
ORIGEN Y EVOLUCIÓN DEL REGALO NUPCIAL EN ARAÑAS LICOSOIDEAS Y SU CONGRUENCIA CON LA FILOGENIA



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Imagen de portada: Macho de *Paratrechalea ornata* (Trechaleidae) con un regalo nupcial en sus quelíceros. Foto: MJ Albo.

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MARIA JOSE ALBO

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“I have been speculating last night what makes a man discoverer of undiscovered things, and a most perplexing problem it is As far as I can conjecture, the art consists in habitually searching for causes or meaning of everything which occurs. This implies sharp observation and requires as much knowledge as possible of the subject investigated”

Charles Darwin, 1871

Letter to his son –Horace

The autobiography of Charles Darwin (1809-1882)

Edited by Nora Barlow, 1958

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The image features a teal background with a light gray border. At the top, there are two horizontal bars: a dark gray one on the left and a light gray one on the right. In the center, the word "ABSTRACT" is written in a bold, black, serif font. At the bottom, there are two more horizontal bars: a light gray one on the left and a dark gray one on the right.

ABSTRACT

RESUMEN

ANTECEDENTES Y OBJETIVOS: Los regalos nupciales son un interesante rasgo sexual usualmente presente en los machos que ha evolucionado en una amplia variedad de formas en diferentes animales. Existe una evidente falta de conocimiento respecto a los sistemas reproductivos con regalo nupcial en arañas, particularmente de aquellos que involucran regalos envueltos en seda. Las presas envueltas en seda han sido intensamente estudiadas en la especie Paleártica *Pisaura mirabilis* (Pisauridae) mientras que poco se sabe del comportamiento de otras arañas donadoras de regalos nupciales, como el caso de la especie Neotropical *Paratrechalea ornata* (Trechaleidae). El aporte de esta tesis es de brindar nuevos conocimientos tanto sobre la especie *P. mirabilis* como sobre la poco estudiada *P. ornata*. Curiosamente, si bien estas especies experimentan diferencias en su ecología, muestran extraordinarias similitudes en su comportamiento sexual, mientras que los estudios filogenéticos sugieren una evolución convergente del rasgo masculino. La inclusión de la especie *P. ornata* en los estudios contenidos en esta tesis permite analizar, comparar y discutir el origen, función y evolución de los regalos nupciales en ambas especies de araña. Para esto se estudiaron diferentes aspectos del comportamiento sexual de dichas especies, incluyendo la función de la envoltura en seda para la elección femenina durante el cortejo, las consecuencias reproductivas causadas por la variación en el contenido del regalo, y cómo la presencia y contenido del regalo influye en la cantidad de esperma almacenado por las hembras. Además, se realizaron estudios moleculares de forma de obtener una nueva filogenia que integre estas familias, y permita discutir sobre el posible origen evolutivo de este tipo de regalo en arañas.

CONCLUSIONES: El regalo nupcial en forma de presa envuelta en seda parece tener múltiples orígenes y/o pérdidas para las familias Trechaleidae y Pisauridae. El origen de los regalos nupciales muy probablemente ha derivado de la adquisición de ventajas en la fecundidad de las hembras con un consecuente beneficio reproductivo para los machos. Las hembras de ambas especies prefieren a los machos que ofrecen regalos durante el cortejo, e incluso en *P. ornata* las hembras parecen ser atraídas por los regalos bien empaquetados y blancos, sugiriendo un importante rol de la envoltura en seda como señal visual durante el cortejo y elección de pareja. Sin embargo, los beneficios para cada sexo pueden ser sensibles a fluctuaciones en la disponibilidad de comida y conducir a una co-evolución antagonista. Cuando la comida es escasa, los machos pueden eventualmente no envolver la presa en seda, comer la presa antes de envolverla, o coleccionar y envolver objetos no nutritivos y de esta forma engañar a las hembras mediante la donación de regalos simbólicos. Dado que pueden existir costos asociados a la producción del regalo, estas estrategias reducen la inversión de los machos. Mediante la ingesta de la presa los machos pueden adquirir más energía y mejorar su condición corporal que es esencial para ser aceptados por las hembras. Pero cuando no hay disponibilidad de presas, envolver objetos no nutritivos es una buena alternativa para obtener una cópula. Sin embargo, muchas veces las hembras pueden contrarrestar esta explotación limitando la cantidad de esperma transferido. Los avances presentados en esta tesis ilustran cómo las condiciones ecológicas, independientemente de las relaciones filogenéticas, juegan entonces un papel central modelando la evolución de los sistemas de apareamiento con regalo nupcial.

PALABRAS CLAVE: condición del macho, disponibilidad de presas, esperma almacenado, *Paratrechalea ornata*, *Pisaura mirabilis*, presas envueltas, regalos simbólicos

ABSTRACT

BACKGROUND AND OBJECTIVES: Nuptial gifts are an intriguing male sexually selected trait that has evolved in a wide range of forms among different animals. Evidently, there is enormous lack of knowledge regarding gift-giving mating systems in spiders, particularly concerning wrapped gifts. Wrapped prey gifts have been intensively studied in the Palearctic species *Pisaura mirabilis* (Pisauridae), while little is known about the behavior of other gift-giving species, as the case of the Neotropical species *Paratrechalea ornata* (Trechaleidae). The goal of this thesis is to contribute with novel knowledge of *P. mirabilis* and *P. ornata*. Interestingly, these species experience differences in their ecology but show remarkable similarities in sexual behavior, while some morphological phylogenies suggest a convergent evolution of the male trait. By including the Neotropical spider *Paratrechalea ornata* from the Trechaleidae family in these studies, I aim to analyze, compare and discuss the origin, function and evolution of nuptial gifts in both spider species. In this thesis I studied different aspects of the sexual behavior in both spider species, including the function of silk wrapping during courtship for female choice, the reproductive consequences of gift content variation, and how the gift presence and content influence the amount of sperm stored by females. In addition, I did molecular studies to obtain a phylogeny including these families, allowing the discussion of the possible origin of the wrapped prey gifts in spiders.

CONCLUSIONS: Wrapped prey gifts seem to have multiple origins and/or losses for the families Trechaleidae and Pisauridae. The origin of nuptial gifts most probably was derived from a fecundity advantage to females and a mating advantage to males. Females from both species prefer males that offer nuptial gifts during courtship. Further, *P. ornata* females seem to be attracted to well wrapped and white gifts, suggesting an important role of silk wrapping as a visual signal during courtship and mate choice. However, the benefits to each sex might be sensitive to fluctuations in food availability leading to antagonistic coevolution. When food is scarce, males may eventually avoid gift wrapping, eat the prey before wrap it in silk or collect and wrap non nutritive items and thus, deceive females by providing worthless gifts. Since there are costs associated to gift production these strategies reduces males' investment. By eating the prey, males can acquire more energy and improve their body condition, which is essential for female acceptance. But, when no prey is available wrap non nutritive items is a successful strategy to obtain a mating. However, in many cases females may counter this exploitation by limiting sperm transfer. The data presented in this thesis illustrate how ecological conditions, aside from the phylogenetic history, play a central role in shaping evolution of gift-giving mating systems.

KEYWORDS: male condition, prey availability, *Paratrechalea ornata*, *Pisaura mirabilis*, sperm storage, wrapped prey, worthless gifts

**GENERAL
INTRODUCTION**

GENERAL INTRODUCTION

THE SUBLIME TREASURES: NUPTIAL GIFTS

Nuptial gifts, where the male offers a gift that is often nutritious to the female in order to mate, are an intriguing sexually selected trait. As with most secondary sexual traits, it is most commonly found in males and only exceptionally in females (Arnqvist et al. 2003). The nuptial gift-giving trait has evolved independently many times and in a wide range of forms among different animal taxa. Studies on nuptial gifts appeared in the beginning of the 1900's with the first descriptions of males offering food to females in Diptera, Mecoptera and Orthoptera (cf. Vahed 1998). In the last decades an increasing number of studies have examined how sexual selection shapes gift-giving behavior in birds, snails and spiders, but with a particular focus on insects (Austad & Thornhill 1986; Vahed 1998, 2007; Mougeot et al. 2006; Burela & Martín 2007; Gwynne 2008; Lewis & South 2012). Nuptial gifts comprise an extensive diversity of donations such as oral food gifts given during courtship, and seminal substances transferred with the sperm during copulation (Vahed 1998, 2007; Gwynne 2008; Lewis & South 2012). A recent classification, which is followed by Lewis and South (2012), distinguishes gifts depending on the method of gift production and on how gifts are absorbed by females. Gifts produced by males themselves are called endogenous gifts, such as glandular and salivary secretions or seminal fluids, while items that males collect from the environment such as prey, seeds or inedible items, are called exogenous gifts. Females receive these donations orally when eating food items or regurgitations, or via the genital tract when males transfer substances together with the sperm.

Nuptial gifts can be given before, during, and after copulation and have positive, neutral or negative effects on both male and female reproductive success (Vahed 1998, 2007; Gwynne 2008; Lewis & South 2012). For instance, in scorpionflies the male's salivary secretion is important during courtship, and males that produce more secretions increase their mating rate. Females adjust mating duration to the number of secretions they receive, which in turn may influence the number of eggs fertilized by the male (Sauer et al. 1998; Engels & Sauer 2006). Among bushcrickets and crickets, females consume a gelatinous spermatophylax associated with the spermatophore during mating, which functions to prevent females from removing the spermatophore before sperm transfer is complete (Gwynne et al. 1984; Sakaluk 1984). Seminal fluids of some fruit fly species contain substances, produced in the male accessory gland, which promote acceleration of female egg production (Wolfner 1997; Heifetz et al. 2001).

NUPTIAL GIFTS IN SPIDERS

Nuptial gifts appear to be an uncommon sexual trait in spiders. Of 43,678 extant species described in the world (Platnick 2013), only few species are known to have males feeding females during courtship and/or mating (Bristowe & Locket 1926; Andrade 1996; Huber 1997; Costa-Schmidt et al. 2008; Uhl & Maelfait 2008). So far, there are no descriptions of females feeding males in spiders. Nuptial gift-giving in spiders take three forms involving endogenous and exogenous gifts orally transferred: male's body, glandular secretions, and wrapped prey items. Seminal gifts have so far not been documented in spiders.

Male body

Sexual cannibalism confers direct benefits to females, and may also allow reproductive advantages for males (Buskirk 1984; Elgar 1998; Schneider & Elgar 2001; Herberstein 2011). If sexual cannibalism occurs before mating (pre-copulatory), it is obviously maladaptive for males but may confer fecundity benefits for females. Sexual cannibalism that occurs during (syn-) or after mating (post-copulatory) may also be adaptive for males. Andrade (1996) showed the adaptive value of male suicidal sexual behavior

in redback spiders (*Latrodectus hasselti*). During mating the male performs a “somersault” by which he presents his abdomen directly to the female’s chelicerae. Sperm transfer continues while the female consumes the male’s abdomen, which results in higher paternity for cannibalized males than for non-cannibalized males. The benefit to males does not derive from the minute nutritional contribution from consumption of their bodies; instead, male sacrifice prolongs sperm transfer and confers an advantage in sperm competition. Male sacrifice evolved as an extreme form of nuptial gift to enhance male’s abilities in sperm competition. However, male sacrifice is unlikely to be the general explanation for sexual cannibalism in spiders (Schneider et al. 2000; Schneider & Elgar 2001; Elgar & Schneider 2004).

Glandular secretions

Another nuptial gift form known in spiders is the external glandular secretions described from species of the families Theridiidae, Linyphiidae and Pholcidae (Lopez 1987; Huber 1997; Uhl & Maelfait 2008). Males of these species often have differentiated head protuberances - knobs, turrets, pits, humps - that are connected to exocrine secretory glands (Vanacker et al. 2003; Michalick & Uhl 2011). During courtship and/or mating the female inserts her chelicerae in the male’s protuberance excreting digestive saliva, and afterwards ingests the fluids (Uhl & Maelfait 2008; Kunz & Uhl 2012). A recent study had shown that males increase mating probability and coupling efficiency when glandular secretion is consumed by the female (Kunz & Uhl 2012). So far, there is no indication that these secretions supply females with nutritional or other benefits, but further research is needed. This type of nuptial gift may be much more common than presently documented.

Wrapped prey items

Nuptial gifts as wrapped prey items are known in two spider families, Pisauridae and Trechaleidae (Bristowe 1958; Costa-Schmidt et al. 2008). Interestingly, fewer than 10 species displaying this behavior have been described, but the behavior may be more widespread in both families. Behavioral research has focused on only two species: the Palearctic *Pisaura mirabilis* (Pisauridae) and the Neotropical *Paratrechalea ornata* (Trechaleidae). In both species, the male captures a prey item (typically an arthropod) and wraps it in silk. The gift is thus a mixed exogenous (prey) and endogenous (silk) one. The result is a round and white package, which the male carries in his chelicerae while searching for a female (Bristowe & Locket 1926; Costa-Schmidt et al. 2008). After finding a female, the male actively courts by vibrating the first pair of legs and pedipalps while offering the nuptial gift. Female mating acceptance happens when she grasps the gift with her chelicerae and begins to feed while the male inserts the pedipalps and initiates sperm transfer. Several possible functions of the nuptial gift have been suggested for these species, including avoidance of sexual cannibalism, paternal investment and mating effort (see below).

FUNCTIONAL EXPLANATIONS OF NUPTIAL GIFTS

The selective forces that favor the origin and maintenance of gift-giving trait may vary in response to ecological conditions or co-evolutionary responses. Consequently, the function of gift-giving behavior may also change in relation to the context under which the trait is maintained. The origin of male donations must have been due to strong female selection for nutrients with the resulting fitness benefit to both partners. Since spiders are often food limited in nature (Wise 1993), fluctuations in food availability during the female lifespan and especially during adulthood would drive these preferences and further evolution of male and female behaviors coupled with inevitable costs of mating. Thus, to maximize their own reproductive success males would originally have benefited by investing in

nutritive nuptial gifts that enhanced female interests. Later, however, they may have potentially changed their investment by reducing the costs of gift production to enhance their own interests.

Sexual cannibalism avoidance

Females of many species kill and consume males before, during or after mating. This phenomenon, known as sexual cannibalism, is relatively common in spiders, and its evolutionary significance depends on the timing of male consumption (Elgar & Schneider 2004; Herberstein 2011). Males of some spider species are consumed during or after mating, but this usually benefits those males since they can prolong matings and hence increase their fertilization success (Schneider & Elgar 2001). However, if males are killed before mating when no sperm transfer has occurred, they obviously lose all potential fitness and only females gain benefits (food). Courtship behavior is particularly important under the risk of precopulatory sexual cannibalism. Spider males have evolved different strategies to avoid female attacks, such as approaching females when they are feeding or molting, so called “opportunistic matings” (Lubin 1986; Fromhage & Schneider 2004). In empidid flies nuptial gifts are suggested to function to prevent males from being eaten by females during courtship (Kessel 1955).

Sexual cannibalism occurs at low frequency in the gift-giving spider species, *P. mirabilis* and *P. ornata* (Bilde et al. 2006; Albo & Costa 2010). Nevertheless, precopulatory cannibalism was observed in a small percentage of staged matings in *P. mirabilis*, which may impose strong selection on cannibalism avoidance in males (Bilde et al. 2006). It is possible that nuptial gift-giving evolved as an anti-cannibalism precaution in this species where males have the chances to get multiple matings, while other functions may occur in concert. When food is scarce and females suffer from low feeding regimes, precopulatory gift stealing by females increases dramatically and sexual cannibalism to some extent (Toft S & MJ Albo unpublished data). Thus, it can also be imagined that the gift may create the opportunity for gift stealing as a substitute for pre-copulatory cannibalism, obviously a better option for the males.

Paternal investment

Parental investment was defined by Trivers (1972) as any investment from the parents that increase offspring survival and fitness. Paternal investment is very important for example in bird species, where males supply food and guard the progeny until they are independent (Stokes & Williams 1971; Mougeot et al. 2006). Ecological conditions such as the level of predation risk or food abundance may determine the relative parental investment of females and males (Gwynne 1990; Simmons & Bailey 1990). It is predicted that males would supply females with resources if food is scarce or if females are under high risk of predation when foraging, thus increasing the likelihood of females succeeding in reproduction and securing male’s own paternity. Alternatively, paternal investment would be low when prey availability is high. Some interesting examples arise from gift-giving species (reviewed in Boggs 1995 and Vahed 1998). Nutrients from the male gift (spermatophylax) are incorporated by female bushcrickets into the developing eggs (Simmons 1990; Simmons & Gwynne 1993), while nuptial gifts also improve female fecundity and longevity in other insect species (Thornhill 1976; Gwynne 1984; Simmons & Parker 1989; Wiklund et al. 1993; Karlsson 1998; Lewis & Cratsley 2008). In the gift-giving spiders, *P. mirabilis* and *P. ornata*, previous studies were unable to demonstrate any effect of the food gift on female fecundity or egg hatching success (Austad & Thornhill 1986; Stålhandske 2001; Albo & Costa 2010). However, it can be argued that these results are a consequence of the experimental designs that were insufficient to detect any effects from male nutrients (Vahed 1998). In fact, recent evidence suggests that females gain direct benefits from mating with males with nutritive gifts, such as accelerated egg production and oviposition in both *P. ornata* and *P. mirabilis* (Albo & Costa 2010;

Tuni et al. 2013). Furthermore, experiments in which females received food only through matings, and thus engaged in high levels of polyandry, showed an enhanced hatching success and only slightly reduced fecundity in *P. mirabilis* (Toft S & MJ Albo unpublished data). Because female spiders simultaneously develop a large number of eggs and lay them in a single clutch (Foelix 2011), the gift nutrients will be divided over all female's eggs. Thus, all males providing a nutritive gift and succeeding in fertilizing part of the eggs, actually contribute with some paternal investment. This differs from the situation in insects, which present a more gradual maturation of eggs that makes possible a direct correspondence between nutrient allocation to and fertilization of specific eggs by each male (cf. Simmons & Parker 1989).

Mating effort

The alternative but non-exclusive mating effort hypothesis suggests that by using gifts males can improve their reproductive success through increasing the number of matings or prolonging the time of copulation, regardless of any nutritive function. In insects, nuptial gifts appear to be maintained by selection to maximize ejaculate transfer and therefore reduce the risk of sperm competition from other males (Simmons & Gwynne 1991; Eady 1995; Wolfner 1997; Heifetz et al. 2001; Sakaluk et al 2006). Similarly, it has been shown that the nuptial gift functions in the context of male mating effort in both *P. ornata* and *P. mirabilis* (Stålhandske 2001; Bilde et al 2007; Albo & Costa 2010). Although males may obtain matings without a gift, the chance of acceptance dramatically increases when a gift is offered. Furthermore, the presence of a gift possibly facilitates the mating position, and a large gift keeps the female occupied for longer and consequently prolongs the mating (Stålhandske 2001; Albo & Costa 2010; Klein et al. 2013).

In accordance with the male mating effort hypothesis, males of *P. mirabilis* often perform a unique behavior during mating, so called "death feigning" or "thanatosis". This remarkable behavior usually occur when the pair is disturbed and the female moves away with the gift, possibly attempting to finish copulation. Then, while grasping the gift with their chelicerae, males "feign dead" (with stretched-out legs) and are dragged through the vegetation until the female stops. Subsequently, the male "revives" and resumes copulation (Bilde et al. 2006; Hansen et al. 2008). Thanatosis increases male mating success, and gives males the opportunity of prolonging copulation and hence increasing sperm transfer (Hansen et al. 2008). So far, there is no evidence of thanatosis in *P. ornata* males, suggesting it is a unique innovation in *P. mirabilis*.

SILK WRAPPING: POTENTIAL FUNCTIONS

The phenomenon of male gift-wrapping the prey in white silk is in itself remarkable. Wrapping of prey caught for their own consumption is common in spiders, and it usually functions to immobilize active and dangerous prey or to fix it in some place for later consumption (Barrantes & Eberhard 2007). Since males capture and kill the gift prey before wrapping it in silk, they could easily offer it without investing energy, time and silk material on wrapping. Despite this, silk wrapping seems to be an important trait in both species. So why do males wrap gift-prey in silk? Silk wrapping is not obligatory for mating to occur and sometimes males offer unwrapped prey to females. However, usually these males wrap the prey after contact with females, in particular if they were initially rejected by the female, subsequently succeeding in mating (Bilde et al. 2007; Albo & Costa 2010). The function of the gift wrapping trait is intriguing, and needs to be analyzed in detail.

Silk wrapping triggered by female silk cues

As in most spider species, contact sex pheromones associated with female silk seem to be important stimuli that elicit male courtship and silk wrapping in *P. mirabilis* and *P. ornata* (Nitzsche 1988; Lang 1996; Albo et al. 2009; Albo et al. 2011a). In *P. ornata*, males initiate nuptial gift construction only after contact with female silk, and indeed female silk seems to be an important stimulus as is the female herself (Albo et al. 2009; Albo et al. 2011a). In contrast, male *P. mirabilis* may produce wrapped gifts without the presence of female sexual stimuli, as in this species gift production may be a spontaneous behavior associated with sexual maturation, although it is still enhanced by female stimuli (Nitzsche 1988; Lang 1996; Albo et al. 2011a). In the field, it is common to find males of both species carrying wrapped gifts (Lang 1996; Albo et al. 2011b; Albo MJ personal observations). Searching for females with a ready-wrapped gift is an advantageous male strategy, because the male can avoid delays in prey capture or wrapping, and court the female immediately on encounter, and thus minimize the risk of a lost mating opportunity.

Female attraction

Sensory exploitation hypothesis

Stålhandske (2002) performed the first experiments attempting to verify the role of the wrapped gift as a visual signal during courtship in *P. mirabilis*. Since the wrapped gift has a visual resemblance to the egg-sac that Pisaurid females carry in their chelicerae, her hypothesis was that gift wrapping evolved through male sensory exploitation of the female maternal instinct. Thus, if wrapped gifts mimic egg-sacs, females are sensory biased to respond to any item resembling an egg-sac (Ryan et al. 1990; Christy 1995; Sakaluk et al. 2000). By mimicking the female's egg-sac with the white wrapped gift males would attract females' attention and increase the chances of mating. Stålhandske (2002) manipulated gift color (brown, natural white and extra-white) and found that females were more attracted and accepted extra-white gifts faster than natural white or brown gifts. Subsequent studies using wrapped and unwrapped prey were unable to verify female preference for silk wrapping, however (Bilde et al. 2007; Andersen et al. 2008). In particular, experiments that varied the gift type (using an egg-sac, a silk-wrapped fly or an unwrapped fly) revealed no female preference between egg-sacs or wrapped gifts. Instead, female hunger level (starved or satiated) predicted mating acceptance (Bilde et al. 2007). Thus, it was instead suggested that males offering food gifts exploit the females' foraging motivation in *P. mirabilis* (Bilde et al. 2007; Prokop & Maxwell 2009).

