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INSTITUTO DE BIOLOGIA

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA, CONSERVAÇÃO E
BIODIVERSIDADE

**A CONSISTÊNCIA NO PAREAMENTO HOMOTÍPICO POR
TAMANHO AO LONGO E ENTRE ESTAÇÕES REPRODUTIVAS NÃO
DEPENDE DA VARIAÇÃO DE TAMANHO NA ARANHA BASÍLICA**

Mecynogea lemniscata (Araneidae)

VÍTOR CAMPOS DE CASTRO

UBERLÂNDIA, MG

JULHO de 2024

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***Mecynogea lemniscata* (Araneidae)**

Dissertação apresentada à Universidade Federal de
Uberlândia, como parte das exigências à defesa de
Mestrado do Programa de Pós-graduação em
Ecologia, Conservação e Biodiversidade.

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Ecologia, Conservação e Biodiversidade.

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Resumo: Identificar padrões de pareamento é crucial para entender com a mudanças genéticas ocorrem. Entretanto, para estimar com precisão o pareamento em uma população, ele deve ser medido dentro e entre estações reprodutivas, porque a consistência dos pareamentos por tamanho em diferentes escalas temporais é frequentemente baixa em animais. Além disso, é importante descrever a variação de tamanho de machos e fêmeas entre essas escalas temporais para evitar o paradoxo de Simpson. Avaliamos o dimorfismo sexual de tamanho, os efeitos da ontogenia, as diferenças de tamanho de cada sexo e a consistência do pareamento por tamanho dentro e entre as estações reprodutivas na aranha basílica, *Mecynogea lemniscata* (Araneidae). Coletamos casais em uma plantação de eucaliptos, localizada na Savana Brasileira, em duas estações reprodutivas (2022 e 2023/24) e em quatro períodos da estação de 2023/24. Medimos a massa e a largura da carapaça das aranhas. Os machos maturaram mais cedo do que as fêmeas e guardaram parceiras subadultas. Embora os adultos tenham sido maiores e mais pesados do que os subadultos, a ontogenia não afetou os padrões de pareamento. As fêmeas foram mais pesadas que os machos na estação de 2023/24, mas não em 2022. No entanto, essas mudanças não influenciaram os pareamentos, pois as cópulas foram aleatórias com base na massa em ambas as estações. Apesar das diferenças de tamanho entre as estações, o pareamento permaneceu homotípico com base na largura da carapaça e dentro da estação de 2023/24. O pareamento homotípico por tamanho foi consistente em diferentes escalas temporais. Ao contrário de outras espécies, a variação temporal no tamanho de *M. lemniscata* não interferiu nos padrões de pareamento. Incentivamos outros pesquisadores a investigarem a variação temporal no tamanho e no pareamento e a examinarem os mecanismos subjacentes a essa consistência.

Palavras-chave: escolha de parceiros, competição intrasexual, acasalamento, seleção sexual.

Abstract: Identifying assortative mating patterns is crucial for understanding genetic changes. However, to accurately estimate assortative mating in a population, it should be measured within and between breeding seasons, because the consistency of size-assortative mating across different temporal scales is often low in animals. In addition, describing size variation of males and females between temporal scales is important to avoid Simpson's paradox. We assessed sexual size dimorphism, the effects of ontogeny, size differences within each sex, and the consistency of size-assortative mating within and between breeding seasons in the basilica spider, *Mecynogea lemniscata*. We collected mating pairs of *M. lemniscata* in a eucalyptus plantation, located in the Brazilian savanna, across two breeding seasons (2022 and 2023/24) and in four periods of the 2023/24 breeding season. We measured body mass and carapace width of spiders. Males matured earlier than females and guarded subadult partners. Although adults were larger and heavier than subadults, ontogeny did not affect assortative mating patterns. Females were generally heavier than males in the 2023/24 breeding season but not in 2022; however, these changes did not influence mate assortments, as pairings were random based on body mass in both seasons. Despite varying size differences between seasons, size-assortative mating remained homotypic based on carapace width across seasons and within the 2023/24 season. Homotypic size-assortative mating was consistent across different temporal scales. Unlike other species, temporal size variation in *M. lemniscata* spiders did not disrupt assortative mating patterns. We urge other researchers to investigate temporal variation in body size and assortative mating, thereby examining the underlying mechanisms that maintain a consistency in size-assortative mating.

Key-words: Mate choice, intrasexual competition, mating, sexual selection.

Consistency in homotypic size-assortative mating within and between breeding seasons is regardless of size variation in the basilica spider *Mecynogea lemniscata* (Araneidae)

Introduction

Assortative mating or pairing is a widespread non-random mating pattern that emerges when sexually active adults form pairs based on the similarity or dissimilarity of phenotypic (or genotypic) traits (Crespi, 1989; Ridley, 1983). Homotypic mate assortment is the most prevalent pattern and can be estimated as a positive correlation between traits of mating pairs, while heterotypic assortative mating is rare and is measured as a negative correlation between these traits (Janicke et al., 2019; Moura et al., 2021). Most cases of assortative mating described in the literature on animals' mating are related to body size of mating pairs (*i.e.*, size-assortative mating) (Moura et al., 2021). Assessments of body size provides important information on mating dynamics because it is a trait related to mate quality and usually sexually selected. Large males usually have advantages in intrasexual competition against small competitors (Vieira & Peixoto, 2013). Moreover, larger females are often more fecund than smaller ones (Honěk, 1993). Large individuals, therefore, are high-quality mates. In addition, sexual size dimorphism is usually a product of intense sexual selection in arthropods and can influence mate dynamics (review: Janicke et al., 2016; Teder and Tammaru, 2005).

Crespi (1989) reviewed three hypotheses to explain the emergence of size-assortative mating in arthropods. Mate competition hypothesis states that mate assortment can emerge due to mate preference for similar-sized individuals or due to a set of conditions: (i) preference for large partners of one or both sexes, (ii) variability in mate quality, and (iii) size advantages in mate competition (Hoefler, 2007; Moura & Gonzaga,

2017; Ridley, 1983). There is evidence that these conditions are common in arthropods (Crespi, 1989; Hoefler, 2007; Moura & Gonzaga, 2017, 2018, 2019). Size-assortative mating can also emerge as a byproduct of the following conditions, even in the absence of mate choice: a physical or physiological restriction during copulation due to size differences between mates (*i.e.*, mating constraints hypothesis; Brown, 1993) or a spatial and temporal variation in mate sizes (Moura & Gonzaga, 2018; Taborsky et al., 2009, 2014). Mate choice hypothesis is usually investigated as an evolutionary cause of mate assortments by size (Moura et al., 2021). However, adults can randomly mate by size even when assumptions of mate choice hypothesis are corroborated (Mobley et al., 2014; Moura & Gonzaga, 2017). Hence, we need to consider how temporal variation in population structure may influence the stability of assortative pairing patterns. Several important conditions favoring the occurrence of assortative pairing may vary in time, such as sex ratio, population density, and the intensity of sexual selection (Montiglio et al., 2016; Moura & Gonzaga, 2017, 2018).

