# Evolución de los regalos nupciales y el canibalismo sexual en arañas del género Trechaleoides

Lic. Mauro Martínez Villar

PEDECIBA Biología, Sub área Ecología y Evolución Montevideo, Uruguay. 2022 **Título:** Evolución de los regalos nupciales y el canibalismo sexual en arañas del género *Trechaleoides* 

**Title:** Evolution of nuptial gifts and sexual cannibalism in spiders of the genus *Trechaleoides* 

Autor: Lic. Mauro Martínez Villar

Tesis presentada con el objetivo de obtener el título de Magister en Ciencias Biológicas en el marco del programa PEDECIBA.

Directora: Dr. María José Albo. Facultad de Ciencias, UdelaR; Instituto de Investigaciones Biológicas Clemente Estable, Uruguay

Co-directora: Dr. Ivanna Tomasco. Facultad de Ciencias, UdelaR, Uruguay.

Co-directora: Dr. Trine Bilde. Aarhus University, Aarhus, Dinamarca

Colaboradores académicos: Dr. Søren Toft y Dr. Jesper Bechsgaard, Aarhus University, Aarhus, Dinamarca



#### Tribunal:

Presidente: Dra: Laura Montes de Oca Instituto de Investigaciones Biológicas Clemente Estable, MEC

Vocales:

Dr. Andrés Parada Laboratorio de Evolución, Facultad de Ciencias, UdelaR

Dr. Benjamin M. Sadd School of Biological Science. Illinois State University, USA

#### Fuentes de financiación

2022. Beca de Finalización del Posgrado Comisión Académica de Posgrado (CSIC), UdelaR, Uruguay.

2020-2022. Beca de Posgrado Nacional Agencia Nacional de Investigación e Innovación (ANII), Uruguay.

- 2022. Beca participación en evento científico del exterior, PEDECIBA, Uruguay.
- 2021. Beca de movilidad para pasantía en el exterior, PEDECIBA, Uruguay.
- 2019. Vincent Roth Research Funds, American Arachnological Society (AAS), USA.



#### Permisos de colecta

Para la colecta de los todos individuos utilizados en esta tesis, se tramitaron y obtuvieron los permisos necesarios otorgados por el Ministerio de Vivienda, Ordenamiento Territorial y Medio Ambiente (MVOTMA) y Dirección Nacional de Medio Ambiente (DINAMA). EXP:2020/14000/000374

#### Contribuciones generadas de la tesis

Artículos científicos:

**Martínez-Villar M.**, M.C. Trillo & M.J. Albo. 2021. Ineffective nuptial gifts suggest female emancipation from sensory exploitation. *Behavioral Ecology and Sociobiology*. 75:1-9

**Martínez Villar M.**, M. Germil, C. Pavón-Peláez, I.H. Tomasco, T. Bilde, S. Toft & M.J. Albo. (In revision) Lack of female preference for nuptial gifts may have changed its function and led to the loss of the male sexual trait. *Evolutionary Biology*.

**Martínez Villar M.**, J. Bechsgaard, T. Bilde, M.J. Albo & I.H. Tomasco (in preparation). Female sexual cannibalism and monogamy may explain reduced heterozygosis and changes in male sexual behavior

Presentaciones en congresos:

**Martínez Villar M**., M. Germil, C. Pavón-Peláez, I.H. Tomasco, T. Bilde, S. Toft, M.J. Albo. 2022. Female emancipation from male sensory exploitation and the subsequent loss of the nuptial gift in spiders. International Society for Behavioral Ecology Congress. Stockholm, Sweden

**Martínez Villar M.**, C. Pavón-Peláez, M. Germil, S. Toft, T. Bilde, I.H. Tomasco & M.J. Albo. 2021. Evolution of nuptial gifts and females' preferences in the spider genus *Trechaleoides* (Trechaleidae). Evolution Virtual Meeting.

**Martínez Villar M.**, C. Pavón-Peláez, M. Germil, S. Toft, T. Bilde, I.H. Tomasco & M.J. Albo. 2021. Female emancipation from male sensory exploitation and the loss of the nuptial gift in spiders. Animal Behavior Society Virtual Meeting

#### Agradecimientos

A Mariana Germil y Camila Pavón-Peláez que estuvieron a mi lado durante casi todo este viaje. Hiciera calor o frío, llueva o truene, siempre estuvieron ahí en el campo como en en el laboratorio desde lo profesional y lo humano, ayudando en lo que fuera con la mejor disposición y buena onda.

A mis tutoras Maria Jose Albo, Ivanna Tomasco y Trine Bilde por el apoyo en cada etapa, por su dedicación y por todas las enseñanzas que me brindaron.

A Søren Toft y Jesper Bechsgaard por su invaluable ayuda, consejos, comentarios y enseñanzas durante la elaboración de esta tesis.

A los miembros del tribunal, a la Dra. Laura Montes de Oca, al Dr. Andrés Parada y al Dr. Benjamin M. Sadd por aceptar ser parte y aportar sus comentarios y sugerencias.

Al Instituto de Investigaciones Biológicas Clemente Estable por brindarme el espacio para mantener a los individuos y hacer los experimentos de comportamiento.

Al laboratorio de Evolución de Facultad de Ciencias por darme el espacio y equipamiento donde poder realizar las extracciones de ARN y análisis moleculares.

Al Departamento de Biología de la Universidad de Aarhus, Dinamarca, por recibirme y hacerme sentir como en casa durante mi estadía.

A Hernán Juan por su ayuda en mis comienzos en la bioinformática y a Facundo Giorello por su ayuda en la finalización de algunos análisis bioinformáticos.

A mi madre y amigos por siempre apoyarme, escucharme, aconsejarme y creer en mi durante toda esta difícil etapa.

Y a todas las agencias que financiaron esta tesis.

### Index

Resumen general:
General summary:
General introduction:
References
Chapter 1 20
Resumen
Abstract
Introduction
Methods
Female choice for nuptial gifts and male mating effort24
Nuptial gift as a shield: female aggression and sexual cannibalism25
Statistical analyses
Results
Female choice for nuptial gifts and male mating effort25
Nuptial gift as a shield: female aggression and sexual cannibalism
Discussion
References
Chapter 2
Resumen
Abstract
Introduction
Materials and Methods
Biological model species
Spider maintenance and experimental design
Results
Presence and function of nuptial gifts in conspecific trials
Heterospecific test of female preference and cannibalism
Discussion
References
Chapter 3
Resumen
Abstract
Introduction
Methods
Spider collections and RNA extractions62

Assembly, assessment of completeness, specific assignment and phylogeny reconstru	uction
	62
Individual heterozygosity and population-species mean heterozygosity	63
Evaluation of selective regimens and genes under positive selection	63
Cross-species differential expression analysis	64
Results	64
Individual heterozygosity and population-species mean heterozygosity	64
Evaluation of selective regimens and genes under positive selection	65
Cross-species differential expression analysis	65
Discussion	65
References	73
Final conclusions and perspectives	77
References	78

"I wish there was a way to know you're in the good old days, before you've actually left them."

Andy Bernard

### **Resumen general:**

La elección femenina es una fuerza poderosa que impulsa la evolución de los rasgos sexuales secundarios conspicuos de los machos. En particular, las preferencias preexistentes de las hembras son relevantes para explicar el origen de varios rasgos sexuales elaborados, como los regalos nupciales. Los regalos nupciales prevalecen en insectos y arañas, y generalmente consisten en comida que los machos ofrecen a las hembras durante el cortejo y/o el apareamiento. Según la hipótesis de la explotación sensorial, la preferencia de las hembras es ancestral al rasgo sexual y, por lo tanto, al ofrecer regalos, los machos explotan la motivación de búsqueda de alimento preexistente de las hembras. Como tal, los machos obtienen ventajas reproductivas al mejorar su acceso al apareamiento y su duración en comparación con los machos que no ofrecen regalos nupciales. Además, los regalos nupciales también pueden funcionar como un escudo contra el canibalismo sexual protegiendo a los machos de las hembras agresivas. Asimismo, las hembras se benefician al recibir regalos alimentarios ya que pueden obtener nutrientes que mejoran su supervivencia y fecundidad. Se ha sugerido que los regalos nupciales son un rasgo ancestral en la familia de arañas Trechaleidae, en la que se reporta que casi la mitad de los géneros tienen machos que producen y ofrecen paquetes envueltos en seda a las hembras. Por lo tanto, se espera que todas las hembras de esta familia tengan preferencia por los regalos nupciales. En esta familia, los machos pueden envolver en seda una presa (regalo nutritivo) pero también ítems no comestibles (regalos simbólicos). Para las hembras recibir regalos simbólicos va en contra de su interés y podría seleccionarlas para cambiar su preferencia por el regalo nupcial, relajando las presiones selectivas y produciendo la consecuente pérdida del rasgo sexual.

En este trabajo, utilizando un enfoque comportamental y molecular, examinamos el comportamiento reproductivo y las diferencias genéticas de dos especies de arañas donadoras de regalos nupciales del género *Trechaleoides*. Este género es muy particular ya que está compuesto por solo dos especies que difieren drásticamente en su comportamiento reproductivo. Estudios previos indican que los machos de *T. keyserlingi* ofrecen regalos tanto nutritivos como simbólicos a las hembras, mientras que se ha sugerido la ausencia de regalo nupcial y la presencia de canibalismo sexual en su especie hermana *T. biocellata*.

En el primer capítulo, examinamos las preferencias de las hembras por los regalos nupciales entre la primer y segunda cópula en la especie *T. keyserlingi*. Encontramos que, aunque los machos ofrecieron regalos nupciales a las hembras, este no confirió a los machos ventajas reproductivas (más y más largas cópulas) o de supervivencia (protección contra el canibalismo sexual). En el segundo capítulo, estudiamos las preferencias de las hembras y la función de los regalos nupciales como esfuerzo de cópula y/o protección en ambas especies utilizando un enfoque de múltiples cópulas. Mostramos que los machos de *T. biocellata* no producen regalos nupciales y confirmamos la ausencia de ventajas reproductivas o de supervivencia para los machos de *T. keyserlingi*. Las hembras de *T. biocellata* demostraron ser muy agresivas, a menudo canibalizando a los machos antes de la cópula. Exploramos si el regalo nupcial alteraba la tasa de canibalismo sexual al exponer a los machos de *T. keyserlingi* que ofrecían regalos a las hembras de *T. biocellata*.

Encontramos que las hembras tampoco tienen preferencias por los regalos y que los regalos no funcionan como un escudo anti canibalismo que protege a los machos. En el tercer capítulo, utilizando transcriptomas, estudiamos las consecuencias del canibalismo sexual comparando los niveles de diversidad genética y la eficacia de selección en ambas arañas. Como era de esperar para una especie altamente caníbal, *T. biocellata* tiene valores más bajos de heterocigosidad y proporciones más altas de sustituciones no sinónimas y sinónimas (dN/dS) en comparación con *T. keyserlingi*, lo que indica una relajación de la selección purificadora en la primera especie.

Los resultados del primer y segundo capítulo indican la ausencia de preferencias de las hembras por los regalos nupciales. Teniendo en cuenta que el rasgo evolucionó a través de la explotación sensorial en una especie ancestral de la familia Trechaleidae, sugerimos una pérdida de las preferencias de las hembras por los regalos nupciales en el ancestro de ambas especies. Esto significa que las hembras han desarrollado resistencia hacia el rasgo sexual del macho emancipándose de la explotación. Además, argumentamos que el cambio en las preferencias de las hembras condujo a la posterior pérdida de la función reproductiva del regalo en *T. keyserlingi* y a la pérdida completa del rasgo sexual precopulatorio ha disminuido la variación genética en *T. biocellata*, reduciendo el tamaño efectivo de la población y la efectividad de la selección purificadora. La baja heterocigosidad encontrada en esta especie

## **General summary:**

Female choice is a powerful force that drives the evolution of conspicuous male secondary sexual traits. Particularly, females' pre-existing preferences are relevant to explain the origin of several elaborated sexual traits, including nuptial gifts. Nuptial gifts are prevalent in insects and spiders, and usually consist of food offered by males to females during courtship and/or mating. Under the sensory exploitation hypothesis, female preference is ancestral to nuptial gift-giving, by offering gifts males exploit females' pre-existing foraging motivation in offering gifts. As such, males gain reproductive advantages by enhancing their mating access and its durations compared to males that do not offer nuptial gifts. In addition, nuptial gifts can also function as a shield against sexual cannibalism protecting males from aggressive females. Females can benefit from receiving food gifts since they can obtain nutrients that improve their survival and fecundity. Nuptial gifts have been suggested to be an ancestral trait in the spider family Trechaleidae, in which almost half of the genera are reported to have males that produce and offer silk wrapped packages to females. Therefore, under the sensory exploitation hypothesis, all Trechaleidae females are expected to have the preference for the nuptial gifts. In this family, males can wrap in silk a prey (nutritive gifts) but also inedible items (worthless gifts). For females, receiving worthless gifts is against their interests and selection could favor females to change their preference for nuptial gifts, relaxing the selective pressures and producing the subsequent loss of the sexual trait.

In this work, using a behavioral and molecular approach, we examined the reproductive behavior and the genetic differences of two gift-giving spider species of the genus *Trechaleoides*. This genus is very particular as it is composed of only two species that differ drastically in their reproductive behavior. Previous studies indicated that *T. keyserlingi* males offer both, nutritive and worthless gifts to females, while it has been suggested that nuptial gift-giving is absent and sexual cannibalism present in *T. biocellata*.

In the first chapter, we examined females' preferences for nuptial gifts between the first and second mating in the species T. keyserlingi. We found that, although the males offered nuptial gifts to females, nuptial gift provisioning does not confer male reproductive (more and longer matings) or survival (protection against sexual cannibalism) advantages. In the second chapter, we studied females' preferences and the function of nuptial gifts as mating effort and/or protection in both species using a multi mating approach. We showed that T. biocellata males do not produce nuptial gifts and we confirmed the absence of reproductive or survival advantages for T. keyserlingi males. Females of T. biocellata were very aggressive often cannibalizing males before mating. We explored whether the nuptial gift altered the probability of sexual cannibalism by exposing T. keyserlingi males offering gifts to T. biocellata females. We found that females neither have preferences for the gifts and that gifts do not function as a protective anti-cannibalism shield for males. In the third chapter, using transcriptomes, we studied the consequences of the sexual cannibalism by comparing the levels of genetic diversity and the efficacy of selection of both spiders. As expected for a highly cannibalistic species, T. biocellata has lower values of heterozygosity and higher proportions of non-synonymous and synonymous

substitutions (dN/dS) compared to *T. keyserlingi*, indicating a relaxation of the purifying selection in the former species.

The results from the first and second chapter indicate the absence of females' preferences for nuptial gifts. Considering that the trait likely evolved via sensory exploitation in an ancestral species of the family Trechaleidae, we suggest a loss of females' preferences for nuptial gifts in the ancestor of the genus *Trechaleoides*. This means that females have evolved resistance towards the male's sexual trait emancipating from exploitation. We additionally argue that this has led to the loss of the gift reproductive function in *T. keyserlingi* and to the complete loss of the sexual trait in *T. biocellata*. The results from the third chapter suggest that pre-mating sexual cannibalism has erode genetic variation in *T. biocellata*, reducing the effective population size and the effectiveness of the purifying selection. The low heterozygosity in this species could be an approximation to understand why the males do not produce nuptial gifts, a possible explanation to the absence of the sexual trait in this species.

## **General introduction:**

Sexual selection is the process defined by Darwin (1871) to explain the origin and development of male's extravagant ornaments and displays. He defined two main precopulatory mechanisms to explain the evolution of these sexual traits: 1) mate competition, in which individuals of the same sex (usually males) compete to access matings; 2) female choice, as the mechanism in which females choose partners (Andersson 1994). Later, Parker et al., (1972) and Eberhard (1996) defined subsequent mechanisms occurring during and/or after mating, known as post-copulatory sexual selection (i.e., sperm competition and cryptic female choice). Today, it is well known that pre- and post-copulatory mechanisms can affect the sexual traits either acting on the same or opposite direction (Jennions & Petrie, 2000; Danielsson, 2001; Supriya et al., 2018; Gasparini et al., 2019). Among these mechanisms, female choice and the existence of female pre- existing preferences are largely relevant to explain the origin of several elaborated sexual traits (Mead & Arnold, 2004; Chaine & Lyon, 2008). The sensory exploitation hypothesis establishes that female preference is ancestral to the sexual trait and it exists also in species in which the trait is absent (Basolo, 1990). But, the particular relationship between the preference and the trait can change over evolutionary time (Lande, 1981; Iwasa et al., 1991). This is possible when the sexual trait is modified by males and result in changes in females' preferences (Wiens, 2001), or when ecological conditions lead females to be less selective for males' trait (i.e. predation risk or parasite detection) (Atwell & Wagner, 2014). Either situation would eventually weaken sexual selection favoring males to produce less elaborate sexual traits (Tinghitella & Zuk, 2009). Female preferences for sexual traits evolving under sensory exploitation are known to be subject of modifications (Bradbury & Vehrencamp, 2000). Males exploit female preexisting sensory biases (Basolo, 1990; Morris et al., 2005; Chaine & Lyon, 2008; Lewis & South, 2012). Because females cannot resist the exploitation, this can result in suboptimal mating rates and thus, females may counter evolve by for instance changing their preferences (Wiens, 2001).

In nuptial gift-giving mating systems, males use food gifts exploiting females foraging motivation (Sakaluk, 2000; Bilde et al., 2007; Albo et al., 2017b). Nuptial gifts are male sexual traits occurring in a wide range of invertebrates, which have evolved independently under diverse forms (Vahed, 1998, 2007; Gwynne, 2008; Lewis & South, 2012; Lewis et al., 2014). They have been defined as materials (beyond gametes) provisioned by males and offered to females (Lewis & South, 2012). Males can offer the nuptial gift before, during or after mating, and its impact can be positive, neutral or negative for either sex (Lewis & South, 2012). Offering nuptial gifts can benefit males because it can function in mate attraction, prolong mating duration, increase the amount of sperm transferred and in some cases improve the quality of the offspring (Lewis & South, 2012). In predator species, the gift can also function as a protection against female's attacks and cannibalism (Kessel, 1955; Bristowe, 1958; Toft & Albo, 2016). For polyandrous females, receiving multiple food gifts increase their fecundity and survival (Vahed, 1998, 2007; Lewis & South, 2012; Toft & Albo, 2015; Pandulli-Alonso et al., 2017). Additionally, females can obtain indirect benefits as assessment of gifts may be a way that females choose genetically high-quality males to fertilize eggs, resulting in better offspring (Jennions &

Petrie, 2000). However, the very interesting cases are those in which the nuptial gifts provide no benefits to females, because such scenario can affect and change female preferences for the sexual trait. For instance, Will & Sakaluk (1994) found that females from *Gryllodes sigillatus* crickets do not increase the number of offspring or mass of eggs when consuming the male gift (i.e., spermatophylax). Likewise, they suggested that the gift is a "sham" as it may only function to lure the female while males complete the sperm transfer.

An analogous case has been reported for gift-giving spiders. Wrapped nuptial gifts are common in the spider families Trechaleidae and Pisauridae (Bristowe, 1958; Costa-Schmidt et al., 2008). Males may wrap in silk a prey item (nutritive gifts), but they can also wrap prey leftovers, small plant parts (worthless gifts) or even produce empty silk gifts (Bristowe, 1958; Albo et al., 2011; Martínez-Villar et al., 2020). It has been shown that nutritive gifts represent an important source of nutrients that improve females' fecundity and survival (Toft & Albo, 2015; Pandulli-Alonso et al., 2017). Because of the negative impact on females' fitness success, the evolution of worthless gifts is a key process that can change females' behavior and even their preferences for the nuptial gifts. For instance, in the Palearctic spider Pisaura mirabilis (Pisauridae), females prefer to mate with males offering nutritive gifts and penalize those offering worthless gifts by reducing the mating duration (Albo et al., 2011). In the Neotropical spider Paratrechalea ornata (Trechaleidae), females are indifferent to the gift content and equally mate with males offering both gift types (Albo et al., 2011; Albo & Peretti, 2015). In fact, females cryptically bias the paternity towards large males but not in relation to the gift content (Albo et al., in revision.). A further step would be the total emancipation from the exploitation (Bradbury & Vehrencamp, 2000), in which females evolve resistance or indifference towards the nuptial gift. The loss of female's preference for the trait would eventually lead to changes in the males' behavior, as the sexual trait would become costly and no longer benefit them.

Contrasting differences in relation to the nuptial gift and sexes reproductive strategies were suggested in the spider genus *Trechaleoides* (Trechaleidae), which includes only two species (Carico, 2005). On one hand, males from *T. keyserlingi* were reported to produce and offer to females both nutritive and worthless gifts (Trillo & Albo, 2019). In contrast, preliminary observations suggested the absence of the sexual trait (Albo *et al.*, 2009) and the presence of pre-mating sexual cannibalism in the species *T. biocellata* (Martinez Villar, personal observations). These differences between the two species are remarkable when considering that wrapped nuptial gifts have been suggested ancestral in Trechaleidae (Albo *et al.*, 2017a) and seven out of 17 genera are already reported having this sexual trait (Costa-Schmidt *et al.*, 2008; Da Silva & Lise, 2009; Lapinski & Tschapka, 2009; Da Silva & Lapinski, 2012; Trillo & Albo, 2019; Martínez-Villar *et al.*, 2020; Rengifo-Gutiérrez *et al.*, 2021, A. Santos and D. Poy personal communication). Therefore, under the sensory exploitation hypothesis the absence of nuptial gift-giving in one species may indicate sexual trait loss and the absence of female preferences for it.

The main goal of this thesis was to study the reproductive strategies and the genetic differences in the two species of the genus *Trechaleoides*. Precisely, we studied the nuptial gift function and females' preferences for nuptial gifts and finally, the genetic variation, effectiveness of purifying selection and differentially expressed genes in both

species. In the first chapter, we studied the male investment in nuptial gift and females' preferences for them in the species *T. keyserlingi* in a first and second mating. In the second chapter, we conducted behavioral experiments to assess the preference for nuptial gifts, the gift function as male mating effort and/or male protection in both species' trough a multi mating approach. Finally, because we verified the presence of pre-mating sexual cannibalism in *T. biocellata*, in the third chapter, we assessed the genetic signatures resulting from the high levels of sexual cannibalism. Specific, using transcriptomes, we evaluated levels of heterozygosity and the efficacy of the purifying selection in both species.

