, 1996). Condition is determined by variance in both the surrounding environmental and genetic quality of an individual; regarding sexually selected traits specifically, high genetic variance in fitness is thought to play the larger role in determining the extent of resource investment into trait development (Rowe and Houle, 1996). This empirically observed phenomenon is less appreciated by the current literature but offers an additional lens to view sexual dimorphism under sex-specific selection.

Dimorphism in Covariance and Allometric Patterns of Scaling

Traits may also be considered in relation to a secondary trait; as one trait changes, so may the other either proportionally or inversely (Gould, 1966). Often, a trait will vary proportionally as a function of overall body size among a population of reproductively mature individuals in a phenomenon known as isometry (Gould, 1966). This effect differs from standard ontogenetic growth in that it occurs among individuals who have acquired their full body size and have reached reproductive maturity (Gould, 1966).

When considering the covariance of a trait, selection may act on the scaling of the trait such that the trait may scale non-linearly with body size due to changes in optimal investment at different body sizes (Gould, 1973; Kodric-Brown *et al.*, 2006). If a trait increases at a higher rate with increasing body size (*i.e.,* the slope of the regression line > 1), it is said to show positive allometry; likewise, if a trait increases at a lower rate with increasing body size it is said to show negative allometry (*i.e.,* the slope of the regression line < 1). Empirical evidence in the current body of literature suggests that the type of allometry exhibited by a trait is correlated to its function (Gould, 1973; Kodric-Brown *et al.*, 2006; Rodríguez and Eberhard, 2019). Current theory predicts that, for sexually selected traits used either as a 'weapon' (*i.e.,* due to intrasexual selection resulting from competition for mating access between individuals of the same sex) or as an 'ornament' (*i.e.,* due to intersexual selection resulting from mate choice by the opposite sex), the rate of optimal investment will increase with larger body sizes and therefore show positive allometry (Gould, 1973; Kodric-Brown *et al.*, 2006; Rodríguez and Eberhard, 2019). For instance, positive allometry occurred among a sample of male Irish elk (*Megaloceros giganteus*), whereby the antler size increased at an exponential rate with body size (Gould, 1973). Male Irish elk utilized antlers in combat as well as in sexual signalling, lending support to the link between positive allometry and sexual selection (Gould, 1973). Conversely, for traits not under sexual selection, theory predicts that optimal investment will covary either isometrically with body size or will decrease in the rate of investment at larger body sizes and show negative allometry (Kodric-Brown *et al.*, 2006). Therefore, investigating the allometry of a trait can lend support to its use as a either a weapon or an ornament, particularly if additional evidence on whether a trait is involved in intrasexual agonistic encounters and intersexual courtship is gathered in addition.

Jumping Spiders (Family Salticidae)

Jumping spiders (Araneae, Salticidae) are a model group for investigating sexual dimorphism and sex-specific selection, as many species possess substantial morphological and behavioural differences between males and females (Jackson, 1981; Masta and Maddison, 2002; Sivalinghem *et al.*, 2010; Zhou *et al.*, 2021). Male jumping spiders often possess modified forelimbs (among other secondary sex differences) which they use in elaborate courtship rituals for females and to ward off other males in agonistic encounters (Clark and Morjan, 2001; Jackson and Cooper, 1991). Additionally, females mate multiple times, resulting in competition among males for continued access to females (Sivalinghem *et al.*, 2010). The ubiquity of both intrasexual selection (via male agonistic encounters for access to females) and intersexual selection (via male courtship rituals and female mate choice) operating on phenotype therefore presents an excellent opportunity to study differences in the mean, variance, and allometric scaling of traits under suspected sexual selection. Recent evidence investigating stoichiometric ratios and compositions of male and female jumping spiders has shown that jumping spiders can vary in their ecological niche space between the sexes (Sobczyk *et al.*, 2020). This provides additional possibility for the existence of sexually dimorphic characters, as traits differing in ecological function between sexes may result in differing trait optima.

Intertidal Jumping Spiders (Terralonus californicus)

The intertidal jumping spider, *Terralonus californicus*, is a local species of jumping spider that solely inhabits the rocks and sands of the intertidal zone and supralittoral zone. Their habitat is unique among jumping spiders, yet they have received little attention in the scientific community apart from their initial description as a species (Banks, 1904). To date, little is known about their ecological adaptations, feeding habits, seasonality, courtship behaviours, and mating behaviours. Despite this, they appear to be a useful candidate species to study mechanisms of sexual dimorphism since they cease to molt after reaching adulthood and therefore become stable in size. Additionally, their unique habitat and behavioural interactions create a life history that is likely to favour differing optimal investments into certain traits between sexes.

Justifications and Predictions

My study provides the first scientific inquiry into *T. californicus*, as well as the first formal investigations of the courtship and mating interactions of males and females using a qualitative approach. Additionally, it investigates and quantifies the sexual dimorphism of forelimb lengths in terms of differences in mean, variance, and allometric scaling. The study therefore tests longstanding and relatively understudied theories of sex-specific in a scientifically novel species.