Homotypic assortative mating can reinforce genetic differences between morphs and promote sympatric speciation from premating isolation (Johannesson et al., 1995; Seehausen, 1997), especially when it is reinforced by ecological or behavioral constraints (Kirkpatrick & Ravigné, 2002; Ritchie, 2007). However, assortative mating needs to be consistent throughout a breeding season and across seasons to reduce gene flow between distinct morphs and generate effective premating isolation (Kondrashov & Shpak, 1998). This is especially important because empirical evidence suggests that the strength of size-assortative mating generally has low consistency across animals, mainly due to lack of repeatability of this mating pattern in Amphibia, Crustacea, and Chelicerata. Among studies on size-assortative mating, researchers reported more than one temporal assessment of assortative mating patterns in only 12.7% of 457 studies (Moura et al.,

2021). In addition, researcher may fall in a statistical trap, called Simpson's paradox, when pooling data from different populations or breeding periods to increase statistical power (Blyth, 1972). This data pooling practice produces a spurious positive correlation when individuals from different populations or periods have distinct average sizes. This practice was adopted in 42.9% of studies (Moura et al., 2021). Therefore, investigating temporal variation in body size of individuals is necessary to interpret size-assortative mating patterns. Furthermore, estimating the consistency of size-assortative mating is essential to assert that this mating patterns indeed influence genetic diversity of a population and, ultimately, speciation and to identify which processes can cause noise in mate assortments.

Large spider males often have advantage in mate competition (Hoefer, 2007; Moura & Gonzaga, 2017), and large spider females are more attractive, probably because they are more fecund than small females (Head, 1995). If large individuals have these impact on mate choice, size-assortative mating should be stronger when large mates are present in the population (*e.g.*, Moura et al., 2020). However, mate assortments may vary temporally if adults of distinct sizes mature at different periods of the breeding season (Crespi, 1989; Miyashita, 1994; Moura et al., 2020). Differences in the timing of female and male maturations can be the driver of this variation (Kvarnemo & Simmons, 2013; Miyashita, 1994; Moura et al., 2020). Protandry, for example, is the a case in which males mature earlier than females in the breeding season and has evolved in many arthropod taxa (Møller et al., 2009). Early-mature males may guard subadult partners until their last molt to assure copulation priority with virgin females (Chelini & Hebets, 2017; Maklakov et al., 2004), and this can generate some degree of temporal asynchrony in individual development (Møller et al., 2009). Spiders provide a great system to investigate how size variation in a population throughout a breeding season can influence mating success and

assortative mating, because adults present asynchronous development (Maklakov et al., 2004), and protandry is observed in many species (Miyashita, 1994; Morbey & Ydenberg, 2001; Moura et al., 2020). Thus, large, or small individuals can become available more often in specific periods of the season and influence the likelihood of size-assortative mating emerging.

Mecynogea lemniscata (Walckenaer, 1841) is an Araneidae with a wide distribution, from USA to Argentina (WSC, 2024). Females are usually larger than males and build three-dimensional web structures with a dense, dome-shaped web at the base, in which the spider remains most of the time (Levi, 1997). Size is important to determine female fecundity, in which large well-fed females produce more eggs (Wise, 1979). As observed in mate choice of a closely related species, *Manogea porracea* (C. L. Koch, 1838) (Araneidae), females are limited to the number of males enticed by their webs and do not refuse mating with extra partners (Moura & Gonzaga, 2017). In *M. lemniscata*, pairings also reflect mating success, because the male usually copulates when the female accepts his presence on her dome-shaped web (Robinson & Robinson, 1980). In addition, female maturation in *M. porracea* is asynchronous and influence mate choice and assortative mating, in which a homotypic pattern emerges only when females become adults (Moura et al., 2020). Thus, a similar effect of protandry and asynchrony in female ontogeny may influence assortative mating in *M. lemniscata*. In this study, we described sexual size dimorphism in two breeding seasons of *M. lemniscata* and how individual size and the strength of size-assortative mating vary throughout a breeding season and across seasons. We also assess the effects of ontogeny on assortative mating. Regarding the effects of temporal size variation in *M. lemniscata*, we hypothesized that (1) individuals differ in size between seasons and throughout the breeding season, and (2) size-assortative mating is stronger when most large individuals mature.

Material and Methods

Study area

We conducted data collection in Estrela do Sul, Minas Gerais, Brazil (18°49'27"S, 47°51'47"W). The study area is a *Eucalyptus* plantation, surrounded by native Cerrado vegetation. The spiders have two breeding cohorts: the longest occurs between August and December and the shorter occurs between January and April (Moura et al. 2017). The property is divided into rectangular blocks with eucalyptus groves at different stages of growth. The trees are spaced 3 m apart. Spiders build their webs at the base of the trees, attaching silk threads to the trunk, thin branches, and leaf litter.

Study species

The genus *Mecynogea* (Simon, 1903) is composed of nine valid species. In Brazil, we found six of them, *M. bigibba*, *M. buique*, *M. erythromela*, *M. infelix*, *M. lemniscata*, and *M. sucre* (WSC, 2024). We have few information on mating behaviors of *M. lemniscata*. In Panama, adults mature in early July and mate until early October. During courtship, males use the mating thread as part of the vibratory courtship. Upon making contact, the female adopts a receptive position, suspending herself from the dome-shaped web. The mating process is lengthy, characterized by prolonged copulations and multiple insertions, all occurring without any additional courtship rituals (Robinson & Robinson, 1980). In the study area, individuals build three-dimensional webs by attaching support threads to eucalyptus branches and leaf litter, close to the ground. The spider's breeding season runs from November to January, as we observed in field.

Data collection

We collected paired couples of *M. lemniscata* in female webs between November and January, during two breeding seasons. The first collection completed in one day, in 2022. The second one was carried out over six field visits between late 2023, and early 2024. We visually check and found males in female sheet-webs, which indicates that females allowed them to approach, court and, probably, mate. In the first breeding season, we visually inspected spider webs and manually collected 62 paired individuals on December 12, 2022. In the second season, we collected 52 paired couples between December 2023 and February 2024. We considered only 5 surveys with intervals of approximately 20 days, because the third and fourth visits happened small interval. We stored all individuals separately in 15mL Falcon tubes with an identification and transport them to the Laboratório de Aracnologia in the Universidade Federal de Uberlândia, MG, Brazil.

We weighted the live individuals on the scale (0.00001 g precision). We then froze the spiders and subsequently stored them in 80% alcohol. We measured the carapace width (mm) of all spiders using a Leica 250 C stereomicroscope equipped with a Leica DFC camera and the LAS Application Suite software. The biological material is deposited in the collection of the Laboratório de Aracnologia (M.O. Gonzaga, curator).