#### REFERENCES

- Albo, M.J., Bidegaray-Batista, L., Bechsgaard, J., Da Silva, E.L.C., Bilde, T. & Pérez-Miles, F. 2017a. Molecular phylogenetic analyses show that Trechaleidae and Lycosidae are sister groups. *Arachnology* 17: 169–176.
- Albo, M.J., Costa-Schmidt, L.E. & Costa, F.G. 2009. To feed or to wrap? Female silk cues elicit male nuptial gift construction in a semiaquatic trechaleid spider. J. Zool. 277: 284–290.
- Albo, M.J., Macías-Hernández, N., Bilde, T. & Toft, S. 2017b. Mutual benefit from exploitation of female foraging motivation may account for the early evolution of gifts in spiders. *Anim. Behav.* 129: 9–14.
- Albo, M.J., Pavón-pelaez, C., Martínez Villar, M., Buzzatto, M.. & Tomasco, I. Stressful environments favor deceptive alternative mating tactics to become dominant (in revision). *BMC Biol.*
- Albo, M.J. & Peretti, A. V. 2015. Worthless and nutritive nuptial gifts: Mating duration, sperm stored and potential female decisions in spiders. *PLoS One* **10**: 1–15.
- Albo, M.J., Winther, G., Tuni, C., Toft, S. & Bilde, T. 2011. Worthless donations: Male deception and female counter play in a nuptial gift-giving spider. *BMC Evol. Biol.* **11**: 1–8.
- Atwell, A. & Wagner, W.E. 2014. Female mate choice plasticity is affected by the interaction between male density and female age in a field cricket. *Anim. Behav.* 98: 177–183. Elsevier Ltd.
- Basolo, A.L. 1990. Female preference predates the evolution of the sword in swordtail fish. *Science (80-.)*. **250**: 808–810.
- Bilde, T., Tuni, C., Elsayed, R., Pekar, S. & Toft, S. 2007. Nuptial gifts of male spiders: sensory exploitation of the female's maternal care instinct or foraging motivation? *Anim. Behav.* 73: 267– 273.
- Bradbury, J.W. & Vehrencamp, S.L. 2000. Economic models of animal coomunication. *Anim. Behav.* **59**: 259–268.
- Bristowe, W.S. 1958. The World of Spiders. London: Collins.
- Carico, J.E. 2005. Descriptions of two new spider genera of Trechaleidae (Araneae, Lycosoidea) from South America. *J. Arachnol.* **33**: 797–812.
- Chaine, A.S. & Lyon, B.E. 2008. Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. *Science (80-.)*. 319: 459–462.
- Costa-Schmidt, L.E., Carico, J.E. & De Araújo, A.M. 2008. Nuptial gifts and sexual behavior in two species of spider (Araneae, Trechaleidae, *Paratrechalea*). *Naturwissenschaften* **95**: 731–739.
- Da Silva, E.L.C. & Lapinski, W. 2012. A new species of *Trechalea* thorell, 1869 (Araneae: Lycosoidea: Trechaleidae: Trechaleinae) from Costa Rica, with notes on its natural history and ecology. *Zootaxa* 64: 58–64.

- Da Silva, E.L.C. & Lise, A.A. 2009. New record of nuptial gift observed in *Trechalea amazonica*. (Araneae, Lycosoidea, Trechaleidae). *Rev. Peru. Biol.* **16**: 119–120.
- Danielsson, I. 2001. Antagonistic pre- and post-copulatory sexual selection on male body size in a water strider (*Gerris lacustris*). Proc. R. Soc. B Biol. Sci. 268: 77–81.
- Darwin, C. 1871. The descent of man, and selection in relation to sex. Princeton.
- Eberhard, W.G. 1996. *Female control: sexual selection by cryptic female choice*. Princeton, NJ: Princeton University Press.
- Gasparini, C., Devigili, A. & Pilastro, A. 2019. Sexual selection and ageing: Interplay between pre- and post-copulatory traits senescence in the guppy. *Proc. R. Soc. B Biol. Sci.* **286**: 20182873.
- Gwynne, D. 2008. Sexual conflict over nuptial gifts in insects. Annu. Rev. Entomol. 53: 83-101.
- Iwasa, Y., Pomiankowski, A. & Nee, S. 1991. The evolution of costly mate preferences. II. The "handicap" principle. *Evolution (N. Y).* **45**: 1431–1442.
- Jennions, M.D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic bene ts. *Biol. Rev. Camb. Philos. Soc.* **75**: 21–64.
- Kessel, E.L. 1955. The mating activities of balloon flies. Syst. Zool. 4: 97-104.
- Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proc. NatL Acad. Sci.* **78**: 3721–3725.
- Lapinski, W. & Tschapka, M. 2009. Erstnachweis von Brautgeschenken bei *Trechalea sp*. (Trechaleidae, Araneae) in Costa Rica. *Arachne* 14: 4–13.
- Lewis, S. & South, A. 2012. The evolution of animal nuptial gifts. In: *Advances in the Study of Behavior*, pp. 53–97.
- Lewis, S., Vahed, K., Koene, J.M., Engqvist, L., Bussière, L.F., Perry, J.C., *et al.* 2014. Emerging issues in the evolution of animal nuptial gifts. *Biol. Lett.* **10**: 20140336.
- Martínez-Villar, M., Germil, M., Pavon-Pelaz, C., Costa-Schmidt, L.E. & Albo, M.J. 2020. Empty nuptial gifts: A further step in the evolution of deception in spiders? J. Arachnol. 48: 214–217.
- Mead, L.S. & Arnold, S.J. 2004. Quantitative genetic models of sexual selection. *Trends Ecol. Evol.* **19**: 264–271.
- Morris, M.R., Moretz, J.A., Farley, K. & Nicoletto, P. 2005. The role of sexual selection in the loss of sexually selected traits in the swordtail fish *Xiphophorus continens*. *Anim. Behav.* **69**: 1415–1424.
- Pandulli-Alonso, I., Quaglia, A. & Albo, M.J. 2017. Females of a gift-giving spider do not trade sex for food gifts: A consequence of male deception? *BMC Evol. Biol.* 17: 1–8.
- Parker, G.A., Baker, R.R. & Smith, V.G.F. 1972. The origin and evolution of gamete dimorphism and the male-female phenomenon. J. Theor. Biol. 9: 529–553.
- Rengifo-Gutiérrez, L., Albo, M.J. & Santa, L.D. 2021. The unknown *Enna* (Araneae: Trechaleidae), new species and first record of wrapped nuptial gifts in the genus. J. Arachnol. 48: 242–248.
- Sakaluk, S.K. 2000. Sensory explotation as an evolutionary origin to nuptial food gifts in insects. *Proc. R. Soc. London. Ser. B Biol. Sci.* 267: 339–343.
- Supriya, K., Prince, D.T. & Rowe, M. 2018. Positive correlations between pre- and post-copulatory sexual traits in warblers. *J. Avian Biol.* **49**: jav-01694.
- Tinghitella, R.M. & Zuk, M. 2009. Asymmetric mating preferences accommodated the rapid evolutionary loss of a sexual signal. *Evolution (N. Y).* **63**: 2087–2098.
- Toft, S. & Albo, M.J. 2015. Optimal numbers of matings: The conditional balance between benefits and costs of mating for females of a nuptial gift-giving spider. *J. Evol. Biol.* **28**: 457–467.
- Toft, S. & Albo, M.J. 2016. The shield effect: Nuptial gifts protect males against pre-copulatory sexual

cannibalism. Biol. Lett. 12: 20151082.

- Trillo, M.C. & Albo, M.J. 2019. Another one bites the gift: sexual behaviour in a *Trechaleoides* species. *Arachnology* **18**: 250–252.
- Vahed, K. 2007. All that glisters is not gold: Sensory bias, sexual conflict and nuptial feeding in insects and spiders. *Ethology* **113**: 105–127.
- Vahed, K. 1998. The function of nuptial feeding in insects: A review of empirical studies. *Biol. Rev.* **73**: 43–78.
- Wiens, J.J. 2001. Widespread loss of sexually selected traits: How the peacock lost its spots. *Trends Ecol. Evol.* **16**: 517–523.
- Will, M.W. & Sakaluk, S.K. 1994. Courtship feeding in decorated crickets: Is the spermatophylax a sham? *Anim. Behav.* **48**: 1309–1315.

## **Chapter 1**

## Ineffective nuptial gifts suggest female emancipation from sensory exploitation

Martínez-Villar M, M.C. Trillo & M.J. Albo. 2021. Ineffective nuptial gifts suggest female emancipation from sensory exploitation. *Behav. Ecol. Sociobiol.* **75**: 1-9.

#### Resumen

La hipótesis de explotación sensorial en donde los machos explotan el sesgo gustativo preexistente de las hembras se ha propuesto para explicar el origen de los regalos nupciales en insectos y arañas. Este rasgo sexual puede haber sido beneficioso para ambos sexos, brindando ventajas de cópula y supervivencia a los machos y proporcionando recursos nutricionales para las hembras. Sin embargo, la evolución de los regalos simbólicos engañosos va en contra de los intereses de las hembras y puede desencadenar un cambio coevolutivo en las preferencias de las mismas. En este trabajo, evaluamos las preferencias de las hembras por los regalos nupciales y la función adaptativa del regalo en la araña Trechaleoides keyserlingi. Este género pertenece a la familia neotropical poco estudiada Trechaleidae en la que la presencia de regalo nupcial está extendida. Está compuesto por solo dos especies, y el regalo parece estar ausente en la especie hermana, lo que crea un escenario relevante para comprender procesos coevolutivos. En el laboratorio, encontramos que, aunque los machos invirtieron más en regalos nupciales cuando se encontraban con hembras copuladas en comparación con las no copuladas, éstos tenían acceso y duración de cópula similares a los machos que carecían de regalo. Asimismo, encontramos ausencia de elección de las hembras entre los machos que ofrecen regalos nutritivos y simbólicos. Pocas hembras fueron agresivas y canibalizaron a los machos, y no encontramos evidencia de que el regalo protegiera a los machos del canibalismo. En el campo, el 50% de los regalos fueron simbólicos. Esto es congruente con los hallazgos de laboratorio donde los machos que ofrecen regalos simbólicos parecen atraer mejor a las hembras, lo que discutimos en el contexto de la explotación del sesgo gustativo de las hembras. Por lo tanto, proponemos que las hembras pudieron haber desarrollado indiferencia por el regalo y que ofrecer regalos en esta especie representaría un comportamiento remanente que actualmente no es funcional.

**Palabras clave:** Preferencia de las hembras, inversión en seda del macho, regalos nupciales, rasgo sexual secundario, selección sexual

#### Abstract

Male sensory exploitation of female gustatory pre-existing bias has been proposed for the origin of nuptial gifts in insects and spiders. This sexual trait may have been beneficial to both sexes, giving mating and survival advantages to males and providing nutritional resources for females. However, the evolution of deceptive worthless gifts is against females' interests and may trigger a co-evolutionary change in females' preferences. We evaluated females' preferences for nuptial gifts and the adaptive function of the gift in the spider Trechaleoides keyserlingi. The genus belongs to the understudied Neotropical family Trechaleidae in which nuptial gifts are widespread. It is composed of only two species, and the gift seems to be absent in the sister species creating a relevant scenario for understanding co-evolutionary processes. In the laboratory, we found that although males invested more in nuptial gifts when encountering mated females compared to unmated, they had similar mating access and duration to males lacking a gift. We also found an absence of female choice between males offering nutritive and worthless gifts. Few females were aggressive and cannibalized males, and we did not find evidence that the gift protected males from cannibalism. In the field, 50% of the gifts were worthless items. This is congruent with the laboratory findings where males offering worthless gifts seem to better attract females, which we discuss in the context of exploitation of female gustatory bias. We therefore propose females may have evolved indifference for the gift and that gift-giving in this species represent a currently non-functional remnant of a behaviour.

Key words: female preferences, male silk effort, nuptial gifts, secondary sexual traits, sexual selection

#### Introduction

The reason why exaggerated and costly male traits are maintained is intimately related to females' preferences for such traits and the reproductive advantages gained by males in terms of descendants (Darwin 1871). A relevant concept for the origin of female preferences is the Sensory Exploitation Hypothesis, which states that novel male traits are likely to become established when they exploit a pre-existing sensory bias in females (Ryan et al. 1990; Christy 1995). This explanation has been proposed for several sexual traits in diverse taxa (Basolo 1990; Proctor 1991; Christy et al. 2003; Madden and Tanner 2003), including for the origin of nuptial gifts in insects and spiders, which involves males exploiting female gustatory preexisting bias (Sakaluk 2000; Bilde et al. 2007; Albo et al. 2017). For spiders, it is suggested that originally the nuptial gift-giving behaviour results in mutual benefits for both sexes (Albo et al. 2017), as females receive food and males receive access to matings and/or protection from sexual cannibalism. Indeed, nuptial gifts can currently represent additional food resources for females functioning as a paternal investment resource (Thornhill 1976; Gwynne 1984; Simmons and Parker 1989), and this is especially important as their fecundity increases when more food is acquired (Toft and Albo 2016; Pandulli-Alonso et al. 2017). By offering a nuptial gift, males increase their mating effort (Simmons and Gwynne 1991; Wolfner 1997; Heifetz et al. 2001; Sakaluk et al. 2006) by acquiring more and prolonged matings, compared to males lacking a gift (Stålhandske 2001; Prokop and Maxwell 2009; Albo and Costa 2010; Albo et al. 2011, 2014a; Maxwell and Prokop 2018). As females usually become reluctant to mate after the first copulation, males' courtship effort increases when encountering mated females (Albo et al. 2014a). At the same time, spiders are predators and females may be aggressive and cannibalize males during courtship or mating (Elgar et al. 1990; Foellmer and Fairbairn 2004; Fromhage and Schneider 2005). Thus, males can use the nuptial gift as a shield for protection against attacks from aggressive females (Kessel 1955; Bristowe 1958; Toft and Albo 2016).

However, the current function of nuptial gifts may no longer be the same as that when this trait originally evolved. For instance, males can benefit from modifying their investment in the gift and reduce costs associated with its maintenance according to their own attributes in relation to the environment, as suggested for other sexual traits (Piersma and Drent 2003; Cornwallis and Uller 2010). Traces of evolutionary changes in the content of nuptial gifts from genuine nutritive (fresh prey) to deceptive gifts, such as worthless (prey leftovers) and even empty silk packages, have been observed in some genera of the spider family Trechaleidae (Costa-Schmidt et al. 2008; Albo et al. 2009; Da Silva and Lise 2009; Lapinski and Tschapka 2009; Da Silva and Lapinski 2012; Trillo and Albo 2019; Martínez-Villar et al. 2020; Rengifo-Gutiérrez et al. 2021). The diversity of gift types may result in the co-occurrence of different male mating tactics in the population (Endler 1995; Gross 1996; Brockmann 2001; Engqvist and Taborsky 2015), and is evidence of a change in the function of the sexual trait as a source of nutrients. If females' preferences for nuptial gifts (even the modified form) are maintained, males will still maximize their fitness success. For instance, in the trechaleid spider Paratrechalea ornata, males offering either a genuine nutritive or a deceptive worthless gift achieve similar mating access and duration (Albo et al. 2014a). Thus, while the males increase their mating effort, females do not necessarily receive nutrients. If male exploitation by worthless gifts is costly for females, this can lead to suboptimal matings, consequently reducing their fitness (Arnqvist 2006; Mokkonen and Lindstedt 2015; Chapman 2018). It is known that worthless gifts reduced female fecundity in P. ornata in relation to nutritive ones (PandulliAlonso et al. 2017). Yet, the maintenance of worthless gifts is widespread across populations, ranging in frequency from 45 to 96% (MJ Albo unpublished data), suggesting sexual conflict may be occurring (Arnqvist and Rowe 2005).

Alternatively, females can potentially co-evolve, changing their preferences for the gift (Arnqvist 2006; Mokkonen and Lindstedt 2015). Females can evolve resistance to the nuptial gift as a sexual stimulus. This means they can evolve insensitivity or indifference towards the gift resulting in the emancipation from the exploitation and consequently elimination of the sensory bias (Bradbury and Vehrencamp 2000; Arnqvist 2006). Yet, there is limited empirical data supporting this evolutionary scenario (Garcia and Ramirez 2005; Ryan and Cummings 2013). The spider genus Trechaleoides (Trechaleidae) offers an opportunity to study the coevolution of a male sexual trait (the gift) and females' preferences for it. This is because nuptial gift-giving behaviour has been recently described in T. keyserlingi F.O.P.-Cambridge (Trillo and Albo 2019), while its absence has been suggested for T. biocellata Mello-Leitão (Albo 2009). The genus is composed of these two species that are morphologically identical, distinguished only by their genitalia (Carico 2005), and therefore the sexual trait was either lost or never gained in one of the species. The scarce information from laboratory experiments (Trillo and Albo 2019) and field observations (MJA unpublished data) indicates a low frequency of male gift production (silk wrapping of the prey) in T. keyserlingi. Additionally, preliminary data showed that unmated females are highly receptive to mating (80%, N = 15) and most (90%) mated without being offered a nuptial gift (MMV unpublished data). Considering that the sister species lacks the sexual trait, this information creates an intriguing background for studying possible changes in the female preferences and the current function of the nuptial gift in the genus.

Here, we evaluated females' preferences for males based on the nuptial gift presence, content and their own reproductive status (mated or not), and the adaptive role of the nuptial gift as a male mating effort and/or shield against sexual cannibalism in *T. keyserlingi*. First, if females exert choice for nuptial gifts and the gift functions as male mating effort, then we expect an increase in male mating success when offering a gift than when no gift is presented. Further, males' effort in the use of an item and in silk investment should increase when courting mated females. Second, if females favour males offering nutritive over those with worthless gifts, then we expect the former acquiring more and longer matings. Finally, if the gift protects males from aggressive females, then we expect higher survival of males offering gifts than those courting without one.

#### Methods

*Trechaleoides keyserlingi* is a riparian species living associated with streams and rivers, where individuals perch on stones near the edge of the watercourse (Carico 2005). As other trechaleid species, *T. keyserlingi* has crepuscular/nocturnal activity and thus, we always performed the collections in the night. During 2013-2018, we collected a total of 71 immature individuals of this species for laboratory experiments in the locality of Quebrada de los Cuervos ( $32^{\circ}55'39''S$   $54^{\circ}27'25''W$ ) Treinta y Tres, Uruguay. Additionally, we searched for males carrying nuptial gifts and when available, we collected and saved the gifts in Eppendorf tubes for analyses in the laboratory. Individuals were transported to the laboratory and raised individually in plastic jars (8 cm width and 7 cm height) in a warm room averaging 25.0 C° (± 0.31 SE) to accelerate

their development. We fed spiders three times a week with two houseflies (*Musca domestica*) and provided water *ad libitum*. Once they reached sexual maturity, we relocated spiders to an experimental room at 21.0 C° ( $\pm$  0.17 SE) and continued the same feeding regimen. Following previous protocols established for another gift-giving spider from the same family (Klein et al. 2012), we used individuals 20 days after maturity in the experiments. The day before the trials all individuals were fed a housefly to secure satiation and avoid males eating the prey, thus favouring the use of it as a nuptial gift. We performed the experiments in a glass terrarium (20 x 29 x 15 cm) with small pebbles as a substrate and water provided in a Petri dish. We placed the females individually in the terraria 24hs before the trials, during which time they could deposit silk functioning as sexual stimuli for males to start courtship (Lang 1996; Albo et al. 2009). On the day of the trial each male was added to the terrarium with a female. A living housefly was gently provided with tweezers when he started to vibrate and court (except in the *Worthless Gifts* group, where we used inedible items; see our two experiments below). All trials last maximum of four hours.

We recorded the following behaviours: occurrence of using a prey or an inedible item for courting females, and whether the prey or item was wrapped in silk (silk investment). We measured the number and duration of wrapping bouts to measure the investment in preparing the gift, and calculated total silk wrapping duration (min) as the sum of all wrapping bouts durations. We additionally measured mating success as the occurrence and duration of mating. We recorded the number and duration of male pedipalp insertions, and we calculated total mating duration (min) as the sum of all insertion durations (= duration of sperm transfer). Finally, we noted female aggression towards males by recording occurrence of attacks during courtship and sexual cannibalism. We performed two experiments under laboratory conditions and examined field data.

#### Female choice for nuptial gifts and male mating effort

#### Unmated and Mated Females

In this first experiment, we examined the male mating success during a first and second encounter with two different females. We evaluated the nuptial gift-giving behaviour (use of a prey item and silk investment) by males when mating with unmated females (N = 26). Then we recorded the mating success of a second group of males, which courted the same females used during the unmated female settings (mated females). We measured the same proxies during both trial groups. Unmated females mated in 100% of the trials. Only 19 out of the 26 initial females survived for the second trial (for unknown reasons) while the interval between the first and the second trial lasted three days on average.

#### Nutritive and Worthless Gifts

For the second experiment, we combined field and laboratory data and examined whether males are able to grab and use inedible items and offer those worthless gifts to females. We also measured the mating success of males offering such gifts comparing to males offering nutritive gifts. In the field, we collected 13 nuptial gifts carried by males in their chelicera. We weighed and dissected them under a stereomicroscope to register the content, classifying each one as nutritive (fresh prey) or worthless gifts (prey leftovers, plant parts or other non-nutritive items) (Albo et al. 2011). In the laboratory, we performed controlled behavioural experiments in two groups. In the *Nutritive Gifts* (N = 13), males with a captured housefly were exposed to females. In the *Worthless Gifts* (N = 13), males had the opportunity to grab and use an inedible item consisting either of a cotton ball, an exoskeleton from a *Tenebrio molitor* larvae or a mud ball. All three items were placed randomly along the terrarium (two of each type). For these experiments, males and females were used only once.

#### Nuptial gift as a shield: female aggression and sexual cannibalism

For each of the two experiments described above, we analysed the occurrence of female attacks and sexual cannibalism in relation to the presence/absence of nuptial gift and its content (nutritive/worthless). Because of low frequency of aggressive and cannibalistic behaviour, we pooled the data from the two experiments.

#### Statistical analyses

We performed the statistical analyses using free software R (R Team Core 2019). We used Chi-square and Fisher exact probability (when low sample size, < 10) tests to analyse differences in the frequencies of the following behaviours: items used by males, silk wrapping, mating access (within the *Mated Females* group), female aggression and sexual cannibalism. For the remaining variables, in the first experiment, we used Generalized Linear Mixed Models (GLMM) for examining the effect of prey presence/absence and group (fixed effects) on mating access, mating duration and number of pedipalp insertions with Binomial (b), Gaussian (g) and Poisson (p) distribution respectively, including female identity as a random effect. We also used GLMM with a Gamma distribution to examine the effect of group (*Unmated and Mated Females*) on silk wrapping duration, while a Poisson distribution was used for the number of silks wrapping bouts. For the second experiment, we performed Generalized Linear Models comparing mating access (GLM(b)), mating duration (GLM(g)) and number of pedipalp insertions (GLM(p)) between groups (*Nutritive and Worthless Gifts*).

#### Results

#### Female choice for nuptial gifts and male mating effort

#### Unmated and Mated Females

Males were highly invested in producing a nuptial gift when encountering mated females. We found that they more often used the prey and wrapped it in silk when exposed to mated females

than when they encountered unmated females. However, silk wrapping duration and number of silks wrapping bouts did not differ between groups (Table 1).

Only 47% of the males succeeded in mating with mated females. Considering the mating cases in the *Unmated and Mated Females* groups, mating occurred in similar frequency for males offering a nuptial gift or not ( $z_{32} = 1.30$ , p = 0.19; Fig. 1a). In fact, when comparing mating access in the *Mated Females* group, all males without a gift succeed in mating, while 53% did not succeed when offering a gift ( $X^2_2 = 5.63$ , p = 0.01). Mating duration and number of insertions were similar between groups whether the male offered a gift or not (Fig. 1b, c; Table 2). This suggests that males invest differentially in the nuptial gift when encountering mated females but this does not increase their mating success.

#### Nutritive and Worthless Gifts

In the field, we found and collected 13 males carrying wrapped nuptial gifts. Forty-six percent of the gifts contained only inedible items wrapped in silk such as prey leftovers, small plant parts and even mud. Worthless gifts averaged 8.0 mg ( $\pm$  2.0 SE) and nutritive gifts 22.0 mg ( $\pm$  6.0 SE).

In the laboratory, we found that in the *Worthless Gifts* group, half of the males grabbed an inedible item (always the cotton ball) but none of them wrapped it in silk (Table 3). In this group, except in one case, females accepted all the males offering a gift, whereas none of the males without a gift succeeded in mating. In the *Nutritive Gifts* group, about half of the males (N = 7) wrapped the prey item in silk; few males succeeded in mating, though all of them offered a gift (wrapped or unwrapped) (Table 3). We did not find differences between groups in mating duration or number of pedipalp insertions. This suggests that males increase their effort by offering worthless gifts when no prey is available and so they may increase their access to mate.