So far, the function of silk wrapping has not been well studied in *P. ornata*, but recent evidence suggests many similarities with *P. mirabilis*. For instance, males' silk investment is independent of prey size (indicated in *P. mirabilis* by Lang 1996), thus with similar silk wrapping duration small prey become white whereas big prey become grey. In spite of this, white gift color seems to have a positive effect on female acceptance (Klein et al. 2013).

Phagostimulants

It has been tested and refuted that gift silk might be a potential source of protein for females in *P. mirabilis* (Nitzsche 1988). Silk has also been suggested as a source of chemical substances used to attract females in this species (Lang 1996; Bilde et al. 2007). In fact, recent studies indicate that male pheromones associated with gift silk play a major role in female attraction in *P. ornata* (Brum et al. 2012). The role of chemical substances as phagostimulants enclosed in nuptial gifts has been reported in insects (Warwick et al. 2009). Whether substances with such effects are involved in wrapped gifts needs to be studied in spiders.

Mating control

In accordance with the male mating effort hypothesis, silk wrapping increases the time that *P. mirabilis* female spends feeding on the gift and therefore the time in copula (Lang 1996). In addition, since males maintain a hold on the gift with the claws of the third pair of legs during pedipalp insertion, silk wrapping allows the male to secure the mating position and prevents the female from escaping with the gift prior sperm transfer. Indeed, *P. mirabilis* females have higher success in stealing the prey when it is unwrapped than if wrapped (Andersen et al. 2008). Similarly, *P. ornata* males also clasp the gift with the third pair of legs and it has been observed that during face-to-face position unwrapped prey may be split in two and each sex remains with one prey piece, consequently ending the mating (Albo MJ & FG Costa unpublished data). These results indicate that silk wrapping has a significant function for maintaining male mating control.

WRAPPED PREY GIFTS IN AN ECOLOGICAL AND EVOLUTIONARY CONTEXT

Like other trechaleid species, *P. ornata* is a semi-aquatic spider associated with freshwater courses in southern Brazil, northern Argentina and Uruguay (Carico 2005). These spiders have crepuscular/nocturnal habits and are able to walk on the water surface. In the field, adults and large juveniles are usually observed during the night perching on stones and pebbles emerging from the freshwater riffles and capturing flying prey (e.g. Ephemeroptera) (Silva et al. 2006; Costa-Schmidt et al. 2008). In contrast, *P. mirabilis* occupies terrestrial habitats, typically living in meadows and has a Palearctic distribution (Bristowe & Locket 1926). It has diurnal habits, generally perching on vegetation approximately 25 cm above the ground, from where it captures prey such as Diptera, Hemiptera and Araneae (Nitzsche 1988).

Trechaleidae and Pisauridae together with Lycosidae, Ctenidae and 8 additional families compose the superfamily Lycosoidea (Griswold 1993; Coddington 2005), which comprise wandering spiders with global distributions. The classification and phylogeny of the species belonging to these families have been extensively discussed, so far based on morphological and some behavioral data (Dondale 1986; Sierwald 1990; Griswold 1993; Carico 2005). Phylogenetic analyses propose that Trechaleidae is a sister group of Lycosidae (Griswold 1993), which suggests that the nuptial gift is an independent trait in Pisauridae and Trechaleidae. If the nuptial gift is a convergent trait, it had appeared at least twice in the evolution of the Lycosoidea lineage. However, the family Trechaleidae was mainly created by species from Pisauridae, suggesting a closer relationship between these two families and bringing questions regarding a common origin of this trait. Sexual selection combined with particular ecological conditions may thus shape the evolution of adaptive sexual behaviors.

A broad understanding of selective pressures acting separately on both females and males needs substantial ecological and evolutionary frameworks. In fact, the way in which environmental constraints shape reproductive behaviors, aside from phylogenetic history, is a central subject of research. Courtship and mating behaviors are often species specific and highly ritualized, to the point of being useful systematic characters, while other aspects of reproductive behavior vary individually and are influenced by environmental factors.

Phylogenetic constraints: ritualized behaviors

From a phylogenetic perspective, courtship and mating behaviors are ritualized and conserved traits (Stratton 1996). Spiders show a wide diversity of courtship and mating behaviors, but patterns are largely consistent within families (Foelix 2011). In the particular case of gift-giving species, the male needs to present the prey gift to the female during courtship, and patterns of gift construction are very similar within and among species (Bristowe 1958; Costa-Schmidt et al. 2008; Albo et al. 2009). Once the

male has captured a prey, it attaches silk threads on the substrate and starts spinning a thin basal plate, then the male deposits the prey onto the basal plate and covers it with silk. The male finally removes the package from the substrate with the chelicerae, and shapes it into a round form rolling it with the legs. The whole silk wrapping process can be repeated several times, while adding more silk.

Gift offering postures differ slightly between *P. mirabilis* and *P. ornata*. *Paratrechalea ornata* males lift and fold their legs above the cephalothorax when presenting the gift (Costa-Schmidt et al. 2008; Fig. 1A). *Pisaura mirabilis* males present the gifts while waving the pedipalps and vertically raising the whole body (Bristowe & Locket 1926; Fig. 1B). In both species, the female then grasps the gift and the couple remain in a face-to-face position pulling the gift from either side (Fig. 1C,D). The female may steal the gift at this stage and run off with it, in which case no mating will follow (Andersen et al. 2008).



Figure 1. (A) Male gift-offering position with male on the right and female on the left in *Paratrechalea ornata*; (B) male gift-offering position with male on the left and female on the right in *Pisaura mirabilis* (photo by M.J. Albo); (C) female on left and male on right in the face-to-face position grasping the gift, during mating in *Paratrechalea ornata*; (D) female on left and male on right in the face-to-face position grasping the gift, during mating in *Pisaura mirabilis*. Photos: A) by M.C. Trillo; B, C, D) by M.J. Albo.

If the female accepts the gift (and the male) the spiders adopt the mating position, which is also stereotyped and differs between both species (Bristowe & Locket 1926; Costa-Schmidt et al. 2008). The typical mating position of *P. ornata* is similar to the one performed by lycosid spiders: the male mounts the female by first climbing over her prosoma (male and female in opposite directions), then turning towards one side of the female's abdomen and performing a pedipalp insertion. Once the insertion ends the male returns to the face-to-face position and again grasps the gift with his chelicerae. These behavioral sequences may be repeated up to four times with changing use of the pedipalps (Costa-Schmidt et al. 2008). The mating position in *P. mirabilis* differs from the one in *P. ornata* in that the male does not climb on top of the female cephalothorax. Instead, he pushes the female body upwards from the ventral side to perform pedipalp insertions (Bristowe & Locket 1926). Similarly to *P. ornata*, once

the pedipalp insertion ends the male returns to the face-to-face position and again grasps the gift with his chelicerae. Up to four pedipalp insertions can also occur here (Albo et al. 2011b).

Ecological constraints: variable behaviors

Some aspects of sexual behavior are quite variable and are likely shaped by ecological factors that influence the degree of sexual selection (Emlen & Oring 1977). One of the important drivers of this type of selection is the spatial and temporal distribution of resources. For instance, when food is scarce in the habitat individuals are forced into heavier competition, increasing the selective forces acting on both males and females. For females, the amount of food not only determines their lifespan but also the number and quality of eggs and offspring, hence directly influences reproductive success (Wise 1975, 2006). In contrast, although males in poor feeding condition may suffer a reduction in reproduction (Mappes et al. 1996; Andrade & Mason 2000; Ahtiainen et al. 2002; Kotiaho 2002; Engqvist & Sauer 2003; Hunt et al. 2004; Engels & Sauer 2006; Lomborg & Toft 2009), acquiring food during the reproductive season is less essential for them. For example, sexually mature males of many spider species stop feeding to search for mates (Foelix 2011).

The relative costs and benefits of providing and receiving nuptial gifts may be experienced differently by males and females (Arnqvist & Nilsson 2000; Vahed 2007; Gwynne 2008). Food availability is an ecological major issue in gift-giving mating systems, where males have a particular interest in acquiring nutrients to offer to females. Females may not only obtain valuable nutrients from nuptial gifts, but they can also be released from the time and energetic costs as well as the dangers of foraging. They are therefore likely to prefer males offering large and high quality gifts, thus imposing strong selection for nuptial gifts (Leimar et al. 1994; Arnqvist & Nilsson 2000; Vahed 2007). Hence, females may eventually benefit from multiple matings with males that offer nutritive gifts, as this may increase their feeding rate, fecundity and the hatching success of their eggs (Arnqvist & Nilsson 2000). Several studies have shown that differences in food intake by females during their lifetime, resulting from changes in prey availability or differences in individual foraging skills, can determine mating frequencies in gift-giving species, so that food-limited females engage in more matings (Boggs 1990; Gwynne 1990; Simmons & Bailey 1990). In the classic example of the katydids, Gwynne (1981, 1984, 1990) showed that when food is scarce, females compete intensively for gifts and males become the choosy sex, thus leading to a sex role-reversed mating system. There is evidence to believe that *P. mirabilis* males exploit females' foraging motivation, since food deprived females are more willing to accept mates than satiated ones (Bilde et al. 2007; Prokop & Maxwell 2009). Thus, as in other gift-giving species (Arnqvist & Nilsson 2000) direct benefits obtained by females via gift consumption could lead to the evolution and maintenance of polyandry. Unfortunately, little is known from the Neotropical *P. ornata*, except that females do mate multiple times (Pandulli I unpublished data) and likely benefit from that if males offer nutritive gifts.

Evidently, there is enormous lack of knowledge regarding gift-giving mating systems in spiders, particularly concerning wrapped gifts. Until now the only well studied species is *P. mirabilis*, which is a Palearctic spider belonging to Pisauridae family. The aim of this thesis is to contribute with knowledge of this and another gift-giving species, this last not only distributed on a different continent but also inhabit a different environment. By including the Neotropical spider *Paratrechalea ornata* from the Trechaleidae family in these studies, I aim to analyze, compare and discuss the function and evolution of nuptial gifts in both spider species. I present similarities and differences in sexual behavior, and discuss how both ecological conditions and phylogenetic history seem to shape variation in nuptial gift-giving behavior of males and females.

THESIS OUTLINE – HYPOTHESES AND OBJECTIVES –

GENERAL HYPOTHESIS: Males from gift-giving spider species are under strong sexual selection to wrap prey in silk and offer gifts to females. The donation is an independent trait in Pisauridae and Trechaleidae and had been originated at least twice in Lycosoidea superfamily.

GENERAL OBJECTIVE: To study and discuss the reproductive strategies, especially the sexual behavior regarding the nuptial gift, in *P. ornata* (Trechaleidae) and *P. mirabilis* (Pisauridae). To perform phylogenetic analyses with representative specimens from these families using molecular characters; including also species belonging to Lycosidae and Ctenidae (this last to root the tree). To map the behavioral character and obtain an approximation whether the nuptial gift constitutes an independent acquisition or contrary, is a synapomorphy in those taxa.

To meet the general objective I divide the thesis in four chapters with specific thematic approaches. The first three chapters discuss about nuptial gifts and sexual selection pressures in both *P. ornata* and *P. mirabilis* species, while the fourth chapter brings information about the phylogenetic relationships of the two families studied. In the first chapter (Chapter 1) I explore whether silk wrapping provides information on male foraging state as an indicator of male condition, and if females can choose males depending on gift characteristics, that will be reflected on the mating quality and the descendents. I analyze courtship, mating and offspring when expose females to well and poorly fed males. In Chapter 2, I investigate the hypothesis that males of gift-giving species are under strong sexual selection to offer a gift and could exploit female preference for the nuptial gift to gain reproductive advantages offering worthless gifts when no prey is available. Thus, I study nuptial gift content in the field and investigate the possible use of worthless gifts by males and its effect on male reproductive success. Since in both species nuptial gifts function as male mating effort, in chapter 3 I test whether prolonged copulations are correlated with more sperm stored by females and consequently higher paternity. In addition, I explore whether the gift itself is a selected trait and females can vary the amount of sperm storage according the gift presence and/or content. For this idea, I calculate and analyze the amount of sperm stored in the female spermathecae, from different mating groups that differ in gift content and presence. Finally, in Chapter 4 I explore the hypothesis that the nuptial gift was acquired independently by Trechaleidae and Pisauridae. According with this, both families do not constitute a monophyletic group, with the following structure: ((Trechaleidae, Lycosidae) Pisauridae). I perform DNA extractions and amplify mitochondrial and nuclear DNA fragments (*COI*, *12S*, *16S-L1*, *ND1*, *18S*, *28S* and *H3*), obtain the sequences and perform a phylogenetic reconstruction integrating Trechaleidae, Pisauridae and Lycosidae, using also the family Ctenidae as outgroup. Then, I map the behavioral trait- nuptial gift presence or absence- onto the resulting phylogeny.

GENERAL METHODS

During 2010-2012, I collected individuals of *Paratrechalea ornata* from Santa Lucía River (Paso del Molino, Lavalleja, 34°16'40.10"S 55°14'00.80"W), Yermal Chico stream (Treinta y Tres Province 32°53'50.65"S 54°27'35.33"W) and from Queguay River (Paysandú 32°10'37.98"S; 57°14'15.30"W), all places in Uruguay. I also collected *Pisaura mirabilis* individuals in grasslands from Mols eastern Jutland, Denmark (56°11'30.44"N; 10°40'55.80"E). I housed spiders in the laboratory according their size and ecological requirements. They were sexed and maintained individually, *P. ornata* were kept in glass jars (8.5 cm inter diameter and 7.5 cm high) containing small pebbles and water, simulating a natural environment, while *P. mirabilis* individuals were kept in vials (30 ml) moist moss (*Sphagnum* spp.). Water was provided regularly to maintain humidity. I raised immature spiders until adulthood in a climate room to accelerate development; molts were daily checked. In general, I fed individuals three times a week: those of *P. ornata* with fruit flies and those of *P. mirabilis* with houseflies, excepting when the experimental design required other feeding regimes.

Experiments were carried out under lab condition during 2010-2013. I used two types of experimental cages according the size and ecological requirements of the species: glass cages of 30x14x20 cm *P. ornata* species (Fig. 2A) and plastic cages of 22x17x6 cm for *P. mirabilis* species (Fig. 2B).

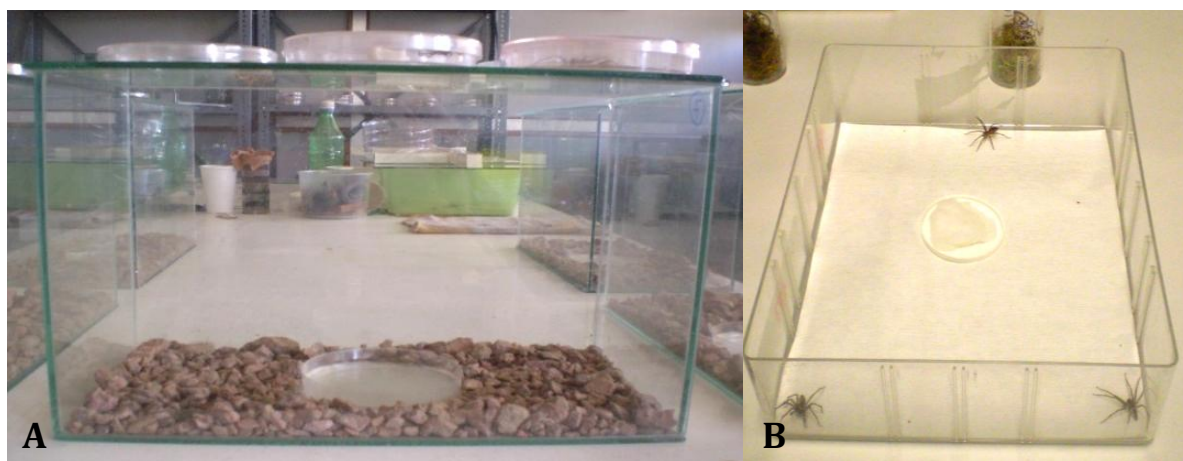
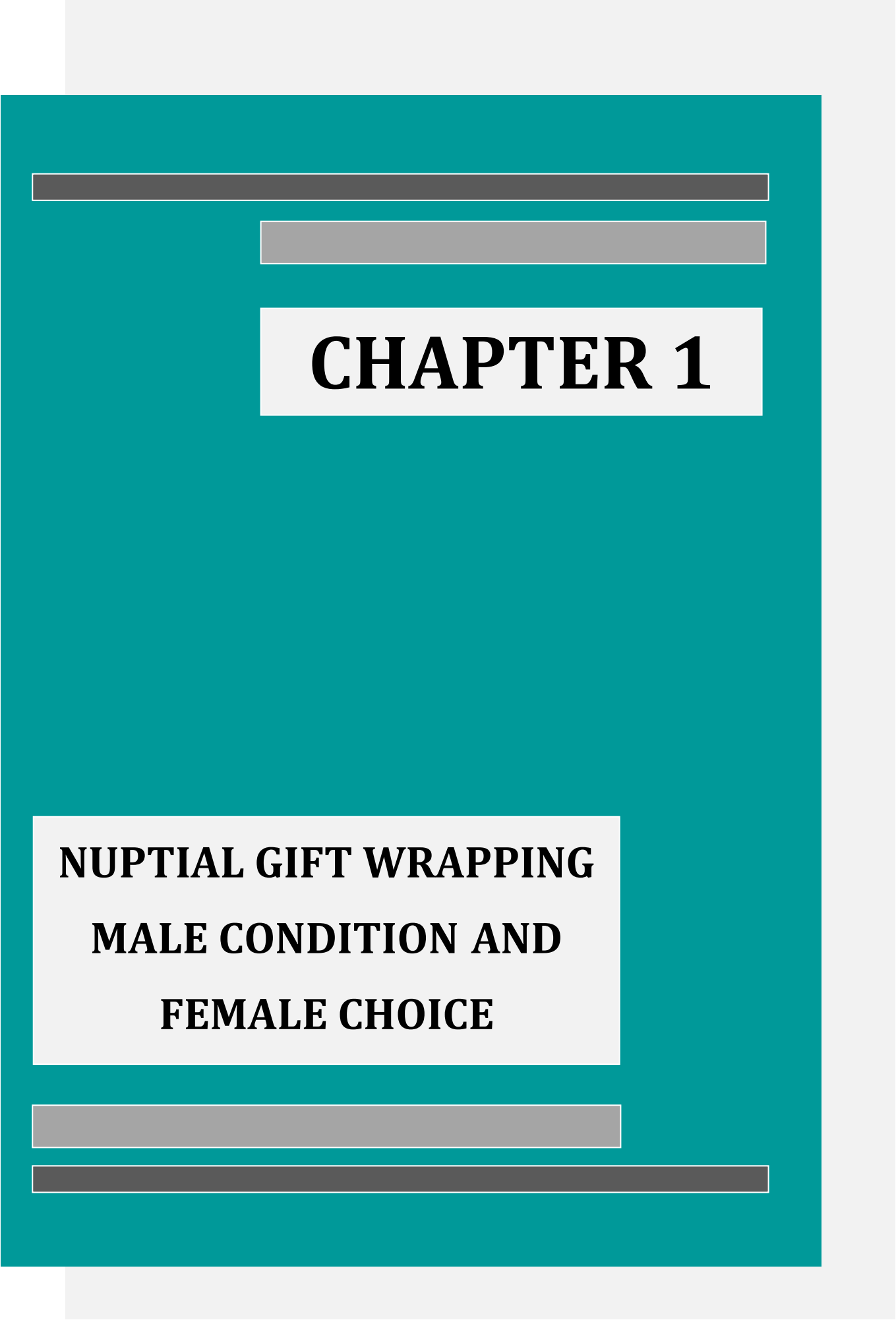


Figure 2. A) Experimental cage used for *P. ornata* species (Photo by A.L. Klein). B) Experimental cage used for *P. mirabilis* species (Photo by M.J. Albo)

STATISTICAL ANALYSES

Statistical analyses were performed using JMP 7.0 software (SAS Institute) and Past Paleontological Statistics version 1.18 (Hammer et al. 2003). Data were inspected for the normal distribution of residuals and homogeneity of variance with Shapiro-Wilk and Levene tests, respectively. Data were log or sqrt transformed whenever necessary to meet parametric assumptions. Continuous variables were analyzed using one-way ANOVA or Kruskal Wallis test when assumptions for parametric tests were not met. Student t-test or Mann-Whitney test were used for pair wise comparisons. Two-way ANOVA was used to examine potential effects of several variables and their interaction on a dependent one. ANCOVA and General linear model of Poisson and binomial family (GLM-p and GLM-b) were used to examine the potential interaction among variables. Nominal Logistic Fit was performed to analyze discrete variables. Frequencies were analyzed by both Chi-square and Fisher exact probability test. Linear regressions and correlations were performed to analyze the effect of independent variables on dependent variables. All tests were two-tailed.



CHAPTER 1

**NUPTIAL GIFT WRAPPING
MALE CONDITION AND
FEMALE CHOICE**

CAPÍTULO 1- ENVOLTURA DEL REGALO NUPCIAL, CONDICIÓN DE LOS MACHOS Y ELECCIÓN FEMENINA

ANTECEDENTES: Las preferencias femeninas pueden promover la evolución de caracteres conspicuos en los machos de forma que éstos se vuelven más atractivos e incrementan su éxito reproductivo. En muchas especies los caracteres sexuales secundarios son condición dependiente y por lo tanto indicadores honestos de la condición del macho. Consecuentemente, las hembras obtienen beneficios indirectos eligiendo las parejas de mejor calidad. Las hembras de las especies *Pisaura mirabilis* y *Paratrechalea ornata* prefieren machos que transportan regalos nupciales en sus quelíceros- usualmente presas envueltas en seda blanca- durante el cortejo. Se investigó cómo los regalos blancos envueltos en seda afectan la elección de la hembra, y también cómo la condición del macho influye en el comportamiento de envoltura.