Statistical analysis

We used two operational variables for spider body size: carapace width, as a conservative measure of size that does not vary within a given developmental stage (subadult and adult), and body mass, as a more variable measure within each stage (Moura & Gonzaga, 2017). To investigate size differences among sexes between breeding seasons, we built general linear mixed models (LMM). When the data violated the model

assumptions, we used generalized linear mixed models (GLMM) with negative binomial or Gamma error distribution and log link function. We evaluated model assumptions and overdispersion using “DHARMA” package (Hartig, 2022). We built linear mixed models using “lme4” package (Bates et al., 2015). We built GLMMs with negative binomial distribution using “MASS” package (Venables & Ripley, 2002). We assessed multicollinearity among predictors with the variance-inflation factor (VIF) and considered 3 as a more conservative cutoff value (Thompson et al., 2017).

To describe sexual size dimorphism, as well as ontogenetic and seasonal differences, we compared body size (as the response variable) between sexes, ontogenetic stages, and breeding seasons (2022 and 2023/24), as well as their interactions, as the predictor variables. We added web identity as the random variable. To investigate size-assortative pairing across seasons, we tested the relationship of female size (response variables) with male mass, breeding season, and its interaction (predictor variables). We considered female size as the response variable because females are limited to males that are attracted to their webs. Therefore, pairing is more likely to emerge through male mate choice.

Within the 2023/24 breeding season, we built general linear models to compare body size (response variables) for each sex separately between breeding days (1, 20, 40, 60), as predictor variable. We then tested the relationship of female size (response variable) with male size, breeding days, and its interaction (predictor variables). We designed graphs using R base (R Core Team, 2023) and “ggplot2” packages (Wickham, 2009).

To investigate size-assortative pairing between breeding days of the 2023/24 season, we assessed how female size (response variable) is related to male size (predictor variable). We also added breeding days and female ontogenetic stage as predictor

variables, in addition to the interaction between breeding days and male size. When within breeding season models do not attend model assumptions, we conducted generalized linear models with the appropriate error distribution and link function. We calculated P -values using “car” packages (Fox & Weisberg, 2019). We conducted post hoc tests using “emmeans” package (Lenth, 2023). We performed data analysis and graphical design in R, version 4.2.3 (R Core Team, 2023).

Results

Sexual size dimorphism and seasonal differences

We found 62 and 52 mating pairs in the 2022 and 2023/24 breeding seasons, respectively. There were four and 27 subadult females in the 2022 and 2023/24 breeding seasons, respectively, and five and 10 subadult males in the 2022 and 2023/24 breeding seasons, respectively. There were 35 and 48 adult females in the 2022 and 2023/24 breeding seasons, respectively, and 57 and 42 adult males in the 2022 and 2023/24 breeding seasons, respectively. Among the mating pairs, four and three were made from subadult individuals in the 2022 and 2023/24 breeding seasons, respectively. In 2022, 22 mating pairs had subadult females and adult males, while only one was found in the 2023/24 breeding season, indicating that this breeding season may have started before our first collection.

There was a medium correlation between body mass and carapace width ($r = 0.61$, $t_{226} = 11.67$, $P < 0.0001$). Therefore, both measures do not precisely capture the same processes. In the models comparing breeding seasons, the interaction between sex, breeding season, and ontogeny was greater than 3 for body mass model (VIF = 6.5) and carapace width model (VIF = 5.6). Hence, we excluded this interaction from the models. For body mass model, we detected an interaction between sexes and breeding seasons

(negative binomial GLMM: $\chi^2_1 = 10.63$, $P = 0.001$) and between ontogeny and breeding seasons ($\chi^2_1 = 12.30$, $P = 0.0004$). Female mass was, on median, 1.8 heavier greater than the male mass in 2023/24 breeding season (Table 1, Fig. 2A). Adults were 1.4 and 2.6 times heavier than subadults in 2022 and 2023/24 breeding season, respectively (Table 1, Fig. 2B). For carapace width model, there were interactions between sex and breeding seasons (LMM: $F_{1,124.9} = 5.04$, $P = 0.026$) and between sex and ontogeny ($F_{1,180.9} = 3.91$, $P = 0.049$). Females were smaller than males in the 2022 breeding season, but they were 1.1 times larger than males in the 2023/24 breeding season (Fig. 2C). Adults were 1.3 times larger than subadults in both sexes (Fig. 2D).

Size-assortative mating between breeding seasons

Between breeding seasons, pairings were random based on body mass (LM: $F_{1,110} = 0.51$, $P = 0.476$; Fig. 3B). There were differences in female mass between seasons ($F_{1,110} = 22.06$, $P < 0.0001$; Fig. 3A), but we did not detect an interaction between male mass and breeding season ($F_{1,110} = 1.82$, $P = 0.179$; Fig. 3B). There was homotypic assortative pairing based on carapace width (LM: $F_{1,110} = 9.35$, $P = 0.003$; Fig. 3D). Female carapace width was 1.2 times larger in the 2023/24 breeding season ($F_{1,110} = 9.35$, $P = 0.003$; Fig. 3C), but there was no interaction between male mass and breeding season ($F_{1,110} = 0.33$, $P = 0.567$; Fig. 3D).

Size variation and assortative mating within breeding season

There was no difference in male mass (LM: $F_{3,48} = 1.54$, $P = 0.216$, Fig. 4A) and female mass (LM: $F_{3,48} = 0.27$, $P = 0.850$, Fig. 4B) between breeding days. We detected differences in carapace width of males between breeding periods (LM: $F_{3,48} = 6.41$, $P = 0.001$). Males were smaller in day 20 in comparison with days 40 and 60 (Fig. 4C).

However, we did not detect differences in carapace width of females within the breeding season (Gamma log GLM: $\chi^2_3 = 7.48$, $P = 0.060$).

Assortative pairing was random based on body mass in the 2023/24 breeding season (LM: $F_{1,43} = 3.59$, $P = 0.065$, Fig. 5B). Average female mass did not differ between breeding days ($F_{3,43} = 0.207$, $P = 0.891$, Fig. 5A), but it differed between ontogeny ($F_{1,43} = 6.13$, $P = 0.017$). There was no interaction between female mass and breeding periods ($F_{3,43} = 0.152$, $P = 0.928$, Fig. 5B).

For carapace width, assortative pairing was homotypic (LM: $F_{1,43} = 17.37$, $P = 0.0001$, Fig. 5D). There were no differences in female carapace width between breeding days ($F_{3,43} = 1.39$, $P = 0.259$, Fig. 5C), but adult females were larger than subadults ($F_{3,43} = 0.101$, $P = 0.959$). There was no interaction of breeding periods with male carapace width ($F_{3,44} = 0.39$, $P = 0.759$, Fig. 5D).