From current evolutionary theory on sex-specific selection and from preliminary intraspecies behavioural observations, I made four predictions about sexual dimorphism in forelimb length (FL). My first prediction was that male FL would differ significantly from female FL and would be longer in length overall due to its observed use in agonistic and courtship encounters I observed. My second prediction was that male FL would differ in overall sample variance from female FL and that the variance would be greater in males. My third prediction was that male FL would differ in its scaling with body size to female FL; specifically, male FL would exhibit positive allometry, while female FL would exhibit either isometric growth or negative allometry. My fourth prediction was that male FL would exhibit greater positive allometry relative to male hindlimb length (HL), a control limb that did not appear to differ in function between sexes.

Methods

Study Site, Laboratory Conditions, and Assigning Sex

Terralonus californicus individuals (Figure 1) were collected from the intertidal zone and supralittoral zone of Finlayson Point, Victoria, British Columbia (48.406878 N, 123.364612 W), between May of 2021 and January of 2022. Collections were generally performed in sunny, clear, and warm conditions with little wind and a low tide. Individuals ranged in age from juveniles to reproductively mature adults. Spiders were housed individually in clear polypropylene vials (2.5 cm in diameter and 9 cm in height) plugged with a foam stopper which were each assigned a unique numerical identifier. Some wild-caught females laid egg sacs in captivity, after which females were moved to a clean vial. After hatching, 2nd or 3rd instar juveniles were transferred to individual vials and reared to adulthood in the manner previously described with notation kept on the identity of the biological mother. Spiders were maintained on a 14 L:10 D light cycle under a full-spectrum grow lamp at room temperature (approximately 20 °C, with a relative humidity of approximately 35%) and provisioned with a minimum of four individual *Drosophila hydei* bi-weekly, reared from culture medium (based on molasses and yeast or banana and yeast, depending on availability).

Reproductively mature males were identified based on the enlarged tips of the pedipalps which were generally black in colouration, along with the lack of an epigynum (a female external genital structure) on the ventral side of the abdomen which served as additional confirmation. Reproductively mature females were identified by the visual confirmation of the presence of an epigynum on the abdomen. If the pedipalps were not enlarged and no epigynum was found, the individual was designated as a juvenile and was omitted from the dataset.

Preliminary Behavioural Examinations

I observed agonistic and courtship behaviours of reproductively mature individuals of *T. californicus* by placing two individuals in a circular arena (*i.e.,* a plastic petri dish 5 cm in diameter) nested in the opening of a white, opaque funnel, with the rim lined with a thin layer petroleum jelly (to discourage escape and limit lasting effects of the petroleum jelly remaining on the limbs after contact). I observed the interactions between individuals in the arena from behind a light source to limit potential observation interference on spider behaviour. Live event-recording of behavioural elements was performed while time was tracked using a timer, but encounters were also recorded from above with a video camera.

To characterize male intrasexual agonistic encounters, two randomly chosen males were transferred into the arena and observed for a maximum of 5 minutes, or until one male was adjudged to be victorious in an agonistic encounter, *i.e*., when one male (the ‘loser’) appeared to back away and/or escape from the other (the ‘winner’) following either mutual chelicerae contact or forelimb and abdomen displaying, whereby an individual protrudes its forelimbs out to either side of its body to form the shape of the letter ‘V’. Each individual male was observed in an encounter with a unique opponent to a maximum of three times. Occasionally, one male would appear to back away and become submissive after initially orientating to an opponent but before any displays were performed by either male. Following this, the male would continue to avoid the opponent and back away when approached for the remainder of the 5-minute period, therefore I adjudged these encounters as a loss for the seemingly submissive male and a victory for the non-submissive male. If no obvious aggressive acts, displays, submission/dominance, or orientation were observed after the 5-minute period, the encounter was marked as inconclusive. For each encounter, I noted the total time to encounter completion, the total number of times each individual displayed its forelimbs, whether the fight escalated to mutual chelicerae contact, and which individual was deemed the 'winner' and the 'loser.'

I additionally observed interactions between male-female individuals of *T. californicus,* which consisted of one randomly selected individual from each sex in the ring to observe the behaviours involved in courtship and copulation. I scored a male as successful in male-female encounters when the male successfully courted and approached a female before mounting the female for the purposes of reproduction. Encounters were observed for a maximum of 10 minutes if the male did not mount the female; the total encounter time was recorded. For each encounter, I additionally noted the total number of forelimb displays by both males and females.