DISEÑO EXPERIMENTAL: En *P. mirabilis* se manipuló la condición del macho (machos saciados y hambrientos) y la envoltura del regalo (regalos bien y pobremente envueltos). Se llevaron a cabo experimentos en un diseño factorial completo de forma de comprender si la envoltura en seda es usada por las hembras como un indicador honesto de la calidad del macho. En *P. ornata* primero se expusieron las hembras a tres grupos de machos (todos sin regalo): 1) machos con los quelíceros pintados de blanco, simulando la presencia de una presa envuelta en seda; 2) machos sin pintura; 3) machos con el cefalotórax ventralmente pintado de blanco (control de la pintura). Luego, se examinó si la envoltura en seda es un rasgo condición dependiente y consecuentemente un potencial indicador de la condición y calidad del macho. Se expusieron machos saciados (buenas condiciones) y hambrientos (malas condiciones) -con una presa- en seda de hembra (contexto sexual) y se registró el color del regalo como un indicador del esfuerzo de envoltura.

RESULTADOS PRINCIPALES: En ambas especies los machos en pobres condiciones intentaron envolver la presa en seda. Sin embargo, estos machos agregaron menos seda a la presa y obtuvieron regalos grises en comparación con los machos en buenas condiciones que agregaron más seda y obtuvieron regalos blancos. En *P. mirabilis* los machos en buenas condiciones fueron más exitosos en obtener cópulas y copularon por más tiempo que los machos en pobres condiciones. Sólo los machos en buenas condiciones tuvieron descendencia. En contraste, la envoltura en seda, sea la presa bien o pobremente envuelta, no afectó significativamente la elección de pareja por parte de la hembra. En *P. ornata*, las hembras contactaron más frecuentemente a los machos con quelíceros blancos y éstos tuvieron un mayor éxito de cópula que el resto machos.

CONCLUSIONES: La envoltura del regalo puede implicar altos costos para los machos, y aquellos en pobres condiciones invierten menos tiempo y menos seda en envolver la presa. Consecuentemente, la envoltura en seda es un indicador honesto de calidad del macho en ambas especies. A pesar de esto, las hembras de *P. mirabilis* no utilizan dicha información para la elección de pareja. Dado que la envoltura en seda parece beneficiar principalmente a los machos, las hembras deberían ignorar la información de la envoltura en seda y en cambio basar la elección de pareja en la condición del macho que inequívocamente beneficia su éxito reproductivo. Similarmente, las hembras de *P. ornata* también basan su elección en la condición del macho y aquellos en mejores condiciones obtienen un mayor éxito reproductivo (capítulo 2). Sin embargo, en esta especie las hembras son atraídas por los machos con

quelíceros blancos, sugiriendo un importante rol de las señales visuales durante el cortejo y elección de pareja. Aparte de la función visual encontrada aquí, también se han reportado rastros químicos asociados a la seda del regalo, ambas señales favorecen a los machos. Sin embargo, es una discusión controversial si la envoltura en seda es una desventaja para las hembras de *P. ornata*. Por ejemplo, cuando las hembras rechazan a los machos, estos muy a menudo envuelven en seda la presa y son luego usualmente aceptados. Consecuentemente, consistente con los resultados presentados aquí es posible que las hembras evalúen la condición de los machos basadas en el despliegue masculino durante el proceso de envoltura en seda y la apariencia del regalo.

CHAPTER 1- NUPTIAL GIFT WRAPPING, MALE CONDITION AND FEMALE CHOICE

BACKGROUND: Female preferences can promote the evolution of conspicuous male traits by which males become more attractive and increase their reproductive success. In many species, condition-dependent secondary sexual traits are important as these can be honest indicators of male condition and thus, females can gain indirect benefits by choosing the best quality partner. Females from both spider species *Pisaura mirabilis* and *Paratrechalea ornata* prefer males carrying nuptial gifts in their chelicerae -usually preys wrapped in white silk- during courtship.

EXPERIMENTAL DESIGN: Here I investigated how white wrapped gift affects female choice, as well as how male condition influences gift wrapping behavior. In *P. mirabilis* I experimentally manipulated male condition (satiated and starved males) and gift wrapping (well- and poorly wrapped gifts). I carried out experiments in a fully factorial design to understand whether silk wrapping is used by females as an honest indicator of male quality. In *P. ornata* I first exposed females to three groups of males (all without a gift): 1) males with their chelicerae painted white, simulating presence of a wrapped prey; 2) males without paint; 3) males with the cephalothorax ventrally painted white (paint control). Thereafter, I examined if silk wrapping is a condition dependent trait and therefore a potential indicator of male condition and quality. I exposed satiated (good condition) and starved (poor condition) males carrying a prey to the female silk (sexual context), and registered the gift color as a proxy for silk wrapping effort.

MAIN RESULTS: In both species even males in poor condition attempted to wrap the prey in silk. However, these males added less silk to the prey than males in good condition, obtaining grey and white gifts, respectively. In *P. mirabilis* males in good condition were more successful in obtaining matings and obtained longer copulations than males in poor condition and only those obtained offspring. In contrast, gift wrapping, whether was well or poor wrapped, did not affect female mate choice. In *P. ornata*, females contacted males with white chelicerae more often and those males obtained higher mating success than other males.

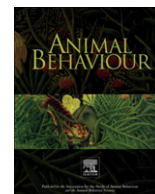
CONCLUSIONS: Gift construction may involve high costs for males, and those in low condition invest less time and silk in wrapping the prey. Consequently, silk wrapping is an honest indicator of male quality in both species. But in spite of this *P. mirabilis* females did not use this information in mate choice. Since in this species it seems that gift wrapping mainly functions to promote male interests, females should therefore evolve to ignore the wrapping information and instead base their mate choice decision on male condition that unequivocally benefits their own reproductive success. Similarly, *P. ornata* females also based their choice on male condition as those in better condition have higher reproductive success (Chapter 2). However, females are attracted to males with white color on their chelicerae, suggesting an important role of visual cues during courtship and mate choice. Aside from the visual function found here, chemical cues also involved in female attraction were recently reported in the gift silk and both signals benefit males. However, whether silk wrapping is against *P. ornata* females' interests is controversial. For instance, when females reject males, they very often start silk wrapping of the prey and interestingly, males are usually accepted afterwards. Hence, consistently with the results presented here it is possible that females evaluate male condition based on silk wrapping performance and gift appearance.

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FEMALE SPIDERS IGNORE CONDITION-DEPENDENT INFORMATION FROM NUPTIAL GIFT WRAPPING WHEN CHOOSING MATES

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Female spiders ignore condition-dependent information from nuptial gift wrapping when choosing mates

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In many species, condition-dependent secondary sexual traits are important for female choice. In the spider *Pisaura mirabilis*, males offer females a nuptial gift (prey wrapped in silk) during courtship. Gift construction may involve high costs for males, and those in low condition invest less time and silk in wrapping the prey. We investigated how male condition and gift wrapping affect male reproductive success and whether females use the wrapped gift as an honest indicator of male condition. We experimentally manipulated male condition (satiated and starved males) and gift wrapping (well- and poorly wrapped gifts) and carried out experiments in a fully factorial design. We found that males in good condition were more successful in obtaining matings and obtained longer copulations than males in poor condition. In contrast, gift wrapping did not affect female mate choice. Only good-condition males obtained offspring. Gift wrapping is an honest indicator of male condition, but in spite of this females did not use this information in mate choice. We argue that because gift wrapping mainly functions to promote male interests, good-condition males would also be better at cheating the female, for example using wrapping to hide a worthless gift. Females should therefore evolve to ignore the wrapping information and instead base their mate choice decision on male traits that unequivocally benefit their own reproductive success. Our results are consistent with this prediction, as females discriminated males based only on their actual feeding condition, and this choice provided them with direct reproductive benefits.

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The ‘handicap hypothesis’ explains how secondary sexual traits, such as ornaments or behaviours, can be costly and honest signals for mate choice (Zahavi 1975; Zahavi & Zahavi 1997). Several examples verify that only males in good condition can efficiently perform and maintain their signals during courtship and mating; as a result males in poor condition are often rejected by females and suffer reduced reproductive success (Mappes et al. 1996; Andrade & Mason 2000; Ahtiainen et al. 2002; Kotiaho 2002; Engqvist & Sauer 2003; Hunt et al. 2004; Engels & Sauer 2006; Lomborg & Toft 2009). Condition-dependent secondary sexual traits may be influenced by food acquisition, as shown in the spider *Schizocosa ocreata* in which hair tufts on the front tibia are negatively affected by a low-quality individual diet (Uetz et al. 2002; Hebets et al. 2008). The tufts are an important part of the visual signal during courtship, and females preferentially select males with well-developed tufts (Uetz et al. 2002; Hebets et al. 2008). Similarly, only males in good feeding

condition can maintain high rates of abdominal ‘drumming’ during courtship in the spider *Hygrolycosa rubrofasciata* (Mappes et al. 1996) indicating high energetic costs of drumming (Kotiaho et al. 1998). As a result females prefer the most actively drumming males (Parri et al. 1997).

In the gift-giving spider *Pisaura mirabilis*, gift construction is affected by male feeding condition (Albo et al. 2011a). The nuptial gift consists of prey wrapped in silk and is constructed when males perceive sexual stimuli as contact pheromones associated with the female silk (Nitzsche 1988; Albo et al. 2011a). In this system, females prefer males that offer a nuptial gift during courtship and those males experience the highest reproductive success (Stålhandske 2001; Prokop 2006; Bilde et al. 2007). Sexual selection for producing nuptial gifts forces males in poor condition to construct a gift, but this behaviour is constrained and performed inefficiently, as these males spend less time and less silk in prey wrapping than good-condition males (Albo et al. 2011a).

Silk wrapping has an important function for males: it facilitates male handling and control over the gift as it allows the male a better grasp of the gift compared with an unwrapped insect (Andersen et al. 2008). A well-wrapped gift reduces the risk of the female running away with it before sperm transfer is completed. In

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addition, silk wrapping provides males with the opportunity to disguise the gift's content, which may vary from genuine nutritious insect prey to worthless items such as empty arthropod exoskeletons, and thus deceive the females (Albo et al. 2011b). Therefore, silk wrapping should act against female interests, unless females use information from the gift wrapping for mate choice. During courtship, females could visually evaluate mate quality by the amount and quality of the silk wrapping and prefer to mate with males that are able to offer well-wrapped gifts as an indication of their good condition. By manipulating gift colour, Stålhandske (2002) found that females are more attracted to bright than dark gifts, revealed by a reduced latency of gift acceptance.

Since silk wrapping has several functions for males, the fact that male condition is a limiting factor for silk wrapping raises the question whether females use gift wrapping as an honest signal of male condition. We experimentally manipulated male diet (to create males in good and poor condition) and gift wrapping (well-wrapped and poorly wrapped gifts), and carried out mating experiments in a fully factorial design. If females during courtship use information on the quality of gift wrapping as an indicator of male condition, males offering well-wrapped gifts should be more successful in obtaining matings than those offering poorly wrapped gifts, when male feeding condition is controlled for. Alternatively, female mate choice could depend on male condition itself or an interaction between both factors.

METHODS

We collected juvenile and subadult *P. mirabilis* spiders in April 2010 and 2011 at the Mols Laboratory in Eastern Jutland, Denmark. In the laboratory, spiders were housed individually in vials (30 ml) containing moist moss (*Sphagnum* spp.) and water was provided two to three times a week to maintain humidity. Males and females were raised at room temperature ($24.0 \pm 0.1^\circ\text{C}$) and natural photoperiod. We fed individuals with house flies, *Musca domestica*, three times per week until maturation.

Male Condition and Gift Wrapping

To examine how male condition and gift wrapping interact and influence reproductive success we designed four experimental male groups, combining male feeding condition (satiated males = good-condition males and starved males = poor-condition males) and gift-wrapping quality (well-wrapped and poorly wrapped, see below). The four experimental groups were: 'GC-WW' consisting of good-condition males offering well-wrapped gifts ($N = 24$); 'GC-PW' consisting of good-condition males offering poorly wrapped gifts ($N = 20$); 'PC-WW' consisting of poor-condition males offering well-wrapped gifts ($N = 24$) and 'PC-PW' consisting of poor-condition males offering poorly wrapped gifts ($N = 25$).

We obtained differences in male condition by exposing males to two different feeding regimes after their maturation to adulthood. Males in good condition were satiated by receiving a house fly every day, while males in poor condition were starved by being fed one house fly in 3 weeks. Male condition index at the time of the experiment was calculated as the residuals of the body weight/cephalothorax width regression (Jakob et al. 1996), and was 0.009 ± 0.001 mg for good-condition males and -0.008 ± 0.001 mg for poor-condition males (mean $\pm$ SE; $F_{1,90} = 144.0$, $P < 0.0001$). As gift wrapping varies with male condition (Albo et al. 2011a) we used gifts constructed by males in good condition as well-wrapped gifts, and those constructed by males in poor condition as poorly wrapped gifts. In the GC-WW and PC-PW groups, we allowed the males to construct and offer their own gift to females. In the GC-PW and

PC-WW groups, we allowed GC and PC males to construct gifts and subsequently switched gifts between the groups, so that good-condition males offered poorly wrapped gifts produced by poor-condition males, and poor-condition males offered well-wrapped gifts produced by good-condition males. In this species, it is easy to remove a gift from a male using forceps, and males readily accept a nuptial gift offered in forceps. Only gifts typical of poor- and good-condition males (see below) were used for switching. We did not switch gifts between individuals of the GC-WW and PC-PW groups because Bilde et al. (2007) found no effects of gift switching on male behaviour. To ensure that gifts differed in wrapping quality between experimental groups, we compared gift construction behaviours between good- and poor-condition males. As indicators of wrapping investment we measured gift construction duration (time between first and last silk wrapping, including periods of gift handling without wrapping), the number and duration of silk-wrapping bouts (including only the time spent wrapping the prey), and gift colour was scored as 'black' or 'white' based on its appearance, reflecting the amount of silk. Gifts were classified as 'black' when the prey was visible through the silk and 'white' if it was not.

Mating experiments were performed in May–June after 3 weeks of the assigned feeding regime and were carried out in transparent plastic cages (22×17 cm and 6 cm high) with paper covering the bottom. A female was placed in the experimental cage at least 1 h prior to the experiment, allowing her to deposit silk threads. We then removed the female and introduced a male, thus exposing him to the female's silk, and the male was provided with a house fly for gift construction. Triggered by silk-borne female cues, males usually initiated gift construction immediately. Ten minutes after wrapping had terminated we assumed the gift construction was completed and carefully reintroduced the female into the experimental cage with the male ($t = 0$). In the gift-switched groups (GC-PW and PC-WW) we gently removed the gift from the male with forceps 10 min after the last silk-wrapping bout and offered him the replacement gift; subsequently the female was transferred to the experimental cage. All individuals used in the experiments were initially virgins and were not reused.

We registered male courtship and mating success. Courtship duration (min) was measured from when the male made contact with the female and offered the gift until the first pedipalp insertion. During mating, the male pushes up the female's abdomen and performs alternate pedipalp insertions into the female's sperm storage organs. After each insertion the male returns to a face-to-face position with the female, grabbing the gift with his chelicerae (Bristowe 1958). Mating duration was measured from the beginning of the first to the end of the last pedipalp insertion and included the time the male and female were in the face-to-face position and handled the gift. We classified individual insertions depending on their duration. 'Long insertions' lasted for at least 0.5 min while 'short insertions' were very brief, that is, less than 1 s. Expansions of the hematochae (the structure that creates the pressure that injects the sperm into the female genitalia) were observed for both long and short insertions. Insertion duration was measured from pedipalp insertion until pedipalp disengagement, and the sum of all long insertion durations was considered the total insertion duration (probably equivalent to the time of sperm transfer). Short insertions were too brief to measure accurately and were not included in the total insertion duration; however, we counted them and compared their frequencies between groups.

Subsequent to the mating experiment, females were kept individually in the same vials and under similar condition as they were raised in, and all females were fed three house flies per week. Vials containing females that had produced an eggsac were placed under light bulbs 20 cm above the vials to increase the temperature to

26.7 ± 0.1 °C for 3 h at noon every day to enhance the hatching success of eggsacs. We measured reproductive fitness by counting the spiderlings emerging from the first eggsac produced by each female.

Statistical Analyses

Statistical analyses were performed using JMP 7.0 software (SAS Institute, Cary, NC, U.S.A.). Response variables were inspected for normal distribution of residuals and homogeneity of variance with Shapiro–Wilk and Levene tests, respectively. Dependent variables were analysed by fully factorial models including male condition, gift wrapping and their interaction. Continuous variables (courtship, mating and total insertion duration) were analysed with two-way ANOVA, number of insertions by two-way ordinal logistic fit, mating success by two-way nominal logistic fit, and number of spiderlings by a general linear model of the Poisson family (GLM-p). Since the data were collected over two seasons, all tests were initially made with year as a third factor, but it was in no case significant ($0.050 \leq P \leq 0.82$ for the variables shown in Table 1) and was therefore excluded in the final models.

RESULTS

Males in good condition spent longer on gift construction and silk wrapping, and they performed more silk-wrapping bouts than males in poor condition. Consequently, gifts of males in good condition appeared completely white compared with gifts of males in poor condition, which appeared black with little or no silk wrapping (Table 1). These results justify our use of the gifts from good-condition males as well wrapped and those from poor-condition males as poorly wrapped, respectively.

Male condition affected mating success significantly, as good-condition males were more successful in mating than poor-condition males ($\chi^2_1 = 14.9$, $P < 0.0001$; Fig. 1). There was no indication of an effect of gift wrapping ($\chi^2_1 = 1.8$, $P = 0.17$) or for a significant interaction between male condition and gift wrapping ($\chi^2_1 = 1.1$, $P = 0.27$). In the four treatment groups all males performed courtship; however, good-condition males courted females for a shorter duration and thus initiated mating earlier than poor-condition males (Table 2, Fig. 2a). Conversely, mating duration, total insertion duration and average insertion duration were longer for males in good condition than for males in poor condition (Table 2, Fig. 2b, c, d). Gift wrapping had no significant effect on courtship duration, mating success or insertion duration (Table 2).

We found that good-condition males performed a significantly higher number of long pedipalp insertions than poor-condition males (Table 2, Fig. 3a). Males in poor condition performed more short insertions (Table 2, Fig. 3b), and they had more difficulty

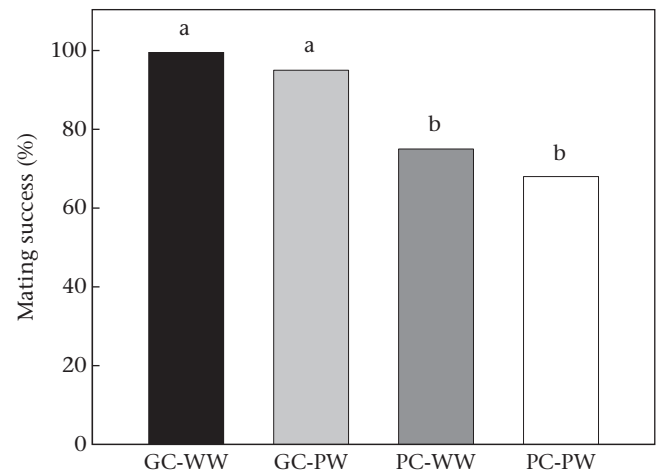


Figure 1. Male mating success in four experimental groups. GC-WW = good-condition males offering well-wrapped gifts to females, GC-PW = good-condition males offering poorly wrapped gifts, PC-PW = poor-condition males offering poorly wrapped gifts, PC-WW = poor-condition males offering well-wrapped gifts. Different letters indicate significant differences ($P < 0.05$) in pairwise chi-square tests.

maintaining the mating position and the hematochoal expansion during each pedipalp insertion than males in good condition. Gift wrapping did not affect male behaviours except for the number of short insertions (Table 2), where males offering poorly wrapped gifts performed significantly fewer short insertions independent of their condition.

Females mated with males in good condition produced on average 38.1 ± 6.3 spiderlings, whereas no female mated to a male in poor condition succeeded in producing spiderlings. There was no effect of gift wrapping on fitness (Table 2).

DISCUSSION

Our results confirmed the findings of Albo et al. (2011a) that gift-wrapping investment is an honest indicator of male condition, which reveals the actual feeding state of the male in *P. mirabilis*, as males in good condition produced well-wrapped gifts compared to males in poor condition. This may indicate that males in poor

Table 1
Nuptial gift construction by males in good and poor condition

	Gifts constructed by good-condition males ($N=48$)	Gifts constructed by poor-condition males ($N=45$)	Statistics
Gift construction duration (min)	10.7±5.5	5.5±3.8	$U=488.5$, $P<0.0001$
Silk-wrapping duration (min)	6.6±2.8	3.6±2.9	$t_{91}=5.85$, $P<0.0001$
No. of silk-wrapping bouts	3.7±1.5	2.5±1.5	$U=565.5$, $P<0.0001$
Gift colour (no. white/no. black)	45/3	1/44	$\chi^2=77.8$, $P<0.0001$

Statistical comparisons were performed using a Mann–Whitney U test, Student's t test and chi-square test.

Table 2
Effects of male condition, gift wrapping and their interaction on courtship and mating duration, total and average pedipalp insertion duration, number of long and short pedipalp insertions, and the average number of spiderlings produced in each experimental group

	N	Male condition ($df=1$)	Gift wrapping ($df=1$)	Interaction ($df=1$)
Courtship duration	78	$F=5.90$, $P=0.02$	$F=1.74$, $P=0.19$	$F=0.16$, $P=0.68$
Mating duration	78	$F=40.15$, $P<0.0001$	$F=0.0003$, $P=0.99$	$F=1.08$, $P=0.30$
Total insertion duration	63	$F=115.9$, $P<0.0001$	$F=0.01$, $P=0.91$	$F=1.83$, $P=0.18$
Average insertion duration	63	$F=6.58$, $P<0.0001$	$F=0.15$, $P=0.89$	$F=1.32$, $P=0.19$
No. of long insertions*	78	$\chi^2=21.96$, $P<0.0001$	$\chi^2=0.07$, $P=0.78$	$\chi^2=0.009$, $P=0.92$
No. of short insertions*	78	$\chi^2=47.78$, $P<0.0001$	$\chi^2=6.61$, $P=0.01$	$\chi^2=0.06$, $P=0.80$
No. of spiderlings*	78	$\chi^2=712.4$, $P<0.0001$	$\chi^2<0.0001$, $P=0.99$	$\chi^2<0.0001$, $P=0.99$

Statistical comparisons were performed using two-way ANOVA, ordinal logistic regression or GLM-p (*). Significant P values are shown in bold.