Discussion

The identification of assortative mating patterns provides important information on the direction of the genetic changes in a population (Janicke et al., 2019; Moura et al., 2021). The emergence of homotypic assortative mating indicates the occurrence of disruptive selection, while heterotypic assortments of mates lead to stabilizing selection (Johannesson et al., 1995; Seehausen, 1997). This narrative is often used to defend the significance of measuring assortative mating in nature. However, it is only reliable if these mating patterns are temporally consistent in a population, because it has generally low consistence in most animal taxa and no consistence in some groups (Moura et al., 2021). A reliable method for estimating assortative mating in a population is to measure it within and between breeding season, as well as describing size variation of males and females between temporal scales to avoid Simpson's paradox (Blyth, 1972; Moura et al., 2021).

In this study, we investigated the temporal consistency of size-assortative mating in the basilica spider *M. lemniscata*. Males were protandrous; thus, maturing earlier than females and guarding subadult partners. Few pairs were formed by subadult individuals, indicating that males may complete their development on female web in some rare cases. Adults were heavier and larger than subadults in both sexes; hence, completing development may influence mate choice and mating patterns, but we did not detect any influence of ontogeny on assortative mating. Females were generally heavier than males in the 2023/24 breeding season, but not in the 2022 breeding season. These changes did not influence mate assortments, because pairings were randomly based on body mass in both seasons. In the 2022 breeding season, females were smaller than their partners, but they were larger than males in the 2023/24 breeding season. These changes also did not influence size-assortative mating, because mate assortments were homotypic based on carapace width across breeding seasons. Within the 2023/24 breeding season, average individual mass did not differ for both sexes, and assortative mating was randomly based on mass. Only males presented a smaller carapace width on day 20 compared to following breeding days. Despite these differences, size-assortative mating was consistent throughout the breeding season. Therefore, homotypic size-assortative mating (for carapace width) was consistent across seasons and within the breeding season in *M. lemniscata*.

Size-assortative mating can emerge because of mate choice and mate competition (Crespi, 1989; Ridley, 1983). However, sexual selection is only one of the mechanisms that can promote assortative mating (Crespi, 1989). Mate assortments can also emerge in the absence of mate choice when phenotypically similar individuals are clustered in heterogeneous habitats (Taborsky et al., 2014) or in different periods of the breeding season (Miyashita, 1994). There are few studies that assessed such temporal and spatial

segregation of individual sizes and accounted for it statistically (Moura et al., 2021). Here, we added important contribution to this literature and reinforce the importance of measuring temporal variation in mate assortments. By conducting multiple assessments within and across breeding seasons, we ensured a robust analysis that accurately reflects the mating patterns of *M. lemniscata*. In a closely related species, *M. porracea*, different effects were observed. On the one hand, size-assortative mating emerged only when females become adults in the beginning of the breeding season. Pairs with protandrous adult males and subadult females were random based on size (Moura et al., 2020). Variation in demography features, such as operational sex ratio (OSR) and density, also influenced size-assortative mating throughout the breeding season in *M. porracea*. Also, mate assortments were homotypic only at the beginning of the breeding season, when OSR was female-biased and population density was higher than in was in the late season (Moura & Gonzaga, 2017). On the other hand, size-assortative mating in *M. lemniscata* was homotypic regardless of ontogeny and size variation between and within breeding seasons. Therefore, it can have significant evolutionary implications to the population of *M. lemniscata*. This pattern can reduce gene flow between morphs of different sizes, potentially leading to genetic differentiation and, ultimately, sympatric speciation.

Mate choice in some species can vary in space and time, which can influence mating patterns. Among populations of the damselfly *Ischnura elegans*, for example, there was a great variation in male size and in female preferences for mates' size (Gosden & Svensson, 2008). It is risky to draw conclusions based on only one temporal measure of assortative mating in this species. Such inconsistency can occur in other species and need to be investigated for different traits. The longest assessment of size-assortative mating between breeding season was performed over 10 years in four populations of the blue tit (*Cyanistes caeruleus*) (Fargevieille et al., 2017). There was great temporal

variation in the strength of assortative mating by color traits (UV-chroma and yellow chroma). The same pattern of assortative mating was rarely observed repeating itself in two consecutive years in the same population and for the same mating trait. In other years, the opposite pattern emerged (Fargevieille et al., 2017). However, it is also important to assess assortative mating throughout a breeding season or a mating aggregation, especially for promiscuous species, because different patterns can emerge (Moura & Gonzaga, 2017, 2018). Size-assortative mating in *M. lemniscata* remained homotypic regardless of changes between seasons and within a breeding season, which shows a robust mating pattern.

The maintenance of assortative mating in *M. lemniscata* suggests that size-based mate choice is a critical factor in the reproductive isolation of the population, but only for carapace width of individuals. Body mass was not an important factor for structuring mate assortments. Homotypic assortative mating emerged regardless of variation in body mass between seasons. Within the breeding season, males exhibited differences in carapace width, while females did not show size differences. This suggests that male size may be more variable within a season, possibly due to differences in growth rates, food availability, or other ecological factors, observed in other spiders (Moura & Gonzaga, 2017; Pitilin et al., 2023). However, this variation did not disrupt the overall pattern of size-assortative mating, indicating a robust mechanism underlying mate choice based on size. Future research should explore the underlying mechanisms driving the temporal consistency in size-assortative mating of *M. lemniscata*. Investigations into mate choice preferences, competitive interactions, and ecological factors influencing body size distribution could provide deeper insights into the stability and evolutionary significance of this mating pattern. Additionally, studying other populations across the species' range

could reveal how environmental variability influences size-assortative mating and its consistency.

In conclusion, our findings reveal that homotypic size-assortative mating in the basilica spider, *M. lemniscata*, remains consistent both throughout individual breeding seasons and across different seasons. This consistent pattern supports the hypothesis that size-assortative mating is a stable mating pattern in this species, contributing to the broader understanding of mating dynamics in *M. lemniscata* and potentially influencing genetic structure of the studied population. Our findings reinforce the importance of measuring assortative mating across temporal scales. The robustness of this mating pattern suggests that size-based mate choice plays a crucial role in shaping the genetic structure and potentially driving speciation within this species. We urge other researchers to investigate temporal variation in body size and assortative mating, thereby examining the underlying mechanisms that maintain a consistency in size-assortative mating.

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Table

Table 1. Descriptive statistics of individual sizes (body mass and carapace width) between sexes and ontogenetic stages. Median ratio was calculated as female size by male size or as adult size by subadult size.