Morphometric Quantification

To quantify the cephalothorax width (CW), total forelimb length (FL), and total hindlimb length (HL), reproductively mature males (n = 34) and females (n = 33) were euthanized and stored in vials containing 40% ethanol solutions. I removed and partially air-dried individuals from the ethanol solution before separating the forelimb and hindlimb from the left side of the body using a ventral view (Figure 2; if a limb on the left side was either degraded or missing, the limb from the right side was used) above the trochanter-femur joint using forceps. The cephalothorax, along with the amputated forelimb and hindlimb, were placed on a glass side that additionally contained calibration squares of a known length (*i.e.*, a hypotenuse of √2 mm from one corner of a square to the other corner). The glass side was placed under a Leica MZ8 dissecting microscope containing an AmScope MD1200A lens camera. Two separate images were taken of each cephalothorax, forelimb, and hindlimb (*i.e.*, 6 images taken in total for each specimen) in the same frame as the calibration squares, with differing orientations relative to the lens camera for each image of the same body part. The resulting images were analyzed using ImageJ software (Schneider *et al.*, 2012). I calibrated the proportion of pixels per millimeter for each image using the calibration squares, from which I quantified measurements in millimetres. Cephalothorax measurements were designated as the width of the cephalothorax measured ventrally between the coxae of the second and third limbs of individuals (Figure 2). Forelimb and hindlimb measurements were designated as the sum of measurements of the femur, tibia (as well as the patella), metatarsus, and tarsus of each corresponding limb (Figure 3). The CW, FL, and HL were calculated for each individual using the mean of the measurements from the two images of the same corresponding cephalothorax, forelimb, and hindlimb.

Statistical Analyses

I investigated summary statistics, performed statistical analyses and data transformations, and created data visualizations in R and RStudio (R Core Team, 2022; RStudio Team, 2022). To test for measurement precision, I calculated Pearson's correlation coefficients between the different sets of measurements for each of the cephalothorax width, forelimb length, and hindlimb length. All statistical analyses were run using a significance value (p) of 0.05 ($\alpha = 0.05$). I performed a Welch's Two-Sample T-Test for each of the CW, FL, and HL between male and female specimens (H_A: males - females $\neq 0$) to assess differences in the sample means of the sexes. To assess differences in CW and limb length variance between males and females, I ran a one-sided F-Test (H_A: males > females) for CW, FL, and HL. I log-transformed the traits CW, FL, and HL and z-standardized each trait (*i.e.,* I subtracted each observed value by the sample mean and divided the resulting number by the sample standard deviation, giving each trait a mean of 0 and a standard deviation of 1) to minimize the effects of differing sample means and variance. With the resulting transformed data, linear models of FL and HL for both males and females (*i.e.*, four regression lines in total) were run with CW as the explanatory variable to represent allometric patterns of limb scaling with body size. Slopes and corresponding confidence intervals of allometric scaling patterns were generated for each regression line. I ran a one-way analysis of covariance (ANCOVA) to assess the effects of CW, sex, and the interaction between CW and sex on FL. I additionally repeated the ANCOVA with HL as the explanatory variable.

Figure 1. Dorsal views of a reproductively mature male (top image) and female (bottom image) of the species *Terralonus californicus*. *T. californicus* appears to exclusively inhabit the intertidal zone and supralittoral zone among bedrock, stones, and sand.