#### Nuptial gift as a shield: female aggression and sexual cannibalism

In the first experiment (*Unmated and Mated Females*), few females (N = 7) aggressively attacked males during courtship (mostly during the second encounter) but this was independent either of the gift presence or group (Fisher: p = 0.14, Fig. 1d). In this case, none of the attacks resulted in sexual cannibalism. Similarly, in the second experiment (*Nutritive and Worthless Gifts*), whether the males offered a nutritive, worthless or no gift, few females were aggressive and attacked males during courtship (Table 3). In the *Nutritive Gifts* group, the four attacked males were offering a gift and females cannibalized two of them. While in the *Worthless Gifts* group, three females attacked males offering a gift and four attacked males without a gift, one of each being cannibalized (Table 3). Sexual cannibalism occurred mainly during courtship and in one case happened during mating.

Pooling both datasets, female attacks occurred in 17% of the cases (N = 12) where males offered nuptial gifts and 8% in the cases (N = 6) where males offered no gift; while sexual

cannibalism occurred in 19% of males offering a nuptial gift and 6% of males without a gift. Thus, we find no evidence that the nuptial gift protects males from aggressive females.

#### Discussion

We examined female preferences for nuptial gifts and its adaptive function in the Neotropical spider *Trechaleoides keyserlingi*. Our findings indicate an absence of female choice between males offering a nuptial gift over those without a gift, and even between nutritive and worthless gifts, leading us to question the function of the nuptial gift as nutritional resource. Overall, we found no evidence that the gift presence and/or content (but see below) either improved male mating success or prevented pre-mating sexual cannibalism.

We verified the preliminary observations that unmated females readily mate with males without a gift. Besides male effort in courtship, females do not become more selective for the nuptial gift after having mated, and we observed in pilot experiments that this choice does not happen even after multiple matings (MMV personal observations). When encountering mated females, males increased their investment in nuptial gifts, significantly using and wrapping more the prey, than when exposed to unmated females. This discrimination of female reproductive status most probably happened while detecting the pheromones associated with her silk. It is known that spider males can adjust their investment during courtship according to different female attributes and conditions, including their reproductive status (Gaskett 2007). For many taxa, male competition and courtship investment increases when females are already mated as these become highly selective (Kvarnemo and Ahnesjö 1996; Jirotkul 1999). Contrary to the prediction of the male mating effort hypothesis (Vahed 1998), when exposed to mated females, males without a nuptial gift acquired higher mating access compared to those offering a gift, both having similar mating duration (a proxy of sperm transfer). The other possible male advantage for the nuptial gift origin and maintenance in species with aggressive females is sexual cannibalism avoidance (Kessel 1955; Bristowe 1958; Vahed 1998). We found that females showed a trend to become more aggressive after the first mating and that the males invested more in gifts. But, unfortunately due to the low number of females that were aggressive the question on whether the nuptial gift functions as a shield protecting males against sexual cannibalism remains unanswered. In few cases females attacked males and even fewer cannibalized them, thus whether the males carrying a nuptial gift were more attacked than those without a gift cannot be resolved with the data presented here (4 cases of sexual cannibalism). The shield effect of the nuptial gift can be difficult to demonstrate because female aggression and sexual cannibalism are generally low, making robust statistical analyses challenging (Toft and Albo 2016). In any case, we can only argue that there is a low risk for T. keyserlingi males of being attacked and cannibalized during courtship.

An intriguing outcome is the successful matings of males offering a worthless item in relation to those lacking a gift. Thus, while we can report for the first time the donation of inedible items to females and confirm this species also has a deceptive alternative mating tactic (Preston-Mafham 1999; LeBas and Hockham 2005; Albo et al. 2011, 2014b, 2019; Ghislandi et al. 2018), the low sample size limits the discussion. We observed that males preferred to use the cotton balls instead of the exoskeletons or mud. A possible explanation may be that males minimized the costs of silk wrapping using the cotton ball -that resembles a wrapped gift- to

visually attract females (Stålhandske 2002; Albo et al. 2011; Trillo et al. 2014), or exploited its texture that may also emulate the texture of the silk wrapped gift to facilitate the grabbing of the offered item (Andersen et al. 2008). In any case, the potential female preference for this type of item, in addition to the large male silk investment on the prey that we found in the first experiment, may suggest a relevant role of the silk during courtship in this species. Further, this result leads to the idea that males might have evolved the silk wrapping to increase the stimulus and strengthen female attraction (Albo et al. 2014b).

The overall lack of female preferences for the nuptial gift and the difficulty of finding any advantage for males in T. keyserlingi, added to the apparent absence of the trait in the sister species (T. biocellata), suggests that different evolutionary process is occurring in this genus compared to others from the family (Albo et al. 2014b). If the gift evolved via male sensory exploitation of the female gustatory pre-existing bias, the absence of female choice may be the result of her emancipation from the exploitation, as suggested by the theoretical model formulated by Bradbury and Vehrencamp (2000). This was empirically demonstrated for Goodeinae fishes, where males have a tail yellow band that resembles food items (worms) functioning as a stimulus to lure females. In some species, the trait is highly conspicuous and females show reduced feeding responsiveness compared to females from species with the poorly developed trait. Thus, the huge exaggeration of the trait allows females to discriminate between sexual and non-sexual feeding responses, and it is suggested that the sexual trait evolved from a sensory trap to an honest signal (Garcia and Ramirez 2005). In an evolutionary scenario, considering the gift-giving species, males originally offered nutritive gifts and used their silk to amplify the exploitation of female bias on white and/or textured items that resembles some food. Females gained direct benefits and the gift became more frequent as females' preferences for them increase (Arnqvist 2006). By accepting multiple mates and nutritive gifts females increase their fecundity (Arnqvist and Nilsson 2000). But, when mating with males offering worthless gifts, the food benefits received by females decrease in relation to the cost of mating, leading them to suboptimal matings (Toft and Albo 2015). Because individual males can either offer a nutritive or a worthless gift (Pavón-Peláez 2019) and females cannot distinguish the gift content before accepting the mating (Albo et al. 2014a), there is no scope for females to differentiate between phenotypes during courtship (Ryan and Cummings 2013). Thus, changes in gift content carried direct cost to females, and in turn females may have co-evolved (at least partially) insensitivity or indifference for the gift (Arnqvist 2006). Presumably, considering the results from the worthless gift group the emancipation from male exploitation is not fixed. Additionally, it may be possible that females exert a cryptic choice according to the nuptial gift after mating (Eberhard 1996) explaining the maintenance of the sexual trait (Moehring and Boughman 2019).

In summary, here we found that *T. keyserlingi* females seem to not favour males offering nuptial gifts over those lacking one. It has been suggested that the nuptial gift appeared ancestrally in this spider family (Albo et al. 2017). If this is correct, female emancipation from exploitation would result in the loss of the nuptial gift by natural selection or drift (Wiens 2001; Arnqvist 2006). We propose that females in this species probably have shifted their preferences towards other male attributes (e.g. body size and/or condition) as better indicators of mate quality (Zahavi 1974) and that the gift-giving in this species may represent a currently non-functional remnant of a behaviour which is widespread in the family.

**Figure 1**. Data showing the median and SE of: a) mating access, b) mating duration (min), c) number of pedipalp insertions, and d) female aggression (measured as number of attacks), between *Unmated and Mated Females* groups from the first experiment (*Female choice for nuptial gifts and male mating effort*).



**Table 1**. Data from the first experiment (*Unmated and Mated Females*) showing the occurrence (in percentage) of males using an item (item used by males), wrapping the item (silk wrapping of the gift), and the mean  $\pm$  SE of silk wrapping duration (min) and number of silk wrapping bouts between groups. Significant values (< 0.05) are shown in bold.

	Unmated Females (N = 26)	Mated Females (N = 19)	Stati	stics
Item used by males	30%	79%	$z_{42} = 3.03$	<i>p</i> = <b>0.002</b>
Silk wrapping of the gift	38%	93%	$z_{20} = 2.76$	<i>p</i> = <b>0.005</b>
Silk wrapping duration	$13.5 \pm 1.5$	14.5 ± 2.2	$t_{15} = 0.54$	<i>p</i> = 0.60
Number of silk wrapping bouts	$3.0 \pm 0.0$	4.7 ± 0.4	$z_{16} = 1.41$	<i>p</i> = 0.15

**Table 2.** Generalized Linear Mixed Models (GLMM) analysing data from the first experiment (*Unmated and Mated Females*): the effect of gift presence/absence, group and the interaction on mating duration and number of insertions. We used female ID as random effect in all models.

	N	Gift presence/absence	Group	Gift*Group
Mating duration (min)	35	$t_{12} = -1.19, p = 0.24$	$t_{12} = -0.86, p = 0.40$	$t_{12} = 0.81, p = 0.42$
Number of insertions	35	$z_{30} = -0.27, p = 0.79$	$z_{30} = -0.84, p = 0.40$	$z_{30} = 0.79, p = 0.43$

**Table 3**. Results from the second experiment (*Nutritive and Worthless Gifts*) showing the frequency (in percent) of males using an item (item use by males), wrapping the item (silk wrapping of the gift), acquiring the mating (mating access), as well as females attacking males (female aggression) and cannibalizing them (sexual cannibalism); mean  $\pm$  SE of time of wrapping (silk wrapping duration, min), number of silk wrapping bouts, mating duration (min) and number of pedipalp insertions, between groups. Statistical comparison between groups in the variable "item use by males" cannot be done due to, according to the experimental design, all males have a housefly in the *Nutritive Gifts* group. Significant p-values (< 0.05) are shown in bold.

	Nutritive Gift	Worthless Gift	Statistics
	( <i>N</i> = <b>13</b> )	( <i>N</i> = 13)	
Item use by males	100%	54%	-
Silk wrapping of the gift	54%	0%	$X^2 = 9.60, p = 0.001$
Silk wrapping duration	$11.7\pm2.5$	-	-
No. silk wrapping bouts	$2.0\pm~0.3$	-	-
Mating access	39%	46%	$X^2 = 0.16, p = 0.70$
Mating duration	$0.64~\pm~0.1$	$0.65 \pm 0.2$	$t_9 = -0.04, p = 0.97$
Number of insertions	$3.0 \pm 0.9$	$3.16 \pm 1.0$	$z_9 = 0.15, p = 0.87$
Female aggression	31%	54%	$X^2 = 1.42, p = 0.23$
Sexual cannibalism	50%	29%	$X^2 = 0.51, p = 0.47$

#### REFERENCES

- Albo, M.J. 2009. Selección sexual y citogenética en arañas donadoras de regalos nupciales (Trechaleidae y Pisauridae). Master Thesis, PEDECIBA. Montevideo, Uruguay.
- Albo, M.J., Costa-Schmidt, L.E. & Costa, F.G. 2009. To feed or to wrap? Female silk cues elicit male nuptial gift construction in a semiaquatic trechaleid spider. *J. Zool.* 277: 284–290.
- Albo, M.J. & Costa, F.G. 2010. Nuptial gift-giving behaviour and male mating effort in the Neotropical spider Paratrechalea ornata (Trechaleidae). *Anim. Behav.* 79: 1031–1036.
- Albo, M.J., Franco-Trecu, V., Wojciechowski, F.J., Toft, S. & Bilde, T. 2019. Maintenance of deceptive gifts in a natural spider population: Ecological and demographic factors. *Behav. Ecol.* 30: 993–1000.
- Albo, M.J., Macías-Hernández, N., Bilde, T. & Toft, S. 2017. Mutual benefit from exploitation of female foraging motivation may account for the early evolution of gifts in spiders. *Anim. Behav.* 129: 9–14.
- Albo, M.J., Melo-González, V., Carballo, M., Baldenegro, F., Trillo, M.C. & Costa, F.G. 2014a. Evolution of worthless gifts is favoured by male condition and prey access in spiders. *Anim. Behav.* 92: 25–31.
- Albo MJ, Pavón-pelaez C, Martínez Villar M, et al Stressful environments favor deceptive alternative mating tactics to become dominant (in revision). *BMC Biol*
- Albo, M.J., Toft, S. & Bilde, T. 2014b. Sexual selection, ecology, and evolution of nuptial gifts in spiders. In: Sexual Selection: Perspectives and Models from the Neotropics (R. Macedo & G. Machado, eds), pp. 183–200.
- Albo, M.J., Winther, G., Tuni, C., Toft, S. & Bilde, T. 2011. Worthless donations: Male deception and female counter play in a nuptial gift-giving spider. *BMC Evol. Biol.* 11: 1–8.
- Andersen, T., Bollerup, K., Toft, S. & Bilde, T. 2008. Why do males of the spider *Pisaura mirabilis* wrap their nuptial gifts in silk: Female preference or male control? *Ethology* 114: 775–781.
- Arnqvist, G. 2006. Sensory exploitation and sexual conflict. Philos. Trans. R. Soc. B Biol. Sci. 361: 375-386.
- Arnqvist, G. & Nilsson, T. 2000. The evolution of polyandry: Multiple mating and female fitness in insects. *Anim. Behav.* 60: 145–164.
- Arnqvist, G. & Rowe, L. 2005. Sexual conflict. Princeton University Press.
- Basolo, A.L. 1990. Female preference predates the evolution of the sword in swordtail fish. *Science*. 250: 808–810.
- Bilde, T., Tuni, C., Elsayed, R., Pekar, S. & Toft, S. 2007. Nuptial gifts of male spiders: sensory exploitation of the female's maternal care instinct or foraging motivation? *Anim. Behav.* 73: 267–273.
- Bradbury, J.W. & Vehrencamp, S.L. 2000. Economic models of animal coomunication. *Anim. Behav.* 59: 259–268.
- Bristowe, W.S. 1958. The World of Spiders. London: Collins.
- Brockmann, H.J. 2001. The evolution of alternative strategies and tactics. Adv. Study Behav. 30: 1–51.
- Carico, J.E. 2005. Descriptions of two new spider genera of Trechaleidae (Araneae, Lycosoidea) from South America. *J. Arachnol.* 33: 797–812.
- Chapman, T. 2018. Sexual conflict: Mechanisms and emerging themes in resistance biology. *Am. Nat.* 192: 217–229.
- Christy, J.H. 1995. Mimicry, mate choice, and the sensory trap hypothesis. Am. Nat. 146: 171-181.
- Christy, J.H., Backwell, P.R.Y. & Schober, U. 2003. Interspecific attractiveness of structures built by courting male fiddler crabs: Experimental evidence of a sensory trap. *Behav. Ecol. Sociobiol.* 53: 84–91.
- Cornwallis, C.K. & Uller, T. 2010. Towards an evolutionary ecology of sexual traits. Trends Ecol. Evol. 25:

145–152.

- Costa-Schmidt, L.E., Carico, J.E. & De Araújo, A.M. 2008. Nuptial gifts and sexual behavior in two species of spider (Araneae, Trechaleidae, *Paratrechalea*). *Naturwissenschaften* 95: 731–739.
- Da Silva, E.L.C. & Lapinski, W. 2012. A new species of *Trechalea thorell*, 1869 (Araneae: Lycosoidea: Trechaleidae: Trechaleinae) from Costa Rica, with notes on its natural history and ecology. *Zootaxa* 64: 58–64.
- Da Silva, E.L.C. & Lise, A.A. 2009. New record of nuptial gift observed in *Trechalea amazonica*. (Araneae, Lycosoidea, Trechaleidae). *Rev. Peru. Biol.* 16: 119–120.
- Darwin, C. 1871. The descent of man, and selection in relation to sex. Princeton.
- Eberhard, W.G. 1996. Female control: sexual selection by cryptic female choice. *Princeton*, NJ: Princeton University Press.
- Elgar, M.A., Ghaffar, N. & Read, A.F. 1990. Sexual dimorphism in leg length among orb-weaving spiders: a possible role for sexual cannibalism. J. Zool. 222: 455–470.
- Endler, J.A. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol. Evol.* 10: 22–29.
- Engqvist, L. & Taborsky, M. 2015. The evolution of genetic and conditional alternative reproductive tactics. *Proc. R. Soc. B Biol. Sci.* 283.
- Foellmer, M.W. & Fairbairn, D.J. 2004. Males under attack: Sexual cannibalism and its consequences for male morphology and behaviour in an orb-weaving spider. *Evol. Ecol. Res.* 6: 163–181.
- Fromhage, L. & Schneider, J.M. 2005. Safer sex with feeding females: Sexual conflict in a cannibalistic spider. *Behav. Ecol.* 16: 377–382.
- Garcia, C.M. & Ramirez, E. 2005. Evidence that sensory traps can evolve into honest signals. *Nature* 434: 501–505.
- Gaskett, A.C. 2007. Spider sex pheromones: Emission, reception, structures, and functions. *Biol. Rev.* 82: 27–48.
- Ghislandi, P.G., Pekar, S., Matzke, M., Schulte-Döinghaus, S., Bilde, T. & Tuni, C. 2018. Resource availability, mating opportunity and sexual selection intensity influence the expression of male alternative reproductive tactics. *J. Evol. Biol.* 31: 1035–1046.
- Gross, M.R. 1996. Tactics : Diversity Within Sexes. Trends Ecol. Evol. 11: 92-98.
- Gwynne, D. 1984. Courtship feeding increases female reproductive success in bushcrickets. *Nature* 307: 361–363.
- Heifetz, Y., Tram, U. & Wolfner, M.F. 2001. Male contributions to egg production: The role of accessory gland products and sperm in *Drosophila melanogaster*. Proc. R. Soc. B Biol. Sci. 268: 175–180.
- Jirotkul, M. 1999. Operational sex ratio influences female preference and male-male competition in guppies. *Anim. Behav.* 58: 287–294.
- Kessel, E.L. 1955. The mating activities of balloon flies. Syst. Zool. 4: 97-104.
- Klein, A.L., Trillo, M.C. & Albo, M.J. 2012. Sexual receptivity varies according to female age in a Neotropical nuptial gift-giving spider. J. Arachnol. 40: 138–140.
- Kvarnemo, C. & Ahnesjö, I. 1996. The dynamics of operational sex ratios and competition for mates. *Trends Ecol. Evol.* 11: 404–408.
- Lang, A. 1996. Silk investment in gifts by males of the nuptial feeding spider *Pisaura mirabilis* (Araneae: Pisauridae). *Behaviour* 133: 697–716.
- Lapinski, W. & Tschapka, M. 2009. Erstnachweis von Brautgeschenken bei *Trechalea sp*. (Trechaleidae, Araneae) in Costa Rica. *Arachne* 14: 4–13.

- LeBas, N.R. & Hockham, L.R. 2005. An invasion of cheats: The evolution of worthless nuptial gifts. *Curr. Biol.* 15: 64–67.
- Madden, J.R. & Tanner, K. 2003. Preferences for coloured bower decorations can be explained in a nonsexual context. Anim. Behav. 65: 1077–1083.
- Martínez-Villar, M., Germil, M., Pavon-Pelaz, C., Costa-Schmidt, L.E. & Albo, M.J. 2020. Empty nuptial gifts: A further step in the evolution of deception in spiders? *J. Arachnol.* 48: 214–217.
- Maxwell, M.R. & Prokop, P. 2018. Fitness effects of nuptial gifts in the spider *Pisaura mirabilis*: examination under an alternative feeding regime. *J. Arachnol.* 46: 404–412.
- Moehring, A.J. & Boughman, J.W. 2019. Veiled preferences and cryptic female choice could underlie the origin of novel sexual traits. *Biol. Lett.* 15: 1–15.
- Mokkonen, M. & Lindstedt, C. 2015. The evolutionary ecology of deception. Biol. Rev. 91: 1020-1035.
- Pandulli-Alonso, I., Quaglia, A. & Albo, M.J. 2017. Females of a gift-giving spider do not trade sex for food gifts: A consequence of male deception? *BMC Evol. Biol.* 17: 1–8.
- Pavón-Peláez, C. 2019. Factores intrínsecos y extrínsecos que modelan las tácticas alternativas de apareamiento en una araña con regalo nupcial. Undergraduate Thesis, Facultad de Ciencias, Universidad de la República. Montevideo, Uruguay. 1-38
- Piersma, T. & Drent, J. 2003. Phenotypic flexibility and the evolution of organismal design. *Trends Ecol. Evol.* 18: 228–233.
- Preston-Mafham, K.G. 1999. Courtship and mating in Empis (*Xanthempis*) trigramma Meig., E. tessellata F. and E. (*Polyblepharis*) opaca F. (Diptera: Empididae) and the possible implications of "cheating" behaviour. J. Zool. 247: 239–246.
- Proctor, H.C. 1991. Courtship in the water mite *Neumania papillator*: Males capitalize on female adaptations for predation. *Anim. Behav.* 42: 589–598.
- Prokop, P. & Maxwell, M.R. 2009. Female feeding regime and polyandry in the nuptially feeding nursery web spider, *Pisaura mirabilis*. *Naturwissenschaften* 96: 259–265.
- R Team Core. 2021. A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. R Foundation for Statistical Computing, Vienna, Austria.
- Rengifo-Gutiérrez, L., Albo, M.J. & Santa, L.D. 2021. The unknown *Enna* (Araneae: Trechaleidae), new species and first record of wrapped nuptial gifts in the genus. *J. Arachnol.* 48: 242–248.
- Ryan, M.J. & Cummings, M.E. 2013. Perceptual Biases and Mate Choice. Annu. *Rev. Ecol. Evol. Syst.* 44: 437–459.
- Ryan, M.J., Fox, J.H., Wilczynski, W. & Rand, A.S. 1990. Sexual selection for sensory exploiation in the frog *Physalaemus pustulosus. Nature* 343: 66–67.
- Sakaluk, S.K. 2000. Sensory explotation as an evolutionary origin to nuptial food gifts in insects. *Proc. R. Soc. London. Ser. B Biol. Sci.* 267: 339–343.
- Sakaluk, S.K., Avery, R.L. & Weddle, C.B. 2006. Cryptic sexual conflict in gift-giving insects: Chasing the chase-away. *Am. Nat.* 167: 94–104.
- Simmons, L.W. & Gwynne, D. 1991. The refractory period of female katydids (Orthoptera: Tettigoniidae): Sexual conflict over the remating interval? *Behav. Ecol.* 2: 276–282.
- Simmons, L.W. & Parker, G.A. 1989. Nuptial Feeding in Insects: Mating Effort versus Paternal Investment. *Ethology* 81: 332–343.
- Stålhandske, P. 2001. Nuptial gift in the spider *Pisaura mirabilis* maintained by sexual selection. *Behav. Ecol.* 12: 691–697.
- Stålhandske, P. 2002. Nuptial gifts of male spiders function as sensory traps. *Proc. R. Soc. B Biol. Sci.* 269: 905–908.

Thornhill, R. 1976. Sexual selection and paternal investmen in insects. Am. Nat. 110: 153-163.