		Body size: Median [1°Q - 3°Q]	Median Ratio
Breeding season	Sex	Mass (mg)	
2022	Female	22.0 [14.4 - 26.9]	Female/Male = 1.2
	Male	20.2 [16.3 - 22.1]	
2023/24	Female	30.8 [20.8 - 37.7]	Female/Male = 1.8*
	Male	16.9 [12.8 - 20.8]	
Breeding season	Ontogeny		
2022	Adult	21.9 [18.7 - 24.9]	Adult/Subadult = 1.4*
	Subadult	15.8 [12.1 - 21.2]	
2023/24	Adult	23.0 [16.9 - 31.5]	Adult/Subadult = 2.6*
	Subadult	8.7 [7.4 - 10.6]	
Breeding season	Sex	Carapace width (mm)	
2022	Female	1.7 [1.6 - 2.1]	Female/Male = 0.9
	Male	2.0 [1.9 - 2.1]	
2023/24	Female	2.1 [1.8 - 2.2]	Female/Male = 1.1
	Male	1.9 [1.7 - 2.0]	
Sex	Ontogeny		
Female	Subadult	2.1 [1.8 - 2.2]	Adult/Subadult = 1.3*
	Adult	1.6 [1.3 - 1.7]	
Male	Adult	2.0 [1.9 - 2.1]	Adult/Subadult = 1.3*
	Subadult	1.6 [1.4 - 1.7]	

Figures



Figure 1. (A) A mating pair of *Mecynogea leminiscata*. (B) An adult male and (C) an adult female.

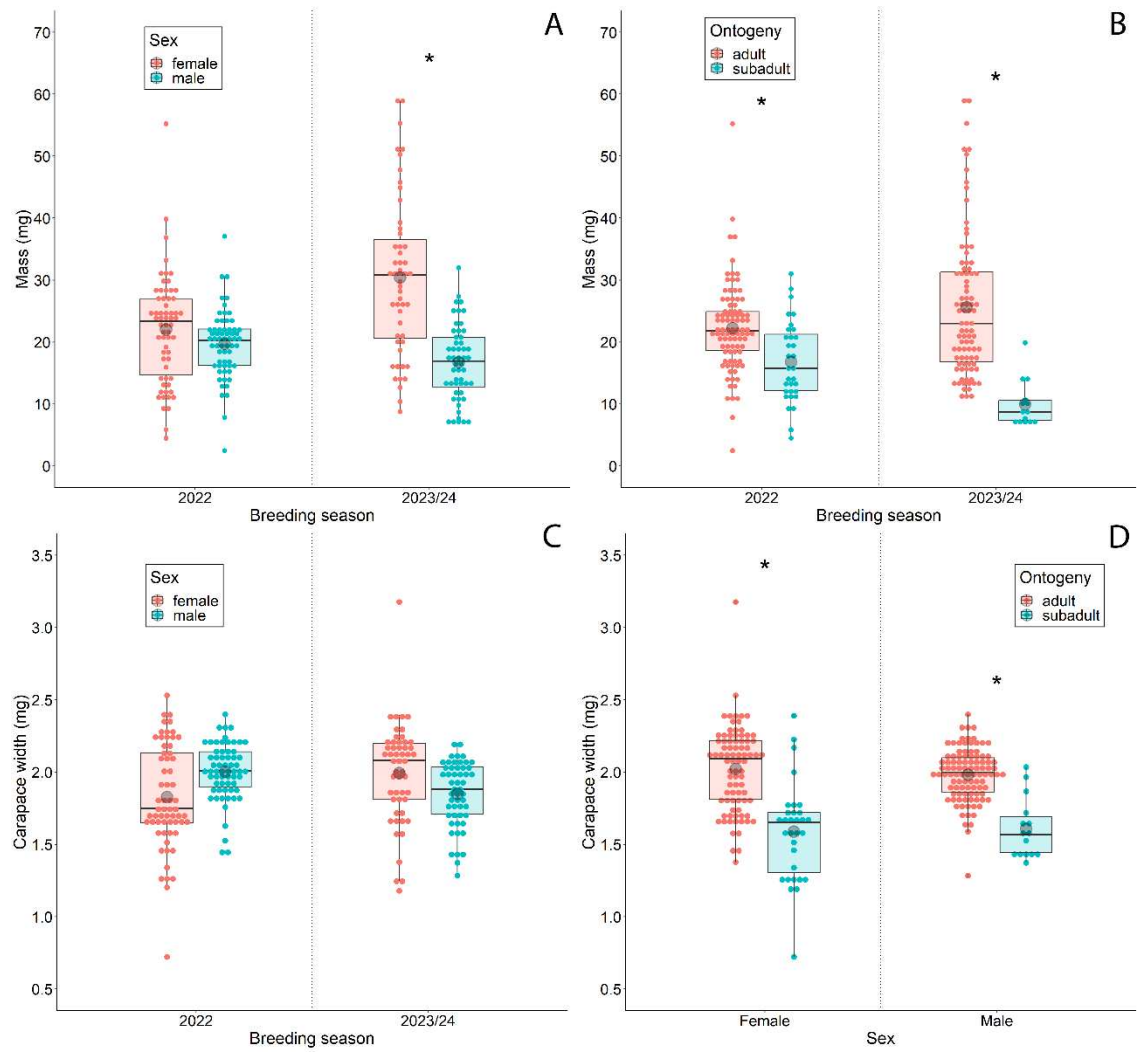


Figure 2. Sex differences in body mass (A) between breeding seasons and (B) ontogenetic stages. Sex differences in carapace width (C) between breeding seasons and (D) ontogenetic stages. Dots represent the samples. * represents $P < 0.05$.