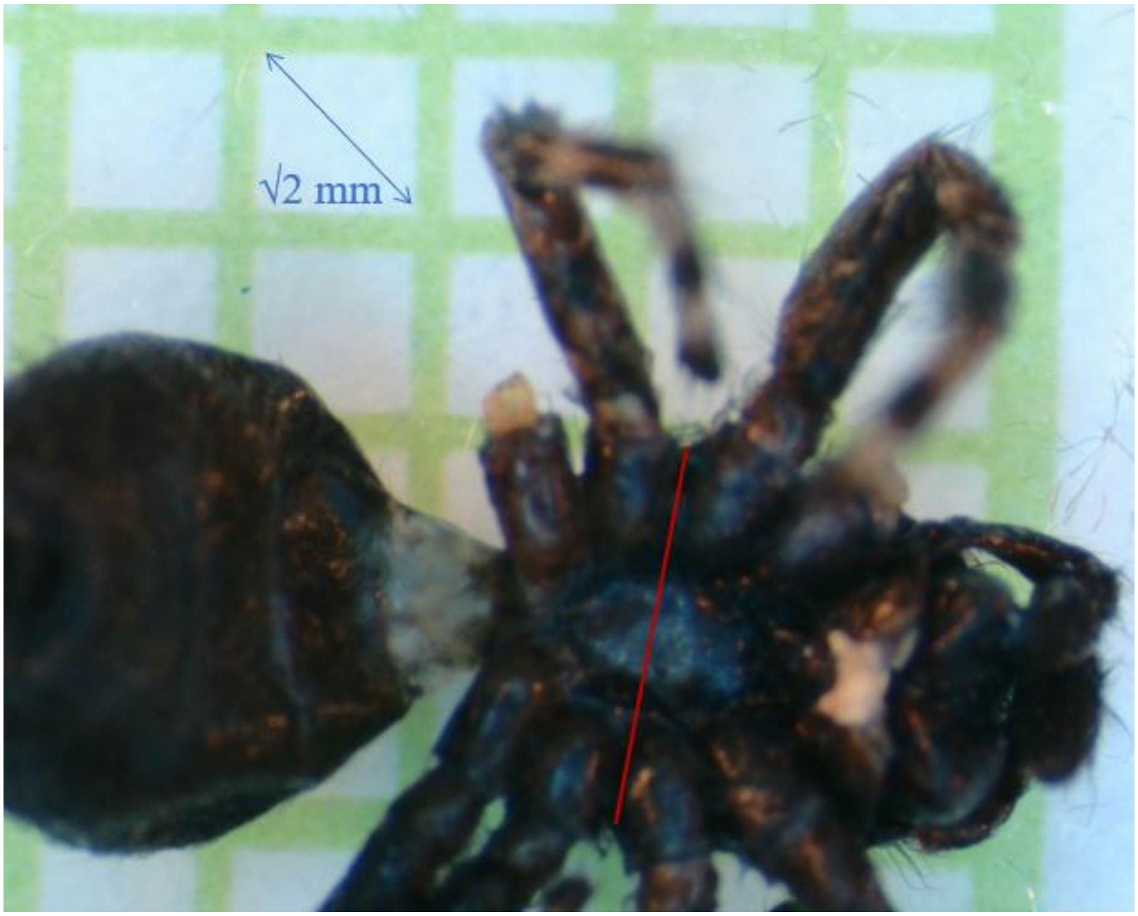

Figure 2. A ventral image of an adult female individual of *Terralonus californicus* (including a calibration square) used to determine the cephalothorax width. The calibration square of known distance (√2 mm) was used to determine the number of pixels per millimeter in the image. The cephalothorax width was defined as the distance (mm) between the coxae of the second and third limbs, delineated above with a red line.

Figure 3. An image of a forelimb dissected from an adult male of *Terralonus californicus* (including a calibration square) used to determine the forelimb length. The calibration square of known distance (√2 mm) was used to determine the number of pixels per millimeter in the image. The forelimb length was defined as the sum of the lengths (mm) of the femur, tibia and patella, metatarsus, and tarsus of each forelimb, delineated above with red lines. This process was additionally repeated for images of hindlimbs from each individual.

Results

Behavioural Examinations

Investigation of behavioural examinations of both male-male agonistic encounters and male courtship attempts towards females yielded qualitative evidence suggesting that males utilize their forelimbs in intrasexual and intersexual contexts as both a 'weapon' and an 'ornament,' respectively. In agonistic encounters, males would first notice and orient towards the other male. They would then lift their forelimbs in unison to form a 'V-shape' from the perspective of the other male before slowly arcing them downwards to then repeat the process. Males would repeat this display throughout the encounter until one male appeared to become submissive to the other male, or until the encounter escalated to chelicerae contact. While a statistical analysis comparing male forelimb length and body size to the rate of ‘victory’ in agonistic contests was not performed due to low sample size, it was observed that males who won tended to be generally larger; substantial differences in overall size usually resulted in one male quickly appearing to act submissive before a contest was escalated.

Male courtship attempts when placed in proximity to females showed little difference in general behavioural tendencies and approach to the agonistic encounters. Much like in an intrasexual interaction, males would initially orient to a female before repeatedly displaying their forearms to the female in a ‘V-shape’ in a slow approach. Only one successful mating attempt was observed throughout the observation process, whereby a female allowed a male to both approach and mount (with relatively little forelimb displaying from the female) to begin the attempt at copulation.

Measurement Error

Results of the test on measurement precision for cephalothorax width yielded a Pearson’s coefficient value (ρ) of 0.964 between each measurement of the same individual, indicating a measurement error of 3.60%. Likewise, the test on forelimb length precision found a correlation of 0.985, while the test on hindlimb length yielded a value of 0.981, resulting in overall measurement errors of 1.50% and 1.90% for forelimb length and hindlimb length, respectively. These results indicated a somewhat low overall percentage of measurement error for each trait and a high degree of measurement repeatability.

Intersexual Differences in Means

The results of the Welch's Two-Sample T-Test comparing male cephalothorax width (CW) and female CW found no significant differences between the two samples (T = -0.0369, df = 65, p = 0.971). Figure 4 visualizes male and female CW as boxplots; median female CW was slightly higher than median male CW, while male CW possessed a larger maximum. The Welch's Two-Sample T-Test comparing male forelimb length (FL) and female FL, visualized in Figure 3, found a significant difference between the two samples (T = 4.17, df = 65, $p < 0.001$, H_0 rejected). As shown in Figure 4, female median FL was smaller than male FL. The maximum female FL was much smaller than maximum male FL. The Welch's Two-Sample T-Test comparing male hindlimb length (HL) and female HL (Figure 4) found no significant differences between the two samples (T = -1.57, df = 65, p = 0.121). Female median HL was higher than median male HL (Figure 4); female HL additionally had a higher maximum and a lower minimum than male HL.

The above statistics indicates that male forelimbs were significantly larger than female forelimbs, consistent with my first prediction. Additionally, males and females did not differ significantly in body size, nor did their hindlimbs differ significantly in length. This lends further support to my prediction since it indicates that sexual dimorphism is specific only to FL rather than as an overall size dimorphism in body width or limb lengths.