- Toft, S. & Albo, M.J. 2015. Optimal numbers of matings: The conditional balance between benefits and costs of mating for females of a nuptial gift-giving spider. *J. Evol. Biol.* 28: 457–467.
- Toft, S. & Albo, M.J. 2016. The shield effect: Nuptial gifts protect males against pre-copulatory sexual cannibalism. *Biol. Lett.* 12: 20151082.
- Trillo, M.C. & Albo, M.J. 2019. Another one bites the gift: sexual behaviour in a *Trechaleoides* species. *Arachnology* 18: 250–252.
- Trillo, M.C., Melo-González, V. & Albo, M.J. 2014. Silk wrapping of nuptial gifts as visual signal for female attraction in a crepuscular spider. *Naturwissenschaften* 101: 123–130.
- Vahed, K. 1998. The function of nuptial feeding in insects: A review of empirical studies. Biol. Rev. 73: 43-78.
- Wiens, J.J. 2001. Widespread loss of sexually selected traits: How the peacock lost its spots. *Trends Ecol. Evol.* 16: 517–523.
- Wolfner, M.F. 1997. Tokens of love: Functions and regulation of *Drosophila* male accessory gland products. Insect Biochem. *Mol. Biol.* 27: 179–192.
- Zahavi, A. 1974. Mate selection-A selection for a handicap. J. Theor. Biol. 53: 205-214.
# **Chapter 2**

# Lack of female preference for nuptial gifts may have changed its function and led to loss of the male sexual trait

**Martínez Villar M**., M. Germil, C. Pavón-Peláez, I.H. Tomasco, T. Bilde, S. Toft & M.J. Albo (in revision). Lack of female preference for nuptial gifts may have changed its function and led to loss of the male sexual trait. *Evol. Biol.* 

### Resumen

La pérdida evolutiva de los rasgos sexuales puede ocurrir si las fuerzas que mantienen estos rasgos se debilitan o desaparecen. Las hembras pueden desarrollar resistencia o un cambio de sus preferencias si el rasgo sexual del macho disminuye su supervivencia y/o reproducción (p. ej., rasgos coercitivos o engañosos). En las arañas donadoras de regalos nupciales, los machos ofrecen comida en forma de regalo envuelto en seda durante el cortejo, tomando ventaja de la motivación de forrajeo de las hembras. Pero, los machos también pueden producir regalos simbólicos, lo que podría favorecer la emancipación del engaño por parte de las hembras y la subsiguiente pérdida de la función del regalo. Este podría ser el caso de las dos especies conocidas del género de arañas Trechaleoides (Trechaleidae). En este trabajo examinamos la preferencia de las hembras por los regalos nupciales y la función del regalo como esfuerzo de cópula de los machos y/o protección para los machos en ambas especies. Los machos de Trechaleoides keyserlingi que ofrecieron regalos obtuvieron significativamente menos cópulas que los machos sin regalos y, por lo tanto, verificamos la ausencia de preferencia de las hembras por el regalo. En T. biocellata los machos nunca produjeron un regalo, aunque experimentaron un alto riesgo de canibalismo pre-copulatorio. Para evaluar si las hembras de T. biocellata poseen un sesgo sensorial preexistente por los regalos nupciales, se les presentaron machos con y sin regalo de T. keyserlingi. No detectamos preferencia de las hembras, y el regalo no protegió a los machos del canibalismo sexual. Siendo que los regalos nupciales envueltos en seda son sugeridos ancestrales en la familia de arañas Trechaleidae, se podría hipotetizar una pérdida basal de preferencia de las hembras por el regalo en el género Trechaleoides. Esto puede haber cambiado posteriormente la función sexual del regalo en T. keyserlingi y condujo a su pérdida completa en T. biocellata.

Palabras clave: Comportamiento de ofrecer regalos nupciales, explotación sensorial, canibalismo sexual, arañas

### Abstract

Evolutionary loss of sexual traits may occur if the forces that maintain those traits weaken or disappear. Females may evolve resistance or a change in preference if the male sexual trait decreases their fitness (e.g., coercive or deceptive traits). In nuptial gift-giving spiders, males offer a food gift wrapped in silk during courtship, taking advantage of female foraging motivation. Males may also produce worthless gifts, which could select for female emancipation from deception and subsequent loss of gift function. This might be the case in the two known species of the spider genus *Trechaleoides* (Trechaleidae). Here, we examined the females' preference for nuptial gifts, and gift function as male mating effort and/or male protection in both species. Trechaleoides keyserlingi males offering gifts acquired significantly fewer matings than males without gifts and thus, we verified no female preference for the gift. In T. biocellata males never produced a gift, although they experienced a high risk of pre-copulatory cannibalism. To assess whether T. biocellata females possess a pre-existing sensory bias for nuptial gifts, they were presented with heterospecific T. keyserlingi males with and without gifts. No female preference was detected, and the gift did not protect males from sexual cannibalism. If silk-wrapped nuptial gifts are ancestral in the spider family Trechaleidae, a basal loss of female preference for the gift in the genus *Trechaleoides* could be hypothesized. This may subsequently have changed the gift's sexual function in *T. keyserlingi* and led to the complete loss of the gift in *T. biocellata*.

Keywords: gift-giving behaviour, sensory exploitation, sexual cannibalism, spiders

### Introduction

Secondary sexual traits are prevalent as a result of intra-sexual and inter-sexual selection across the animal kingdom (Andersson & Simmons, 2006; Clutton-Brock, 2017). Much research has focused on the origin and maintenance of these traits, while their potential loss has received less attention (Wiens, 2001; Morris et al., 2005; Ellers et al., 2012; Starrett et al., 2022). Secondary sexual traits can be lost if conditions change, relaxing sexual selection on the focal trait. This may alter or reduce the benefit to either males or females, ultimately leading to loss of the trait (Wiens 2001). For example, if the trait is extremely difficult or easy to display, it can become obsolete (Reimchen, 1989; Marchetti, 1993); it may disappear if, due to limited resources, individuals cannot afford the costs of developing it (Emlen, 1994; Hill, 1994); or it can be lost if the trait increases detection by predators or parasites (Morris et al., 2005; Zuk et al., 2006). Further, females may become less choosy or lose their preference, if the costs of accepting a male based on the sexual trait are higher than the benefits (Arnqvist & Rowe, 2005), for example, as seen in species where male secondary traits evolved via sensory exploitation (Ryan et al., 1990; Kirkpatrick & Ryan, 1991). In such cases, a male trait exploits the females' pre-existing sensory bias to enhance their mating success (Basolo, 1990; Proctor, 1991; Sakaluk, 2000; Arnqvist, 2006; Vahed, 2007), which may lead to suboptimal mating rates for the females (Arnqvist & Rowe, 2005).

Sensory exploitation has been a central hypothesis proposed for the evolution and maintenance of nuptial gifts (Sakaluk, 2000; Bilde et al., 2007; Albo et al., 2017b). By using food gifts, males can exploit females' pre-existing foraging motivation to increase their mating chances, but it is debated whether the gift always benefits the females (Vahed, 1998; Gwynne, 2008; Lewis & South, 2012). Nuptial gifts are found across a wide range of taxonomic groups and come in various forms (Lewis & South, 2012). The prevailing explanations for the evolution of nuptial gifts include paternal investment, male mating effort, or protection against sexual cannibalism (Vahed, 1998). Some nuptial gifts are endogenous, like glandular secretions or proteins transferred together with the sperm, and may contribute to female fecundity (Lewis & South, 2012; but see Warwick et al., 2009 and Will & Sakaluk, 1994). Other gifts are exogenous and allow males to modify them and deceive the females, creating scope for sexual conflict (Preston-Mafham, 1999; LeBas & Hockham, 2005; Albo et al., 2014b). This seems to be the case in spiders, where males offer females either nutritive gifts (i.e., prey) (Bristowe, 1958; Costa-Schmidt et al., 2008; Trillo & Albo, 2019; Rengifo-Gutiérrez et al., 2021), worthless gifts (i.e., prey leftovers) gathered from the surroundings (Albo et al., 2011, 2014a), or even empty silk gifts (Martínez-Villar et al., 2020). Female spiders benefit from receiving nutritive gifts as they acquire food that increases their fecundity and survival (Toft & Albo, 2015; Pandulli-Alonso et al., 2017). In contrast, receiving a worthless gift seems to be against the females' interests as they suffer mating costs, such as reduced fecundity, without receiving benefits from food (Albo et al., 2011; Toft & Albo, 2015; Pandulli-Alonso et al., 2017). Eventually, if females are subject to suboptimal mating rates with males offering worthless gifts, they may counteradapt to mitigate the costs. For instance, Pisaura mirabilis females reduce mating duration, and thereby sperm transfer, when mating with males offering worthless gifts; this is expected to favour males that offer nutritive gifts in sperm competition (Albo et al., 2011; Ghislandi et al., 2018).

Worthless gifts are expected to exert selection on females to evolve resistance or indifference toward the male trait. This process is known as "emancipation from exploitation" (Bradbury & Vehrencamp, 2000), in which females lose their preference for the exploiting trait, and potentially evolve a preference for other traits that give more reliable information on potential mates. For example, females can shift preference to a trait that confers reliable information about the males or the resources that they hold, i.e., food abundance, territory size, plumage colour, male song (Reid & Weatherhead, 1990; Calkins & Burley, 2003; Chaine & Lyon, 2008; Burley *et al.*, 2018). The loss of a female preference for a secondary sexual male trait should lead to the subsequent loss of the trait's reproductive function for the male (Morris *et al.*, 2005; Tinghitella & Zuk, 2009; Heinen-Kay & Zuk, 2019), and eventually to the complete loss of the trait (Wiens, 2001).

Female emancipation from male sensory exploitation was suggested for the spider Trechaleoides keyserlingi (Martínez Villar et al., 2021), in which males can produce both nutritive and worthless gifts (Trillo & Albo, 2019; Martínez Villar et al., 2021). Yet, from the variables measured (mating access, mating duration and cannibalism protection), it seemed that the nuptial gift does not confer reproductive or survival advantages to the males (Martínez Villar et al., 2021). This is contrary to the hypothesis that the nuptial gift functions as mating effort increasing male mating access or mating duration (Stålhandske, 2001; Prokop & Maxwell, 2009; Albo & Costa, 2010; Albo et al., 2011, 2014b; Maxwell & Prokop, 2018), or as a shield against cannibalistic females (Kessel, 1955; Bristowe, 1958; Toft & Albo, 2016). Therefore, it was suggested that T. keyserlingi females had lost their preference for the gift trait (Martínez Villar et al., 2021). While it is expected that males would subsequently lose the trait, the existence of nuptial gifts in the wild indicates that it is maintained as a remnant nonfunctional trait (Martínez Villar et al., 2021). Interestingly, preliminary field and laboratory data indicated that males of the species T. biocellata do not produce nuptial gifts (Albo, 2009), and that females are very aggressive and often engage in pre-copulatory cannibalism (Martinez Villar, personal observations).

Here, we aimed to assess the possible loss of the nuptial gift as a sexual trait by studying female preference and the functional significance of male nuptial gifts in the genus Trechaleoides. The genus includes only two known species, T. keyserlingi and T. biocellata (Carico, 2005), and belongs to the family Trechaleidae. This family consists of 131 species from 17 genera of which 7 genera have been reported to have males producing silk-wrapped nuptial gifts: Paratrechalea (Costa-Schmidt et al., 2008), Trechalea (Da Silva & Lise, 2009; Lapinski & Tschapka, 2009; Da Silva & Lapinski, 2012), Trechaleoides (Trillo & Albo, 2019), Paradossenus (Martínez-Villar et al., 2020), Enna (Rengifo-Gutiérrez et al., 2021), Dossenus (A. Santos personal communication) and Hesydrus (D. Poy, personal communication). The fact that nuptial gift use is widespread in the family confers an opportunity to discuss the function of the nuptial giftgiving trait, and to understand the role of female preference in driving evolutionary changes of this trait. We studied the reproductive strategies of both species by examining the frequency of male gift production, female attraction to males offering or not offering gifts, and whether males use the gift as protection against sexual cannibalism. We performed two series of experiments. First, we performed conspecific mating trials in both species. To assess whether males produce nuptial gifts, each male was given a prey in a mating context, providing an opportunity to wrap it in silk. If the nuptial gift trait is lost in T. biocellata, we predicted giftproduction to be absent, implying that males would attempt to acquire matings without a gift.

To assess gift function, each male was exposed sequentially to multiple females, to test: 1) whether multiple mating cause females to exert stronger preference for a nuptial gift, as predicted if the gift functions to overcome female resistance to mating; or 2), whether the gift would protect the male from cannibalism, as predicted if females become increasingly aggressive with increasing male encounters. If the nuptial gift trait has lost its function in T. keyserlingi (Martínez Villar et al., 2021) we predict that gift-giving males do not acquire mating benefits (access to and duration of matings) in repeated encounters with females compared with non-gift giving males. Second, we performed a heterospecific experiment to determine whether females of the non-gift giving species T. biocellata show a pre-existing sensory bias (preference) for nuptial gift-giving males. This could only be done by presenting T. keyserlingi males with and without a gift (as T. biocellata males never produced gifts) to T. biocellata females, which allowed to assess whether 1) females show preference for the nuptial gift providing gift-giving males with mating benefits, or 2) the gift protects males against sexual cannibalism. We discuss the findings based on the assumption of the sensory exploitation hypothesis that female preference is ancestral to the male trait (Basolo, 1990; Ryan et al., 1990; Sakaluk, 2000; Albo et al., 2017b). If we detect female preference for gift-giving males, it would suggest that the nuptial gift-giving trait has not evolved in T. biocellata. Under the scenario where the nuptial gift and female preference are ancestral in the spider family or, at least, in a clade within the family (cf. above), the absence of female preference in both species would indicate a loss of both the preference and the gift function in T. keyserlingi, and the loss of the gift itself in *T. biocellata*.

### **Materials and Methods**

### **Biological model species**

The two species from the genus *Trechaleoides* live in the Neotropical riparian forest associated with freshwater courses (Carico, 2005). Individuals of the two species are morphologically similar, mainly distinguished by the genitalia. Both species are found perching on stones, trees, and ravines at the edge of streams and rivers. In Uruguay, T. biocellata has a restricted distribution and is found in only a few localities where it co-occurs with T. keyserlingi: San Miguel (33°41′51′′S, 53°32′00′′W) Rocha, Quebrada de los Cuervos (32°55′39′′S, 54°27′25′′W) Treinta y Tres, and Paso Centurión (32°08′42′′S, 53°47′21′′W) Cerro Largo. In contrast T. keyserlingi is geographically widespread, common in almost all riparian forests of the country (Sección Entomología, Facultad de Ciencias, Universidad de la República, Uruguay). From preliminary fieldwork, we know that both species can occur in the same localities, but they differ in the microhabitats occupied and timing of reproduction (MMV personal observations). The species T. biocellata is only found in the glens near the principal watercourses, and they reproduce from April to October. In contrast, T. keyserlingi is found at strong water currents and reproduction occurs mainly during January to April. The natural mating system of both species has not been studied; therefore, it is unknown whether females are inclined to mate multiply. Further, nothing is known about the reproductive biology of T. biocellata. In T. keyserlingi, as in other gift-giving spiders, male courtship is triggered by silkborne pheromones (Albo *et al.*, 2009; Trillo & Albo, 2019). After wrapping a prey item in silk, the male of *T. keyserlingi* searches for a female while carrying the gift in his chelicerae. Once he finds a female, he offers the gift by adopting a specific position call hyperflexion while simultaneously vibrating forelegs and pedipalps. The female shows acceptance by grabbing the gift and allowing the male to mount and initiate sperm transfer (Costa-Schmidt *et al.*, 2008; Albo & Costa, 2010; Trillo & Albo, 2019).

### Spider maintenance and experimental design

We collected a total of 121 subadults of T. biocellata (N = 50) and T. keyserlingi (N = 71) at the locality of Quebrada de los Cuervos (32°55'39"S 54°27'25"O), Treinta y Tres, Uruguay, during 2018-2019. We collected spiders during the night and transported them to the laboratory. Following standardized protocols (Albo & Costa, 2010), we raised them individually in plastic jars (8.5 cm internal diameter and 7.5 cm height) in a warm room averaging 25.0 °C ( $\pm$  0.31 SE) to accelerate their development to adulthood. Twice a week, we fed all individuals with three houseflies (Musca domestica) and water ad libitum. Once they reached sexual maturity, we relocated the spiders to an experimental room at 21.0 °C ( $\pm$  0.17 SE) and continued the same feeding regimen. Following previous experimental protocols for other gift-giving spiders (Klein et al., 2012), we used the individuals after 20 days of their final moult. We measured cephalothorax width of all individuals as a proxy of individual size using a stereomicroscope. We performed the experiments in a glass cage (20 x 29 x 15 cm) with small pebbles as substrate and water presented in a Petri dish. The day before each trial, we fed all individuals with a housefly, to prevent hungry females from cannibalism and males from eating the prey instead of producing a nuptial gift. We placed the females individually in the experimental cages overnight; during this time they could deposit silk that would provide sexual stimuli for males to initiate courtship and gift production (Albo et al., 2009).

For statistical analyses, we used software R (R Team Core, 2021). We explored the distribution of the raw data for each variable to account for the error distribution, after which we used Generalized Linear Mixed Models (GLMM) and Generalized Linear Models (GLM). All the models were validated by exploring the residual errors with graphical tools (Zuur *et al.*, 2010).

### Presence and function of nuptial gifts in conspecific trials

We first aimed to understand the role of the nuptial gift and its presence in *T. biocellata* and *T. keyserlingi*. We exposed conspecific males and females to each other once a week. Originally, the plan was to expose the same two individuals to each other only one time, with females presented with up to 15 trials in sequential encounters. Due to low numbers of individuals available, as well as mortality during the experiment, some pairs were exposed to each other 2 or 3 times. In total, we obtained 124 trials in *T. biocellata* (11 males and 13 females) and 208 in *T. keyserlingi* (14 males and 17 females). This experimental design allowed us to examine how frequently males produce a nuptial gift and to understand whether females become more selective for nuptial gifts and more aggressive after multiple matings (Toft & Albo, 2015)

On the day of the trial, we added a male to the experimental cage which already contained a female. Once he started to court, we gave him a housefly that he could wrap or not wrap in silk before offering it to the female as a nuptial gift. All trials lasted 80 min. We registered the occurrence of male prey grabbing and nuptial gift-giving, and recorded whether males obtained a mating or not (mating occurrence). We recorded the number and duration of insertions by the male pedipalps (copulatory organs). We calculated total mating duration (min) as the sum of all insertion durations (= proxy of the amount of sperm transferred). Finally, we registered the number of female attacks and occurrence of sexual cannibalism, categorizing both according to whether they occurred before, during or after the mating. As we had few males and wanted to save their lives for more experiments, we judged the occurrence of sexual cannibalism when the male could not escape from a female attack. In such cases, we attempted to prevent the males from being cannibalized by separating them immediately after the attack. Even under this protocol, 7 males died following female attacks during the different mating encounters. Thus, of the 11 *T. biocellata* males, only four males obtained the maximum of 15 encounters.

In both species, females were larger than males, and individuals of *T. biocellata* were larger than those of *T. keyserlingi* (GLM: Intercept: Estimate = 0.64, SE = 0.01, p < 0.0001; Sex: Estimate = -0.03, SE = 0.01, p = 0.003; Species: Estimate = -0.05, SE = 0.01, p < 0.0001). Cephalothorax widths of males of *T. biocellata* averaged 0.58 cm ( $\pm$  0.05 SE) and females averaged 0.64 cm ( $\pm$  0.06 SE), while in *T. keyserlingi* males averaged 0.56 cm ( $\pm$  0.02 SE) and females 0.58 cm ( $\pm$  0.02 SE).

We first analysed the data within each species by using GLMMs with response variables being courtship and mating parameters: gift presence/absence, experimental day and size of both sexes' sizes were used as fixed effects, and female and male ID as random effects. Using GLMM with Binomial distribution family, we examined frequency of mating (mated: 1, unmated: 0), female attacks (attack: 1, no attack: 0) and sexual cannibalism (cannibalism: 1, no cannibalism: 0). Mating duration (min) was analysed using GLMM with Gamma distribution family and number of pedipalp insertions using GLMM with Poisson distribution family. Second, we performed statistical comparisons between species by GLMM (same distributions as before) for the following response variables: mating occurrence, mating duration, number of pedipalp insertions, female attacks and sexual cannibalism, with species and individual sizes used as fixed effects, female and male ID as random effects, and experimental day as covariate. This accounts for the repeated measures structure within males and females for all parameters, controlling for the effect of male and female ID and age.

### Heterospecific test of female preference and cannibalism

Once we verified that the gift is absent in *T. biocellata* and that females are extremely aggressive, we tested whether females might have a preference (i.e., pre-existing bias) for males with nuptial gifts, and whether the gift can protect males from female attacks.

For this, we followed a previous experimental design (Albo *et al.*, 2017b), in which females from a non-gift-giving spider (*Cladygnis insignis*) were exposed to males of a gift-giving species (*Pisaura mirabilis*). The gift represented a novel sexual trait for the females. The results showed that females were attracted to the nuptial gift and increased acceptance of

heterospecific males with gifts, demonstrating that females have an ancestral preference for this novel sexual trait.

In line with this, we performed experiments presenting females T. biocellata to males T. keyserlingi. The individuals used for this experiment differed from the ones used in the previous experiment. The size of T. keyserlingi males averaged 0.64 cm ( $\pm$  0.01 SE) while females T. biocellata averaged 0.68 cm ( $\pm$  0.008 SE) (GLM: Intercept: Estimate = 0.67, SE = 0.01,  $p = \langle 0.001;$  Sex: Estimate = -0.03, SE = 0.01, p = 0.01). We created two groups, where females were exposed to males with and without a nuptial gift, respectively. In the Gift group (N = 16), the males had a housefly wrapped in silk to offer to females, while in the No gift group (N = 15), the males had no prey available. As in Albo *et al.* (2017b), to obtain courting males with wrapped gifts we presented T. keyserlingi males to conspecific females allowing contact but avoiding mating. In the Gift group, once the male started courtship vibrations, we offered him a housefly. After the male had wrapped the gift, we carefully replaced the female with a T. biocellata female. In the No Gift group, we switched females after the male started courting without offering a gift. During the switching, males were separated by a paper barrier to avoid contact and possible attack by the new female. After five minutes, we removed the barrier allowing the male to contact the female. Each individual was used only once and we registered gift acceptance and sexual cannibalism by females, as well as the survival of males in each group. An experiment was finished if the female had attacked 4 times or had cannibalized the male. If a male did not court the female within one hour after physical contact, he was re-exposed to another female two days later.

To analyse the data from this experiment, we used a GLM (Binomial distribution family) with sexual cannibalism (cannibalism: 1, no cannibalism: 0) as the response variable. The initial model included effects of Gift and No Gift in interaction with female and male size as fixed effects. Then we performed model reduction using the Akaike information criterion (AIC) (Akaike, 1973) to obtain the best model.

### Results

### Presence and function of nuptial gifts in conspecific trials

We investigated whether males of both species produce nuptial gifts, and the function of the gift by testing whether multiple mating cause females to exert stronger preference for a nuptial gift, or whether the gift would protect the male from cannibalism. In *T. biocellata*, none of the males offered a gift (Table 1), and half of them never acquired a mating. The average number of matings was 1 per male (range: 0-3) and 1 per female (range: 0-3). In *T. keyserlingi*, all males offered a nuptial gift to females at least once, with an average of 5 matings per male (range: 2-7) and 4 per female (range: 0-12). In total, males of *T. biocellata* achieved significantly fewer matings (10% vs 31%), which were of shorter duration and with fewer pedipalp insertions, than *T. keyserlingi* males (Table 1, Figure 1A-C). Females from *T. biocellata* were much more aggressive and cannibalistic during courtship than females from *T.* 

*keyserlingi* (66% vs. 11% attacks and 60% vs. 18% cannibalism, respectively) (Table 1, Figure 2A-B).