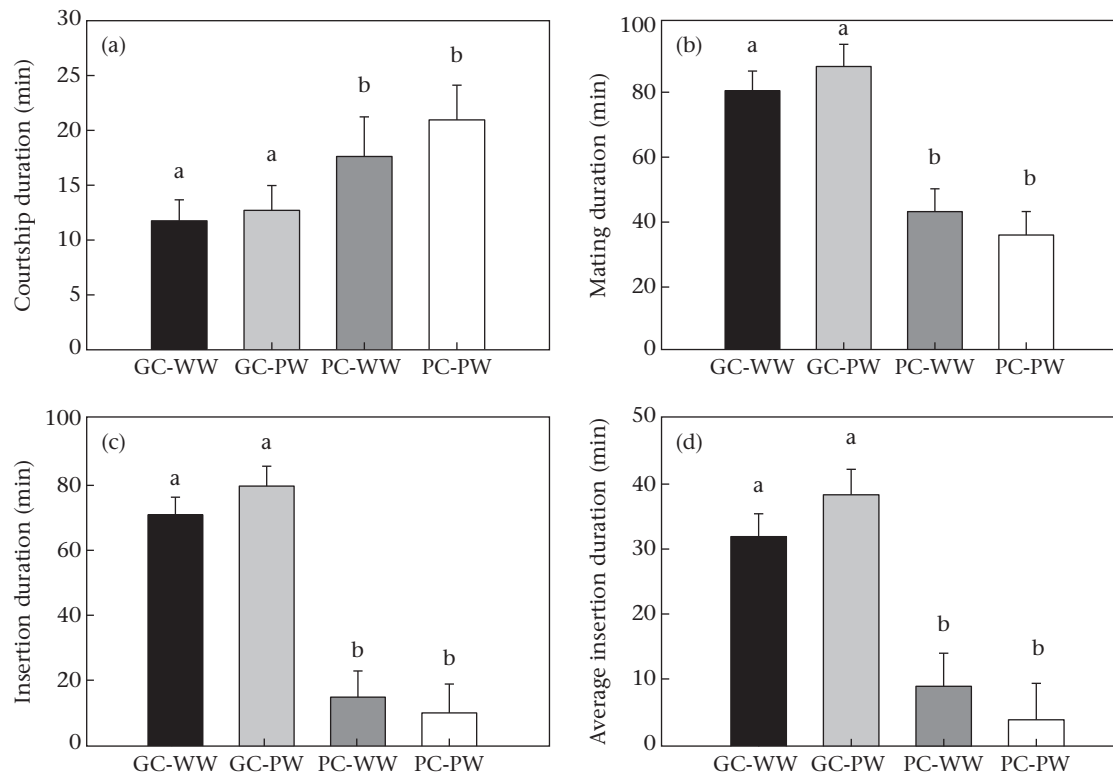


Figure 2. Male mating behaviours in four experimental groups (mean and SE). (a) Courtship duration, (b) mating duration, (c) total insertion duration and (d) average insertion duration per female in GC-WW (good-condition males offering well-wrapped gifts to females), GC-PW (good-condition males offering poorly wrapped gifts), PC-WW (poor-condition males offering well-wrapped gifts) and PC-PW (poor-condition males offering poorly wrapped gifts). Post hoc comparisons were performed using Tukey's test after one-way ANOVAs; different letters indicate significant differences ($P < 0.05$).

condition have high energetic or material costs of silk wrapping and/or are impaired in their ability to perform these behaviours; thus silk wrapping potentially can be used as an honest indicator in mate choice (Zahavi 1975; Zahavi & Zahavi 1997). However, *P. mirabilis* females did not use information revealed by the gift-wrapping trait in mating decisions. Mating success depended solely on male condition.

Previous studies have assigned several roles to the gift-wrapping trait that serve to promote male interests: gift wrapping in *P. mirabilis* may function to disguise a worthless gift (Albo et al. 2011b), facilitate male control over gift possession and thus over mating (Andersen et al. 2008), and prolong copulation duration (Lang 1996). These effects favour male interests and may lead to mating rates and durations that are suboptimal for females (Arnqvist & Rowe 2005). Females should thus evolve resistance to the gift-wrapping information (Bilde et al. 2007) and instead base their mate choice decisions on traits that directly and unequivocally benefit their own reproductive success. Our results are consistent with this argument. Evidently, females mated with good-condition males reproduced successfully, while those mated to poor-condition males did not; hence females gained direct benefits from choosing males in good condition.

Male feeding condition generally has strong effects on male courtship performance and female mate choice in many species such as crickets, scorpionflies, beetles and other spiders (Gwynne 1993; Mappes et al. 1996; Andrade & Mason 2000; Ahtiainen et al. 2002; Kotiaho 2002; Engqvist & Sauer 2003; Hunt et al. 2004; Hoefler et al. 2008; Lomborg & Toft 2009). In fact, males often reveal information about their body condition by courtship intensity, for example by strong vibrations and displays (Mappes et al. 1996; Parri et al. 1997; Kotiaho et al. 1998; Schneider & Lesmono 2009; Byers et al. 2010) or by demonstrating individual

skills (Byers et al. 2010). In our study, males in good condition (whether with well- or poorly wrapped gifts) experienced shorter courtship and started mating earlier than males in poor condition, indicating that female acceptance is probably mediated by the male's active performance. A similar phenomenon was found in a wolf spider, in which female choice depended on aspects of male courtship, irrespective of male ornamentation (Shamble et al. 2009).

Male condition is also related to several advantages for males, for instance short courtships decrease the time exposed to predators and diminish the chances of attracting competitors (Kotiaho et al. 1998; Balsby & Dabelsteen 2005; Hoefler et al. 2008; Wilgers et al. 2009), whereas long matings increase sperm transferred by males and therefore the paternity in a sperm competition context (Simmons 2001). *Pisaura mirabilis* males in good condition obtained longer matings and were more successful than poor-condition males. Although we did not quantify it, we observed that males in poor condition were less able to reach female genitalia and had problems maintaining the hematochoae expanded during pedipalp insertions. Consequently, these males performed fewer and shorter insertions leading to a remarkably low fertilization success of zero. Sperm transfer in *P. mirabilis* is correlated with insertion duration (M. Albo, S. Toft & T. Bilde, unpublished data) and it is probably not accomplished during short insertions. Poor-condition males performed some long insertions although fewer than good-condition males and their average duration was shorter. Accordingly, they must be assumed to have transferred some sperm. Our results may therefore indicate that a threshold duration of long pedipalp insertions is required for enough sperm to be transferred to secure egg fertilization. Other explanations for the low success of poor-condition males are possible, for instance in poor-condition males the ability to transfer sperm may be

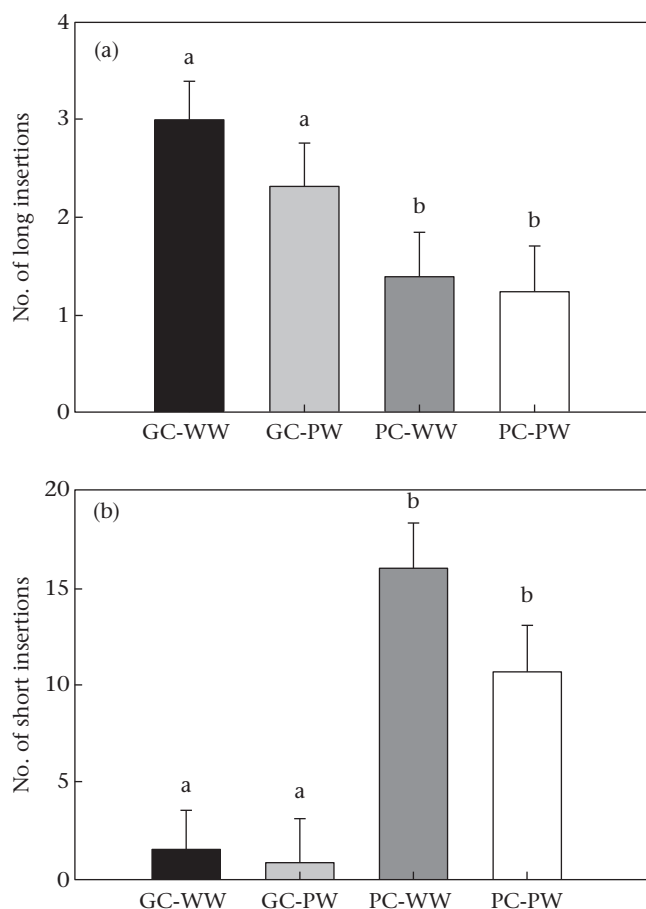


Figure 3. (a) Number of long pedipalp insertions (>0.5 min) and (b) number of short insertions (<1 s, mean $\pm$ SE), in four experimental groups, GC-WW (good-condition males offering well-wrapped gifts to females), GC-PW (good-condition males offering poorly wrapped gifts), PC-WW (poor-condition males offering well-wrapped gifts) and PC-PW (poor-condition males offering poorly wrapped gifts). Post hoc comparisons were performed using Tukey's test after one-way ANOVAs; different letters indicate significant differences ($P < 0.05$).

impaired. They may also suffer from low sperm supply or other physiological deficiencies that restrict their success in sperm transfer (Proctor 1992; Andrade & Mason 2000) even if sperm is available. Alternatively, females may be capable of storing or using the sperm differentially depending on male condition. Whether male mating success was driven by female mate choice for males in good condition or lack of ability for males in poor condition to perform courtship, mating and sperm transfer, we conclude that male success depended on male condition and was independent of gift wrapping.

We note that gift wrapping affected only short insertions, and this effect was independent of male condition. Males with poorly wrapped gifts had fewer short insertion attempts. A possible explanation for this may be derived from the fact that it is more difficult for the males to control a poorly wrapped gift (Andersen et al. 2008). Short insertions indicate that the male also has difficulties with mating. With a poorly wrapped gift the male must divert more of his attention to gift control at the expense of his attention to mating (cf. Dukas 2004). If gift wrapping has any effects of prolonging mating duration or making the gift more attractive to the female (Lang 1996; Stålhandske 2002) these effects were overridden by the effects of differences in condition, which, admittedly, were here extreme. With smaller variation in male condition, as is probably the situation in nature, wrapping might possibly have an effect on male mating success.

In conclusion, offering a wrapped gift in *P. mirabilis* is not in itself sufficient to achieve a successful mating. Instead, it appears that male performance during courtship provides honest signals to the female about male quality, as is the case for many other animal species (Byers et al. 2010).

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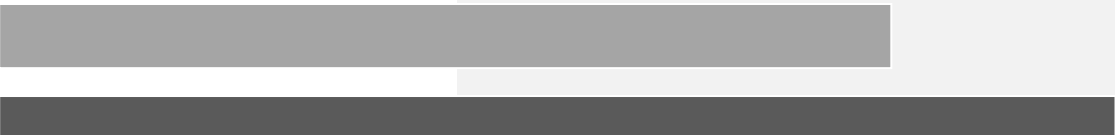
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SILK WRAPPING OF NUPTIAL GIFTS AS VISUAL SIGNAL FOR FEMALE ATTRACTION IN A CREPUSCULAR SPIDER

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Silk wrapping of nuptial gifts as visual signal for female attraction in a crepuscular spider

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Abstract An extensive diversity of nuptial gifts is known in invertebrates, but prey wrapped in silk is a unique type of gift present in few insects and spiders. Females from spider species prefer males offering a gift accepting more and longer matings than when males offered no gift. Silk wrapping of the gift is not essential to obtain a mating, but appears to increase the chance of a mating evidencing a particularly intriguing function of this trait. Consequently, as other secondary sexual traits, silk wrapping may be an important trait under sexual selection, if it is used by females as a signal providing information on male quality. We aimed to understand whether the white color of wrapped gifts is used as visual signal during courtship in the spider *Paratrechalea ornata*. We studied if a patch of white paint on the males' chelicerae is attractive to females by exposing females to males: with their chelicerae painted white; without paint; and with the sternum painted white (paint control). Females contacted males with white chelicerae more often and those males obtained higher mating success than other males. Thereafter, we explored whether silk wrapping is a condition-dependent trait and drives female visual attraction. We exposed good and poor condition males, carrying a prey, to the female silk. Males in poor condition added less silk to the prey than males in good condition, indicating that gift wrapping is an indicator of male quality and may be used by females to acquire information of the potential mate.

Keywords Female visual attraction · Nuptial gifts in spiders · *Paratrechalea ornata* · Condition dependence

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Introduction

Nuptial gift-giving is a relatively common behavior found in different animals, but particularly known in invertebrate species. It comprises a broad diversity of donations that males transfer—orally or together with the sperm—to females during courtship and mating (Vahed 1998, 2007; Gwynne 2008; Lewis and South 2012). Nuptial gifts can be produced by males themselves (endogenous) such as salivary or glandular secretions, but can also include items collected by males from the environment (exogenous) like prey or seeds (Lewis and South 2012). In spiders, there are three described types of nuptial gifts all transferred orally and increasing male mating success (Albo et al. 2014). The male's body, as in spiders from the family Theridiidae were the male offers his abdomen to the female (Andrade 1996). External glandular secretions connected to head protuberances as in the families Theridiidae, Linyphiidae, and Pholcidae (Lopez 1987; Huber 1997; Vanacker et al. 2003; Michalick and Uhl 2011; Kunz et al. 2012). Prey wrapped in silk reported for spiders from the families Pisauridae and Trechaleidae (Bristowe 1958; Costa-Schmidt et al. 2008).

Trechaleidae and Pisauridae belong to the superfamily Lycosoidea (Griswold 1993; Coddington 2005) and the phylogenetic data suggest that the prey gift is a convergent trait. If so, there is a surprisingly set of similarities between species which evidence the influence of sexual selection and particular environmental conditions on the evolution of gift-giving behavior. The studied species in the subject of sexual selection are the Palearctic spider *Pisaura mirabilis* (Pisauridae) (Bristowe 1958) and the Neotropical spider *Paratrechalea ornata* (Trechaleidae) (Costa-Schmidt et al. 2008). Both are hunting spiders but vary in their life style, as they live in different habitats and are active in different moments of the day. *P. ornata* is a semi-aquatic and crepuscular spider associated to streams and rivers, while *P. mirabilis* is a terrestrial and diurnal spider living in meadows.

In these species, females prefer males that offer nuptial gifts as males courting with a gift increase the chances of obtaining a mating and prolong copulation duration compared to males courting without a gift (Stålhandske 2001; Albo and Costa 2010). Silk wrapping of the gift is not essential to obtain a mating, however usually males perform gift wrapping if females initially reject them, which appears to increase the chance of a mating (Bilde et al. 2007; Albo and Costa 2010). While silk wrapping in spiders is usually used to immobilize active and dangerous prey or to fix it in some place for later consumption (Barrantes and Eberhard 2007), the function of silk wrapping is particularly intriguing in gift-giving species. Since males capture and kill the prey or even collect inedible items before adding silk (Albo and Costa 2010; Albo et al. 2011a), gift-wrapping behavior may have additional functions and needs to be studied in other contexts.

The silk wrapping of the gift may be an important trait under sexual selection, if it is used by females as a signal providing information on male quality. In *P. mirabilis*, it has been suggested as a visual signal for luring females during courtship (Stålhandske 2002; Albo et al. 2011b). Stålhandske (2002) found that males with white wrapped gifts are accepted faster than males with dark gifts and suggested males exploit the female maternal care instinct, but recent studies rejected this idea and suggested male exploitation of female foraging motivation (Bilde et al. 2007; Prokop and Maxwell 2009). Thus, females would respond to conspicuous male traits because these traits stimulate their sensory system (West-Eberhard 1984; Basolo 1991), an idea suggested in several species (Ryan et al. 1990; Basolo 1990; Proctor 1991, 1992; Christy 1995; Sakaluk 1984, 2000; Rodd et al. 2002; Christy et al. 2003a; b; Madden and Tanner 2003; Reinhardt et al. 2009). Nevertheless, *P. mirabilis* males in poor condition are usually limited in their ability to construct gifts and perform courtship, experiencing lower reproductive success than males in good condition (Albo et al. 2011b; Albo et al. 2012). Silk wrapping may be an honest indicator of male quality, as females prefer males that reveal their genetic quality via honest and costly signals (Zahavi 1975; Zahavi and Zahavi 1997).

So far, the function of silk wrapping has not been investigated in *P. ornata*. Considering that most of the behavioral information is restricted to the well-known species *P. mirabilis*, the inclusion of data from other gift-giving spider, such as *P. ornata*, gives the possibility for comparisons which is ultimately crucial for understanding mechanisms behind nuptial gift-giving behavior in spiders. In both species, silk wrapping is stimulated by pheromones associated with female silk. Thus, males can construct a gift in anticipation of an encounter with females, leading to several advantages as being ready to mate and minimize risks of female desertion (Albo et al. 2009; Albo et al. 2011b). A recent study in *P. ornata* indicated that males invest similar time in silk

wrapping regardless prey size, thus small gifts are usually whiter than large ones (which are grey) and females accepted white gifts faster than grey ones (Klein et al. 2013). During mate searching and courtship, *P. ornata* males walk towards females with vibrating forelegs and pedipalps, while carrying the gift in their chelicerae. After finding a female, males offer the gift by raising and folding the forelegs above the cephalothorax, a particular visual posture called “hyperflexion” (Costa-Schmidt et al. 2008). Additionally, there is a clear dimorphism in chelicerae size and color between sexes with males’ chelicerae being bigger and more reddish than those of females, and it was suggested that male chelicerae may be a sexually selected trait (Costa-Schmidt and Araújo 2008). Although visual signals are expected to be less frequent than vibrations or chemical signals in nocturnal species, the data available raises the question whether the wrapped white gift can act as a visual signal during courtship. Physiological and behavioral studies in other related night-active species have shown highly developed visual systems and an apparently important role of vision during night (Barth 2002).

The goal of this study was to integrate two different experimental approaches in order to understand whether silk wrapping may be used as visual signal by females during courtship in the spider *P. ornata*. Based on the previous results that suggested some effect of gift color on female acceptance (Klein et al. 2013), (1) we investigated if white color on males’ chelicerae attracts females, exposing females to males (a) with the chelicerae painted white (mimicking a white gift), (b) without paint, (c) with the sternum (ventral cephalothorax) painted white (paint control). Additionally, (2) we examined if silk wrapping is a condition-dependent trait and therefore a potential indicator of male condition and quality. We presented satiated (good condition) and starved (poor condition) males with a prey in a sexual context (on female silk) and registered the gift color as a proxy for silk wrapping effort. If males in good condition are better able to wrap the gift in white silk, the silk wrapping can be used as an honest indicator of male condition. In order to have a more natural approach of nuptial gifts characteristics and corroborate results from this last experiment, we collected gifts from the field analyzing and registering number of silk sheets, color, and number of prey.

Material and methods

We collected large juveniles, sub-adults of both sexes, and adult males of *P. ornata* in 2010–2012, from Santa Lucía River (Paso del Molino, Arequita, Lavalleja, 34°16' S, 55°14' W), Uruguay. We housed and kept spiders in the laboratory where they were sexed and maintained individually in glass jars (8 cm internal diameter and 11 cm high) containing small pebbles and water, simulating a natural

environment. We raised immature spiders until adulthood in a climate room (24.1 ± 1.6 °C) to accelerate development; molts were daily checked. We fed individuals three times a week with a mixed diet of fruit flies (*Drosophila* spp.), crickets (*Acheta domesticus*), and mealworms (*Tenebrio molitor*), providing water daily with wet cotton wool. All adult individuals were kept in a room with an average temperature of 21.3 °C (± 2.3 SD). We fed adult spiders three times a week with fruit flies, and transferred them to Petri dishes (9.5 cm of diameter and 1.5 cm high) to facilitate feeding and manipulation during the trials.

Statistical analyses were performed using JMP 7.0 software (SAS Institute) and Past Paleontological Statistics version 1.18 (Hammer et al. 2003). Data were tested for normal distribution of residuals and homogeneity of variance with Shapiro–Wilk and Levene tests, respectively.

Experiment 1: testing female attraction to white color in a sexual context

To study if white color attracts female and affects male mating success, we presented virgin females with males in three experimental treatments. Males that had the front of their chelicerae painted white ($n=21$); males that were not painted ($n=22$); and males that had the sternum (ventral cephalothorax) painted white ($n=20$), not visible by females and as a control of any paint effect (Fig. 1). We exposed females to males without a gift in order to avoid any chemical or behavioral factor not related to color affecting male and female behaviors, such as substances associated with gift silk (Brum et al. 2012) or male manipulation of the gift. All painted males were treated with white acrylic paint (Liky), at least 2 days before the trial. For painting, we immobilized males between two kitchen sponges and left their chelicerae uncovered; afterwards we painted them using a tiny paintbrush. Immediately after the manipulation and to check for possible paint effects, we returned males to their Petri dish and offered fruit flies ad libitum (*Drosophila* spp.), registering prey capture and feeding. This procedure was performed during feeding days, so all spiders (manipulated and not) received fruit flies and had the same feeding regimen.

Since *P. ornata* is a crepuscular/nocturnal spider, trials were performed in a dark room and under red light (bulb of 40 W),

and were recorded with a digital video camera with night-shot (Sony DCR-SR85). One day before the trials, we placed females in the experimental terraria (containing pebbles and water in a Petri dish) in a room with an average temperature of 22.9 °C (± 3.0 SD), allowing silk deposition, a key stimulus to trigger male courtship (Albo et al. 2009). In the trials, we exposed the male to female silk but isolated from the female by an opaque glass barrier. After 1.5 min we carefully removed the barrier allowing male–female encounter. We ended the trials 15 min after females rejected males (running away without mating) or 5 min after the mating ended. If both sexes remained immobile and no physical interaction between them occurred during 15 min, we discarded the trial and exposed the female and the male to another partner at least 1 day after the first trial. All males and females were virgins, and we used them at an average age of 17.1 days (± 9.9 SD) for females and 20.9 days (± 15.7 SD) for males after the final molt. Male size measured as the cephalothorax width (mean $\pm$ SD) was 4.3 ± 0.32 mm in males with the chelicerae painted white ($N=21$), 4.1 ± 0.27 mm in males without paint ($N=22$), and 4.1 ± 0.32 mm in males with the sternum painted white ($N=20$), with no statistical differences among them (ANOVA: $F_{2,58} = 2.20$, $p=0.12$).