Intersexual Differences in Variance

As visualized in Figure 4, results of the one-sided F-Test comparing the variance of male CW and female CW found no significant difference in variance (F = 1.10, df = 33;32, p = 0.392). Results of the one-sided F-Test comparing the variances of male FL and female FL found that male FL was significantly higher than female FL (F = 1.81, df = 33;32, p = 0.0485). Results of the one-sided F-test comparing the variances of male HL and female HL found that male HL was not significantly higher than female HL (F = 0.477, df = 33;32, p = 0.981).

These statistics indicate that variance in male forelimbs is significantly higher than in female forelimbs, consistent with my second prediction. This variation is only shown in FL since male CW and HL did not exhibit higher variance than female CW and HL, lending further support to my prediction.

Scaling Patterns of FL and HL with CW Between Males and Females

As shown in Figure 5, results of the one-way ANCOVA investigating the effect of the log-transformed and z-standardized CW, sex, and the interaction between CW and sex on FL found that there was a significant overall effect on FL (F = 90.1, $p < 0.001$). The significant effect was found to derive from the effect of CW on FL only ($p < 0.001$), as there was no significant effect of either sex (p = 1.00) or the interaction of CW and sex (p = 0.892) on FL. Similarly, the one-way ANCOVA involving the log-transformed and z-standardized data investigating the effects of CW, sex, and their interaction on HL found a significant overall effect on HL (F = 61.7, $p < 0.001$) but the significance was limited to the effect of CW on HL ($p < 0.001$), while sex (p = 1.00) and the interaction of CW and sex (p = 0.705) were not significant.

Contrary to my third prediction, male forelimbs did not exhibit a significantly more positive scaling pattern with body size than female forelimbs after controlling for differences in mean and variance. This same relationship was found between scaling patterns of male HL and female HL, though this was consistent with my predictions since I did not hypothesize any intersexual dimorphism in HL allometry.

Scaling Patterns in Males and Females Between FL and HL with CW

Comparison of the allometric slopes and their confidence intervals of FL and HL with CW amongst males and females found no effective difference between them as shown in Figure 6. Among males, there was a slight difference in allometric slope between FL and HL, however the confidence intervals of each slope overlapped substantially ($b = 0.91 \pm 0.15$ for FL and 0.84 ± 0.19 for HL). Among females, the difference was less pronounced, as the slopes differed little from each other ($b = 0.89 \pm 0.16$ for FL and 0.84 ± 0.17 for HL).

The lack of observable difference in allometric scaling in FL and HL among males runs contrary to my fourth prediction that male FL would show greater positive allometry than male HL. Although the scaling of male FL was slightly greater than male HL, the difference was not large enough to warrant confirmation of a difference between the two slopes (this was also due to constraints posed by the sample size).

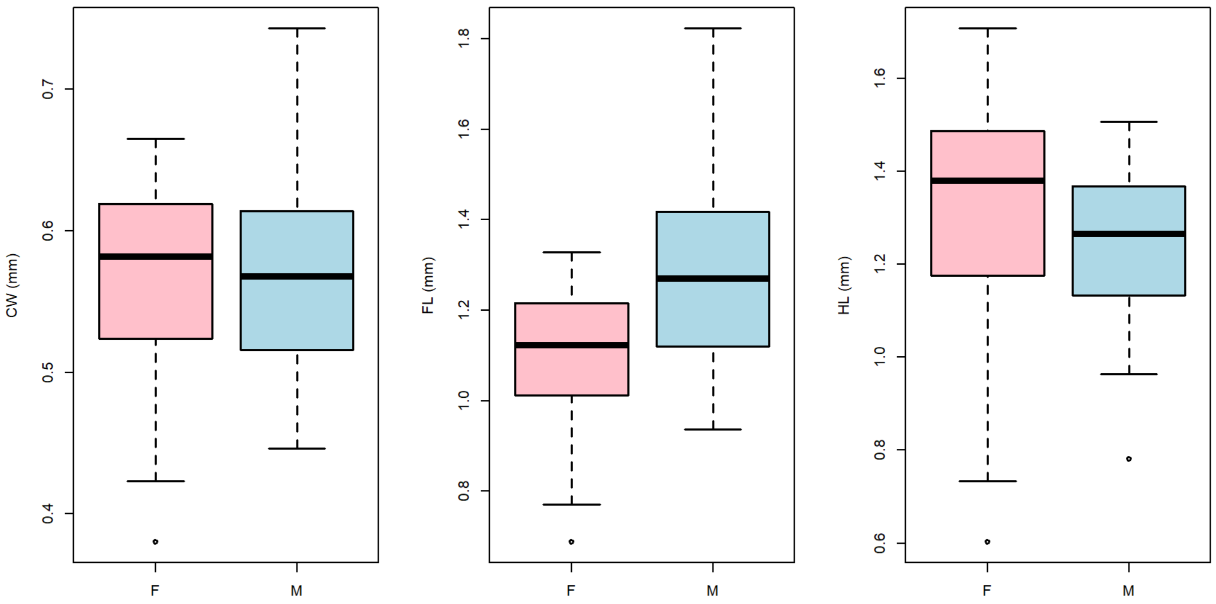

Figure 4. Boxplots visualizing the median, interquartile range, maximum, minum, and outliers for the cephalothorax width (CW), forelimb length (FL), and hindlimb length (HL) of male and female individuals of *Terralonus californicus*. Data were derived from specimens using images taken under a dissecting microscope along with a calibration square of a known length (√2 mm).