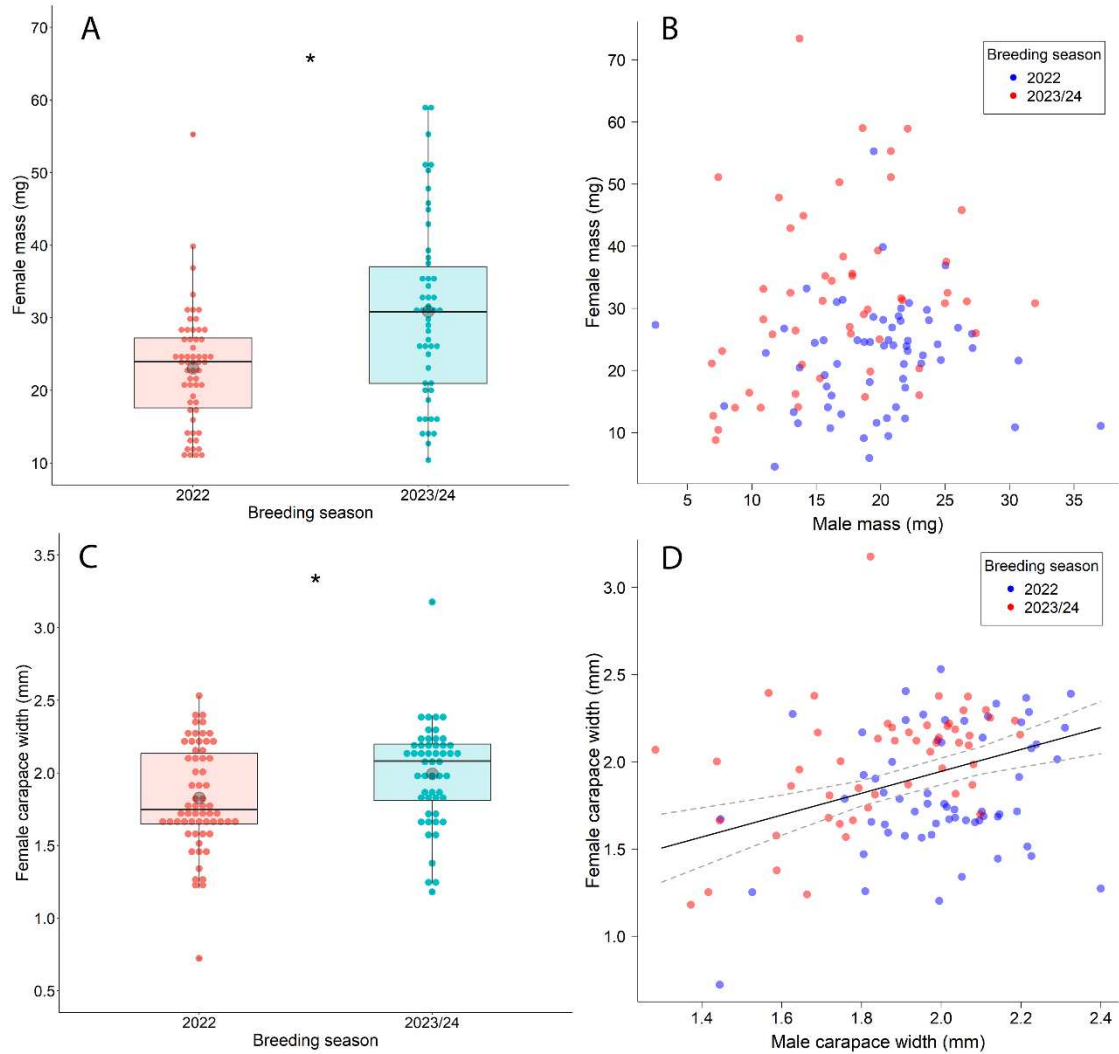


Figure 3. (A) Differences in female mass between breeding seasons. (B) Relationship between male and female mass. (C) Differences in female carapace width between breeding seasons. (D) Relationship between male and female carapace width. Dots represent the samples. In (D), the continuous line corresponds to the mechanistic estimation, and the dashed lines represent 95% confidence intervals. * represents $P < 0.05$.

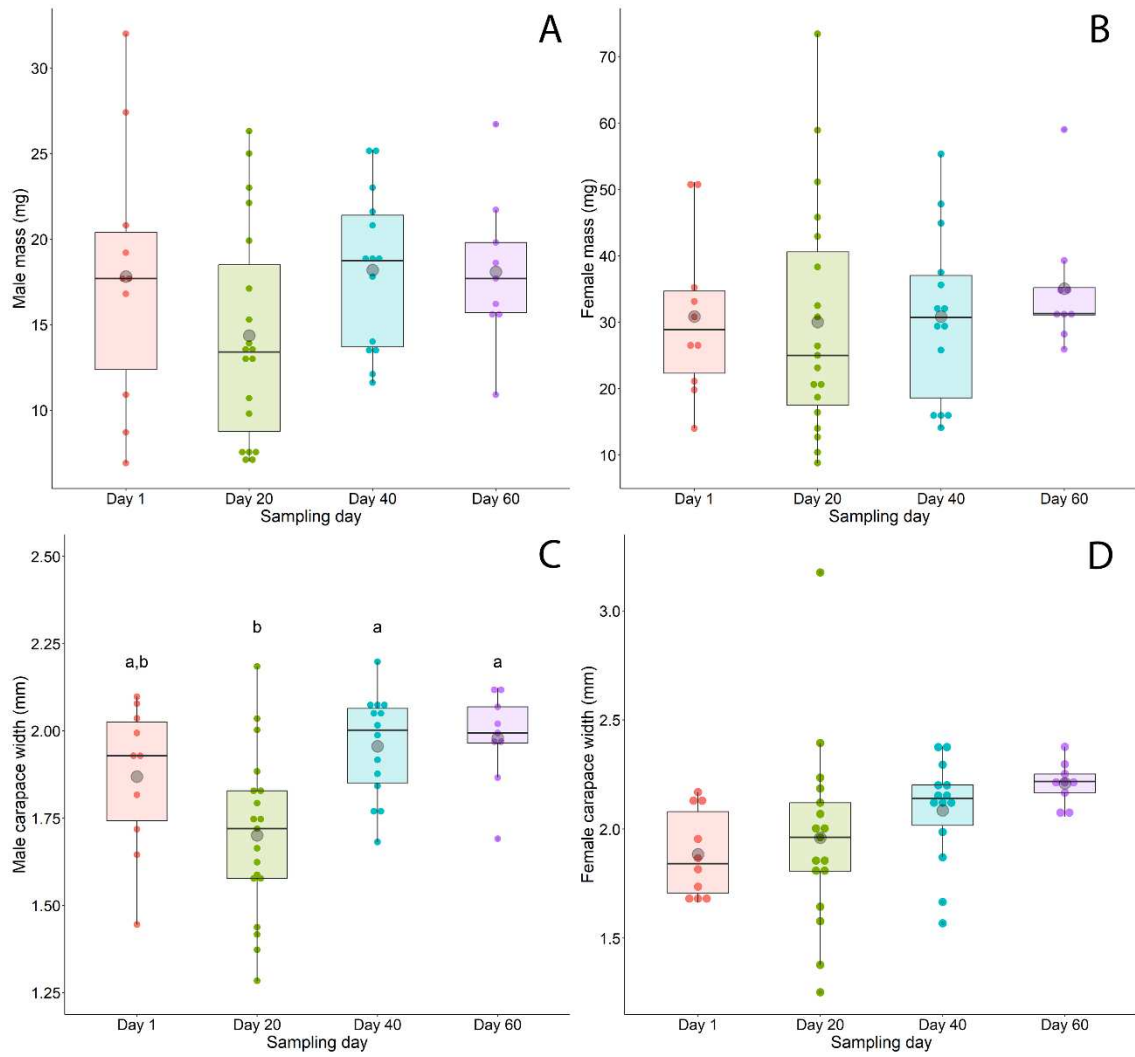


Figure 4. Differences in male mass (A), female mass (B), male carapace width (C), and female carapace width (D) in four periods (day 1, 20, 40, and 60) of the 2023/24 breeding season. Dots represent the samples.