We video recorded and registered latency of female acceptance, mating success and duration, number of mounts (male above female's prosoma), time in face to face position, and the number of times a female contacted males during courtship and after mating. We analyzed videos in detail with the program J-Watcher (Blumstein et al. 2000). The courtship includes searching behavior and vibrations of the first and second pair of legs in the female's direction (Costa-Schmidt et al. 2008). Latency of female acceptance was measured from the beginning of the trial ($t=0$) until the first mount. The mating in this species includes up to four consecutive mounts, each including a single male pedipalp insertion (Costa-Schmidt et al. 2008). Once a pedipalp insertion finishes, the male dismounts and returns to the face to face position, holding the gift, or remaining in front of the female when no gift is present (less than 1 cm distance) (Costa-Schmidt et al. 2008; Albo and Costa 2010). Mating duration was measured from the first mount until the last dismount. We registered the occurrence of female–male contact, particularly observing the occurrence of leg touching, when both sexes were very close.

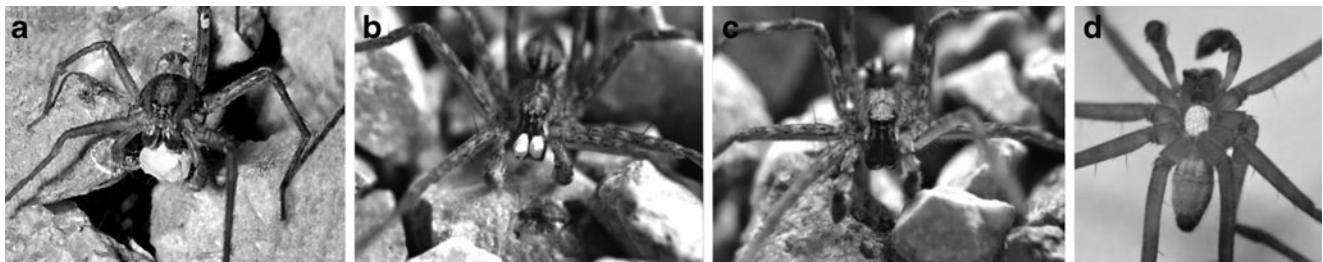


Fig. 1 Pictures of *P. ornata* males with: **a** a wrapped gift; **b** chelicerae painted white; **c** chelicerae without paint; **d** sternum painted white

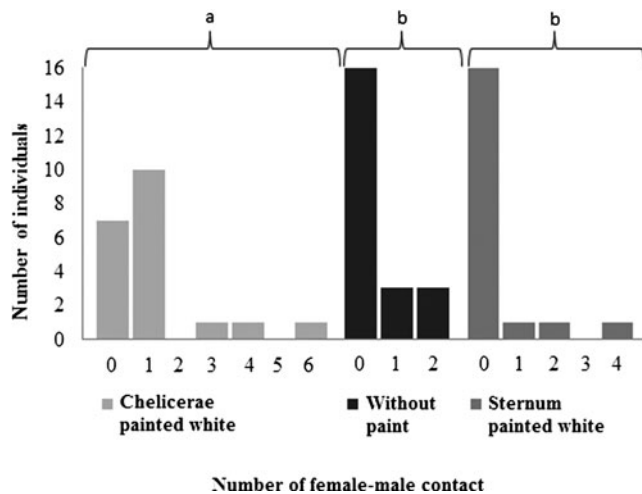


Fig. 2 Number of times a female contacted a male among the three treatments: males with chelicerae painted white; males without paint; and males with sternum painted white. Different letters indicate statistical differences ($p < 0.05$)

We analyzed mating duration using ANOVA, and we used Kruskal–Wallis for analyzing latency of female acceptance, time in face to face position and number of mounts. We analyzed mating frequencies using the Chi-square test for independent samples. As results from Chi-square were statistically marginal and followed the direction expected we analyzed the effect between treatments.

Experiment 2: testing for the silk wrapping as a condition-dependent trait

To examine how male condition affects gift wrapping, we exposed males in good and poor condition (satiated and starved, respectively) carrying a captured fly (*Calliphora* sp.) to female silk (important sexual stimulus). We obtained differences in male condition by exposing adult males to two different feeding regimes after field collection. Males in good condition (GC group, $n=25$) were fed with fruit flies every day during 2 weeks; males in poor condition (PC group, $n=24$) did not receive food in 2 weeks before the trial. Male condition index at the time of the trial was calculated as the residuals of the body weight/cephalothorax width regression

(Jakob et al. 1996) and averaged $0.03 \text{ g} (\pm 0.04 \text{ SD})$ for GC males and $-0.03 \text{ g} (\pm 0.07 \text{ SD})$ for PC males (ANOVA: $F_{1, 42} = 9.47$, $p = 0.004$).

One day before each trial a female was placed in an experimental cage, allowing her to deposit silk threads. We then removed the female and exposed the male with a recently captured fly (averaged fly weight, 0.03 ± 0.01 , $n=20$) to the female's silk. Since gift wrapping behavior is not affected by daylight (authors unpublished data) trials were performed during afternoon and male behaviors were not video recorded but manually registered. Trials ended 10 min after males finished prey wrapping (assuming it was completed), or after males completely ate the prey. We registered occurrence and latency of gift wrapping, gift wrapping duration, number of silk wrapping bouts, and gift color. Latency of gift wrapping was measured from the time the male was placed in female's silk until he initiated silk wrapping behavior. Gift wrapping duration was the time between the start of the first and the end of the last silk wrapping bout (without including periods of gift handling and carrying without wrapping). To estimate the amount of silk deposited in the gift we also registered the number of silk wrapping bouts a male performed, and classified gift color according to its appearance following Albo et al. (2011b): black (no or small amount of silk, fly visible) and white (abundant silk, fly not visible).

We analyzed latency of silk wrapping using ANOVA, and we used Kruskal–Wallis for analyzing gift wrapping duration and silk wrapping bouts. We performed Chi-square test to analyze gift color.

Field data

To describe the characteristics of the gifts constructed in the field, we collected 40 gifts and dissected them in the lab using forceps under the stereomicroscope, registering gift color, number of silk sheets, and number of prey included in each gift. Number of silk sheets represents the amount of silk deposited in the gift; each sheet was carefully separated from the other with forceps. We analyzed the data using Nominal Logistic Regression and Chi-square test.

Table 1 Latency of female acceptance and mating duration, number of mounts and time of face to face position from males with chelicerae painted white; males without paint; and males with sternum painted white

	Males chelicerae painted white	Males without paint	Males sternum painted white	Statistics
Latency of female acceptance (min)	3.2 ± 2.8 ($n=18$)	6.8 ± 4.2 ($n=12$)	3.6 ± 3.0 ($n=13$)	$F_{2,40} = 3.02$ $p = 0.06$
Mating duration (min)	1.3 ± 1.3 ($n=18$)	0.9 ± 0.5 ($n=12$)	1.2 ± 1.0 ($n=13$)	$F_{2,24} = 0.75$ $p = 0.48$
No. of mounts	2.7 ± 1.5 ($n=18$)	2.5 ± 1.5 ($n=12$)	4.0 ± 2.7 ($n=13$)	$H_2 = 1.72$ $p = 0.40$
Time of face to face position (min)	2.4 ± 2.9 ($n=21$)	0.8 ± 1.3 ($n=22$)	1.7 ± 2.1 ($n=20$)	$H_2 = 4.3$ $p = 0.11$

Data are shown as mean $\pm$ standard deviation. Statistical analyses were performed using Kruskal–Wallis test for non-normal distributed data and one-way ANOVA test when data was normally distributed

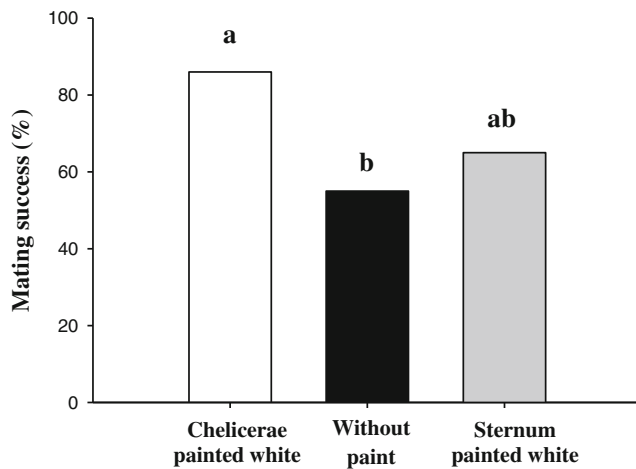


Fig. 3 Mating success from the three male treatments: males with chelicerae painted white ($n=21$); males without paint ($n=22$); and males with sternum painted white ($n=20$). Different letters indicate statistical differences ($p<0.05$)

Results

Experiment 1: testing female attraction to white color in a sexual context

All males searched and courted females. We found that during courtship and mating females contacted males with the chelicerae painted white (performing leg touching) more times compared to females exposed to males without paint or males with the sternum painted white (Kruskal–Wallis: $H=6.45$, $p=0.01$, $df=2$; Fig. 2). Similarly, females showed a tendency to remain more time in a face to face position when exposed to males with the chelicerae painted white than when exposed to males without paint or with the sternum painted white (Table 1); in two cases females with open fangs approached to the male's white chelicerae (like trying to bite it).

Latency of female acceptance tended to be shorter in males with the chelicerae painted white than in males without paint or with sternum painted white, with marginal significance (Table 1). The mating success showed marginal statistical differences among treatments (Chi-square test: $\chi^2=4.96$, $p=$

0.08, $df=2$). As these values followed the direction expected (males with chelicerae painted white more successful than the others) we analyzed the effect between treatments. Males with sternum painted white have similar mating success than males without paint (Fisher test, $p=0.54$, Fig. 3). Males with the chelicerae painted white obtained 31 % more matings than males without paint (Fisher test, $p=0.04$) and 20 % more matings than males with the sternum painted white (Fisher test, $p=0.15$). We did not detect significant differences either in the mating duration or in the number of mounts among treatments (Table 1).

Experiment 2: testing for the silk wrapping as a condition-dependent trait

Fifty-two percent of the males in poor condition wrapped the prey in silk, while 76 % of the males in good condition did it, with marginal statistical differences (Chi-square test: $\chi^2=3.13$, $p=0.07$, $df=1$). Gift wrapping duration was not statistically different between both groups. However, males in poor condition initiated gift wrapping later and performed fewer silk-wrapping bouts compared to good-condition males (Table 2). As a result, gifts of males in poor condition appeared black with little or no silk compared to gifts from good-condition males that appeared white (Table 2).

Field data

From a total of 40 gifts collected in the field, 29 appeared white and 11 corresponded to other colors depending on prey species (i.e., brown, green, and black). This variation was affected by the amount of silk (nominal logistic regression: $\chi^2=35.89$, $p<0.0001$, $df=3$), as most of non-white gifts were wrapped in one silk sheet (11/one sheet; 0/more sheets), while most of white gifts had two or more silk sheets (2/one sheet; 27/more sheets). The 52 % of gifts contained one prey, 40 % included two prey and 8 % included three prey. We did not find any effect of number of prey on gift color (with one prey: 13/white; 8/non-white and with two or more prey: 16/white; 3/non-white; Chi-square test: $\chi^2=2.49$, $p=0.12$, $df=2$).

Table 2 Latency of gift wrapping, gift wrapping duration, silk-wrapping bouts, and gift color by males in good (GC) and poor (PC) condition

	GC males	PC males	Statistics
Latency of gift wrapping (min)	42.75±14.75 ($n=19$)	89.83±18.56 ($n=12$)	$F=10.96$, $p=0.003$
Gifts wrapping duration (min)	3.71±0.91 ($n=19$)	1.49±0.27 ($n=11$) ^a	$H=1.16$, $p=0.28$
Silk-wrapping bouts	1.58±0.22 ($n=19$)	1±0.0 ($n=12$)	$H=5.45$, $p=0.02$
White gift color	7/12	0/12	$\chi^2=5.71$, $p=0.02$

^a There is one missing point from PC group

Data are shown as mean ± standard deviation. Statistical analyses were performed using Kruskal–Wallis test for non normal distributed data, One-way ANOVA test when data was normally distributed or Chi-square test. Significant p values are shown in bold

Discussion

Our findings indicate that females are attracted to males with white color on their chelicerae, suggesting an important role of visual cues during courtship and mate choice in *P. ornata*. Females exposed to males with white chelicerae were more active, showing more physical contact, spending more time in front of them (even trying to bite the male chelicerae), accepting earlier the matings, and with more frequency than those exposed to males without paint. Visual signals are also essential during courtship of other related spider species (Barth 2002; Foelix 2011; Herberstein 2011). In wolf spiders (Lycosidae), males develop conspicuous displays, sometimes showing special ornamentations to attract females (Hebets and Uetz 2000). Visual signals are also present in nocturnal spider species, as in the genera *Leucorchestris* (Nørgaard 2005) and *Cupiennius* where the visual system is highly developed (Barth 2002). Indeed, this last species have a very fine resolution and a particular sensitivity in the green, blue, and UV wavelength regions (Barth 2002). *P. ornata* also belongs to the Lycosoidea clade and it is mostly active during sunset and night. Thus, white wrapped gifts may highlight the male's presence, and amplify his movements. In nature, bright objects are easier to advertise over longer distances compared to black ones. White reflects most of the light received and reduces the edge resolution of the object, which becomes blurry and appears to be greater than it is (cf. Zahavi and Zahavi 1997). Hence, white is especially effective in advertising movements rather than isolated positions. Following this idea, *P. ornata* females may better visually perceive male movements when males carry a white wrapped gift than when males carry an unwrapped gift or no gift, suggested also by previous experiments (Klein et al. 2013).

On the other hand, there were no differences among our experimental treatments in relation to mating characteristics even for those that have white chelicerae, but this is logical as females did not receive any gift. The fact that mating success of males with the sternum painted white had intermediate values suggests that in some cases females may have seen the white paint. For instance, visual observation of a male's ventral part could be done by females if males climbed the glass wall from the terrarium during experiments. Thus, a better control for the painting such as colorless paint would have been more appropriate. In addition, it may be possible that some other variable, not measured here, caused variation in response.

Female attraction to white color may have influenced the evolution of silk wrapping of nuptial gifts by males. Female preferences for wrapped gifts may eventually evolve via good genes hypothesis (Zahavi 1975; Zahavi and Zahavi 1997). In many species, secondary sexual traits are condition-dependent and females assess male condition and quality via these honest signals, consequently favoring matings with the best quality

males (Simmons et al. 1998; Uetz et al. 2002; Engels and Sauer 2006; Parri et al. 1997; Kotiaho 2002; Hebets et al. 2008). Indeed, our findings indicated that gift wrapping is a potential honest indicator of male body condition for female mate choice in *P. ornata* (Zahavi 1975; Zahavi and Zahavi 1997). Males in poor condition seem to not be able to pay the energetic or material cost of silk wrapping. These males performed gift construction inefficiently obtaining poorly wrapped gifts, as was shown in *P. mirabilis* (Albo et al. 2011b). In spite of this physical constraint, these males added silk to the prey, suggesting silk wrapping is in their own interest.

So far, in *P. mirabilis* silk wrapping of the prey seems to benefit males allowing them to control and prolong the mating (Lang 1996; Andersen et al. 2008), also giving the opportunity to hide gift content as non-nutritive items for females (Albo et al. 2011a). In line with this information and even if silk wrapping is an honest trait *P. mirabilis* females ignore this signal and based their mate choice decision on male condition (Albo et al. 2012). In *P. ornata*, aside from the visual function found here, chemical cues also involved in female attraction were recently reported in the gift silk (Brum et al. 2012), and both signals benefit males. However, whether silk wrapping is against *P. ornata* females' interests is controversial. For instance, here we found that silk wrapping also allows males to pack several prey together, which can also benefit females as they will receive more food. Further, when females reject males, they very often start silk wrapping of the prey and interestingly, males are usually accepted afterwards (Albo and Costa 2010). Hence, consistently with the results presented here it is possible that females evaluate male condition based on silk wrapping performance and gift appearance.

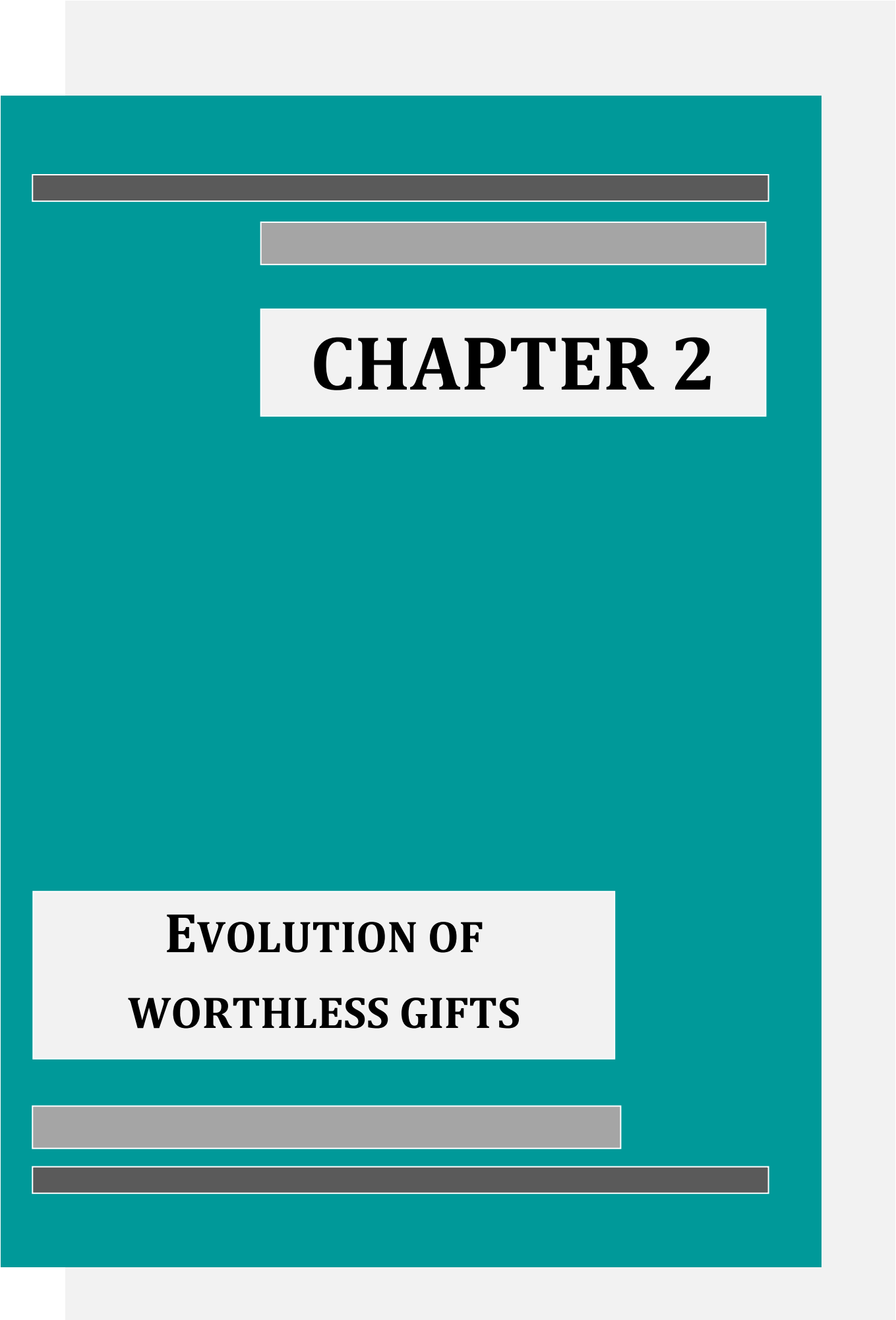
In conclusion, our results show that females are attracted to white color on male's chelicerae during courtship. Silk wrapping is a condition dependent trait and most probably allows *P. ornata* females to acquire information of the potential mate, including body condition and quality.

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CHAPTER 2

EVOLUTION OF WORTHLESS GIFTS

CAPÍTULO 2- EVOLUCIÓN DE LOS REGALOS SIMBÓLICOS

ANTECEDENTES: Los machos de especies donadoras de regalos nupciales necesitan obtener comida para ofrecer a las hembras y por lo tanto la disponibilidad de alimento determinará su éxito reproductivo. Consecuentemente, los beneficios de obtener pareja mediante un regalo nupcial pueden determinar que los machos ofrezcan regalos sin valor nutritivo, o “simbólicos”. Por ejemplo, cuando la comida es limitada los machos pueden comer la presa antes de ofrecerla a las hembras o coleccionar objetos no nutritivos de los alrededores. Se investigó el efecto de los regalos simbólicos en el éxito reproductivo de los machos en las arañas *Pisaura mirabilis* y *Paratrechalea ornata*. Durante el cortejo, los machos de estas especies ofrecen a las hembras presas frescas (regalos genuinos) pero también pueden ofrecer restos de presas o partes de plantas (regalos simbólicos).

DISEÑO EXPERIMENTAL: Primero se examinó el contenido de regalo en el campo y se clasificó los regalos en “frescos” o “restos no frescos”. Luego, se investigó cómo el contenido del regalo afecta el éxito de cópula y su duración en el laboratorio. En *P. mirabilis* se realizaron los siguientes grupos experimentales: regalo con mosca rica en proteínas (PG), regalo con mosca común (FG), regalo simbólico (WG), y no regalo (NG). En *P. ornata* se examinó la condición corporal del macho y el peso del regalo de machos y regalos coleccionados en el campo. En el laboratorio, se expusieron hembras a machos ofreciendo regalo con FG, WG y NG en dos grupos de machos con régimen alimenticio diferencial (buenas condiciones; malas condiciones), totalizando seis tratamientos-grupos experimentales. Además, se analizó si el estado reproductivo de la hembra afecta las decisiones del macho en el uso de regalo, exponiendo hembras copuladas a machos en buenas condiciones ofreciendo regalo simbólico o no.