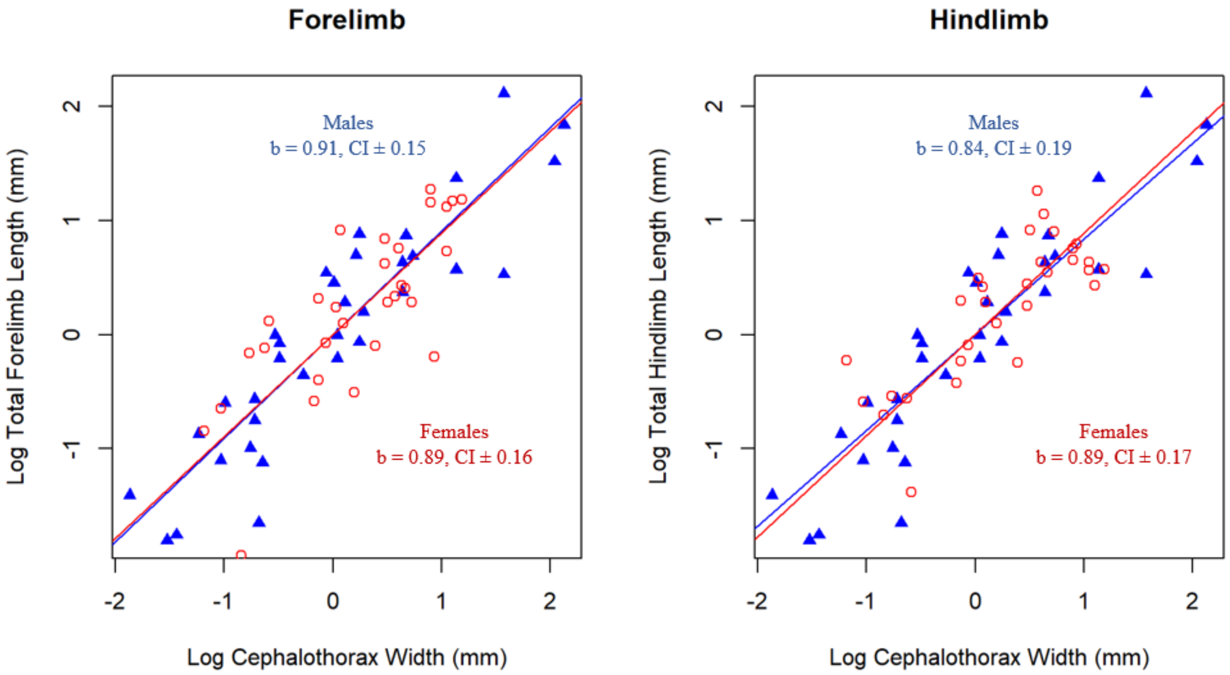

Figure 5. Visualizations of the allometric scaling patterns of log-transformed and z-standardized data (which controls for differences in the mean and variance between groups; each group is adjusted to mean = 0 and standard deviation = 1) of forelimb and hindlimb lengths (mm) against cephalothorax width (mm) for males and females of *Terralonus californicus*. Scaling patterns, slopes (b) and confidence intervals (CI) were calculated using general linear models. An ANCOVA was run to compare the pattern of male and female forelimbs and found no significant effect of the interaction between sex and body size (p = 0.892), inconsistent with my prediction that male forelimbs would scale more positively with body size relative to female forelimbs.