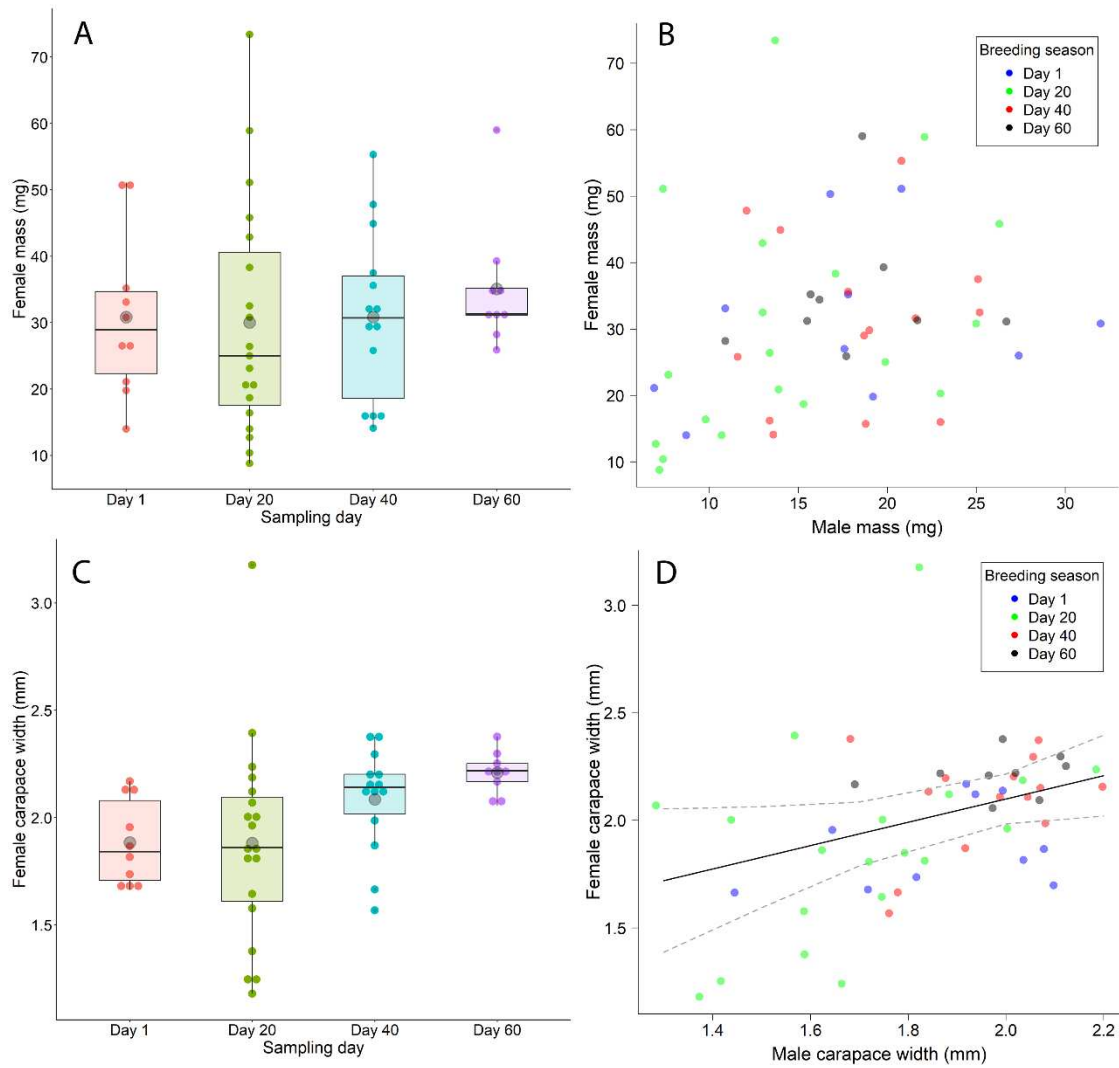


Figure 5. Comparison of female mass (A) and relationship between female and male mass (B). Comparison of female carapace width (C) and relationship between female and male carapace width (D). Dots represent the samples. In D, the continuous line corresponds to the mechanistic estimation, and the dashed lines represent 95% confidence intervals.

References

- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Blyth, C. R. (1972). On Simpson's paradox and the sure-thing principle. *Journal of the American Statistical Association*, 67(338), 364–366.
- Brown, W. D. (1993). The cause of size-assortative mating in the leaf beetle *Trirhabda canadensis* (Coleoptera: Chrysomelidae). *Behavioral Ecology and Sociobiology*, 33(3), 151–157. <https://doi.org/10.1007/BF00216595>
- Chelini, M.-C., & Hebets, E. (2017). Field evidence challenges the often-presumed relationship between early male maturation and female-biased sexual size dimorphism. *Ecology and Evolution*, 7(22), 9592–9601. <https://doi.org/10.1002/ece3.3450>
- Crespi, B. J. (1989). Causes of assortative mating in arthropods. *Animal Behaviour*, 38(6), 980–1000. [https://doi.org/10.1016/S0003-3472\(89\)80138-1](https://doi.org/10.1016/S0003-3472(89)80138-1)
- Fargevieille, A., Grégoire, A., Charmantier, A., del Rey Granado, M., & Doutrelant, C. (2017). Assortative mating by colored ornaments in blue tits: space and time matter. *Ecology and Evolution*, 7(7), 2069–2078. <https://doi.org/10.1002/ece3.2822>
- Fox, J., & Weisberg, S. (2019). *An {R} companion to applied regression* (Second Edition). Sage. <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>
- Gosden, T. P., & Svensson, E. I. (2008). Spatial and temporal dynamics in a sexual selection mosaic. *Evolution*, 62(4), 845–856. <https://doi.org/10.1111/j.1558-5646.2008.00323.x>

- Hartig, F. (2022). *DHARMA: residual diagnostics for hierarchical (multi-level / mixed) regression models*. R package version 0.4.5.
- Head, G. (1995). Selection on fecundity and variation in the degree of sexual size dimorphism among spider species (Class Araneae). *Evolution*, 49(4), 776. <https://doi.org/10.2307/2410330>
- Hoefler, C. D. (2007). Male mate choice and size-assortative pairing in a jumping spider, *Phidippus clarus*. *Animal Behaviour*, 73(6), 943–954. <https://doi.org/10.1016/j.anbehav.2006.10.017>
- Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*, 66(3), 483. <https://doi.org/10.2307/3544943>
- Janicke, T., Häderer, I. K., Lajeunesse, M. J., & Anthes, N. (2016). Darwinian sex roles confirmed across the animal kingdom. *Science Advances*, 2(2), e1500983. <https://doi.org/10.1126/sciadv.1500983>
- Janicke, T., Marie-Orleach, L., Aubier, T. G., Perrier, C., & Morrow, E. H. (2019). Assortative mating in animals and its role for speciation. *The American Naturalist*, 194(6), 865–875. <https://doi.org/10.1086/705825>
- Johannesson, K., Rolan-Alvarez, E., & Ekendahl, A. (1995). Incipient reproductive isolation between two sympatric morphs of the intertidal snail *Littorina saxatilis*. *Evolution*, 49(6), 1180. <https://doi.org/10.2307/2410443>
- Kirkpatrick, M., & Ravigné, V. (2002). Speciation by natural and sexual selection: Models and experiments. *The American Naturalist*, 159(S3), S22–S35. <https://doi.org/10.1086/338370>
- Kondrashov, A. S., & Shpak, M. (1998). On the origin of species by means of assortative