From the 208 experimental cases obtained with *T. keyserlingi*, the males grabbed the prey 120 times. Nuptial gift-giving behaviour was recorded in 92% of the cases (103 wrapped in silk / 7 unwrapped). The frequency of gift-giving did not change over time, averaging 7 gifts out of the 15 experiments performed per day (range: 3-11; Estimate = -0.01, SE = 0.03, p = 0.55). Only in 31% of all the cases did males acquire a mating (Table 2). Males approaching females without a gift acquired a mating more than 1.5 times as often as males offering a nuptial gift (Table 2, Figure 1A). Mating occurrence decreased with time for males with a gift, but not for those without a gift, indicated by the significant interaction between gift and experimental day (Table 2). Males without gifts acquired similar mating duration and number of insertions as males with gifts (Figure 1B-C) and this effect was constant over time (Table 2). Independently on whether males offered a gift or not, or on experimental day, during courtship females were aggressive and attacked the male on 14% of the trials (Table 2, Figure 2A). Four attacks resulted in sexual cannibalism, all towards males lacking a gift (Fisher test: p = 0.12; Figure 2B).

### Heterospecific test of female preference and cannibalism

The aim of the experiment was to investigate whether *T. biocellata* females have a preference for males offering a gift, which would indicate a sensory bias. All females approached all males whether they had a gift or not, thus females did not appear to prefer a nuptial gift. The gift did not increase male survival probability compared to males without a nuptial gift (Estimate = 0.31, SE = 1.21, p = 0.79). Seven males (47%) from the *No gift group* and 6 (35%) from the *Gift group* were attacked and cannibalized by females. All cases of cannibalism occurred during courtship, and no trials led to mating attempts. Sexual cannibalism was independent of male size, but its probability increased marginally with female size (Estimate = 20.72, SE = 10.95, p = 0.058; Figure 3).

### Discussion

Female preferences for exaggerated sexual traits have been extensively documented across taxa (Basolo, 1990; Kirkpatrick & Ryan, 1991; Hill, 1994; Hebets & Uetz, 2000; Ödeen & Björklund, 2003; Morris *et al.*, 2005; Servedio & Boughman, 2017), whereas changes in these preferences are less frequently reported (Heinen-Kay & Zuk, 2019). There is scope for selection to act on female preference when the females suffer fitness costs associated with suboptimal mating rates (Wiens, 2001; Morris *et al.*, 2005; Tinghitella & Zuk, 2009; Heinen-Kay & Zuk, 2019), for example, in gift-giving mating systems where the nuptial gift has evolved through exploitation of female feeding motivation (Sakaluk, 2000; Fromhage & Schneider, 2005; Bilde *et al.*, 2007; Albo *et al.*, 2017b). By studying the genus *Trechaleoides*, we documented the absence of female preference for nuptial gifts in the two species. Given the existence of nuptial gifts in one of these species, the lack of a preference for the gift is remarkable. Under the sensory exploitation hypothesis, female preference is ancestral to the

male sexual trait (Basolo, 1990; Ryan *et al.*, 1990; Sakaluk, 2000; Albo *et al.*, 2017b), therefore, our findings corroborate the idea that females have evolved resistance to male exploitation by ignoring the nuptial gift (Bradbury & Vehrencamp, 2000; Martínez Villar *et al.*, 2021). This is further supported by the absence of any reproductive benefit to the male by the gift in *T. keyserlingi*. In contrast to the expectations even after multiple encounters (Toft & Albo, 2015), nuptial gifts did not appear to improve mating success or to protect males against cannibalism under the conditions used in our experiments. We hypothesize that the absence of male use of a nuptial gift in *T. biocellata* is an example of an evolutionary loss of a sexual trait. Next, we will substantiate this idea.

Nuptial gifts in the form of silk-wrapped food items are widespread in the spider family Trechaleidae, occurring in almost half of the genera (Costa-Schmidt et al., 2008; Da Silva & Lise, 2009; Lapinski & Tschapka, 2009; Da Silva & Lapinski, 2012; Trillo & Albo, 2019; Martínez-Villar et al., 2020; Rengifo-Gutiérrez et al., 2021; A. Santos and D. Poy, personal communication). This has led to the suggestion that the nuptial gift might have appeared once in an ancestral species of the family (Albo et al., 2017a), or in an early clade within the family (Piacentini & Ramírez, 2019). Because we lack the complete phylogeny, we do not know whether the genus *Cupiennius* is sister group to the remaining taxa and shares a gift-giving ancestor with the trait being lost in this lineage or if gift-giving evolved following the divergence of the Cupiennius lineage and the others. However, for the other species the most parsimonious scenario suggests that at least one loss of the gift-giving trait has occurred in this clade after the trait evolved, in T. biocellata (Figure 4). A possible evolutionary path is that initially Trechaleoides females have lost their preference for the nuptial gift. As both species show lack of preference for the gift, they may have lost it before the species diverged. The absence of female preference may subsequently have led to the loss of reproductive function of the gift for males in T. keyserlingi and to the complete loss of the gift in T. biocellata. There are only a few examples in the literature indicating that the absence of female preference may lead to the loss of the sexual traits that were previously preferred. For instance, Omland (1994, 1997) showed that some populations of female mallard ducks lack the preference for the males' dichromatic plumage, and suggested that this had led to the loss of this sexual trait in the males. Similarly, in a swordtail fish, it was proposed that relaxation of female preference for large males has led to relaxed selection on male size (Morris et al., 2005). Alternatively, the phylogenetic pattern of nuptial gift use in the spider family Trechaleidae could have resulted from repeated evolution of the trait, from the natural capture of insect prey to opportunistic exploitation of the female foraging motivation (Sakaluk, 2000; Fromhage & Schneider, 2005; Bilde et al., 2006; Albo et al., 2017b). The convergent evolution of this sexual trait is less parsimonious, however, as it would imply at least 3 independent acquisitions of the trait or 6 when including the species with unknown position in the current phylogeny (Figure 4). But most importantly, this hypothesis requires evidence of female preference for the nuptial gift in the non-gift-giving species T. biocellata. In contrast, we verified that in this species females lack a preference for the gift.

Overall, there is large potential in gift-giving spider mating systems for studying the function of the gift trait and the concomitant changes in female preferences. One of the functions of the nuptial gift is to increase mate acquisition and sperm competition success, thereby enhancing male reproductive success in a polyandrous mating system (Vahed, 1998; Sakaluk, 2000; Rowe & Arnqvist, 2002; Lewis & South, 2012). This is because females gain direct benefits and

increase their fecundity when accepting multiple food gifts (Arnqvist & Nilsson, 2000). We know from some gift-giving spiders that females accept the first male regardless of whether he offers a gift or not, but they will only accept subsequent males if they offer a gift, showing strong sexual selection on the gift-giving trait (Stålhandske, 2001; Albo & Costa, 2010; Albo *et al.*, 2014b). Here, we verified the absence of female preference for nuptial gifts in *T. keyserlingi* over multiple encounters including the first (when the female was virgin), reinforcing the hypothesis that the gift has lost the reproductive function as male mating effort and may represent a non-functional remnant trait (Martínez Villar *et al.*, 2021). Moreover, according to our findings, offering a nuptial gift is against the males' interest, as offering a gift led to fewer matings than not offering a gift. This is intriguing because why would males produce a nuptial gift that decreases their mating success?

The nuptial gift may also function as a shield to protect males against sexual cannibalism (Kessel, 1955; Bristowe, 1958; Toft & Albo, 2016). Sexual cannibalism represents the ultimate form of sexual conflict (Parker, 1979; Bilde et al., 2006), and its evolutionary significance depends on the time at which it occurs (Elgar, 1992; Arnqvist & Henriksson, 1997). Precopulatory sexual cannibalism is disadvantageous for males, while females may still benefit from consuming the males' bodies or from male mate choice. But, if females are extremely cannibalistic, they may die without mating (Schneider, 2014). Interestingly, we found that females from the non-gift-giving species were cannibalistic even if they were very well fed. The aggressiveness of these females, when exposed to either conspecific males without gifts or gift-giving heterospecific T. keyserlingi males, indicates that the gift is useless as a shield for protecting the males, and thus may explain the absence of the gift in T. biocellata. In contrast, although females rarely killed males in the gift-giving species, the risk of sexual cannibalism exists. Toft and Albo (2016) argued that due to often low frequency of female aggression, it has been hard to obtain statistical evidence for the gift functioning as a shield against sexual cannibalism. This could be the case in T. keyserlingi, as the 4 cannibalized males were all courting without a gift. However, the shield hypothesis does not apply to T. biocellata as cannibalism was not significant differently when a gift was present or not. Hence, it seems unlikely that the gift would be maintained for male protection in this species.

The literature generally suggests female-biased sexual size dimorphism in cannibalistic species (Miller, 2007; Wilder & Rypstra, 2008; Kuntner et al., 2009, 2015; Assis & Foellmer, 2019). This is also the case for the non-gift-giving and cannibalistic species T. biocellata, as females are larger than males. Further, our results indicate that larger females are more likely to cannibalize males. Pre-copulatory sexual cannibalism could represent a maladaptive trait (Arnqvist & Henriksson, 1997; Johnson & Sih, 2005), since females reduce the number of potential mating partners before copulation, and some of them might die without mating. In fact, in our experiments, 6 females did not mate, 3 mated once, 3 two times and only one mated three times. It seems that these aggressive females are at risk of being unfertilized, or become monandrous, as they acquired on average one mating even when having multiple mating opportunities. Since the experiments were performed in the lab, we cannot necessarily extrapolate to natural conditions, although we have observed cases of sexual cannibalism in the field. Another possibility is that female cannibalism is a form of mate choice, eliminating males of inferior quality (Prenter et al., 2006; Kralj-Fišer et al., 2012). However, according to the amount of cannibalism observed, it would imply that most males were low-quality individuals. Nevertheless, selective pressures on males can lead to the antagonistic evolution of traits that reduce the risk of being cannibalized (Fromhage & Schneider, 2005; Bilde *et al.*, 2006; Burke & Holwell, 2021). Behavioural examples of how spider males avoid being cannibalized range from death feigning (Bilde *et al.*, 2006), mating while females consume part of the male (Neumann & Schneider, 2020), catapulting from females after mating (Zhang *et al.*, 2022), mating while females are foraging or moulting (Robinson & Robinson, 1980; Foellmer & Fairbairn, 2004; Fromhage & Schneider, 2005) to males using gifts as a shield (Bristowe, 1958; Toft & Albo, 2016). Here, we found that *T. biocellata* males have rapid matings with a single and short pedipalp insertion, which might be an adaptation for minimizing the time they spend in contact with females. A similar example is from the spider genus *Dolomedes* (Pisauridae), reported males having a single pedipalp insertion (Wojcicki, 1992) and short mating duration with highly cannibalistic females (Schoenberg *et al.*, 2022).

Secondary sexual traits can be lost if there is a loss or a relaxation in the pressures that maintain those traits (Morris *et al.*, 2005; Maughan *et al.*, 2006; Lahti *et al.*, 2009; Ellers *et al.*, 2012). We propose that the most parsimonious evolutionary scenario for our findings is that the nuptial gift appeared ancestrally in the spider family Trechaleidae or an early clade within the family (Albo *et al.*, 2017a), and a basal loss of female preference for the gift occurred in the genus *Trechaleoides*. This has led to the loss of the gift function in *T. keyserlingi* and the complete loss of the gift in *T. biocellata*. Given the lack of benefits detected in our study, it is surprising that *T. keyserlingi* males produce nuptial gifts. One possible explanation is that the male trait may persist for some time after the female preference disappeared if gift production is not too costly for the males. Alternatively, it may reflect that there are other functions of the nuptial gift that were not detected under laboratory conditions, or that functional benefits are expressed in specific ecological contexts. Further research on paternity success, ideally under ecologically relevant conditions, is needed to better understand the maintenance of nuptial gift production in this spider species.

**Figure 1. Mating success in conspecific encounters**. A) Mating occurrence, B) mating duration in min, and C) number of pedipalp insertions for *T. biocellata* males without nuptial gifts (this species does not produce gifts) and *T. keyserlingi* males with and without nuptial gifts. Boxplots: the black bold horizontal line represents the median, the box represents the first and third quartile, the whiskers represent minimum and maximum.



**Figure 2. Female responses in conspecific encounters.** A) Percentage of female attacks, B) percentage of sexual cannibalisms toward for *T. biocellata* males without nuptial gifts (this species does not produce gifts) and *T. keyserlingi* males with and without nuptial gifts.



**Figure 3**. **Female size and occurrence of sexual cannibalism in heterospecific encounters.** In these trials *T. biocellata* females were presented to *T. keyserlingi* males. The logistic regression plot shows the effect of female size on the probability of sexual cannibalism. The initial model included group (males with and without nuptial gift) in interaction with female and male size (mm) as fixed effects.



**Figure 4.** Diagram showing the currently most complete phylogeny of the spider family Trechaleidae, which includes 7 genera (Piacentini & Ramírez, 2019). Separately from the phylogeny, we have added the remaining 9 genera with unknown position in the cladogram.



**Table 1. Presence and function of nuptial gifts in conspecific encounters**. Data and statistical analysis for the comparison of the reproductive strategies of *T. biocellata* and *T. keyserlingi* showing the use of gift, mating occurrence (total number of matings), mean and SE of mating duration (min), number of pedipalp insertions, as well as the total of female attacks and cases of sexual cannibalism. N represents the sample size for the variables in each species. We used GLMM including species, female and male size (mm) as fixed effects, and experimental day, female and male ID as random effects. Note that comparison from the use of gift between species cannot be done due to the lack of gift in *T. biocellata*. Significant p-values are shown in bold.

						Fixed effects				Random effects				
					Species		Female size		Male size		Days Female ID		Days Male ID	
	N	Trechaleoides biocellata	N	Trechaleoides keyserlingi	Estimate	р	Estimate	р	Estimat	e p	Intercept Std Dev	Intercept Day	Intercept Std Dev	Intercept Day
Use of gift	124	0	208	110										
Mating occurrence	124	12	208	64	1.45	0.03	-5.37	0.04	0.87	0.85	1.16	0.17	1.39	0.15
Mating duration	12	$0.18\pm0.05$	64	$0.92\pm0.11$	0.84	0.006	-2.78	0.33	5.04	0.04	0.22	0.004	0.26	0.04
Number of insertions	12	$1\pm 0$	64	$2.41 \pm 0.30$	0.94	0.01	-1.59	0.63	4.22	0.12	0.06	0.03	0.06	0.02
Female attacks	124	83	208	29	-2.94	0.0001	8.17	0.21	2.70	0.55	0.92	0.02	0.89	0.04
Sexual cannibalism	83	50	29	4	-2.79	0.04	1.85	0.77	6.57	0.20	1.08	0.39	0.01	0.003

**Table 2**. **Data on** *Trechaleoides keyserlingi* in relation to presence/absence of nuptial gift. Total number of matings, mean and SD of mating duration (min), number of pedipalp insertions, as well as the total number of female attacks and sexual cannibalism. N represents the sample size for each variable. We performed GLMM including gift (presence/absence), experimental day (the day of experiment), the interaction between gift and experimental day, and female and male sizes (mm) as fixed effects. We used female and male ID as random effect. Significant p-values are shown in bold.

				Fixed effects						Random effects					
T. keyserlingi	N	Gift presence	Gift absence	Gift		Experimental day		Gift*Experimental day		Female size		Male size		Female ID	Male ID
				Estimate	e p	Estimate	р	Estimate	р	Estimate	р	Estimate	р	Intercept Std Dev	Intercept Std Dev
Mating occurrence	64	23	41	-2.71	0.0003	-0.24	0.0001	0.21	0.01	-5.69	0.60	3.0	0.66	0.86	0.003
Mating duration	64	$0.94 \pm 0.19$	$0.91 \pm 0.14$	-0.32	0.35	0.01	0.71	0.04	0.33	4.05	0.41	-7.66	0.11	0.21	0.21
Number of insertions	64	$2.55\pm0.53$	$2.34\pm0.36$	-0.06	0.56	0.003	0.57	0.003	0.79	1.14	0.50	-2.23	0.06	0.09	0.05
Female attacks	208	14	15	-0.64	0.45	-0.07	0.27	0.06	0.53	-11.8	0.20	-8.86	0.37	0.27	0.60
Sexual cannibalism	29	0	4	372.06	0.92	1.52	0.22	-189.5	0.93	-533.07	0.92	-758.08	0.21	0.62	0.09

#### REFERENCES

- Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. In: 2nd International Symposium on Information Theory (BN Petrov; F Csaki, ed), pp. 267–281. *Akadémiai Kiadó*.
- Albo, M.J. 2009. Selección sexual y citogenética en arañas donadoras de regalos nupciales (Trechaleidae y Pisauridae). Master Thesis, PEDECIBA. Montevideo, Uruguay.
- Albo, M.J., Bidegaray-Batista, L., Bechsgaard, J., Da Silva, E.L.C., Bilde, T. & Pérez-Miles, F. 2017a. Molecular phylogenetic analyses show that Trechaleidae and Lycosidae are sister groups. *Arachnology* 17: 169–176.
- Albo, M.J., Costa-Schmidt, L.E. & Costa, F.G. 2009. To feed or to wrap? Female silk cues elicit male nuptial gift construction in a semiaquatic trechaleid spider. *J. Zool.* 277: 284–290.
- Albo, M.J. & Costa, F.G. 2010. Nuptial gift-giving behaviour and male mating effort in the Neotropical spider *Paratrechalea ornata* (Trechaleidae). Anim. Behav. 79: 1031–1036.
- Albo, M.J., Macías-Hernández, N., Bilde, T. & Toft, S. 2017b. Mutual benefit from exploitation of female foraging motivation may account for the early evolution of gifts in spiders. *Anim. Behav.* 129: 9–14.
- Albo, M.J., Melo-González, V., Carballo, M., Baldenegro, F., Trillo, M.C. & Costa, F.G. 2014a. Evolution of worthless gifts is favoured by male condition and prey access in spiders. *Anim. Behav.* 92: 25–31.
- Albo, M.J., Toft, S. & Bilde, T. 2014b. Sexual selection, ecology, and evolution of nuptial gifts in spiders. In: Sexual Selection: Perspectives and Models from the Neotropics (R. Macedo & G. Machado, eds), pp. 183– 200.
- Albo, M.J., Winther, G., Tuni, C., Toft, S. & Bilde, T. 2011. Worthless donations: Male deception and female counter play in a nuptial gift-giving spider. *BMC Evol. Biol.* 11: 1–8.
- Andersson, M. & Simmons, L.W. 2006. Sexual selection and mate choice. Trends Ecol. Evol. 21: 296–302.
- Arnqvist, G. 2006. Sensory exploitation and sexual conflict. Philos. Trans. R. Soc. B Biol. Sci. 361: 375-386.
- Arnqvist, G. & Henriksson, S. 1997. Sexual cannibalism in the fishing spider and a model for the evolution of sexual cannibalism based on genetic constraints. *Evol. Ecol.* 11: 255–273.
- Arnqvist, G. & Nilsson, T. 2000. The evolution of polyandry: Multiple mating and female fitness in insects. *Anim. Behav.* 60: 145–164.
- Arnqvist, G. & Rowe, L. 2005. Sexual conflict. Princeton University Press.
- Assis, B.A. & Foellmer, M.W. 2019. Optimal ultra-short copulation duration in a sexually cannibalistic spider. *Behav. Ecol. Sociobiol.* 73: 1–8.
- Basolo, A.L. 1990. Female preference predates the evolution of the sword in swordtail fish. Science. 250: 808-810.
- Bilde, T., Tuni, C., Elsayed, R., Pekar, S. & Toft, S. 2006. Death feigning in the face of sexual cannibalism. *Biol. Lett.* 2: 23–25.
- Bilde, T., Tuni, C., Elsayed, R., Pekar, S. & Toft, S. 2007. Nuptial gifts of male spiders: sensory exploitation of the female's maternal care instinct or foraging motivation? *Anim. Behav.* 73: 267–273.
- Bradbury, J.W. & Vehrencamp, S.L. 2000. Economic models of animal coomunication. Anim. Behav. 59: 259-268.
- Bristowe, W.S. 1958. The World of Spiders. London: Collins.
- Burke, N.W. & Holwell, G.I. 2021. Male coercion and female injury in a sexually cannibalistic mantis: Coercive mating in a sexual cannibal. *Biol. Lett.* 17.
- Burley, N.T., Hamedani, E. & Symanski, C. 2018. Mate choice decision rules: Trait synergisms and preference shifts. *Ecol. Evol.* 8: 2380–2394.
- Calkins, J.D. & Burley, N.T. 2003. Mate choice for multiple ornaments in the California quail, *Callipepla californica*. *Anim. Behav.* 65: 69–81.
- Carico, J.E. 2005. Descriptions of two new spider genera of Trechaleidae (Araneae, Lycosoidea) from South America. *J. Arachnol.* 33: 797–812.
- Chaine, A.S. & Lyon, B.E. 2008. Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. *Science* 319: 459–462.
- Clutton-Brock, T. 2017. Reproductive competition and sexual selection. *Philosophical Transactions of the Royal* Society B: Biological Sciences.
- Costa-Schmidt, L.E., Carico, J.E. & De Araújo, A.M. 2008. Nuptial gifts and sexual behavior in two species of spider (Araneae, Trechaleidae, *Paratrechalea*). *Naturwissenschaften* 95: 731–739.
- Da Silva, E.L.C. & Lapinski, W. 2012. A new species of *Trechalea thorell*, 1869 (Araneae: Lycosoidea: Trechaleidae: Trechaleinae) from Costa Rica, with notes on its natural history and ecology. *Zootaxa* 64: 58–64.

Da Silva, E.L.C. & Lise, A.A. 2009. New record of nuptial gift observed in Trechalea amazonica. (Araneae,

Lycosoidea, Trechaleidae). Rev. Peru. Biol. 16: 119-120.