RESULTADOS PRINCIPALES: Se disecaron 16 regalos que eran transportados por machos de la especie *P. mirabilis* en el campo. Se encontró que 62% contenía presas frescas mientras que 38% contenía exoesqueletos vacíos de artrópodos. En el laboratorio, los machos que ofrecieron regalos simbólicos tuvieron similar éxito de cópula comparado con los machos que ofrecieron regalos genuinos, mientras que los que no tenían regalo tuvieron un reducido éxito de cópula. Las hembras que copularon con regalo simbólico terminaron las cópulas antes, probablemente reduciendo la cantidad de esperma transferido. Por otro lado, en *P. ornata* se disecaron 63 regalos de campo y se encontró que el 30% contenía presas frescas mientras que el 70% contenía restos vacíos de presas. Los regalos con presas frescas fueron más pesados que los que contenían restos vacíos, y el peso del regalo se correlacionó positivamente con la condición del macho. Los machos en buena condición corporal obtuvieron más frecuentemente cópulas comparado con los machos en mala condición y además las primeras fueron más largas. Los machos en buena condición que ofrecieron regalo (genuino o simbólico) a hembras vírgenes obtuvieron similar éxito y duración de cópula; estos resultados tampoco difirieron significativamente cuando se comparó con los machos sin regalo. En contraste, las hembras copuladas se comportaron diferente. Los machos sin regalo fueron consistentemente rechazados por las hembras copuladas, mientras que aquellos que ofrecieron regalo simbólico copularon.

CONCLUSIONES: En ambas especies existen fuertes presiones selectivas en el ofrecimiento del regalo nupcial que facilitan el engaño mediante la donación de regalos simbólicos. Las preferencias de las hembras por los regalos genuinos combinadas con el control sobre la duración de la cópula

contrarrestan el engaño del macho, probablemente imponiendo desventajas en la competencia espermática para los machos de *P. mirabilis*. Pero, este no es el caso de *P. ornata* ya que en esta especie existen ventajas para los machos de ofrecer un regalo sin importar el contenido y aparentemente las hembras parecen no penalizar las cópulas con regalos simbólicos. Los datos de campo combinados con los datos de laboratorio muestran cómo el régimen alimenticio del macho y la condición consecuentemente influyen en la calidad del regalo nupcial.

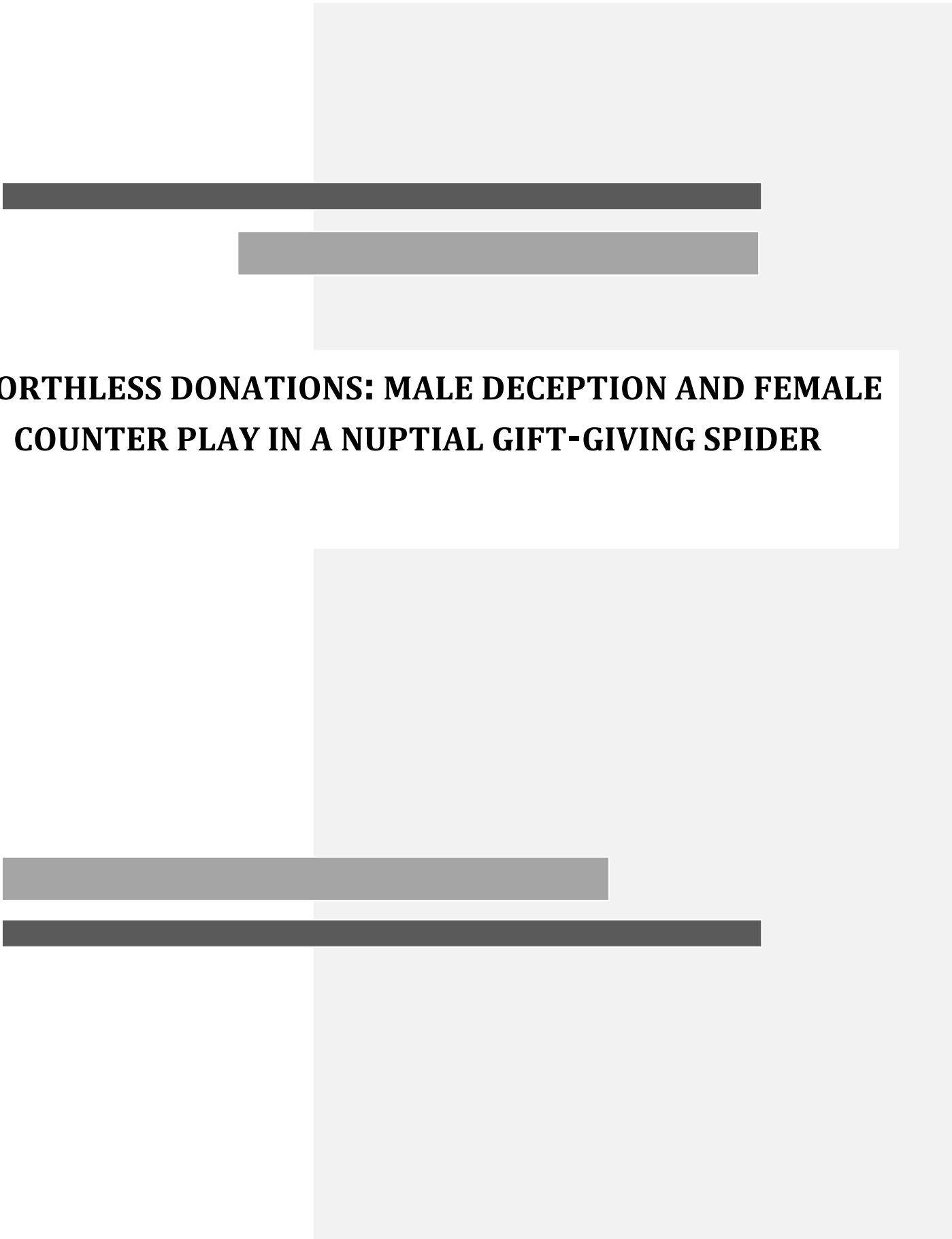
CHAPTER 2- EVOLUTION OF WORTHLESS GIFTS

BACKGROUND: Males from gift-giving species have the particular goal of obtaining food to offer to females and therefore food availability would determine their reproductive success. Thus, benefits of acquiring a mate may select for male deception by donation of worthless gifts. For instance under food limited conditions males may eat the prey before offering it to females or offer inedible items collected from the surroundings. Here I investigated the effect of worthless gifts on male reproductive success in the spiders *Pisaura mirabilis* and *Paratrechalea ornata*. During courtship, males from these species offer to females fresh prey (genuine gifts), but also prey leftovers (worthless gifts).

EXPERIMENTAL DESIGN: I first examined gift content in the field and classified gifts as “fresh” or “leftovers”. Afterwards, I investigated how gift content affects male mating success and duration under lab conditions. In *P. mirabilis* I tested male mating success in the following experimental groups: protein enriched fly gift (PG), regular fly gift (FG), worthless gift (WG), or no gift (NG). In *P. ornata* I examined male body condition, gift weight in males and gifts from the field. In the lab, I exposed virgin females to males carrying a FG, a WG and NG in two male feeding groups (good condition; poor condition), totalizing six experimental treatment-groups. To examine whether female reproductive status affect male decisions on the use of nuptial gift, I exposed mated females to males in good condition offering worthless or no gifts.

MAIN RESULTS: Dissection of 16 gifts carried by *P. mirabilis* males in the field showed that 62% contained fresh prey, while the remaining 38% contained empty arthropod exoskeletons. In the lab, males that offered worthless gifts acquired similar mating success as males offering nutritional gifts, while males with no gift experienced reduced mating success. Females terminated matings faster when males offered worthless donations, probably reducing sperm transfer. On the other hand, in *P. ornata* the dissection of 63 gifts from the field showed that 30% of the gifts were fresh prey, while 70% were prey leftovers. Fresh gifts were heavier than leftover ones, and gift weight related positively with male condition. Males in good condition obtained more and longer matings compared to males in poor condition. Males offering gifts (genuine or worthless) to virgin females enjoyed similar mating success and duration; neither differed significantly when compared to males without gift. In contrast, mated females behaved differently. Males without gifts were consistently rejected by mated females, while those that offered worthless gifts achieved matings.

CONCLUSIONS: Strong selection on the nuptial gift-giving trait facilitates male deception by donation of worthless gifts in both species. Female preference for genuine prey gift combined with control over mating duration counteracts the male deception a probable disadvantage in sperm competition for males in *P. mirabilis*. But, this is not the case in *P. ornata* as in this species there are advantages for males to offer gifts regardless it's content and so far, females do not seem to penalize matings with worthless gifts. Additionally, the combined data from the field and the lab show how male feeding regimes and body condition consequently influence nuptial gift quality.



WORTHLESS DONATIONS: MALE DECEPTION AND FEMALE COUNTER PLAY IN A NUPTIAL GIFT-GIVING SPIDER

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RESEARCH ARTICLE

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Worthless donations: male deception and female counter play in a nuptial gift-giving spider

Maria J Albo^{1,2*}, Gudrun Winther¹, Cristina Tuni¹, Søren Toft¹ and Trine Bilde¹

Abstract

Background: In nuptial gift-giving species, benefits of acquiring a mate may select for male deception by donation of worthless gifts. We investigated the effect of worthless gifts on mating success in the spider *Pisaura mirabilis*. Males usually offer an insect prey wrapped in silk; however, worthless gifts containing inedible items are reported. We tested male mating success in the following experimental groups: protein enriched fly gift (PG), regular fly gift (FG), worthless gift (WG), or no gift (NG).

Results: Males that offered worthless gifts acquired similar mating success as males offering nutritional gifts, while males with no gift experienced reduced mating success. The results suggest that strong selection on the nuptial gift-giving trait facilitates male deception by donation of worthless gifts. Females terminated matings faster when males offered worthless donations; this demonstrate a cost of deception for the males as shorter matings lead to reduced sperm transfer and thus give the deceiving males a disadvantage in sperm competition.

Conclusion: We propose that the gift wrapping trait allows males to exploit female foraging preference by disguising the gift content thus deceiving females into mating without acquiring direct benefits. Female preference for a genuine prey gift combined with control over mating duration, however, counteracts the male deception.

Background

Differences in the evolutionary interests between the sexes over maximizing reproductive success commonly lead to inter-sexual conflict [1,2]. This conflict influences the opportunity, form and intensity of sexual selection that drives the evolution of traits that enhance mating success [3]. Traits that differ in optimum between males and females may include the number of mates, copulation duration, fertilization success as well as parental investment in offspring [1]. Male-male competition may be a particular strong driver of sexually antagonistic traits; thus to enhance their success in sperm competition, males may be under selection to manipulate females to mate at a suboptimal rate [2,4].

In species where males provide females with a nuptial gift during mating, there is particular scope for males to manipulate females to acquire matings and prolong copulation to enhance their fertilization success [4-6]. Female choice for males with nuptial gifts could lead to

the evolution of male “deception” by the use of token gifts. For instance, males can decrease the costs of mating by re-using gifts or by offering worthless gifts [7,8]. Males of some dance flies (*Empis* spp.) may deceive females by offering inadequate or false gifts [8]. Although males that offer inedible gifts run a higher risk of being rejected and may suffer from shorter matings compared to males offering edible gifts, the chance of acquiring an extra mating should make deception an attractive strategy for males. Hence, males of the dance fly *Rhamphomyia sulcata* that use inedible token gifts to obtain mates are as successful as males offering small genuine gifts [9].

In spiders, nuptial gifts in the form of prey are restricted to a few species from two families belonging to the superfamily Lycosoidea: Pisauridae and Trechaleidae [10-17]. In both families, the male courts the female by offering a prey wrapped in silk and mating occurs while the female consumes the gift [15,18]. In the species *Pisaura mirabilis* (Pisauridae) the gift functions as a mating effort that increases male mating success [19]; a similar function was recently suggested for the trechaleid spider, *Paratrechalea ornata* [20]. In both species, males can obtain mating without a gift, but male mating success

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increases dramatically when a gift is offered [19,20]. In *P. mirabilis*, the male pushes up the female during mating and performs alternate pedipalp insertions into the female sperm storage organs placed ventrally on the abdomen. After each insertion the male returns to a face-to-face position with the female, grabbing the gift in the chelicerae [21]. Females usually control mating duration and they often attempt to run away with the gift upon terminating the copulation [19]. During each insertion the male and the female remain motionless. However, if the female moves and attempts to terminate copulation, the male may perform “thanatosis” which is a “death feigning” behaviour (with stretched-out legs) unique to this species. The male ends the insertion and grasp the gift with his chelicerae. The female moves away while holding the nuptial gift and the male is dragged along until the female stops. Subsequently he “revives” and resumes mating [22-24]. Thanatosis functions as a mating effort that increases the male’s chances of completing or prolonging the copulation [23]. Silk wrapping facilitates male handling and control over the gift [25], as it facilitates a stronger hold of the silk covered package, both with the chelicerae and the feet claws, compared to an unwrapped insect. As a result, females are less likely to succeed in stealing a wrapped than an unwrapped gift [26,27]. In addition, Stålhandske [28] showed that relatively brighter gifts, i.e. gifts that were wrapped in plenty of white silk, were more attractive to females, suggesting an additional function of silk wrapping that directly influences mate choice. The prey gift and silk wrapping thus provide males with opportunities to exploit female foraging preferences in a sexual context [29].

Male spiders have a unique opportunity for gift manipulation through the gift wrapping trait, for example by preventing female assessment of the gift content. By disguising the gift content males may deceive females to copulate, while the female attempts to consume the gift. In *P. ornata*, males were observed wrapping prey carrion and occasionally inedible items such as plant seeds [20]. In *P. mirabilis*, males have been reported to carry gifts containing empty arthropod exoskeletons or plant parts [21,30,31], gifts that are of no nutritional value. Dissection of 16 gifts carried by males in the field showed that 62% contained fresh prey, while the remaining 38% contained empty arthropod exoskeletons, i.e. prey already sucked out probably by the male itself [M.J. Albo, unpublished data]. Such evidence suggests that males of *P. mirabilis* may exploit the female’s preference for prey gifts to gain a reproductive advantage without providing the nutritional benefit of fresh prey from which the gift-giving trait must ultimately have evolved [29]. The costs of prey capture and gift construction may thus interact with male condition to favour the evolution of male deceit by donation of worthless gifts [32].

Here we tested male use of worthless gifts and investigated their effect on male and female reproductive success in a Scandinavian population of *P. mirabilis*. We explored how the gift content affects reproductive success in experimental trials where males offered either genuine prey gifts, worthless gifts, or no gift to females. Males are under strong selection to provide a gift [19]; therefore, we expected males to offer a worthless (non-nutritive) item if no prey is available. Males offering worthless gifts may initially be accepted by females, however during feeding the females should realize the low nutritional value of the gift and respond appropriately, e.g. by interrupting the mating prematurely. As a consequence, males would experience shorter matings, lower sperm transfer and ultimately lower reproductive success than males offering genuine gifts.

Results

Courtship and mating

In the worthless gift group (WG), 13 males (70%) produced a worthless gift for courtship and 12 out of these mated, whereas the remaining 6 males (30%) courted without a gift and only one mated (Fisher exact test: $p = 0.002$). Comparing all treatment groups, males that offered a worthless gift (WG) were equally successful in obtaining mating as those offering a genuine gift (PG and FG), whereas males with no gift (NG) experienced significantly reduced mating success (Chi-Square test: $\chi^2_{\text{yates}} = 24.8$, $p < 0.0001$, $df = 3$; Figure 1). The total mating duration was significantly shorter for NG males compared with males that offered a gift ($F = 10.03$, $p < 0.0001$, $df = 3$, Figure 2A). Although WG males experienced a 20% shorter mating duration compared with PG and FG males, this effect was not statistically significant (Figure 2A).

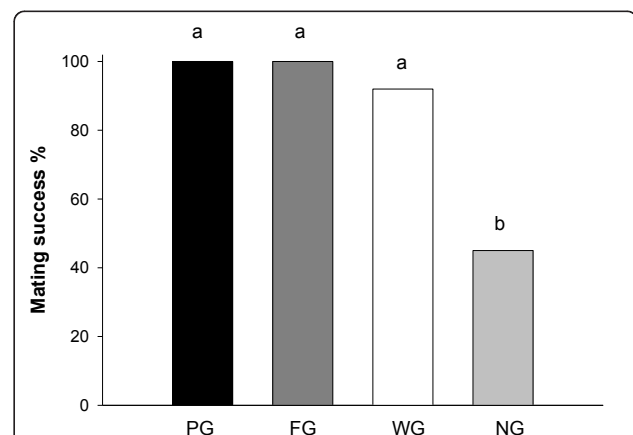
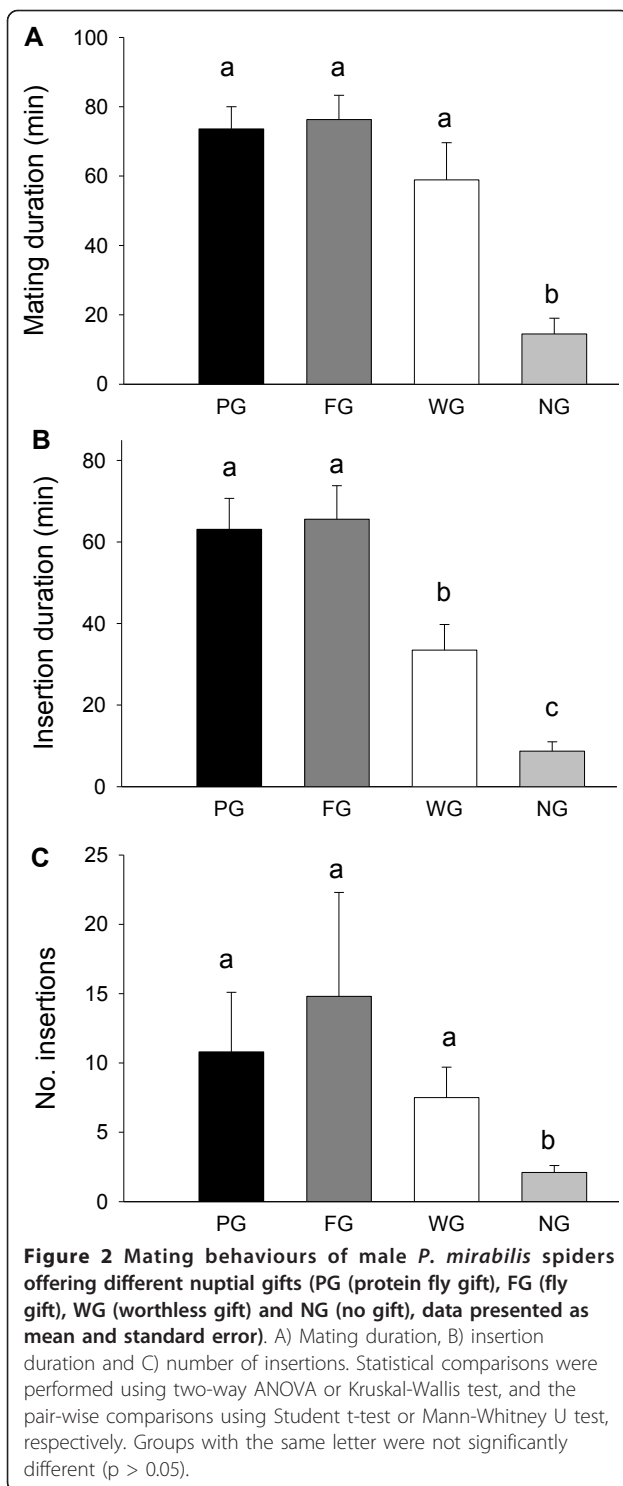


Figure 1 Mating success of male *P. mirabilis* spiders offering different nuptial gifts, PG (protein gift), FG (fly gift), WG (worthless gift) and NG (no gift). Different letters indicate significant differences from pair-wise Chi-square tests.



Insertion duration, which reflects the duration of actual sperm transfer, was significantly shorter for WG males compared with males offering genuine gifts, and shortest for males with no gift ($H = 41.32$, $p < 0.0001$, $df = 3$, Figure 2B). The number of pedipalp insertions followed a similar pattern ($H = 7.8$, $p = 0.05$, $df = 3$, Figure 2C).

Male thanatosis and gift control

Male death feigning (thanatosis) occurred in more than 50% of the trials in the PG and FG groups, with only one occurrence in the WG group and none in the NG group (Table 1). In the PG and FG groups, females terminated mating and retained the gift in all mating trials, in 11 out of 40 cases females and males were observed fighting over the gift. In the WG group, all females that accepted the non-nutritive gift actively manipulated it, moving the item with the chelicerae and pedipalps in the same way as females from the PG and FG groups. Cotton balls were wet after handling, suggesting that the females had regurgitated digestive fluids on them in an attempt to feed on the gift. We did not observe fights between males and females over the non-nutritive items.

Worthless items and gift construction

In the WG group, the males preferred cotton balls over prey leftovers and flower heads (Chi-Square test: $\chi^2_{\text{yates}} = 14.8$, $p = 0.001$, $df = 3$, Figure 3). Two males wrapped and combined two items: one male combined a prey leftover and a flower head, while the other combined a prey leftover and a cotton ball. To measure male silk investment in worthless gifts, we compared the duration of gift wrapping for WG and FG gifts. Males with worthless gifts spent less time on gift construction (mean $\pm$ SE, 3.9 ± 0.5 min, $N = 12$) than those with fly gifts (5.3 ± 0.5 min, $N = 20$) (Student t-test: $t = 2.04$, $p = 0.049$) and showed a tendency to perform fewer wrapping bouts (3.0 ± 0.4) than males with fly gifts (4.1 ± 0.3) (Student t-test: $t = 1.80$, $p = 0.08$). Males with cotton ball gifts (preferred item by WG males) performed a significantly lower number of wrapping bouts (2.5 ± 0.3 , $N = 8$) compared to males with fly gifts (4.1 ± 0.3 , $N = 20$) (Mann-Whitney U-test: $U = 37$, $p = 0.03$).