- mating. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265(1412), 2273–2278. <https://doi.org/10.1098/rspb.1998.0570>
- Kvarnemo, C., & Simmons, L. W. (2013). Polyandry as a mediator of sexual selection before and after mating. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1613), 20120042. <https://doi.org/10.1098/rstb.2012.0042>
- Lenth, R. V. (2023). emmeans: estimated marginal means, aka least-squares means. *R Package*, 1.8.6. <https://cran.r-project.org/package=emmeans>
- Levi, H. W. (1997). The American orb weavers of the genera *Mecynogea*, *Manogea*, *Kapogea* and *Cyrtophora* (Araneae: Araneidae). *Bulletin of the Museum of Comparative Zoology*, 155, 215–255.
- Maklakov, A. A., Bilde, T., & Lubin, Y. (2004). Sexual selection for increased male body size and protandry in a spider. *Animal Behaviour*, 68(5), 1041–1048. <https://doi.org/10.1016/j.anbehav.2004.02.010>
- Miyashita, T. (1994). Size-related mating and mate guarding in the orb-web spider *Nephila clavata* (Araneae, Araneidae). *Journal of Insect Behavior*, 7(3), 289–296. <https://doi.org/10.1007/BF01989736>
- Mobley, K. B., Abou Chakra, M., & Jones, A. G. (2014). No evidence for size-assortative mating in the wild despite mutual mate choice in sex-role-reversed pipefishes. *Ecology and Evolution*, 4(1), 67–78. <https://doi.org/10.1002/ece3.907>
- Møller, A. P., Balbontín, J., Cuervo, J. J., Hermosell, I. G., & de Lope, F. (2009). Individual differences in protandry, sexual selection, and fitness. *Behavioral Ecology*, 20(2), 433–440. <https://doi.org/10.1093/beheco/arn142>
- Montiglio, P.-O., Wey, T. W., Chang, A. T., Fogarty, S., & Sih, A. (2016). Multiple

- mating reveals complex patterns of assortative mating by personality and body size. *Journal of Animal Ecology*, 85(1), 125–135. <https://doi.org/10.1111/1365-2656.12436>
- Morbey, Y. E., & Ydenberg, R. C. (2001). Protandrous arrival timing to breeding areas: a review. *Ecology Letters*, 4(6), 663–673. <https://doi.org/10.1046/j.1461-0248.2001.00265.x>
- Moura, R. R., & Gonzaga, M. O. (2017). Temporal variation in size-assortative mating and male mate choice in a spider with amphisexual care. *Science of Nature*, 104(3–4). <https://doi.org/10.1007/s00114-017-1448-6>
- Moura, R. R., & Gonzaga, M. O. (2018). Sex ratio and density may affect temporal variation in size-assortative mating in a Neotropical ant species. *Zoologischer Anzeiger*, 276, 50–56. <https://doi.org/10.1016/j.jcz.2018.07.001>
- Moura, R. R., & Gonzaga, M. O. (2019). Spatial variation in sex ratio and density explains subtle changes in the strength of size-assortative mating in *Edessa contermina* (Hemiptera: Pentatomidae). *Acta Oecologica*, 95, 86–92. <https://doi.org/10.1016/j.actao.2018.12.003>
- Moura, R. R., Vasconcellos-Neto, J., & Gonzaga, M. O. (2020). Female sexual maturity as a determining factor of size-assortative pairing in the protandrous spider *Manogea porracea* (Araneae, Araneidae). *Zoologischer Anzeiger*, 284, 1–6. <https://doi.org/10.1016/j.jcz.2019.11.003>
- Moura, R. R., Gonzaga, M. O., Pinto, N. S., Vasconcellos-Neto, J., & Requena, G. S. (2021). Assortative mating in space and time: patterns and biases. *Ecology Letters*, 24(5), 1089–1102. <https://doi.org/10.1111/ele.13690>

- Pitilin, R. B., Moura, R. R., & Gonzaga, M. O. (2023). The effect of diet on fecundity and survival of *Tidarren haemorrhoidale* (Bertkau, 1880) (Araneae, Theridiidae). *Journal of Arachnology*, in press.
- R Core Team, R. (2023). *R: A language and environment for statistical computing* (4.2.3). <https://www.r-project.org/>
- Ridley, M. (1983). *The explanation of organic diversity: The comparative method and adaptations for mating*. Clarendon Press.
- Ritchie, M. G. (2007). Sexual selection and speciation. *Trends in Ecology and Evolution*, 38, 79–102. <https://doi.org/10.1146/annurev.ecolsys.38.091206.095733>
- Robinson, M. H., & Robinson, B. (1980). *Comparative studies of the courtship and mating behavior of tropical araneid spiders*. Pac. Insects Monogr. No. 36, Bishop Museum.
- Seehausen, O. (1997). Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science*, 277(5333), 1808–1811. <https://doi.org/10.1126/science.277.5333.1808>
- Taborsky, B., Guyer, L., & Demus, P. (2014). “Prudent habitat choice”: A novel mechanism of size-assortative mating. *Journal of Evolutionary Biology*, 27(6), 1217–1228. <https://doi.org/10.1111/jeb.12398>
- Taborsky, B., Guyer, L., & Taborsky, M. (2009). Size-assortative mating in the absence of mate choice. *Animal Behaviour*, 77(2), 439–448. <https://doi.org/10.1016/j.anbehav.2008.10.020>
- Teder, T., & Tammaru, T. (2005). Sexual size dimorphism within species increases with body size in insects. *Oikos*, 108(2), 321–334. <https://doi.org/10.1111/j.0030->

1299.2005.13609.x

- Thompson, C. G., Kim, R. S., Aloe, A. M., & Becker, B. J. (2017). Extracting the variance inflation factor and other multicollinearity diagnostics from typical regression results. *Basic and Applied Social Psychology*, 39(2), 81–90. <https://doi.org/10.1080/01973533.2016.1277529>
- Venables, W. N., & Ripley, B. D. (2002). *Modern Applied Statistics with S* (Fourth Edi). Springer.
- Vieira, M. C., & Peixoto, P. E. C. (2013). Winners and losers: a meta-analysis of functional determinants of fighting ability in arthropod contests. *Functional Ecology*, 27(2), 305–313. <https://doi.org/10.1111/1365-2435.12051>
- Wickham, H. (2009). *ggplot2: elegant graphics for data analysis*. Springer-Verlag. <http://ggplot2.org>
- Wise, D. H. (1979). Effects of an experimental increase in prey abundance upon the reproductive rates of two orb-weaving spider species (Araneae: Araneidae). *Oecologia*, 41(3), 289–300. <https://doi.org/10.1007/BF00377433>
- WSC. (2024). *World Spider Catalog. Version 25.0*. Natural History Museum Bern. <https://doi.org/10.24436/2>. Visited in: 06/22/2024