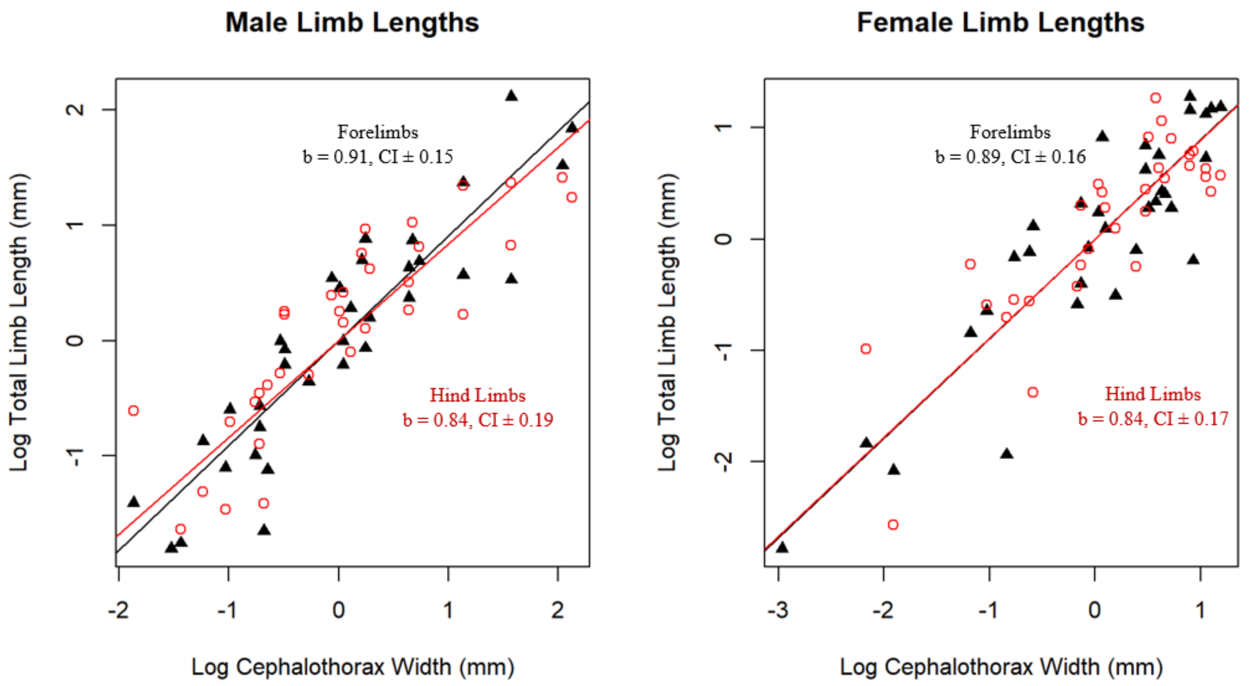

Figure 6. Visualizations of the allometric scaling patterns of log-transformed and z-standardized data (which controls for differences in the mean and variance between groups; each group is adjusted to mean = 0 and standard deviation = 1) of forelimb and hindlimb lengths (mm) against cephalothorax width (mm) for males and females of *Terralonus californicus*. Scaling patterns, slopes (b) and confidence intervals (CI) were calculated using general linear models. The confidence intervals of the allometric slopes between male forelimbs and hindlimbs overlapped by a large margin. This indicated that limb scaling does not differ in males, inconsistent with my prediction that male forelimbs would scale positively with body size relative to male hindlimbs.

Discussion

Summary of Results

From behavioural examinations of intrasexual agonistic encounters and intersexual interactions between reproductively mature individuals of *Terralonus californicus*, I found qualitative evidence that males utilize their forelimbs in displays pertaining to both intrasexual aggression (*i.e.*, as a weapon) and in courtship attempts (*i.e.*, as an ornament). From my observations, I made a series of predictions pertaining differences in the forelimb length (FL) between males and females that was consistent with theory on sexual selection mechanisms. My first prediction was that mean FL would differ between males and females. Results of a Welch's Two-Sample T-Test found that mean male FL differed significantly from mean female FL ($T = 4.17$, $df = 65$, $p < 0.001$), providing support for my first prediction. My second prediction was that the variation in male FL would show a greater degree of variation than female FL. Results of a one-sided F-Test on the variances of male FL and female FL found that male FL was significantly greater than female FL, lending support to my second prediction as well. My third prediction was that male FL covariance with cephalothorax width (CW) would differ in scaling from both HL and female FL covariance with body size, while my fourth prediction was that male FL would scale more positively with CW than male HL. Results of one-way ANCOVAs found that, while CW had a significant effect on FL ($F = 90.1$, $p < 0.001$), there was no significant effect of sex or the interaction of CW and sex. Therefore, allometric scaling of FL did not differ between males and females as I predicted. Similarly, there was no effective difference between allometric slopes between male FL and HL ($b = 0.91 \pm 0.15$ for FL and 0.84 ± 0.19 for HL), indicating that male FL did not scale more positively than male HL as my fourth prediction theorized.

Support for Sexual Dimorphism in Forelimb Length

I found a significant difference between male FL and female FL, which suggests that the forelimbs of *Terralonus californicus* are a sexually dimorphic trait. The observed dimorphism is likely driven by sex-specific selection acting on forelimb length in which longer forelimbs are selected for in males while limbs of a moderate length (*i.e.*, a stabilizing selection) are selected for in females. Furthermore, the fact that there was no significant difference between either male and female CW or male and female HL provides additional support for my prediction, as it shows that male FL varied independently of differing overall body sizes. This result is also consistent with the wide body of literature on sexual selection acting on trait means (Darwin, 1871; Masta and Maddison, 2002), indicating that sexual selection may be the type of evolution acting on FL, though ecological niche selection may also serve as the source of the observed dimorphism.

F-Tests comparing the sample variances of male and female FL found a significant difference between the two, lending support to my second prediction and existing theory on sexual selection leading to higher variability of a trait due to the either the selection on variability itself or due to the dependence of sexually dimorphic characters on condition (Rowe and Houle, 1996; Punzalan *et al.*, 2008).