- Elgar, M.A. 1992. Sexual cannibalism in spiders and other invertebrates. *Cannibalism Ecol. Evol. among Divers.* taxa 128–155.
- Ellers, J., Toby Kiers, E., Currie, C.R., Mcdonald, B.R. & Visser, B. 2012. Ecological interactions drive evolutionary loss of traits. *Ecol. Lett.* 15: 1071–1082.
- Emlen, D.J. 1994. Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proc. R. Soc. B Biol. Sci.* 256: 131–136.
- Foellmer, M.W. & Fairbairn, D.J. 2004. Males under attack: Sexual cannibalism and its consequences for male morphology and behaviour in an orb-weaving spider. *Evol. Ecol. Res.* 6: 163–181.
- Fromhage, L. & Schneider, J.M. 2005. Safer sex with feeding females: Sexual conflict in a cannibalistic spider. *Behav. Ecol.* 16: 377–382.
- Ghislandi, P.G., Pekar, S., Matzke, M., Schulte-Döinghaus, S., Bilde, T. & Tuni, C. 2018. Resource availability, mating opportunity and sexual selection intensity influence the expression of male alternative reproductive tactics. *J. Evol. Biol.* 31: 1035–1046.
- Gwynne, D. 2008. Sexual conflict over nuptial gifts in insects. Annu. Rev. Entomol. 53: 83-101.
- Hebets, E.A. & Uetz, G.W. 2000. Leg ornamentation and the efficacy of courtship display in four species of wolf spider (Araneae: Lycosidae). *Behav. Ecol. Sociobiol.* 47: 280–286.
- Heinen-Kay, J.L. & Zuk, M. 2019. When does sexual signal exploitation lead to signal loss? *Front. Ecol. Evol.* 7: 1–11.
- Hill, G.E. 1994. Geographic variation in male ornamentation and female mate preference in the house finch: A comparative test of models of sexual selection. *Behav. Ecol.* 5: 64–73.
- Johnson, J.C. & Sih, A. 2005. Precopulatory sexual cannibalism in fishing spiders (*Dolomedes triton*): a role for behavioral syndromes. *Behav. Ecol. Sociobiol.* 58: 390–396.
- Kessel, E.L. 1955. The mating activities of balloon flies. Syst. Zool. 4: 97-104.
- Kirkpatrick, M. & Ryan, M.J. 1991. The evolution of mating preferences and the paradox of the lek. *Nature* 350: 33–38.
- Klein, A.L., Trillo, M.C. & Albo, M.J. 2012. Sexual receptivity varies according to female age in a Neotropical nuptial gift-giving spider. J. Arachnol. 40: 138–140.
- Kralj-Fišer, S., Schneider, J.M., Justinek, Ž., Kalin, S., Gregorič, M., Pekar, S., et al. 2012. Mate quality, not aggressive spillover, explains sexual cannibalism in a size-dimorphic spider. *Behav. Ecol. Sociobiol.* 66: 145– 151.
- Kuntner, M., Agnarsson, I. & Li, D. 2015. The eunuch phenomenon: adaptive evolution of genital emasculation in sexually dimorphic spiders. *Biol. Rev.* 90: 279–296.
- Kuntner, M., Coddington, J.A. & Schneider, J.M. 2009. Intersexual arms race? Genital coevolution in nephilid spiders (Araneae, Nephilidae). *Evolution* 63: 1451–1463.
- Lahti, D.C., Johnson, N.A., Ajie, B.C., Otto, S.P., Hendry, A.P., Blumstein, D.T., et al. 2009. Relaxed selection in the wild. *Trends Ecol. Evol.* 24: 487–496.
- Lapinski, W. & Tschapka, M. 2009. Erstnachweis von Brautgeschenken bei *Trechalea sp*. (Trechaleidae, Araneae) in Costa Rica. *Arachne* 14: 4–13.
- LeBas, N.R. & Hockham, L.R. 2005. An invasion of cheats: The evolution of worthless nuptial gifts. *Curr. Biol.* 15: 64–67.
- Lewis, S. & South, A. 2012. The evolution of animal nuptial gifts. In: Advances in the Study of Behavior, pp. 53–97.
- Marchetti, K. 1993. Dark habitats and bright birds illustrate the role of the environment in species divergence. *Nature* 362: 149–152.
- Martínez-Villar, M., Germil, M., Pavon-Pelaz, C., Costa-Schmidt, L.E. & Albo, M.J. 2020. Empty nuptial gifts: A further step in the evolution of deception in spiders? J. Arachnol. 48: 214–217.
- Martínez Villar, M., Trillo, M.C. & Albo, M.J. 2021. Ineffective nuptial gifts suggest female emancipation from sensory exploitation. *Behav. Ecol. Sociobiol.* 75: 61.
- Maughan, H., Callicotte, V., Hancock, A., Birky, C.W., Nicholson, W.L. & Masel, J. 2006. The population genetics of phenotypic deterioration in experimental populations of *Bacillus subtilis*. *Evolution* 60: 686–695.
- Maxwell, M.R. & Prokop, P. 2018. Fitness effects of nuptial gifts in the spider *Pisaura mirabilis*: examination under an alternative feeding regime. *J. Arachnol.* 46: 404–412.
- Miller, J.A. 2007. Repeated evolution of male sacrifice behavior in spiders correlated with genital mutilation. *Evolution* 61: 1301–1315.
- Morris, M.R., Moretz, J.A., Farley, K. & Nicoletto, P. 2005. The role of sexual selection in the loss of sexually

selected traits in the swordtail fish Xiphophorus continens. Anim. Behav. 69: 1415–1424.

- Neumann, R. & Schneider, J.M. 2020. Males sacrifice their legs to pacify aggressive females in a sexually cannibalistic spider. *Anim. Behav.* 159: 59–67.
- Ödeen, A. & Björklund, M. 2003. Dynamics in the evolution of sexual traits: Losses and gains, radiation and convergence in yellow wagtails (*Motacilla flava*). *Mol. Ecol.* 12: 2113–2130.
- Omland, K.E. 1994. Character congruence between a molecular and a morphological phylogeny for dabbling ducks (ANAS). *Syst. Biol.* 43: 369–386.
- Omland, K.E. 1997. Examining two standard assumptions of ancestral reconstructions: Repeated loss of dichromatism in dabbling ducks (*Anatini*). *Evolution* 51: 1636–1646.
- Pandulli-Alonso, I., Quaglia, A. & Albo, M.J. 2017. Females of a gift-giving spider do not trade sex for food gifts: A consequence of male deception? *BMC Evol. Biol.* 17: 1–8.
- Parker, G.A. 1979. Sexual selection and sexual conflict. In: Sexual Selection and Reproductive Competition in Insects (M. B. S. & N. B. A., eds), pp. 123–163. Academic press, New York.
- Piacentini, L.N. & Ramírez, M.J. 2019. Hunting the wolf: A molecular phylogeny of the wolf spiders (Araneae, Lycosidae). *Mol. Phylogenet. Evol.* 136: 227–240. Elsevier Inc.
- Prenter, J., MacNeil, C. & Elwood, R.W. 2006. Sexual cannibalism and mate choice. Anim. Behav. 71: 481-490.
- Preston-Mafham, K.G. 1999. Courtship and mating in *Empis (Xanthempis) trigramma* Meig., *E. tessellata F. and E. (Polyblepharis) opaca* F. (Diptera: Empididae) and the possible implications of "cheating" behaviour. *J. Zool.* 247: 239–246.
- Proctor, H.C. 1991. Courtship in the water mite Neumania papillator: Males capitalize on female adaptations for predation. *Anim. Behav.* 42: 589–598.
- Prokop, P. & Maxwell, M.R. 2009. Female feeding regime and polyandry in the nuptially feeding nursery web spider, *Pisaura mirabilis*. *Naturwissenschaften* 96: 259–265.
- R Team Core. 2021. A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. R Foundation for Statistical Computing, Vienna, Austria.
- Reid, M.L. & Weatherhead, P.J. 1990. Mate-choice criteria of Ipswich sparrows: the importance of variability. *Anim. Behav.* 40: 538–544.
- Reimchen, T.E. 1989. Loss of nuptial color in threespine sticklebacks (*Gasterosteus aculeatus*). Evolution 43: 450–460.
- Rengifo-Gutiérrez, L., Albo, M.J. & Santa, L.D. 2021. The unknown *Enna* (Araneae: Trechaleidae), new species and first record of wrapped nuptial gifts in the genus. *J. Arachnol.* 48: 242–248.
- Robinson, M.H. & Robinson, B. 1980. Comparative studies of the courtship and mating behavior of tropical araneid spiders. *Comp. Stud. Court. mating Behav. Trop. araneid spiders* 36.
- Rowe, L. & Arnqvist, G. 2002. Sexually antagonistic coevolution in a mating system: Combining experimental and comparative approaches to address evolutionary processes. *Evolution* 56: 754–767.
- Ryan, M.J., Fox, J.H., Wilczynski, W. & Rand, A.S. 1990. Sexual selection for sensory exploiation in the frog *Physalaemus pustulosus. Nature* 343: 66–67.
- Sakaluk, S.K. 2000. Sensory explotation as an evolutionary origin to nuptial food gifts in insects. *Proc. R. Soc. London. Ser. B Biol. Sci.* 267: 339–343.
- Schneider, J.M. 2014. Sexual cannibalism as a manifestation of sexual conflict. Cold Spring Harb. *Perspect. Biol.* 6: 1–16.
- Schoenberg, D., Hebets, E.A. & Sullivan-Beckers, L. 2022. Mating and cannibalism dynamics of the fishing spider Dolomedes scriptus Hentz, 1845 (Araneae: Pisauridae). J. Arachnol. 50: 55–56.
- Servedio, M.R. & Boughman, J.W. 2017. The role of sexual selection in local adaptation and speciation. *Annu. Rev. Ecol. Evol. Syst.* 48: 85–109.
- Stålhandske, P. 2001. Nuptial gift in the spider *Pisaura mirabilis* maintained by sexual selection. *Behav. Ecol.* 12: 691–697.
- Starrett, J., McGinley, R.H., Hebets, E.A. & Bond, J.E. 2022. Phylogeny and secondary sexual trait evolution in *Schizocosa* wolf spiders (Araneae, Lycosidae) shows evidence for multiple gains and losses of ornamentation and species delimitation uncertainty. *Mol. Phylogenet. Evol.* 169.
- Tinghitella, R.M. & Zuk, M. 2009. Asymmetric mating preferences accommodated the rapid evolutionary loss of a sexual signal. *Evolution* 63: 2087–2098.
- Toft, S. & Albo, M.J. 2015. Optimal numbers of matings: The conditional balance between benefits and costs of mating for females of a nuptial gift-giving spider. *J. Evol. Biol.* 28: 457–467.
- Toft, S. & Albo, M.J. 2016. The shield effect: Nuptial gifts protect males against pre-copulatory sexual cannibalism. *Biol. Lett.* 12: 20151082.

- Trillo, M.C. & Albo, M.J. 2019. Another one bites the gift: sexual behaviour in a *Trechaleoides* species. *Arachnology* 18: 250–252.
- Vahed, K. 2007. All that glisters is not gold: Sensory bias, sexual conflict and nuptial feeding in insects and spiders. *Ethology* 113: 105–127.
- Vahed, K. 1998. The function of nuptial feeding in insects: A review of empirical studies. *Biol. Rev.* 73: 43–78.
- Warwick, S., Vahed, K., Raubenheimer, D. & Simpson, S.J. 2009. Free amino acids as phagostimulants in cricket nuptial gifts: Support for the "Candymaker" hypothesis. *Biol. Lett.* 5: 194–196.
- Wiens, J.J. 2001. Widespread loss of sexually selected traits: How the peacock lost its spots. *Trends Ecol. Evol.* 16: 517–523.
- Wilder, S.M. & Rypstra, A.L. 2008. Sexual size dimorphism predicts the frequency of sexual cannibalism within and among species of spiders. *Am. Nat.* 172: 431–440.
- Will, M.W. & Sakaluk, S.K. 1994. Courtship feeding in decorated crickets: Is the spermatophylax a sham? *Anim. Behav.* 48: 1309–1315.
- Wojcicki, J. 1992. A description of the reproductive biology of the fishing spider *Dolomedes triton* (Walck.) (Araneae: Pisuridae) in central Alberta.
- Zhang, S., Liu, Y., Ma, Y., Hao, W., Yao, Z., Matjaž, K., et al. 2022. Male spiders avoid sexual cannibalism with a catapult mechanism. *Curr. Biol.* 32: R354–R355.
- Zuk, M., Rotenberry, J.T. & Tinghitella, R.M. 2006. Silent night: Adaptive disappearance of a sexual signal in a parasitized population of field crickets. *Biol. Lett.* 2: 521–524.
- Zuur, A.F., Ieno, E.N. & Elphick, C.S. 2010. A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.* 1: 3–14.

# **Chapter 3**

# Female sexual cannibalism and monogamy may explain reduced heterozygosis and changes in male sexual behavior

**Martínez Villar M.**, J. Bechsgaard, T. Bilde, M.J. Albo & I.H. Tomasco (in preparation). Female sexual cannibalism and monogamy may explain reduced heterozygosis and changes in male sexual behavior.

### Resumen

El canibalismo sexual puede disminuir el tamaño efectivo de la población (Ne) y la heterocigosidad (Hobs) porque los machos adultos son eliminados del pool reproductivo. Esta reducción es extrema en el canibalismo sexual pre-copulatorio porque algunos machos y hembras pueden permanecer sin aparearse al final de la temporada, sesgando la reproducción a unos pocos individuos. Las reducciones en Ne y Hobs conducen a una disminución en la supervivencia y/o reproducción individual y la continuidad de la población y, eventualmente, pueden limitar la capacidad de los machos para exponer rasgos sexuales. Nuestro objetivo fue comprender las diferencias genéticas entre las dos especies del género de arañas Trechaleoides. Estas arañas difieren notablemente en sus estrategias de apareamiento, ya que T. biocellata es una especie cuyos machos no ofrecen regalos nupciales y las hembras incurren en altas tazas de canibalismo pre-copulatorio, lo que conduce a un sistema de apareamiento monógamo. Por el contrario, T. keyserlingi es una especie cuyos machos ofrecen regalos nupciales y sus hembras no son caníbales, siendo un sistema de apareamiento polígamo. Usando transcriptomas, evaluamos y comparamos los niveles de Hobs, y cuantificamos la selección de purificación a través de relaciones de sustitución no sinónimas y sinónimas (relación dN / dS). Encontramos que T. biocellata tiene valores más bajos de variación genética, lo que es compatible con los rasgos de comportamiento (canibalismo pre-copulatorio y monogamia). Además, en esta especie encontramos proporciones más altas de dN/dS en comparación con T. keyserlingi sugiriendo una débil selección hacia las mutaciones levemente deletéreas. Además, evaluamos genes que se expresan diferencialmente entre las dos especies y encontramos diferencias en aquellos relacionados con la producción de energía metabólica, lo que sugiere que ambas especies difieren en sus tasas tasas metabólicas. En general, los resultados genéticos son consistentes con la existencia de canibalismo sexual, que puede desempeñar un papel principal en la reducción del éxito en la reproducción y la ausencia del regalo nupcial del macho en esta especie.

Palabras clave: canibalismo sexual pre-copulatorio, efectividad de la selección, variación genética

### Abstract

Sexual cannibalism can decrease the effective population size (Ne) and heterozygosity (Hobs) because adult males are being removed from the reproductive pool. This reduction is extreme in pre-mating sexual cannibalism because some males and females can remain unmated at the end of the season, skewing reproduction to a few individuals. Reductions in the Ne and Hobs lead to a decrease in individual and population fitness and can eventually limit males' ability to display sexual ornaments. Here, we aimed to understand the genetic differences between the two species of the spider genus Trechaleoides. These spiders markedly differ in their mating strategies, as T. biocellata is a non-gift-giving species with extreme premating cannibalism leading to a monogamous mating system. In contrast, T. keyserlingi is a gift-giving and non-cannibalistic species with a polyandrous mating system. Using transcriptomes we evaluated and compared the levels of Hobs, and quantified purifying selection through non-synonymous and synonymous substitution ratios (dN/dS ratio). We found that T. biocellata have lower values of genetic variation, which is compatible with the behavioral traits (pre-mating cannibalism and monogamy) and higher ratios of dN/dS compared to T. keyserlingi, suggesting reduced selection against weakly deleterious mutations. Additionally, we assessed genes that are differentially expressed between the two species and we found differences in those related the production of metabolic energy, suggesting that both species differs in their metabolic rates. Overall, the genetic results are consistent with the existence of sexual cannibalism, which may play a main role in leading to a reduction in fitness and the absence of the male nuptial gift in this species.

Key words: pre-mating sexual cannibalism, effectiveness of selection, genetic variation

# Introduction

Female sexual cannibalism is common during courtship and mating of several predator species (Lawrence, 1992; Schneider & Elgar, 2001; Johnson & Sih, 2005; Persons & Uetz, 2005; Walker & Holwell, 2015). This behavior occurs when a female consumes a male before, during or after mating. Because commonly males are polygamous, regardless of the timing in which it occurs, an adult male is permanently removed from the population, which over time will reduce the number of available reproductive males (Lawrence, 1992; Hurd *et al.*, 1994; Fisher *et al.*, 2018). This effect is highly dramatic in species with pre-mating sexual cannibalism, as not only is the male removed from the population but also the female misses a mating opportunity and consequently potentially reduces egg fertilization (Elgar & Schneider, 2004; Fisher *et al.*, 2018). Therefore, pre-mating sexual cannibalism increases the proportion of adults that die without mating and can extremely decreases individual mating rates, to the point that in some species females may be monogamous (Fisher *et al.*, 2018).

Cannibalistic and monogamous species are therefore, expected to have reduced effective population sizes (Ne), which consequently reduce the population genetic diversity. This is because reproduction is skewed to a few individuals (Montano, 2016) and creates a double fold effect, as genetic diversity will be affected by the lack of potential mates and the low genetic diversity of the existent ones. In this scenario, and depending the numbers of mating upon polygamy, Ne is predicted to be approximately two-thirds the size in monogamous species compared to polygamous species (Balloux & Lehmann, 2003) that increase the genetic variation across generations (Sugg & Chesser, 1994; Karl, 2008; Taylor et al., 2014). Reductions in genetic variation by the increases of the intensity of genetic drift in fixing/eliminating alleles (Kimura, 1983; Hill & Zhang, 2004; Frankham, 2012) can lead to the accumulation of weak deleterious mutations and decline variations in individuals' phenotype and fitness success (Charlesworth & Charlesworth, 1987; Lynch & Gabriel, 1990; Kempenaers, 2007; Bolund et al., 2010; Holman & Kokko, 2013). In particular, low heterozygosity has been shown to directly impact reproduction by affecting the expression of costly sexual traits and mating success, as well as survival through individuals' condition, disease and parasite resistance, and the expression of deleterious recessives alleles connected to developmental stability (Coltman et al., 1999; Daniels & Walters, 2000; Slate et al., 2000; Kruuk et al., 2002; MacDougall-Shackleton et al., 2005; Kempenaers, 2007; Reid et al., 2007; Cohas et al., 2009; Mainguy et al., 2009; Ferrer et al., 2015).

In this way, highly cannibalistic species with reduced genetic variation, can experience modifications in the expression of reproductive traits. In fact, the good-genes as-heterozygosity hypothesis (Brown, 1997) predicts that the ability of males to produce and display ornaments is positively correlated with genetic diversity as such traits may reflect males' heterozygosity (reviewed in Kempenaers, 2007). A reduction in genetic diversity has been related to a decrease in males' sexual traits, such as sperm quality and testis size in wild rabbits (Gage et al., 2006), song rate, beak color and body size in zebra finches (Bolund et al., 2010), as well as total sperm number, tail size and iridescent coloration in guppies (Zajitschek & Brooks, 2010). The fitness reduction can be assessed by the effectiveness of selection throughout the nonsynonymous and synonymous substitutions (dN/dS) ratios (Strohm et al., 2015; Biswas et al., 2016; Bechsgaard et al., 2019; Lin et al., 2019; Martinez-Gutierrez & Aylward, 2019). Basically, a reduction in Ne increases the effect of genetic drift and reduces the effectiveness of purifying selection on weakly deleterious mutations. This increases the dN/dS ratio because more slightly deleterious variants are fixed by drift. For instance, higher dN/dS ratios were found in social versus subsocial spiders (Settepani et al., 2016), and in selfing plants species compared to outcrossing ones (Slotte et al., 2010; Qiu et al., 2011; Brandvain et al., 2013; Hazzouri et al., 2013).

Here, we used the spider genus *Trechaleoides* to explore the degree of genetic differentiation between the two single species, *T. keyserlingi* and *T. biocellata*, that markedly differ in their sexual behavior. First, *T. biocellata* females are very aggressive and cannibals. Under multiple mating opportunities, these females very often attempt pre-mating sexual cannibalism reducing both sexes' mating numbers (range: 0-3). This suggests that females and males are almost monogamous (Martinez Villar et al. in rev). In contrast, this is not the case for *T. keyserlingi* males and females that mate multiple times, having a polyandrous mating system (Martínez Villar *et al.*, 2021; in

revision.). Second, *T. keyserlingi* males produce nuptial gifts, but not *T. biocellata* males (Martínez Villar *et al.*, in revision; Trillo & Albo, 2019). This is outstanding as the genus belongs to the family Trechaleidae known to have species with males producing wrapped nuptial gifts (Costa-Schmidt *et al.*, 2008; Da Silva & Lise, 2009; Lapinski & Tschapka, 2009; Da Silva & Lapinski, 2012; Trillo & Albo, 2019; Martínez-Villar *et al.*, 2020; Rengifo-Gutiérrez *et al.*, 2021), and the sexual trait has been suggested ancestral in the family (Albo et al. 2017). Third, females from both species lack the preference for nuptial gifts (Martínez Villar *et al.*, in revision). This is, *T. keyserlingi* males offer nuptial gifts but gain no reproductive or survival benefit compared to males without a gift (Martínez Villar *et al.*, 2021; in revision.). On the other hand, a test exposing *T. biocellata* females to gift-giving males showed that these females are not attracted to nuptial gifts (no pre-existent bias). Hence, the absence of female preferences in the genus and the absence of nuptial gifts in the cannibalistic species *T. biocellata* have been proposed as evolutionary losses by relaxed selection on female choice (Martínez Villar *et al.*, in revision).

We obtained transcriptomes from a set of males of both *Trechaleoides* species and the spider *Paratrechalea ornata* as outgroup to evaluate levels of genetic diversity, the intensity of selective processes, and the differentially expressed genes between the two species. Our main objectives were to: 1) compare the heterozygosity of both species to assess the impacts of pre-mating sexual cannibalism on the genetic diversity and discuss possible implications on the males sexual behavior; 2) evaluate whether there is a relaxation of the strength of the purifying selection between the two species, by estimating the ratio of non-synonymous (dN) and synonymous substitutions (dS); 3) identify the and the genes under positive selection in both species and link them to the known differences between them; and 4) assess the genes underlying the differentiation of the two species through a cross-species differential gene expression analysis. Finally, we studied the putative functionalization of the positively selected and differential expressed genes via gene ontology categories (e.g., biological process).

# Methods

### Spider collections and RNA extractions

We collected 14 adult males from the three populations where *T. keyserlingi* and T. *biocellata* cooccur (Figure 1) in Uruguay. This allowed us to have a representation of the intraspecific variation with six individuals per species, two per population. We additionally collected two individuals from *Paratrechalea ornata* as an outgroup. Once in the laboratory, we sacrificed spiders by freezing them for two minutes at -80°C, then immersed them in liquid N2 to preserve RNA and then stored individuals in -80°C until the RNA extraction day. We extracted RNA from half of the cephalothorax using RNAasy Kit–QIAGEN following the manufacturer's recommendations. Then, we stabilized the extractions in an ethanol (EtOH) precipitation and finally, we sent them to Macrogen Inc. for paired-end sequencing on Illumina TruSeq platform (101 bp; 40 – 50 million reads).

### Assembly, assessment of completeness, specific assignment and phylogeny reconstruction

We evaluated raw reads quality and content using FastQC and discarded the low-quality ones. De novo assemblies were performed in Trinity v2.12.0 (Grabherr *et al.*, 2011) for each individual and species using default parameters. We used BUSCOv5.2.2 (Benchmarking Universal Single-Copy Orthologous) (Manni *et al.*, 2021) to evaluate the proportion and quality (complete, fragmented and duplicated) of the spider orthologs genes present in each *de novo* assembly (*Parasteatoda tepidariorum* reference). We then, used CD-HIT (Li & Godzik, 2006; Fu *et al.*, 2012) EST mode with a threshold of 0.95 on each individual and species assembly to reduce sequence redundancy and improve the performance of the downstream analysis. From now on, we will refer to these last CD-HIT outputs assemblies as just assembly. FastQC results showed that the mean of all raw read bases was > 30, length mean was 101 and no Illumina adapters were found. Individual and species assembly statistics are shown in Table S1. Individuals BUSCO completeness average of 78.8%

(rang 64.2% - 94%), while for species averaged 95.4% (range 94.9% - 95.8%). Full BUSCO results for individuals and species are shown in Figure S1A, B.