Oviposition, spiderling emergence and female life span

We found no significant differences in the proportion of mated females that constructed an egg-sac among groups; 19 (20) PG females, 20 (20) FG females, 12 (12) WG females and 8 (9) NG females constructed an egg-sac (Chi-Square test: $\chi^2_{\text{yates}} = 0.20$, $p = 0.97$, $df = 3$). The time until egg-sac hatching was similar among groups: 18.1 days (± 0.4 SE) in PG, 18.9 days (± 0.8 SE) in FG, 17.0 days (± 1.2 SE) in WG and 17.5 days (± 0.5 SE) in NG (ANOVA: $F = 0.85$, $p = 0.48$, $df = 3$). Egg hatching success differed among groups, and was higher when females had received a gift (PG, FG and WG) compared with no gift (Table 2). We found no significant differences in clutch-size or the number of spiderlings that emerged from the egg-sac among groups (Table 2). Spiderling size differed among groups, the largest spiderlings appeared to be found in the WG group, however a significant treatment by clutch-size

Table 1 Frequencies of male thanatosis and female gift control in PG (protein fly gift), FG (fly gift), WG (worthless gift) and NG (no gift) groups.

	PG (n = 20)	FG (n = 20)	WG (n = 12)	NG (n = 9)	Statistics		
					χ^2	P	DF
Male thanatosis	12 a	11 a	1 b	0	12.1	= 0.006	3
Female gift control	20 a	20 a	1 b	-	40.0	< 0.00001	2

interaction and a significant positive co-variation between clutch size and spiderling size make a simple interpretation of these data difficult (Table 2).

Mated females did not differ in adult lifespan among groups (ANOVA: $F = 0.93$, $p = 0.42$, $df = 3$; PG females $102.9 (\pm 10.6 \text{ SE})$ days, FG females $91.6 (\pm 6.4 \text{ SE})$ days, WG females $113.9 (\pm 10.0 \text{ SE})$ days, and NG females $107.9 (\pm 14.4 \text{ SE})$ days, $N = 61$).

Discussion

We showed that *P. mirabilis* males that offered worthless gifts acquired similar copulation success as males offering genuine nutritional gifts. In contrast, males with no gift experienced a significantly reduced mating success. Previous studies have demonstrated strong female preference for a prey gift (genuine gift), suggesting direct benefits to females of receiving a nuptial gift [29]. Together these findings support the hypothesis that strong selection on the nuptial gift-giving trait facilitates the evolution of male deception through donation of worthless gifts. We suggest that the gift wrapping trait allows males to take advantage of the female preference for a gift by disguising the gift content, thus deceiving females into mating without acquiring a direct benefit. Probably gift wrapping was crucial for deceit to evolve, while gift wrapping itself evolved due to additional

advantages to the male: it increases male control over the gift and mating [25,27], and mating duration increases with the amount of silk invested in wrapping [26].

Male deceit may be costly for females if it results in a higher than optimal mating rate, without conferring females a direct benefit [2,4]. If deception with worthless gifts is common [[21,30,31], M.J. Albo personal observations], females should evolve the ability to discriminate nutritive and non-nutritive gifts and avoid mating with males offering worthless gifts. Indeed we found that females terminated copulations with males that offered worthless donations sooner than copulations with males offering genuine gifts. This supports the hypothesis that the female can not evaluate the gift's value before having fed on it for some time. Males with worthless donations experienced shorter insertion duration and thus had less time to transfer sperm. This reveals a cost of deception for males, as shorter copulations and reduced sperm transfer disadvantages males in sperm competition [33]. Females that received a worthless gift did not retain the gift post-mating while they always retained genuine prey gifts, indicating that the nutritional value of the gift was actually revealed to the female during gift consumption and mating. Female preference for a genuine prey gift combined with her control over mating duration therefore counteracts male deception. However, males appear to hold the upper hand in the co-evolutionary cycle, since deception is only discovered after the copulation is initiated.

Males offering worthless gifts were less likely to feign dead (thanatosis), which is puzzling, as thanatosis functions as a male mating effort [22,23]. However, males often feign death if the female attempts to steal the gift without mating [23]. As the females did not try to run away with the worthless gifts, there was less scope for males to use thanatosis to prolong the copulation.

We did not detect strong negative effects of worthless donations on female reproductive fitness. Females receiving no gift, however, experienced reduced egg hatching success. This may suggest that successful sperm transfer (and hence egg hatching) is tightly coupled to whether the female receives a gift or not, but not to actual gift content (genuine or worthless). Males that mated without a gift experienced shorter copulations and fewer pedipalp insertions, indeed indicating that the gift facilitates sperm

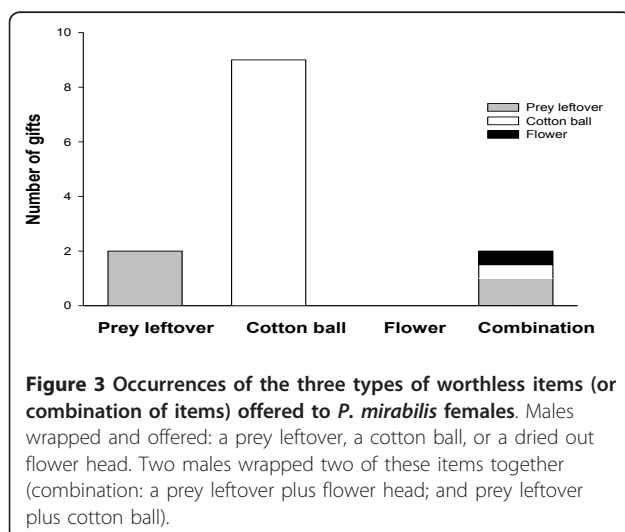


Table 2 Female fitness traits across nuptial gift mating treatments, PG (protein gift), FG (fly gift), WG (worthless gift) and NG (no gift), data are presented as means and standard error

	PG	FG	WG	NG	Statistics	P	DF
% Egg hatching success	0.66 ± 0.10 a (n _{es} = 12)	0.50 ± 0.15 a (n _{es} = 11)	0.48 ± 0.13 a (n _{es} = 8)	0.33 ± 0.33 b (n _{es} = 3)	$\chi^2_{\text{yates}} = 91.4$	<0.0001	3
Clutch-size	45.5 ± 4.5 a (n _{es} = 12)	38.0 ± 4.7 a (n _{es} = 11)	43.8 ± 5.5 a (n _{es} = 8)	27.3 ± 9.0 a (n _{es} = 3)	F = 1.30	0.29	3
Number of spiderlings per egg-sac	31.7 ± 6.1 a (n _{es} = 12)	18.2 ± 6.5 a (n _{es} = 11)	23.7 ± 7.6 a (n _{es} = 8)	6.3 ± 12.3 a (n _{es} = 3)	F = 1.45	0.24	3
Size of spiderlings (mm+SE)	0.649 ± 0.003 a (n _{sp} = 211; n _{es} = 12)	0.672 ± 0.004 b (n _{sp} = 107; n _{es} = 7)	0.674 ± 0.004 b (n _{sp} = 103; n _{es} = 7)	n/a	$\chi^2_{\text{overall}} = 86.92$ $\chi^2_{\text{group}} = 56.11$ $\chi^2_{\text{clutch-size}} = 52.49$ $\chi^2_{\text{group*clutch-size}} = 27.56$	<0.0001 <0.0001 <0.0001 <0.0001	5 2 1 2

n_{sp} = spiderlings sample size; n_{es} = egg-sac sample size.

Spiderling size was analyzed with GLM with clutch size as covariate. Groups with the same letter were not significantly different (p > 0.05).

transfer, at least through its effect on mating duration. This produces a strong incentive on males to present any type of gift rather than no gift. While copulation is possible without a gift, the short mating and insertion durations make mating without a gift unsuccessful. To some extent this also holds for mating with worthless gifts. We found an effect of gift type on spiderling size, though not in the direction expected if worthless gifts had negative effects on spiderling size. Instead, females receiving protein gifts which were presumably of the highest nutritional quality produced the smallest spiderlings; this effect was partly explained by the positive co-variation between clutch size and spiderlings size. Positive nutrient effects of the gift might be revealed in female adult life span [34,35], however, female longevity was similar among groups. Overall we found no indication of a cost of worthless donations on female reproductive fitness.

As females in our study received only a single gift, it is possible that the small amount of nutrients obtained through one gift is insufficient to detect an effect of nuptial gift quality on female fitness traits. *Pisaura mirabilis* females are polyandrous [36,37], and may thus benefit from receiving multiple nuptial gifts. Nuptial feeding may be particularly beneficial for females in poor feeding condition, and such females would benefit from the ability to choose males with a nutritive gift [7,38]. The cost for females of receiving worthless gifts is expected to vary with natural mating rates and to be high under poor foraging conditions.

Among the non-nutritive items, males showed preference for using the cotton ball rather than the other inedible items as a nuptial gift. Since the cotton ball visually appears to resemble a silk wrapped gift, it is possible that males took advantage of this characteristic. The preference may be for both colour (white) and shape (round): Stålhandske [28] showed increased female preference the whiter the gift, and Andersen et al. [27] showed male preference for a round gift compared with an elongate one,

probably because the latter mechanically inhibited his access to the female's genitalia. These features suggest that nuptial gift-giving spiders may be more restricted in the availability and suitability of worthless items to use as gifts compared to, for example, the empidid dance flies (9). Empidid dance flies offer a variety of token gifts that are not disguised, for example seed tufts, leaves, or small twigs, these gifts should be less costly to acquire than those of spiders, as they do not require prey capture and gift construction [7-9]. In contrast, spider males are more conservative, they wrap insect exoskeletons which could be prey remains and only occasionally use plant material to construct gifts. Hence, even worthless donations are costly for the male to produce as they require investment in silk and time for gift production. Indeed gift production was shown to be costly, as *P. mirabilis* males in poor condition are constrained in their ability to construct nuptial gifts [32].

Conclusions

There are large benefits to males of achieving a mating with a worthless donation, favouring male deception and the evolution of worthless gifts. However, the potential disadvantage in sperm competition through shorter copulations effected by the females coupled with costs of gift construction counteracts deceit. This may explain the maintenance of both mating tactics in the population and the prevalence of matings with genuine gifts.

Methods

We collected *Pisaura mirabilis* (Pisauridae) juveniles and subadults in April 2009 on grasslands surrounding the Mols Laboratory close to Aarhus, eastern Jutland, Denmark. In the laboratory, spiders were housed individually in vials (30 ml) containing water and moss (*Sphagnum* spp.) for maintaining humidity. The spiders were kept at an average room temperature of 23.1°C (± 0.3 SE) and a natural photoperiod. We raised spiders until adulthood,

registering the day of each moult to maturity. Individuals were fed three times per week with houseflies (*Musca domestica*). Mating experiments were conducted from 7 to 27 May, 2009. Observations were made in transparent plastic terraria (22 × 17 × 6 cm), with the bottom covered with paper and containing a dish (5 cm diameter) with wet cotton wool. All individuals used during the mating trials were virgins; males were of an average adult age of 19.5 (± 0.6 SE) days and females 19.9 (± 0.6 SE) days. Individuals were used only once.

To investigate the use and reproductive consequences of worthless donations in *P. mirabilis* we carried out experiments where females were exposed to males offering one of three gift types, or no gift: PG (protein fly gift), FG (normal fly gift), WG (worthless gifts), NG (no gift) (20 trials for each group). Protein enriched flies were chosen to examine the effect of gift nutrient quality on female reproductive success [39]. We assumed the following order of gift quality: PG > FG > WG = NG for females ranked according to nutritional value, and PG ≥ FG > WG > NG for males in terms of cost of gift production. Normal flies (*Musca domestica*) were raised on standard housefly medium, whereas protein flies were *Musca domestica* obtained by raising the larvae in a medium consisting of 40% standard house fly medium and 60% casein. Since PG flies were smaller than FG flies (mean ± SD weight, 70.8 ± 2.2 mg and 129.2 ± 3.8 mg, respectively), we supplied PG males with two PG flies to provide approximately the same mass of prey. Worthless gifts consisted either of a cotton wool ball, a dry flower head (*Malus* sp.), or a prey leftover (housefly) previously eaten by a female. The three WG items were of approximately similar size compared to a normal housefly (FG). Flower heads and prey leftovers have been reported as wrapped gifts in gift-giving spider species [[21,30,31], M.J. Albo unpublished data] whereas the cotton wool ball was chosen following the protocol of LeBas & Hockham [9] and because of its visual similarity to the natural gift wrapped in silk.

Males and females were randomly assigned to treatments. For each trial a female was placed in the experimental terrarium one hour before initiating the mating experiment, allowing her to release draglines, which are important stimuli for the male in mate search, and for inducing courtship behaviour and gift construction [30]. Immediately before the trials, we removed the female from the terrarium and allow the male contact with female silk. In the FG and PG groups, once the male initiated courtship we offered the prey with forceps, and the male grasped it and wrapped it in silk. No gift was provided in the NG group. In the WG group, we sequentially and in random order offered one of each worthless gift type to each male (a flower head, a prey leftover, and a small cotton ball); males could chose to wrap one or more items in

the same silk package ("combination"). This procedure allowed us to see which non-nutritive gift type was preferred by males. If the male did not grasp and/or wrap any of these items within 15 min, we provided a live housefly to assure that the male was able to wrap. Males that did not accept and wrap this fly were discarded; those that accepted and wrapped had the fly removed and were re-exposed to the same non-nutritive items (this time dispersed on the floor of the terrarium). Therefore, in the WG group some females were exposed to males carrying a gift and others not carrying one. Males without an item could grasp one after contact with the female, or they could copulate without offering a gift. The latter were excluded from comparative analysis; hence only males from the WG group that obtained a mating with a worthless gift were included in comparative analysis across treatment groups. The experiments started by returning the female to the terrarium and were terminated 10 min after the end of copulation or after 30 min if no interactions between the male and the female occurred.

Gift wrapping may occur in separate bouts of adding silk [31] both before and after the male encounters a female. To estimate differences in male investment in gift production between worthless gifts (WG) and normal gifts (FG), we measured the duration of gift construction and counted the number of gift wrapping bouts. Courtship duration (in min) was measured from when the males initiated courtship until copulation was initiated. We registered the occurrence of male thanatosis, i.e. males "feigning death" during courtship and mating [22] and the occurrence of females retaining the gift after mating ("female gift control"). Mating duration was measured from the beginning of the first to the end of the last pedipalp insertion and included the time the male and female were in face-to-face position and handled the gift. Insertion duration was measured from pedipalp insertion until the pedipalp disengagement, and the sum of all insertion durations was considered the total duration of sperm transfer. Courtship and mating data from the WG group included only males that offered an item.

Mated females were subsequently kept individually in the same vials they were raised in and continued with the same feeding regime. This took place at room temperature; however light bulbs were placed 20 cm above the vials raising the temperature to 25.0 °C (± 0.2 SE) during 3 hours at noon, to enhance the hatching success of the egg-sacs. We registered the latency of egg-sac hatching (period from egg-sac construction to the emergence of spiderlings), clutch-size of the first egg-sac (spiderlings + unhatched eggs), egg hatching success, and the number and size of spiderlings among experimental groups. Cephalothorax (prosoma) width of 20 randomly selected spiderlings from each egg-sac was measured under the

stereo microscope, to evaluate the effect of gift type on offspring size. Adult female life span was compared between mated females of each group.

Statistical analyses were performed using JMP 7.0 software (SAS institute) and Past [40]. Assumptions of parametric tests were examined using Shapiro-Wilk tests for normal distribution of residuals, and Levene's test for homogeneity of variances. Data were log or sqrt transformed whenever necessary to meet parametric assumptions. For continuous data, we performed ANOVA using Student t-test for planned pair wise comparisons. Non-parametric tests (Kruskal-Wallis and Mann-Whitney) were applied to ordinal data and when assumptions for parametric tests were not met. Spiderling size was analysed by ANCOVA (GLM modelling) with the number of eggs in the egg sac (clutch-size) as covariate. Frequencies were analysed with Chi-square tests (with Yates correction) or Fisher's exact probability test. All tests were two-tailed.

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Authors' contributions

MJA, CT, ST and TB contributed with the conceptual development of the work and the writing of the manuscript. MJA and GW carried out the experiments; MJA and TB performed data analyses. All authors read and approved the final version of the manuscript.

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EVOLUTION OF WORTHLESS GIFTS IS FAVORED BY MALE CONDITION AND PREY ACCESS IN SPIDERS

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Evolution of worthless gifts is favoured by male condition and prey access in spiders

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Abstract

Males from gift-giving species attempt to obtain food to offer to females. Therefore food access may affect both their body condition and their reproductive success. In some species, males reduce the costs associated with giving gifts by reusing gifts, or by offering inedible items. Males from the spider *Paratrechalea ornata* (Trechaleidae) offer fresh prey or “genuine gifts” to females, but also offer prey leftovers or “worthless gifts”. We examined gift weight and content, and their relation to male condition in the field. We also investigated how gift content, male condition and female reproductive status (virgin/mated) affect male mating success. In the field, most gifts were worthless; genuine gifts were heavier than worthless ones; and gift weights (both genuine and worthless gifts combined) correlated positively with male condition. In the lab, males in good condition had higher mating success than males in poor condition. Males offering gifts (genuine or worthless) to virgin females enjoyed similar mating success and duration; neither differed significantly when compared to males without gift. In contrast, mated females behaved differently. Males without gifts were consistently rejected by mated females, while those that offered worthless gifts achieved matings. The interplay between male condition, prey access, female mating history and female preferences for gifts can favour the evolution of worthless gifts under certain conditions. Females do not penalize males with non-nutritive items by not mating or by reducing mating duration, but penalties may be potentially exerted via post-copulatory process.

Key words: female mate choice, male body condition, nuptial gift content, *Paratrechalea ornata*.

Introduction

Sexual conflict and a coevolutionary arms race may occur when sexes' interests diverge (Arnqvist & Rowe 2002, 2005). In such cases, adaptations in one sex may be disadvantageous for individuals of the other sex, favouring counteradaptations to minimize costs. The degree of conflict can be influenced by ecological conditions, such as the spatial and temporal distribution of resources (Emlen & Oring 1977; Gwynne 1990; Simmons & Bailey 1990). Indeed, the amount of food during the reproductive season strongly influences female reproductive success in spiders, as it determines the number and quality of eggs and offspring (Wise 1975, 2006). In contrast, obtaining food during this period is not as relevant for males (Foelix 2011) unless they have a particular interest in acquiring nutrients to offer to females as nuptial gifts.

In gift-giving species, females should favour those males that offer nutritional resources. In some bird species, males supply food and guard the progeny until they are independent, while potentially reducing the risk of female extrapair copulations (Stokes & Williams 1971; Mougeot et al. 2006). Male nuptial feeding is also a common behaviour in many invertebrates (Austad & Thornhill 1986; Vahed 1998, 2007; Burela & Martín 2007; Gwynne 2008; Lewis & South 2012). In general, males benefit from offering food gifts to females as these usually increase mating success, copulation duration and number of offspring. However, males can also suffer from costs of gift production (Gwynne 1990; Engels & Sauer 2006; Vahed 2007; Immonen et al. 2009; Albo et al. 2011a). Just, as in many other secondary sexual traits, nuptial gifts can be condition-dependent. For example, in some scorpionflies food availability regulates male salivary secretion, and well-fed males produce more secretion and are able to obtain more matings than poorly fed ones (Engels & Sauer 2006).

Thus, contrary to females' interests males may evolve to minimize costs of gift production when food is scarce, reducing gift quality while maximizing their reproductive chances. In some insect and spider species, males offer prey gifts and they have been observed to reuse gifts after one mating (Thornhill 1976) or to offer inedible items, so-called worthless gifts (Preston-Mafham 1999; LeBas & Hockham 2005; Albo et al. 2011b). Such deceptive behaviours may allow males to increase the number of matings compared to males without gifts, and minimize costs of prey searching and capturing compared to males with genuine gifts. In the spider *Pisaura mirabilis* (Pisauridae), the male captures a prey (typically an arthropod), wraps it in silk, and offers it to the female during courtship (Bristowe 1958). Field studies indicate that approximately 38% of the gifts are inedible items, such as prey leftovers, offering these worthless gifts have been suggested to constitute an alternative male mating tactic in this species (Albo et al. 2011b). Males with worthless gifts not only increase chances of being accepted by females but also prolong mating time compared to males without a gift. However, females prefer genuine nutritive gifts, and penalize males offering worthless gifts by reducing time in mating and probably the number of sperm stored (Albo et al. 2011b; Albo et al. 2013).

The evolution of worthless gift-giving is potentially favoured when the male is in poor condition and/or has low access to food, because males may reduce the costs of gift production by eating the prey before offering it to females or by offering inedible items. We tested these hypotheses using the nuptial gift-giving spider *Paratrechalea ornata* (Trechaleidae). In this species, males also offer wrapped prey and gift-giving males experience higher mating success and longer mating duration than males without a gift (Albo & Costa 2010). Despite the benefits of gift-giving, males sometimes attempt to mate without offering a gift, but they may wrap worthless items as prey leftovers and occasionally plant parts when are rejected by females (Albo & Costa 2010; see video in supporting information). Additionally, males in poor condition usually delay wrapping the prey in silk compared to males in good condition, suggesting that males may eat the prey before turning it into a gift and offering it to the female (Trillo et al. 2014). Due to this plasticity in behaviour this species is a particularly good model for studying sexual conflict over gift nutritional quality and for discussing the evolutionary processes involved.

We predicted that males in poor body condition would feed on their prey before wrapping it in silk, resulting in worthless gifts, while males in better condition would offer fresh genuine gifts. Since females are able to recognize gift content only after they have grasped the gift and mating has started, we expected females to equally accept matings with males offering gifts, regardless of the content (genuine or worthless). But if feeding on the gift is positively correlated to mating duration, we also expected the females to reduce mating duration for males offering worthless gifts as a counteraction to male deception. In addition, we expected that males in good condition would experience higher mating success and longer mating duration than males in poor condition independently of the gift.

Material and Methods

Paratrechalea ornata is a crepuscular/nocturnal spider associated with rivers and streams in South America (Carico 2005). During field surveys we collected males with gifts from three different sites in Uruguay: Santa Lucía River (Paso del Molino, Arequita, Lavalleja, 34°16'40.10"S 55°14'00.80"W), Yermal Chico stream (Treinta y Tres Province 32°55'30.50"S 54°27'33.10"W) and Queguay River (Rincón de Perez, Paysandú Province, 32°10'37.98"S; 57°14'15.30"W). For the behavioural experiments we collected immature individuals from Santa Lucía River.