Complications of Theory and Prior Methodology on Patterns of Allometric Scaling

Results of analyses on the allometric scaling of FL with body size found no significant differences of scaling of FL between the sexes; likewise, there was no observable difference between the scaling of FL and HL among males. These results indicate that male FL did not show positive allometry relative to female FL, nor did male FL show greater positive allometry than male HL. This runs contrary to my third and fourth predictions which postulated that the allometric scaling would vary between the FL of males and females and between the FL and HL of males, respectively. This also runs contrary to theory on allometric scaling theorizing that sexually dimorphic traits acting as either a weapon or an ornament will exhibit positive allometry (Gould, 1973; Kodric-Brown *et al.*, 2006). However, rather than imply that male forelimbs are not used as either weapons or ornaments, this may simply elucidate that sexual selection does not always create positive scaling in allometry for sexually selected characters. One recent paper on allometric scaling in sexually selected traits found that only some weapons exhibit positive allometry, while even fewer ornaments scale proportionally at all with body size (Rodríguez and

Eberhard, 2019). This recent evidence contradicts and complicates much of the theory on the correlations between allometry and function and may indicate that much of the current body of evidence is biased towards traits with highly exaggerated dimorphisms and does not include a full representation of dimorphic traits. Additionally, most studies of allometry do not z-standardize their data as performed in this study. Therefore, much of the supposed evidence of dimorphic allometric scaling may be due to differences in mean or variance since they were not controlled for. Bivariate analyses may also be too constraining to tell the full story of trait relationships; in reality, traits likely co-vary with more than only one other trait that only multivariate analyses can investigate. The lack of positive allometry in male forelimb lengths may therefore be due to several factors; perhaps males do not need to invest relatively more with greater body size as with some other sexually dimorphic traits, or perhaps there is either a developmental, genetic, or biomechanical constraint on forelimb length that caps total length at larger body sizes. For example, since males appear to raise their forelimbs in a display, there must be a constraint to their total weight so that they do not become too heavy to effectively form a ‘V-shape’ in either mating or intrasexual contests. Additionally, males and females likely use their forelimbs to latch onto a vertical surface when approaching from a horizontal surface; forelimbs that are very large would cause this process more difficult which may cause constraints on overall length.

Study Limitations

This study was the first to investigate individuals of *T. californicus* and therefore involved several exploratory methods that were not all effective. Juvenile mortality in captivity was high and most did not molt to reach reproductive maturity, possibly due to nutritional deficits or a lack of external signals to initiate molting. As a result, the resulting sample size (n =

67) from which I gathered behavioural observations and morphological data was substantially smaller than the initial number of specimens in captivity. There were also few adult individuals present at the collection site after the month of October, so the sample could not be replenished at a satisfactory rate. Furthermore, many males who had spent time in the test tubes appeared to grow lethargic, slow, or unresponsive. Therefore, a dataset large enough to quantitatively investigate both male-male encounters and male-female interactions could not be gathered, as most encounters concluded inconclusively due to a lack of behavioural reactions from one or both males. Due to this, I limited my behavioural data to qualitative observations to support my predictions of the use of forelimbs as a weapon and ornament by males.

Implications and Future Research

This study tested various theories of sex-specific selection mechanisms on an unstudied species of jumping spider that inhabits a unique ecological habitat with unique challenges relative to other jumping spiders. My methodology regarding the morphometric analysis appeared to provide a reliable and precise way to quantify morphological traits in jumping spiders, as the correlation coefficients were high between each measurement. Therefore, the methodology I employed and described can be applied to the measurement of a wide variety of characters suspected of being weapons or ornaments in both jumping spiders and other small invertebrates. The results of my research provided support for my hypothesis that forelimb length is a sexually dimorphic trait that is longer in males and more moderate in females. The study also provided results that contradicted existing theory on sexually selected characters and positive allometry. Therefore, additional study is warranted, ideally involving a larger sample size conducted during months that individuals can be collected directly from the wild to avoid specimen mortality and lethargy. This would allow for a more robust sample size regarding both morphometric analysis and the quantification of behavioural trends for both intrasexual and intersexual encounters. Testing for correlations between male FL and success in intrasexual and intersexual encounters would yield evidence that FL is a trait under sexual selection. A future study that included a large behavioural dataset could compare the rates of both victory in intrasexual encounters and successful copulations in intersexual encounters to forelimb length to test whether forelimb length plays a role in affecting agonistic outcomes and female mate choice. Further investigation into their ecological niche space such as their habitat, diet, adaptations to the intertidal zone, and degree of intraspecies competition would likely provide evidence that could determine whether the empirically observed dimorphism is due to differences in ecological niches between the sexes. Further research approaching *T. californicus* from different angles of study, whether they be evolutionary, behavioural, or ecological in nature, would likely provide intriguing findings on a unique and understudied local species and expand on the work that I have initiated with this research.

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