We confirmed the correct specific assignment of the individuals analyzed and corroborated the specific status of each species by using different complementary methods: phylogenetic reconstruction, Principal Component Analysis (PCA), and an ADMIXTURE analysis. For phylogenetic reconstruction, we first retrieved the orthologs of all individuals. We used Transdecoder v5.5.0 (Haas & Papanicolaou, 2016) on the individual's assembly to predict the longest isoforms and the nucleotide coding sequences (CDS), then we used these CDS to identify putative "1 to 1" nucleotide orthologs shared between all individuals using Orthofinder v 2.5.4 (Emms & Kelly, 2019). From the 277008 coding transcripts from the 14 individuals submitted to OrthoFinder to identify orthologous groups, OrthoFinder identified 268185 genes (96.8% of the total) to 24514 orthogroups (containing both orthologs and paralogs). There were 6680 (27.2%) orthogroups with all 14 individuals present, and 1527 of these were 1-to-1 orthologs between all species, all of which were used for the phylogenetic reconstruction. We aligned the sequences using MUSCLE v 3.8 (Edgar, 2004) and performed Maximum Likelihood trees for each gene in IQ-TREE v1.6.12 (Nguyen et al., 2015) with ultrafast bootstrap approximation (Nguyen et al., 2015; Hoang et al., 2018) and ModelFinder method (Kalyaanamoorthy et al., 2017). Finally, we used ASTRAL (Rabiee et al., 2019) with default parameters to infer the population tree based on these gene trees. The phylogeny successfully groups each species as monophyletic (Figure S2).

We then performed the PCA component analysis in R software (R Team Core, 2022) using the base function. For admixture, we firstly mapped all 14 individuals to our *P. ornata* assembly reference using bowtie2 (Langmead & Salzberg, 2012). Secondly, we merged all vcf files with bcftools, filtered it by quality and depth (>20) using VcfFilter and selected one random SNP per contig to avoid skewing the analysis towards longer genes. We finally converted the final vcf file to a ped file, using vcftools and Plink1.9 (Purcell *et al.*, 2007) and carried out the analysis using *ADMIXTURE* (Alexander *et al.*, 2009) with K = 7 (7 populations). The PCA analysis grouped the individuals in their corresponding species. The first and second PCA accounted for 22.2% and 21.7% of total variation, respectively (Figure S3A). The PC1 component separated the species *P. ornata* from the species *T. biocellata*, while PC2 component separated *T. biocellata* from *T. keyserlingi*. In line with previous results, admixture at K=3 grouped individuals according to the assigned species, which is maintained for larger K, with no evidence of introgression between species. (Figure S3B)

### Individual heterozygosity and population-species mean heterozygosity

We aimed to assess individual heterozygosity ( $H_{obs}$ ) and the mean heterozygosity for populations and species. First, we mapped all individuals to their corresponding species assembly using bowtie2 (Langmead & Salzberg, 2012). Second, we performed the SNP calling using Bcftools (Danecek *et al.*, 2021) package with a combination of mpileup and call methods. Based on all sites, we only used those with at least 20 of coverage and quality. We estimated each  $H_{obs}$  as the number of heterozygote sites divided by the number of total sites. To test the difference in  $H_{obs}$  between populations and species, we used a Generalized Linear Model (GLM) with Gaussian distribution with species as a fixed effect in interaction with population and log.  $H_{obs}$  was square-root transformed to fulfil assumptions of parametric analysis.

### Evaluation of purifying selection and genes under positive selection

To evaluate selective regimens and genes under positive selection, we worked at the species level. For this, we obtained the orthologs for the three species, using Orthofinder and the same procedure described above but using species' assembly. Afterwards, we translated the nucleotide sequences to their corresponding amino acid sequences and aligned them using MUSCLE v 3.8 (Edgar, 2004). We created nucleotide sequence alignments using the protein alignment as a reference in PAL2NAL v14 (Suyama *et al.*, 2006) and performed a test of positive selection at the species level

using the Codeml program implemented in the PAML package (Yang, 2007). First, we evaluated the evolutionary rate of the branches in the species tree, by concatenating all individuals' genes alignments and estimating rates of nonsynonymous (dN) substitutions, synonymous (dS) substitutions, and their dN/dS ratio for all branches. With PAML, we bootstrapped the sequence (n = 1000) and using the branch-free ratio model (model = 1) we estimated an independent dN, dS and dN/dS for each branch in the phylogeny (Yang & Nielsen, 1998). We, then, estimated 95% confidence limits of dN, dS, dN/dS for each branch using R software. Second, to detect genes under positive selection in the branch leading to each Trechaleoides species, we applied the branch-site model for all genes separately, using each species alternatively as the foreground branch. We ran codeml for model 2 (branch-site estimation), NSites 2 (selection), and fix\_omega 0 (for dN/dS estimation). We evaluated model significance by comparing this model with the branch-site null model (model 2, NSites 2, and fix\_omega 1) by using log-likelihood ratios  $(2x\Delta \ln L)$  (LRT) with strict Bonferroni correction for multiple testing. A significant result from the branch-sites model is indicative that a subset of the sites in the coding gene has undergone episodic positive selection, with the selected sites providing a molecular adaptation for the species studied. For all models, we used the phylogenetic tree inferred by Orthofinder and anormal dN, dS and dN/dS values were checked and removed. We functionally annotate and perform the GO enrichment analysis for the selection test and for the cross-species differential gene expression analysis. We obtained the genes GO accession numbers for the gene sequences that passed the LRT for possible positive selection from the branch-site model and T. biocellata and T. keyserlingi full transcript CDS sequences using eggNOG-mapper v2 webserver (Cantalapiedra et al., 2021) with default settings. Finally, we assessed the enrichment analysis using AgriGo (Tian et al., 2017) with default settings at the biological process level.

### Cross-species differential expression analysis

We assessed differential gene expression between *T. biocellata* and *T. keyserlingi*, using the genome of the spider *Parasteatoda tepidariorum* as a reference (GCF\_000365465.1). We extracted the *P. tepidariorum* coding sequences from the gff file and using GffRead (Pertea & Pertea, 2020) and we blasted each *Trechaleoides* species *de novo* assembly to *P. tepidariorum* CDS using tBlastx. We retained those best hits matches that were common for the two species and mapped each individual to the corresponding species assembly following Trinity's guide and using bowtie2 (Langmead & Salzberg, 2012) and RSEM method (Li & Dewey, 2011). With this procedure we quantified transcript abundance for each individual and constructed the raw count and gene length matrix.

We tested for cross-species differential gene expression using Deseq2 (Love *et al.*, 2014) v1.32.0. We treated the six individuals from each species as replicates and used the raw count matrix and gene length matrix as inputs. We consider single-copy genes with an absolute log-fold change (log-ratio of a gene or transcript expression values in two different conditions) greater than or equal to 0.5 and alpha of 0.05 as differentially expressed. We also estimated shrunken log-fold changes using the lfcShrink function with apeglm (Zhu *et al.*, 2019) method and lfcTreshold = 0.5, these last genes were used for ontology enrichment. For the differentially expressed genes, we obtained the sequences GO numbers and *P. tepidariorum* CDS GO numbers and performed the GO enrichment analysis as for the positively selected genes.

# Results

Individual heterozygosity and population-species mean heterozygosity

The  $H_{obs}$  was significant different among *Trechaleoides* species (Table 1), being lowest in *T. biocellata* (range: 0.00037 to 0.00058) compared to *T. keyserlingi* (range: 0.00070 to 0.00087) while in *P. ornata* ranged from 0.00037 to 0.00087 (Figure 2). At the population level, the San Miguel population has the lowest  $H_{obs}$  in both species compared with other two populations (Table 1; Figure 2).

### Evaluation of purifying selection and genes under positive selection

From the 80774 coding transcripts submitted to OrthoFinder to identify orthologous groups, it identified 70183 genes (86.9% of the total) and 20355 orthogroups (containing both orthologs and paralogs). There were 13873 (17.1%) orthogroups shared among the three species, and 8037 of these were 1-to-1 orthologs all of which were analyzed for positive selection tests. The free ratios model indicated that the mean dN/dS ratio of the two *Trechaleoides* species is higher than the one from *P. ornata*. Within *Trechaleoides*, *T. biocellata* had higher values of dN/dS compared to that of *T. keyserlingi*, although with no significative differences in the dN and dS estimates. Full dN, dS and dN/dS values for each branch are reported in Table 2.

In the branch-site model, we found statistical support for increased positive selection in 119 and 125 orthogroups in the branch leading to *T. biocellata* and *T. keyserlingi*, respectively (1.4% and 1.5% of the total orthogroups analyzed). In *T. biocellata*, we retrieved the annotation and GO number of 96 genes clustered in the biological processes related to cellular morphogenesis, anatomical structure morphogenesis and cellular developmental components (Table S2). While in *T. keyserlingi* we retrieved the annotation and GO number of 87 genes that most were clustered in the biological processes (Table S2).

### Cross-species differential expression analysis

From the 9942 "1 to 1" orthologs from the two *Trechaleoides* species and the reference *P*. *tepidariorum*, 900 were differentially expressed (log-fold change  $\geq 0.5$  and alpha = 0.05) (Figure 3), of which 438 were upregulated and 462 were downregulated. We annotated and retrieved the GOs number of 525 sequences and we found differences mainly in the RNA processing and in the energy production (electron transport chain) (Table S3). Some upregulated genes included troponins, transcriptional repressors, hydrolytic activity, mitochondrial ribosomal protein, and electron chain transports. Downregulated genes included functions like protein dimerization activity, methyltransferase activity, ion channel activity, cholesterol metabolic process, extracellular matrix organization and transmembrane transporter activity.

### Discussion

Our findings reveal that the cannibalistic and predominantly monogamous *T. biocellata* has significantly lower heterozygosity (about two-thirds) and lower relaxation of purifying selection compared to her non-cannibalistic and polyandrous sister species *T. keyserlingi*. This agrees with our prediction based on the differences in behavioral traits and mating systems (Martínez Villar *et al.*, in revision). Additionally, the reduction in the observed heterozygosity estimates in the species *T. biocellata* is consistent with the hypothesis that this could limit their ability to produce or display sexual ornaments (Brown, 1997). Several sexual traits are reported to be positively correlated with male heterozygosity, such as feathers length and color, song repertoires (Aparicio *et al.*, 2001; Foerster *et al.*, 2003; Ferrer *et al.*, 2015). Here, we discuss the potential implications of the genetic differences on species reproduction.

Mathematical models predict that high levels of pre-mating sexual cannibalism can lead to low population growth (Fisher *et al.*, 2018). On the other side, the Ne of strict monogamous species is predicted to be two-thirds size compared to polygamous ones (Balloux & Lehmann, 2003). Both phenomena reduce genetic variability because reproduction is skewed to fewer individuals. When contrasting the H<sub>obs</sub> values, we observed that those from the outgroup *P. ornata* are intermediate between the two *Trechaleoides* species. This a very interesting result and fits with recent results found in *P. ornata*, in which using molecular markers it was corroborated that double-mated females bias the paternity towards one male (Albo *et al.*, in revision), at least in the first clutch. Thus, to some extent, these polyandrous females are able to reduce the genetic diversity of the

clutch. At last, cryptic female choice is the main mechanism shaping genetic diversity of offspring (Eberhard, 1994, 2004; Welke & Schneider, 2009; Firman *et al.*, 2017), and thus, from our genetic results it is expected *T. keyserlingi* females exert the weakest paternity bias. This effect in the heterozygosity can be also strengthened by the action of demographic (i.e., life history) or ecological (i.e., habitat fragmentation) effects (Martinez *et al.*, 2018; Schlaepfer *et al.*, 2018; Degen *et al.*, 2021). For instance, at the population level we observed that both species have the lowest heterozygosity in San Miguel. We do not know which factor is affecting individuals in this locality, but it is seeming that this additionally erodes genetic variability, maybe due to increased random genetic drift, inbreeding, and reductions in gene flow (Frankham, 2012).

We found evidence of higher dN/dS ratio in T. biocellata than in T. keyserlingi, which would reflect a relaxation of the intensity of the purifying selection in the former species. Purifying selection is the dominant force responsible for the evolution of protein-coding sequences such as those studied here. It implies that, under the Neutral Theory, positive selection is expected to be rare and beneficial alleles that appear in a population rapidly go to fixation making more of the comparable variation lost (Jensen et al., 2019). Assuming that the cannibalistic and monogamous T. biocellata could have reduced effective population size (Ne), these small populations are expected to decrease the efficacy of selection against weakly deleterious mutations increasing their accumulation compared with larger populations (Charlesworth & Wright, 2001; Charlesworth & Meagher, 2003; Settepani et al., 2016). The accumulation of deleterious mutation can decline population fitness and lead the population to the extinction vortex or mutational meltdown (Gilpin & Soulé, 1986; Lynch & Gabriel, 1990). Interestingly, the values of dN/dS ratios were greater in the Trechaleoides species than in P. ornata. A possible interpretation would be that P. ornata could be under regimens of stronger purifying selection than Trechaleoides species. In small populations, drift might also have random effects on female choice, possibly causing changes in female preferences for sexual traits (Wiens, 2001). The fact that the Trechaleoides species present a relaxation in purifying selection pressures, could be a clue to understand the loss of female preferences for nuptial gifts in the genus (Martínez Villar et al., in revision.). Although we found 244 (119 in T. biocellata and 125 in T. keyserlingi) genes under positive selection between species, we could not find a function associated with the known behavioral differences. At this point, we can only state that these genes have a role in determining different morphological structures related to cellular development, cellular communication and transcription factors in T. biocellata and metabolic process in T. keyserlingi.

When analyzing the differential gene expression, we found that most genes were related to the production of metabolic energy in both species, although we do not know which of the two species has a higher metabolism. Both Trechaleoides species are morphologically identical and live in riparian forests associated with freshwater courses perching on stones and vegetation (Carico, 2005) and thus, share similar ecological conditions (i.e., similar prey availability). A plausible scenario is that the cannibalistic *T. biocellata* may have faster metabolic rates than *T. keyserlingi*. Higher metabolic rates, may be linked to females' voracity and the occurrence of sexual cannibalism and additionally would allow males to escape fastest from females' attacks. Despite we performed this study focusing only on males, we do not find a reason to believe that females would differ from males in this type of result linked to survival effects. Studies in cannibalistic spider species show that females usually attempt to increase rates of sexual cannibalism when they are starved (Arnqvist & Henriksson, 1997; Wilder & Rypstra, 2008). Thus, females in poor nutritive habitats may cannibalize males more frequently (Fisher et al., 2018). In our case, T. biocellata females still cannibalized males even when they are well fed (Martínez Villar et al., in revision.), thus a more eco-evolutionary approach is needed to understand the presence of premating sexual cannibalism in this species.

In conclusion, we found evidence of a genetic variation reduction, low effective population size, and the possible relaxation of the purifying selection in a sexual cannibalistic and monogamous species compared to a non-cannibalistic and polyandrous species. We interpret that the low heterozygosity may have possibly limited the ability of males to produce sexual traits, giving a potential explanation for the absence of nuptial gifts in this species. It is known that males from polyandrous species differ in their rate of molecular evolution than monogamous species and specifically, genes that are related to testis or sperm function can experience weakest selection in monogamous ones (Johnson *et al.*, 2022). Further studies considering tissue-specific molecular

analyses (i.e. gonads) would bring more detailed information on differential selective pressures between the two contrasting species of the genus *Trechaleoides*.

**Figure 1. Distribution map of** *Trechaleoides* **genus in Uruguay**. Numbers correspond to the three sampled populations where they co-occur: 1) San Miguel, Rocha; 2) Quebrada de los Cuervos, Treinta y Tres and 3) Centurión, Cerro Largo. Grey circles represent individuals from *T. biocellata* and black triangles from *T. kyeserlingi*.



**Figure 2. Individual heterozygosity per population and species**. Grey boxplot corresponds to the species *P. ornata*, black boxplots correspond to the species *T. biocellata* and white boxplots corresponds to the species *T. keyserlingi*. The x axis shows the different populations where individuals were collected: Centurión (C), Quebrada de los Cuervos (QC) and San Miguel (SM). The y axis shows H<sub>obs</sub>.



Figure 3. Volcano plot for gen expression in *Trechaleoides* species. Data from the 900 differentited expressed genes for a p value = 0.05 and  $\log_2$  fold change = 0.50. Red dots represents the genes that meet p-value and  $\log_2$  fold change conditions between *T. biocellata* and *T. keyserlingi* and are considered differentially expressed. The x axis shows the  $\log_2$  fold change and the y axis shows the negative value in base 10 of the p value.



Table 1. Generalized lineal model analyzing heterozygosity ( $H_{obs}$ ). We performed a GLM analyzing individual heterozygosis ( $H_{obs}$ ) with species and population as fixed effects. Significant p-values are shown in bold.

		Fixed effects							
	Speci	es	Populat	ion					
	Estimative	р	Estimative	р					
Hobs	-1.179e-4	< 0.01	-1.144e-4	0.002					

**Table 2. Estimates of dN, dS and dN/dS from the branch free-ratios model.** It shows the mean for each one and the low and high 95% confident interval in brackets for *T. biocellata*, *T. keyserlingi* and *P. ornata*. Significance differences are met when the 95% CI do not overlap.

	dN	dS	dN/dS
Species	mean (95% CI)	mean (95% CI)	mean (95% CI)
T. biocellata	0.0034 (0.00341 - 0.00341)	0.0177 (0.01777 - 0.01778)	0.1921 (0.1920 - 0.1922)
T. keyserlingi	0.0032 (0.00326 - 0.00327)	0.0178 (0.01783 - 0.01784)	0.1833 (0.1832 - 0.1834)
P. ornata	0.0101 (0.01016 - 0.01016)	0.0696 (0.06968 - 0.06970)	0.1458 (0.1458 - 0.1458)
## REFERENCES

- Albo, M.J., Bidegaray-Batista, L., Bechsgaard, J., Da Silva, E.L.C., Bilde, T. & Pérez-Miles, F. 2017. Molecular phylogenetic analyses show that Trechaleidae and Lycosidae are sister groups. *Arachnology* 17: 169–176.
- Albo, M.J., Pavón-pelaez, C., Martínez Villar, M., Buzzatto, M.. & Tomasco, I. Stressful environments favor deceptive alternative mating tactics to become dominant (in revision). *BMC Biol*.
- Alexander, D.H., Novembre, J. & Lange, K. 2009. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* 19: 1655–1664.
- Aparicio, J.M., Cordero, P.J. & Veiga, J.P. 2001. A test of the hypothesis of mate choice based on heterozygosity in the spotless starling. *Anim. Behav.* **62**: 1001–1006.
- Arnqvist, G. & Henriksson, S. 1997. Sexual cannibalism in the fishing spider and a model for the evolution of sexual cannibalism based on genetic constraints. *Evol. Ecol.* **11**: 255–273.
- Balloux, F. & Lehmann, L. 2003. Random mating with a finite number of matings. *Genetics* 165: 2313–2315.
- Bechsgaard, J., Schou, M.F., Vanthournout, B., Hendrickx, F., Knudsen, B., Settepani, V., *et al.* 2019. Evidence for faster X chromosome evolution in spiders. *Mol. Biol. Evol.* **36**: 1281–1293.
- Biswas, K., Chakraborty, S., Podder, S. & Ghosh, T.C. 2016. Insights into the dN/dS ratio heterogeneity between brain specific genes and widely expressed genes in species of different complexity. *Genomics* **108**: 11–17. Elsevier Inc.
- Bolund, E., Martin, K., Kempenaers, B. & Forstmeier, W. 2010. Inbreeding depression of sexually selected traits and attractiveness in the zebra finch. *Anim. Behav.* **79**: 947–955. Elsevier Ltd.
- Brandvain, Y., Slotte, T., Hazzouri, K.M., Wright, S.I. & Coop, G. 2013. Genomic identification of founding haplotypes reveals the history of the selfing species *Capsella rubella*. *PLoS Genet.* **9**: e1003754.
- Brown, J.L. 1997. A theory of mate choice based on heterozygosity. Behav. Ecol. 8: 60-65.
- Cantalapiedra, C., Hernández-Plaza, A., Letunic, I., Bork, P. & Huerta-Cepas, J. 2021. eggNOG-mapper v2: Functional Annotation, Orthology Assignments, and Domain Prediction at the Metagenomic Scale. *Mol. Biol. Evol.* **38**: 5825–5829.
- Carico, J.E. 2005. Descriptions of two new spider genera of Trechaleidae (Araneae, Lycosoidea) from South America. *J. Arachnol.* **33**: 797–812.
- Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst. Vol. 18* **18**: 237–268.
- Charlesworth, D. & Meagher, T.R. 2003. Effects of inbreeding on the genetic diversity of populations. *Philos. Trans. R. Soc. B Biol. Sci.* **358**: 1051–1070.
- Charlesworth, D. & Wright, S.I. 2001. Breeding systems and genome evolution. *Curr. Opin. Genet. Dev.* **11**: 685–690.
- Cohas, A., Bonenfant, C., Kempenaers, B. & Allainé, D. 2009. Age-specific effect of heterozygosity on survival in alpine marmots, *Marmota marmota*. *Mol. Ecol.* 18: 1491–1503.
- Coltman, D.W., Pilkington, J.G., Smith, J.A. & Pemberton, J.M. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution (N. Y).* **53**: 1259–1267.
- Costa-Schmidt, L.E., Carico, J.E. & De Araújo, A.M. 2008. Nuptial gifts and sexual behavior in two species of spider (Araneae, Trechaleidae, *Paratrechalea*). *Naturwissenschaften* **95**: 731–739.
- Da Silva, E.L.C. & Lapinski, W. 2012. A new species of *Trechalea* thorell, 1869 (Araneae: Lycosoidea: Trechaleidae: Trechaleinae) from Costa Rica, with notes on its natural history and ecology. *Zootaxa* **64**: 58–64.
- Da Silva, E.L.C. & Lise, A.A. 2009. New record of nuptial gift observed in *Trechalea amazonica*. (Araneae, Lycosoidea, Trechaleidae). *Rev. Peru. Biol.* **16**: 119–120.
- Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O., *et al.* 2021. Twelve years of SAMtools and BCFtools. *Gigascience* **10**: 1–4. Oxford University Press.
- Daniels, S.J. & Walters, J.R. 2000. Inbreeding depression and its effects on natal dispersal in Red-cockaded Woodpeckers. *Condor* **102**: 482–491.
- Degen, B., Yanbaev, Y., Ianbaev, R., Bakhtina, S. & Sultanova, R. 2021. When does habitat fragmentation lead to changes in populations gene pool of pedunculate oak (*Quercus robur L.*)? For. Ecol. Manage. 499: 119617. Elsevier B.V.
- Eberhard, W.G. 1994. Evidence for Widespread Courtship During Copulation in 131 Species of Insects and Spiders, and Implications for Cryptic Female Choice. *Evolution (N. Y).* **48**: 711.

- Eberhard, W.G. 2004. Why Study Spider Sex: Special Traits of Spiders Facilitate Studies of Sperm Competition and Cryptic Female Choice. J. Arachnol. **32**: 545–556.
- Edgar, R.C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**: 1792–1797.
- Elgar, M.A. & Schneider, J.M. 2004. Evolutionary significance of sexual cannibalism. *Adv. Study Behav.* **33**: 135–163.
- Emms, D.M. & Kelly, S. 2019. OrthoFinder: Phylogenetic orthology inference for comparative genomics. *Genome Biol.* **20**: 1–14. Genome Biology.
- Ferrer, E.S., García-Navas, V., Bueno-Enciso, J., Sanz, J.J. & Ortego, J. 2015. Multiple sexual ornaments signal heterozygosity in male blue tits. *Biol. J. Linn. Soc.* 115: 362–375.
- Firman, R.C., Gasparini, C., Manier, M.K. & Pizzari, T. 2017. Postmating Female Control: 20 Years of Cryptic Female Choice. *Trends Ecol. Evol.* **32**: 368–382. Elsevier Ltd.
- Fisher, A.M., Cornell, S.J., Holwell, G.I. & Price, T.A.R. 2018. Sexual cannibalism and population viability. *Ecol. Evol.* **8**: 6663–6670.
- Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J.T. & Kempenaers, B. 2003. Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature* 425: 714–717.
- Frankham, R. 2012. How closely does genetic diversity in finite populations conform to predictions of neutral theory? Large deficits in regions of low recombination. *Heredity (Edinb)*. **108**: 167–178. Nature Publishing Group.
- Fu, L., Niu, B., Zhu, Z., Wu, S. & Li, W. 2012. CD-HIT: Accelerated for clustering the next-generation sequencing data. *Bioinformatics* 28: 3150–3152.
- Gage, M.J.G., Surridge, A.K., Tomkins, J.L., Green, E., Wiskin, L., Bell, D.J., *et al.* 2006. Reduced heterozygosity depresses sperm quality in wild rabbits, *Oryctolagus cuniculus. Curr. Biol.* **16**: 612–617.
- Gilpin, M.E. & Soulé, M.E. 1986. Minimum viable populations: the processes of species extinctions. *Conserv. Biol. Sci. scarcity Divers.* 19– 34.
- Grabherr, M., Haas, B.J., Yassour, M., Levin, J., Thompson, D.A., Amit, I., *et al.* 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* **29**: 644–652.
- Haas, B. & Papanicolaou, A.J.S. 2016. TransDecoder (find coding regions within transcripts).
- Hazzouri, K.M., Escobar, J.S., Ness, R.W., Killian Newman, L., Randle, A.M., Kalisz, S., *et al.* 2013. Comparative population genomics in collinsia sister species reveals evidence for reduced effective population size, relaxed selection, and evolution of biased gene conversion with an ongoing mating system shift. *Evolution (N. Y).* 67: 1263–1278.
- Hill, W.G. & Zhang, X.-S. 2004. Genetic variation within and among animal populations. *BSAP Occas. Publ.* **30**: 67–84.
- Hoang, D.T., Chernomor, O., Von Haeseler, A., Minh, B.Q. & Vinh, L.S. 2018. UFBoot2: Improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* 35: 518–522.
- Holman, L. & Kokko, H. 2013. The consequences of polyandry for population viability, extinction risk and conservation. *Philos. Trans. R. Soc. B Biol. Sci.* **368**: 20120053.
- Hurd, A.L.E., Eisenberg, R.M., Fagan, W.F., Tilmon, K.J., Snyder, W.E. & Datz, J.D. 1994. Cannibalism reverses male-biased sex ratio in adult mantids: female strategy against food limitation? *Oikos* 69: 193–198.
- Jensen, J.D., Payseur, B.A., Stephan, W., Aquadro, C.F., Lynch, M., Charlesworth, D., *et al.* 2019. The importance of the Neutral Theory in 1968 and 50 years on: A response to Kern and Hahn 2018. *Evolution (N. Y).* **73**: 111–114.
- Johnson, B.D., Anderson, A.P., Small, C.M., Rose, E., Flanagan, S.P., Hendrickson-Rose, C., *et al.* 2022. The evolution of the testis transcriptome in pregnant male pipefishes and seahorses. *Evolution (N. Y).* **76**: 2162–2180.
- Johnson, J.C. & Sih, A. 2005. Precopulatory sexual cannibalism in fishing spiders (*Dolomedes triton*): a role for behavioral syndromes. *Behav. Ecol. Sociobiol.* **58**: 390–396.
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Von Haeseler, A. & Jermiin, L.S. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* **14**: 587–589. Nature Publishing Group.
- Karl, S.A. 2008. The effect of multiple paternity on the genetically effective size of a population. *Mol. Ecol.* **17**: 3973–3977.
- Kempenaers, B. 2007. Mate choice and genetic quality: A review of the heterozygosity theory. *Adv. Study Behav.* **37**: 189–278.

Kimura, M. 1983. The neutral theory of molecular evolution. Cambridge University Press.

- Kruuk, L.E.B., Sheldon, B.C. & Merilä, J. 2002. Severe inbreeding depression in collared flycatchers (*Ficedula albicollis*). Proc. R. Soc. B Biol. Sci. 269: 1581–1589.
- Langmead, B., & Salzberg, S. L. 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods 9: 357-359.
- Lapinski, W. & Tschapka, M. 2009. Erstnachweis von Brautgeschenken bei *Trechalea sp*. (Trechaleidae, Araneae) in Costa Rica. *Arachne* 14: 4–13.
- Lawrence, S.E. 1992. Sexual cannibalism in the praying mantid, *Mantis religiosa*: a field study. *Anim. Behav.* **43**: 569–583.
- Li, B. & Dewey, C.N. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* **12**: 1–16.
- Li, W. & Godzik, A. 2006. Cd-hit: A fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 22: 1658–1659.
- Lin, J.J., Bhattacharjee, M.J., Yu, C.P., Tseng, Y.Y. & Li, W.H. 2019. Many human RNA viruses show extraordinarily stringent selective constraints on protein evolution. *Proc. Natl. Acad. Sci. U. S. A.* 116: 19009– 19018.
- Love, M. I., Huber, W., & Anders, S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*. 15: 1-21.
- Lynch, M. & Gabriel, W. 1990. Mutation load of small populations. 44: 1725–1737.
- MacDougall-Shackleton, E.A., Derryberry, E.P., Foufopoulos, J., Dobson, A.P. & Hahn, T.P. 2005. Parasitemediated heterozygote advantage in an outbred songbird population. *Biol. Lett.* 1: 105–107.
- Mainguy, J., CÔtÉ, S.D. & Coltman, D.W. 2009. Multilocus heterozygosity, parental relatedness and individual fitness components in a wild mountain goat, *Oreamnos americanus* population. *Mol. Ecol.* **18**: 2297–2306.
- Manni, M., Berkeley, M.R., Seppey, M., Simão, F.A. & Zdobnov, E.M. 2021. BUSCO Update: Novel and Streamlined Workflows along with Broader and Deeper Phylogenetic Coverage for Scoring of Eukaryotic, Prokaryotic, and Viral Genomes. *Mol. Biol. Evol.* 38: 4647–4654.
- Martinez-Gutierrez, C.A. & Aylward, F.O. 2019. Strong Purifying Selection Is Associated with Genome Streamlining in Epipelagic Marinimicrobia. *Genome Biol. Evol.* **11**: 2887–2894.
- Martínez-Villar, M., Germil, M., Pavon-Pelaz, C., Costa-Schmidt, L.E. & Albo, M.J. 2020. Empty nuptial gifts: A further step in the evolution of deception in spiders? *J. Arachnol.* **48**: 214–217.
- Martinez, A.S., Willoughby, J.R. & Christie, M.R. 2018. Genetic diversity in fishes is influenced by habitat type and life-history variation. *Ecol. Evol.* 8: 12022–12031.
- Martínez Villar, M., Germil, M., Pavón-Peláez, C., Tomasco, I., Bilde, T., Toft, S., *et al.* Lack of female preference for nuptial gifts may have changed its function and led to loss of the male sexual trait (in revision). *Evol. Biol.*
- Martínez Villar, M., Trillo, M.C. & Albo, M.J. 2021. Ineffective nuptial gifts suggest female emancipation from sensory exploitation. *Behav. Ecol. Sociobiol.* 75: 61.
- Montano, V. 2016. Coalescent inferences in conservation genetics: Should the exception become the rule? *Biol. Lett.* **12**: 20160211.
- Nguyen, L.T., Schmidt, H.A., Von Haeseler, A. & Minh, B.Q. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* **32**: 268–274.
- Persons, M.H. & Uetz, G.W. 2005. Sexual cannibalism and mate choice decisions in wolf spiders: Influence of male size and secondary sexual characters. *Anim. Behav.* **69**: 83–94.
- Pertea, G. & Pertea, M. 2020. GFF Utilities: GffRead and GffCompare. F1000Research 9: 1-19.
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., et al. 2007. PLINK: A tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81: 559–575.
- Qiu, S., Zeng, K., Slotte, T., Wright, S. & Charlesworth, D. 2011. Reduced efficacy of natural selection on codon usage bias in selfing *Arabidopsis* and *Capsella* species. *Genome Biol. Evol.* **3**: 868–880.
- R Team Core. 2022. A language and environment for statistical computing. *R Found. Stat. Comput. Vienna, Austria.* R Foundation for Statistical Computing, Vienna, Austria.
- Rabiee, M., Sayyari, E. & Mirarab, S. 2019. Multi-allele species reconstruction using ASTRAL. *Mol. Phylogenet. Evol.* **130**: 286–296.
- Reid, J.M., Arcese, P., Keller, L.F., Elliott, K.H., Sampson, L. & Hasselquist, D. 2007. Inbreeding effects on immune response in free-living song sparrows (*Melospiza melodia*). Proc. R. Soc. B Biol. Sci. 274: 697–706.

- Rengifo-Gutiérrez, L., Albo, M.J. & Santa, L.D. 2021. The unknown *Enna* (Araneae: Trechaleidae), new species and first record of wrapped nuptial gifts in the genus. *J. Arachnol.* **48**: 242–248.
- Schlaepfer, D.R., Braschler, B., Rusterholz, H.P. & Baur, B. 2018. Genetic effects of anthropogenic habitat fragmentation on remnant animal and plant populations: a meta-analysis. *Ecosphere* **9**: e02488.
- Schneider, J.M. & Elgar, M.A. 2001. Sexual cannibalism and sperm competition in the golden orb-web spider *Nephila plumipes* (Araneoidea): Female and male perspectives. *Behav. Ecol.* **12**: 547–552.
- Settepani, V., Bechsgaard, J. & Bilde, T. 2016. Phylogenetic analysis suggests that sociality is associated with reduced effectiveness of selection. *Ecol. Evol.* **6**: 469–477.
- Slate, J., Kruuk, L.E.B., Marshall, T.C., Pemberton, J.M. & Clutton-Brock, T.H. 2000. Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). Proc. R. Soc. B Biol. Sci. 267: 1657–1662.
- Slotte, T., Foxe, J.P., Hazzouri, K.M. & Wright, S.I. 2010. Genome-wide evidence for efficient positive and purifying selection in *Capsella grandiflora*, a plant species with a large effective population size. *Mol. Biol. Evol.* 27: 1813–1821.
- Strohm, J.H.T., Gwiazdowski, R.A. & Hanner, R. 2015. Fast fish face fewer mitochondrial mutations: Patterns of *dN/dS* across fish mitogenomes. *Gene* **572**: 27–34.
- Sugg, D.W. & Chesser, R.K. 1994. Effective population sizes with multiple paternity. Genetics 137: 1147–1155.
- Suyama, M., Torrents, D. & Bork, P. 2006. PAL2NAL: Robust conversion of protein sequence alignments into the corresponding codon alignments. *Nucleic Acids Res.* **34**: 609–612.
- Taylor, M.L., Price, T.A.R. & Wedell, N. 2014. Polyandry in nature: a global analysis. *Trends Ecol. Evol.* 29: 376–383. Elsevier Ltd.
- Tian, T., Liu, Y., Yan, H., You, Q., Yi, X., Du, Z., *et al.* 2017. AgriGO v2.0: A GO analysis toolkit for the agricultural community, 2017 update. *Nucleic Acids Res.* **45**: W122–W129.
- Trillo, M.C. & Albo, M.J. 2019. Another one bites the gift: sexual behaviour in a *Trechaleoides* species. *Arachnology* **18**: 250–252.
- Walker, L.A. & Holwell, G.I. 2015. Sexual cannibalism in a facultative parthenogen: The springbok mantis (*Miomantis caffra*). *Behav. Ecol.* **0**: 1–6.
- Welke, K. & Schneider, J.M. 2009. Inbreeding avoidance through cryptic female choice in the cannibalistic orb-web spider *Argiope lobata*. *Behav. Ecol.* **20**: 1056–1062.
- Wiens, J.J. 2001. Widespread loss of sexually selected traits: How the peacock lost its spots. *Trends Ecol. Evol.* **16**: 517–523.
- Wilder, S.M. & Rypstra, A.L. 2008. Sexual size dimorphism predicts the frequency of sexual cannibalism within and among species of spiders. *Am. Nat.* **172**: 431–440.
- Yang, Z. 2007. PAML 4: Phylogenetic analysis by maximum likelihood. Mol. Biol. Evol. 24: 1586–1591.
- Yang, Z. & Nielsen, R. 1998. Synonymous and nonsynonymous rate variation in nuclear genes of mammals. *J. Mol. Evol.* **46**: 409–418.
- Zajitschek, S.R.K. & Brooks, R.C. 2010. Inbreeding depression in male traits and preference for outbred males in *Poecilia reticulata. Behav. Ecol.* **21**: 884–891.
- Zhu, A., Ibrahim, J.G. & Love, M.I. 2019. Heavy-Tailed prior distributions for sequence count data: Removing the noise and preserving large differences. *Bioinformatics* 35: 2084–2092.

## **Final conclusions and perspectives**

Courtship and mating can be risky for many spider males since females can be aggressive and cannibals (Foelix, 2011). In several species, males have developed strategies to lure and distract females while courting and/or completing sperm transfer (Robinson & Robinson, 1980; Foellmer & Fairbairn, 2004; Fromhage & Schneider, 2005; Neumann & Schneider, 2020; Zhang et al., 2022). Many of these strategies involve dissuading females with food, and to some extent exploiting females foraging motivation (Sakaluk, 2000; Bilde et al., 2007; Albo et al., 2017b). Thus, female preferences for food seem to be an ancestral trait in spiders. In some species males use nuptial gifts exploiting these female preferences and leading to benefits for both sexes, with females increasing their fecundity and males acquiring matings (Albo et al., 2017b). Yet, silk wrapped nuptial gifts are rare in spiders and only widespread in the family Trechaleidae as 7 out of the 17 genera in the family reported to have males offering nuptial gifts to females (Costa-Schmidt et al., 2008; Albo et al., 2009; Da Silva & Lise, 2009; Lapinski & Tschapka, 2009; Da Silva & Lapinski, 2012; Trillo & Albo, 2019; Martínez-Villar et al., 2020; A. Santos and D. Poy, personal communication). This information suggests the most parsimonious scenario is that the trait appeared once in an ancestor in the family or in a clade within the family (Albo et al., 2017a). Because of these peculiarities the family is an ideal model to study evolutionary changes in female preferences for food and the male sexual trait, nuptial gift-giving.

By using the spider genus *Trechaleoides* (Trechaleidae) which includes a gift-giving (*T. keyserlingi*) and a non-gift-giving (*T. biocellata*) species this thesis exemplified how the nuptial gift can change its adaptive function, even being lost if the selective pressures that maintain the trait are relaxed or absent.

The conclusion from this thesis is that there has been a basal loss of the female preference for the nuptial gifts in the genus. This could be possible due to males having modified the gift content by offering worthless gifts (Martínez Villar et al., 2021), and thus, females have counteracted the deception by emancipating themselves from the exploitation (Martínez Villar et al., 2021). This led to changes in the benefits for males offering nuptial gifts, that possibly become costly to produce. There are no apparent benefits maintaining the nuptial gift in T. keyserlingi, and thus, its presence may indicate that is a vestigial trait that may be lost over time, or there are benefits maintaining it that are beyond the ones measured here. For instance, it is known that female spiders can storage sperm from several males and they can cryptically bias paternity by selecting sperm to fertilize eggs (Eberhard, 1996; Albo et al., 2013; Albo & Peretti, 2015). Thus, it may be possible that after multiple matings T. keyserlingi females may potentially benefit gift-giving males throughout post-copulatory mechanisms. However, the fact that the genetic variation (H<sub>obs</sub>) found in this species is higher compared to species in which females are either monogamous (T. *biocellata*) or can bias paternity towards a male (*P. ornata*) suggests limited post copulatory biases. Future studies assessing paternity success of males mating with nutritive, worthless and without nuptial gifts would improve our understanding on this fascinating question.

We interpret the absence of the sexual trait in *T. biocellata* as an evolutionary loss. The intrigue remains about the presence of the pre-mating sexual cannibalism in this species. One possibility is that sexual cannibalism appeared before the loss of the sexual trait. The absence of female preference for nuptial gifts added to the cannibalism could accelerate the loss of the gift, as we showed it was useless for male protection. Alternatively, sexual cannibalism could appear after the loss of the sexual trait. In any case, selection on females may have increased their aggressiveness towards males to obtain nutrients. This type of sexual cannibalism seems to be a maladaptive trait as females seems to not be able to distinguish potential mates from prey. Some explanations for this include the spillover hypothesis, where the pre-mating sexual cannibalism is a genetic constraint (Arnqvist & Henriksson, 1997; Kralj-Fišer *et al.*, 2012). This means, there is a genetic correlation in both sexes and the same genes that cause high levels of aggression in juvenile stages (males and females) to increase the nutrient intake, are the same that cause non-adaptive high levels of aggression in adult females. Since female spiders are homogametic (Araujo *et. al.*, 2012; Kořínková & Král, 2012), they receive a double dose of those genes, pre-mating sexual cannibalism is an "evolutionary trap" dictated by the genetic design of the regulation for

aggression. Further studies on prey/mate recognition and levels of aggression between juveniles and adult females in both species of the genus would improve our understanding of the pre-mating sexual cannibalism in *T. biocellata*.

Finally, we conducted the first comparative molecular study using transcriptomes for the two species of the genus *Trechaleoides*, which share several ecological conditions but highly differ in their reproductive behavior. We found marked differences in genetic variation, effectiveness of purifying selection and gene expression between species. Further studies on estimating the effective population size in both species, as well as studying if *T. biocellata* experienced bottle neckless will bring valuable information about the evolutionary history of these two species. Lastly, regardless the type of genomic data, further studies should focus on retrieve genomic data together with behavioral data (female preferences and male nuptial gift) from several species of the family. This will allow to build a complete and comprehensive phylogeny using a multi locus approach and perform further comparative evolutionary studies.

## REFERENCES

- Albo, M.J., Bidegaray-Batista, L., Bechsgaard, J., Da Silva, E.L.C., Bilde, T. & Pérez-Miles, F. 2017a. Molecular phylogenetic analyses show that Trechaleidae and Lycosidae are sister groups. *Arachnology* **17**: 169–176.
- Albo, M.J., Bilde, T. & Uhl, G. 2013. Sperm storage mediated by cryptic female choice for nuptial gifts. *Proc. R. Soc. B Biol. Sci.* 280.
- Albo, M.J., Costa-Schmidt, L.E. & Costa, F.G. 2009. To feed or to wrap? Female silk cues elicit male nuptial gift construction in a semiaquatic trechaleid spider. *J. Zool.* **277**: 284–290.
- Albo, M.J., Macías-Hernández, N., Bilde, T. & Toft, S. 2017b. Mutual benefit from exploitation of female foraging motivation may account for the early evolution of gifts in spiders. *Anim. Behav.* **129**: 9–14.
- Albo, M.J. & Peretti, A. V. 2015. Worthless and nutritive nuptial gifts: Mating duration, sperm stored and potential female decisions in spiders. *PLoS One* **10**: 1–15.
- Araujo, D. S. M. C., Schneider, M. C., Paula-Neto, E., Cella, D. M., & Swan, A. 2012. Sex chromosomes and meioesis in spiders: a review. In: Swan A (ed) Meiosis: molecular mechanisms and cytogenetic diversity. IntechOpen, London.
- Arnqvist, G. & Henriksson, S. 1997. Sexual cannibalism in the fishing spider and a model for the evolution of sexual cannibalism based on genetic constraints. *Evol. Ecol.* **11**: 255–273.
- Bilde, T., Tuni, C., Elsayed, R., Pekar, S. & Toft, S. 2007. Nuptial gifts of male spiders: sensory exploitation of the female's maternal care instinct or foraging motivation? *Anim. Behav.* **73**: 267–273.
- Costa-Schmidt, L.E., Carico, J.E. & De Araújo, A.M. 2008. Nuptial gifts and sexual behavior in two species of spider (Araneae, Trechaleidae, *Paratrechalea*). *Naturwissenschaften* **95**: 731–739.
- Da Silva, E.L.C. & Lapinski, W. 2012. A new species of *Trechalea* thorell, 1869 (Araneae: Lycosoidea: Trechaleidae: Trechaleinae) from Costa Rica, with notes on its natural history and ecology. *Zootaxa* **64**: 58–64.
- Da Silva, E.L.C. & Lise, A.A. 2009. New record of nuptial gift observed in *Trechalea amazonica*. (Araneae, Lycosoidea, Trechaleidae). *Rev. Peru. Biol.* **16**: 119–120.
- Eberhard, W.G. 1996. *Female control: sexual selection by cryptic female choice*. Princeton, NJ: Princeton University Press.
- Foelix, R.F. 2011. Biology of Spiders, 3rd ed. Oxford University Press, New York.
- Foellmer, M.W. & Fairbairn, D.J. 2004. Males under attack: Sexual cannibalism and its consequences for male morphology and behaviour in an orb-weaving spider. *Evol. Ecol. Res.* **6**: 163–181.
- Fromhage, L. & Schneider, J.M. 2005. Safer sex with feeding females: Sexual conflict in a cannibalistic spider. *Behav. Ecol.* **16**: 377–382.
- Kořínková, T., & Král, J. 2013. Karyotypes, sex chromosomes, and meiotic division in spiders. *Spider* ecophysiology, 159-171.
- Kralj-Fišer, S., Schneider, J.M., Justinek, Ž., Kalin, S., Gregorič, M., Pekar, S., et al. 2012. Mate quality, not aggressive spillover, explains sexual cannibalism in a size-dimorphic spider. Behav. Ecol. Sociobiol. 66: 145– 151.

Lapinski, W. & Tschapka, M. 2009. Erstnachweis von Brautgeschenken bei Trechalea sp. (Trechaleidae, Araneae)

in Costa Rica. Arachne 14: 4–13.

- Martínez-Villar, M., Germil, M., Pavon-Pelaz, C., Costa-Schmidt, L.E. & Albo, M.J. 2020. Empty nuptial gifts: A further step in the evolution of deception in spiders? *J. Arachnol.* **48**: 214–217.
- Martínez Villar, M., Trillo, M.C. & Albo, M.J. 2021. Ineffective nuptial gifts suggest female emancipation from sensory exploitation. *Behav. Ecol. Sociobiol.* **75**: 61.
- Neumann, R. & Schneider, J.M. 2020. Males sacrifice their legs to pacify aggressive females in a sexually cannibalistic spider. *Anim. Behav.* **159**: 59–67.
- Robinson, M.H. & Robinson, B. 1980. Comparative studies of the courtship and mating behavior of tropical araneid spiders. *Comp. Stud. Court. mating Behav. Trop. araneid spiders* **36**.
- Sakaluk, S.K. 2000. Sensory explotation as an evolutionary origin to nuptial food gifts in insects. *Proc. R. Soc. London. Ser. B Biol. Sci.* 267: 339–343.
- Trillo, M.C. & Albo, M.J. 2019. Another one bites the gift: sexual behaviour in a *Trechaleoides* species. *Arachnology* **18**: 250–252.
- Zhang, S., Liu, Y., Ma, Y., Hao, W., Yao, Z., Matjaž, K., *et al.* 2022. Male spiders avoid sexual cannibalism with a catapult mechanism. *Curr. Biol.* **32**: R354–R355.