Spiders were sexed and kept in the laboratory in plastic jars (8.5 cm internal diameter and 7.5 cm high); experiments were performed in glass cages (30 x 14 cm base and 20 cm height), containing a layer of small pebbles and water, simulating their natural environment. Immature spiders were raised in a warm room (24.8 ± 1.9 °C) to accelerate development, and were checked daily for moults. We fed individuals three times a week with fruit-flies (*Drosophila* spp.), and provided water in a wet cotton wool. Once individuals reached adulthood, we placed them in a room averaging 20.6 °C (± 3.3 SD). Adult females received fruit-flies two times a week; adult males were included in one of two different feeding groups (see below). Vouchers were deposited in the Arachnological collection of the Facultad de Ciencias, Montevideo, Uruguay.

Statistical analyses were performed using JMP 9.0 software (SAS Institute, Cary, NC, U.S.A.). Response variables were examined for normal distribution of residuals and homogeneity of variance with Shapiro-Wilk and Levene tests, respectively.

FIELD DATA: Quantifying gift content and male condition

We examined how nuptial gift content varies in nature and whether there is a correlation between gift content and male body condition. To quantify male condition and gift content, in the field, we collected males carrying gifts on 8 dates during three reproductive seasons (2010, 2011 and 2012) and from the three different localities mentioned above. In order to avoid manipulation of the gift by the males, we immediately removed the gift from each male's chelicerae and placed it in an eppendorf tube together with the corresponding male in a plastic bag. Within 24 hours after field collection, we weighed and measured males. We calculated an index of male condition (*residual index*) as the residuals of the body weight/cephalothorax width regression (Jakob et al. 1996). We also weighed the gifts, dissected them by using forceps under the stereomicroscope, and registered the content. We classified the gifts according to Albo et al. (2011a) as "genuine" (containing fresh prey) or "worthless" (containing prey leftovers, i.e. empty exoskeletons and/or plant parts or other non-nutritive items) (Figure 1 A,B). In the field, the number of prey in a gift package varied from one to five (Trillo et al. 2014), but for the aim of this study we did not quantify the prey. Thus, if a gift contained both genuine and worthless prey, we counted the gift as genuine. After silk wrapping males search for a female and during that time they do not feed on the prey, as they would need to dissolve their own silk package. Additionally, males seem not to carry gifts longer than 24 hs (MJ Albo personal observations), avoiding the drying out of the gifts

over time. Thus, gift content is good evidence of what the male added before silk wrapping and what the female is going to receive.

We analyzed gift weight with one-way ANOVA and we also calculated a correlation between gift weight and body condition. We analyzed the effects on the gift content from the collecting date and the site using ANCOVA.

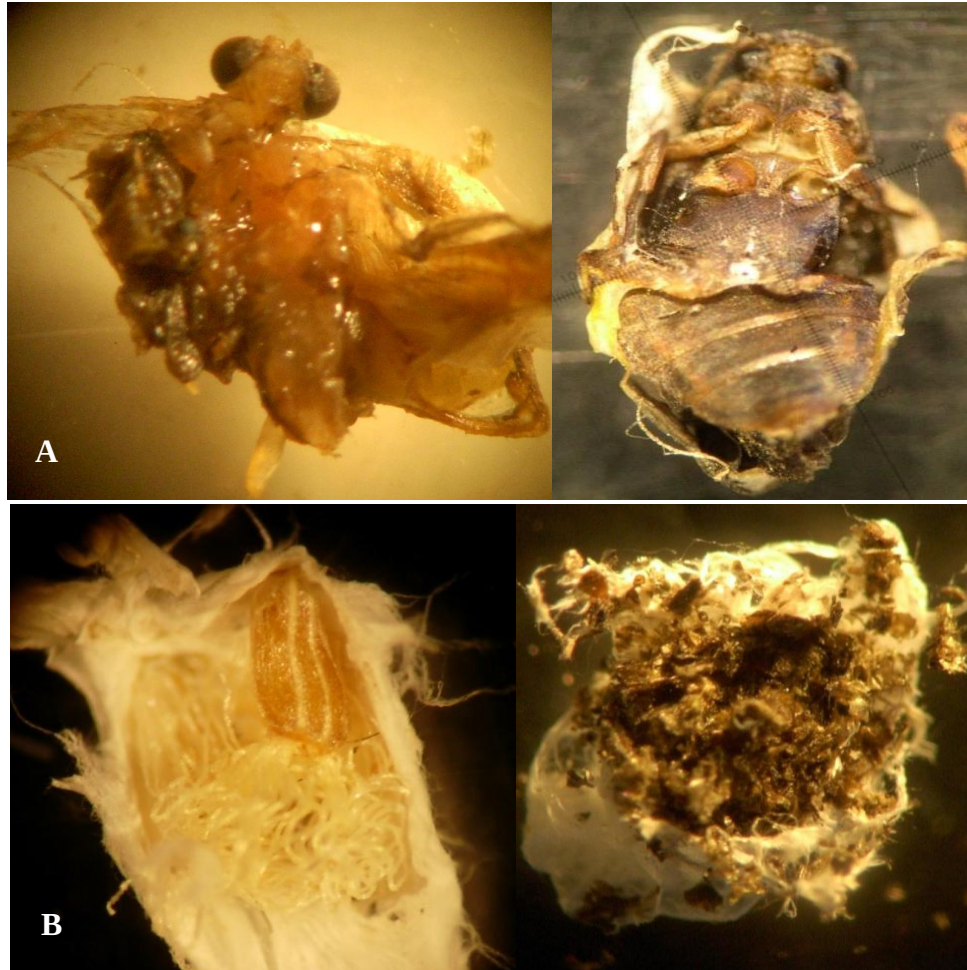


Figure 1. Pictures of A) genuine gift (fresh prey) and B) worthless gift (prey leftovers and plant seed) carried by males from the field. Photos: M.J. Albo.

EXPERIMENT 1: Worthless gifts, male condition and reproductive consequences

We designed experiments in the lab to investigate how both gift content and male condition affect male mating success. The typical courtship behaviour of a *P. ornata* male – whether carrying a gift on his chelicerae or not – consists of nonlinear locomotion towards the female and foreleg vibrations. The male offers the gift to the female in a particular posture called “hyperflexion” (Costa-Schmidt et al. 2008). Female mating acceptance occurs when she grasps the gift, allowing the male to mount and initiate sperm transfer via pedipalp insertion into the female genitalia. Males can perform up to four insertions during a mating. Between two pedipalp insertions the male returns to a face-to-face position and grasps the gift with his chelicerae (Costa-Schmidt et al. 2008; Albo & Costa 2010).

We exposed virgin females to males in three experimental treatments: FG, males carrying a genuine gift (a recently captured *Musca domestica* fly); WG, males carrying a worthless gift (a dry exoskeleton from *Tenebrio molitor* larva of approximately similar size as a housefly); and males with no gift (NG). We tested if male condition influences the use of gifts by repeating these three treatments in two male groups: males in good condition (GC) and males in poor condition (PC). To achieve the difference in male condition, we kept males into two different feeding regimes after the final moult: “GC” males (n = 86) received fruit flies every day and “PC” males (n = 51) did not receive food during the last 3 weeks before the experiment. We weighed males before the experimental trial and measured them once they naturally died. We calculated male condition index (*residual index*) in a subset of males resulting in 0.01 (± 0.01 SE) for good-condition males and -0.01 (± 0.01 SE) for poor-condition males (ANOVA: $F = 4.00$ $p = 0.04$, $N_{GC} = 56$, $N_{PC} = 47$). In total we had six experimental treatment-groups: 1) males in good condition offering a fly gift (FG-GC, n = 20); 2) males in good condition offering a worthless gift (WG-GC, n = 42); 3) males in good condition offering no gift (NG-GC, n = 24); 4) males in poor condition offering a fly gift (FG-PC, n = 17); 5) males in poor condition offering a worthless gift (WG-PC, n = 17); 6) males in poor condition offering no gift (NG-PC, n = 17). We controlled for individuals age to avoid any additional effect on the results (Klein et al. 2012), using males at average 24.9 days of adulthood (± 1.2 SE) and females at averaged 33.9 days (± 2.7 SE); no individuals were used more than once.

One day before each experiment we allowed females to deposit silk threads in the experimental cages, in order to provide stimulus to elicit male courtship and gift construction (Albo et al. 2009). Each trial consisted of two steps. First, we removed the female and placed a male in the experimental cage for 20 minutes. In FG, we first offered a housefly to the male; once he had captured it, we placed him in the experimental cage. In WG, the male was exposed to five dry exoskeletons randomly distributed in the experimental cage, giving him the opportunity to use one or not. In NG, the male was exposed to the female silk without any prey or inedible item. Second, regardless of whether silk wrapping occurred, we re-introduced the female, and registered all sexual behaviours. Therefore, in FG some males were carrying wrapped houseflies while other males carried unwrapped houseflies; similarly in the WG males carried wrapped or unwrapped dry exoskeletons. In WG, males without an item could also grasp one after contact with the female, or they could court and copulate without offering a gift. Data from the WG cases in which no gift was offered were excluded from the statistical analyses. We terminated experiments 10 min after the end of mating or at 30 min after the female was re-introduced if no mating occurred.

We registered when mating occurred (mating success), latency to mate and mating duration. Latency to mate (in min) was measured from when the males first touched the females until the beginning of the first pedipalp insertion. Latency to mate sometimes included time during which the male manipulated the prey or worthless item and wrapped it in silk. Mating duration (min) was measured as the sum of all insertion durations and was considered the total duration of possible sperm transfer. We analyzed mating frequencies using Chi-square tests for independent samples. We log transformed data of latency to mate and mating duration to accomplish normality, and analyzed them using two-way ANOVAs.

EXPERIMENT 2: Worthless gifts and female choice

In *P. ornata*, virgin females are usually less reluctant to mate than mated females (Klein et al. 2013). Additionally, it is likely that in the field males more often encounter mated females than virgins. Thus, in order to examine whether female reproductive status also influenced a male’s decision regarding the use of worthless gift and mating success, we first exposed the female (n = 20) to a mating with a male in good condition offering a fly gift (*Musca domestica*). Two days later, these mated females were exposed

to different males in good condition (*residual index*: 0.01 ± 0.03) offering worthless gifts (WG-GC). Similarly to Exp. 1, the experimental cage had five dry exoskeletons from *T. molitor* larva randomly distributed, and males had the opportunity to use one or not. Thus, males could mate with or without offering a gift, resulting in two male groups WG-GC and NG-GC. The feeding regime of males and females as well as the experimental procedure were identical to the one followed in Exp. 1. We registered the use of worthless gift and mating success. We analyzed mating frequencies using Chi-square tests for independent samples.

Results

FIELD DATA: Quantifying gift content and male condition

We collected 53 males carrying gifts in the field, 70% were worthless gifts and 30% were genuine gifts. Variation in gift content was not influenced either by the collecting date or by the site (ANCOVA: $F_{2,7} = 3.45$, $P = 0.63$ collecting date; $F_{2,2} = 1.84$, $P = 0.40$ collecting site). We found that genuine gifts were heavier than worthless ones (ANOVA: $F_{1,42} = 14.26$, $P = 0.0005$; Figure 2A) and that gift weight correlated positively with male body condition ($r = 0.42$, $P = 0.007$, $n = 40$; Figure 2B).

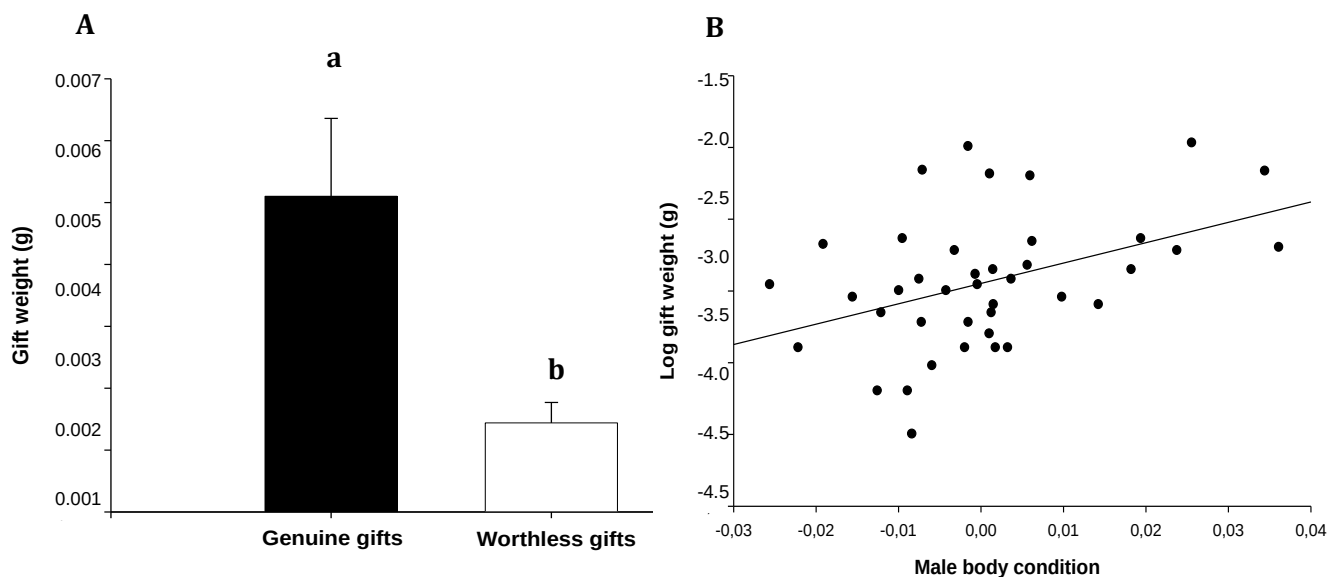


Figure 2. Data of males and gifts from the field. A) Weight from genuine gifts (fresh prey) and worthless gifts (prey leftovers), B) Correlation between male condition and gift weight. Different letters indicate significant differences from Chi-square tests

EXPERIMENT 1: Worthless gifts, male condition and reproductive consequences

Regardless of gift treatment, males in good condition had mated more often (82%) than males in poor condition (36%) (Chi-Square test: $X^2_1 = 23.79$, $P < 0.0001$, Figure 3).

Among males in good condition in the WG treatment, 19 used a worthless gift whereas the remaining 23 courted without a gift. For good condition males, mating success did not differ between FG, WG and NG treatments (Chi-Square test: $X^2_2 = 3.94$, $P = 0.14$, Figure 3). In the poor condition group, six out of 17 males used a worthless gift, while the rest of the males courted without gift in the WG treatment. There were no significant differences in mating success in poor condition males among FG, WG and NG (Chi-Square test: $X^2_2 = 0.18$, $P = 0.91$, Figure 3).

Males in good condition had shorter latencies to mate, reaching the first pedipalp insertion faster than males in poor condition; this latency was not affected by whether males offered a gift or not (FG, WG and NG) (Table 1, Figure 4A). Mating duration was longer for males in good condition than for those in poor condition, with no statistical differences among FG, WG and NG treatments (Table 1; Figure 4B).

Figure 3. Data from Experiment 1. Mating success (percentage) from males in good and poor condition (GC, PC), offering different nuptial gifts types: fly gift (FG), worthless gift (WG) and no gift (NG). Different letters indicate significant differences from Chi-square tests. Small letters indicate comparisons among gift treatments (FG, WG, NG); capital letters indicate comparisons between feeding groups (GC, PC).

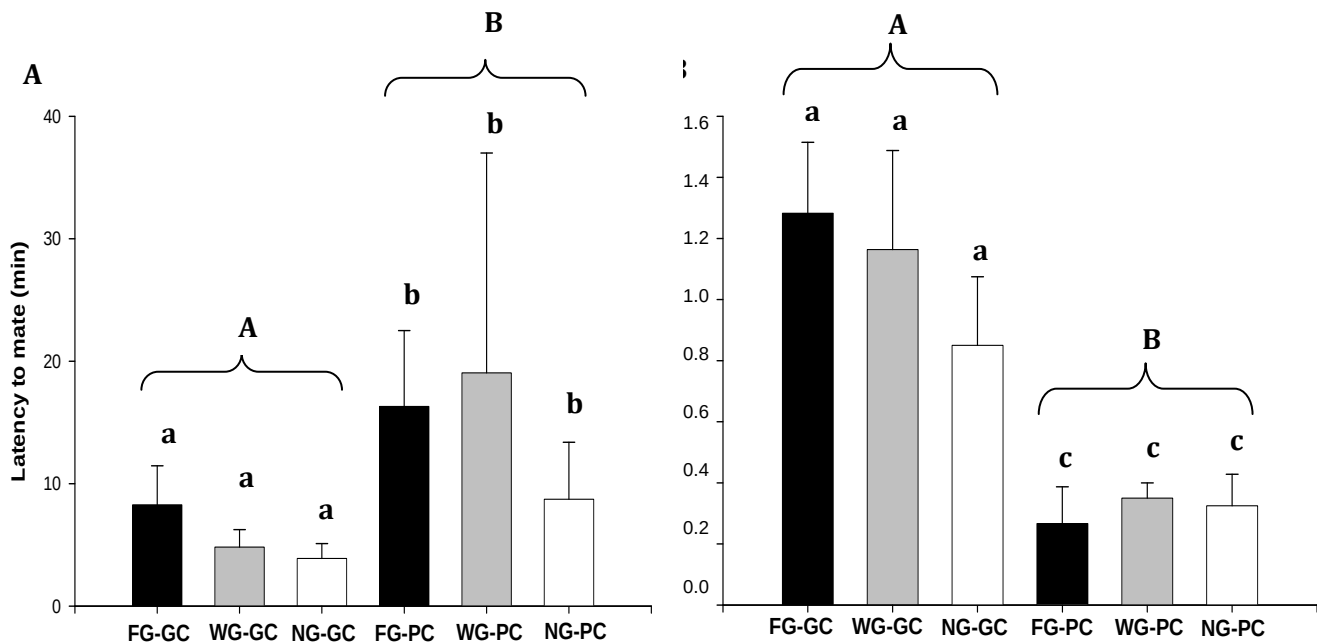
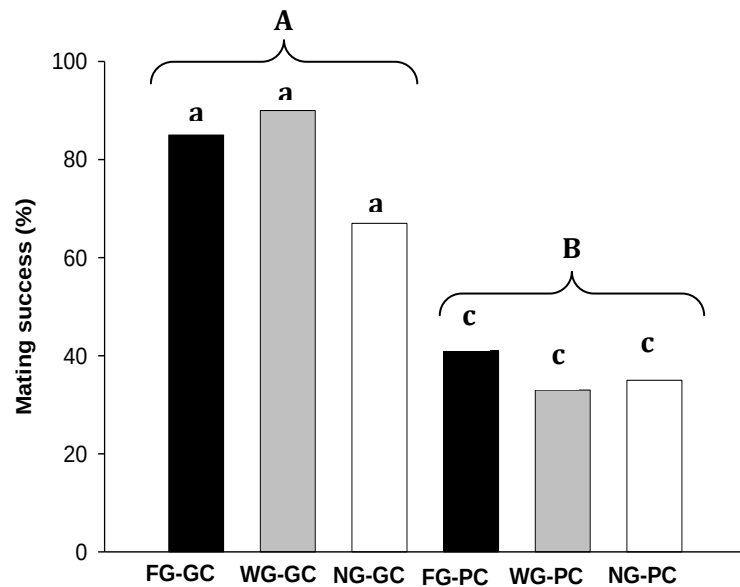


Figure 4. Data from Experiment 1. A) Latency to mate (min), B) mating duration (min) from males in good and poor condition (GC, PC), offering different nuptial gifts types: fly gift (FG), worthless gift (WG) and no gift (NG). Different letters indicate significant differences: small letters indicate comparisons among gift treatments (FG, WG, NG); capital letters indicate comparisons between feeding groups (GC, PC).

Table 1. Effects of male condition (good and poor), gift treatments (FG, WG, NG) and their interaction on latency to mate and mating duration.

	Male condition (df = 1)	Gift treatment (df = 2)	Interaction (df = 2)
Latency to mate (min) (N = 58)	F = 4.21 P = 0.04	F = 1.36 P = 0.26	F = 0.15 P = 0.85
Mating duration (min) (N = 51)	F = 13.35 P = 0.001	F = 0.21 P = 0.81	F = 0.68 P = 0.51

Statistical comparisons were performed using two-way ANOVA.

EXPERIMENT 2: Worthless gifts and female choice

Sixty-five percent of the males used a worthless gift (WG) and the remaining 35% of males courted without a gift (NG). Only males that used a worthless gift were accepted by females and obtained a mating (Chi-Square test: $X^2_1 = 6.24$, $P = 0.01$; Figure 5).

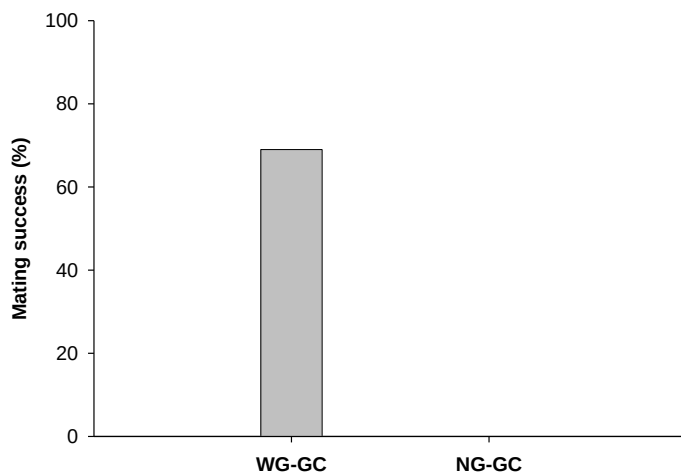


Figure 5. Data from Experiment 2. Mating success (percentage) from males in good condition offering a worthless gift (WG) and no gift (NG).

Discussion

The evolutionary origin of nuptial gift-giving by males most probably occurred because female selection for acquiring extra meals and a consequent reproductive advantage for males. However, because producing a nuptial gift may be costly in some circumstances, males may have evolved to vary nuptial gift content as an alternative tactic that reduces investment. Our findings show that *P. ornata* males more often carried worthless gifts than genuine gifts in the field, indicating that this alternative tactic is now an established strategy in the population. The occurrence of worthless gifts seems to be affected by male condition, as males in lower condition were usually carrying prey leftovers (worthless gifts) while those in better condition usually carried fresh prey gift (genuine gifts). Thus, when males are in poor body condition, they can eat the prey before turning it into a gift by wrapping it in silk. Indeed, in the lab males in poor condition sometimes reduce the original prey weight by 80% before beginning to wrap it in silk (M.J. Albo & V. Melo-Gonzalez unpublished data). By eating the prey a male can acquire energy to enhance his body condition, which according to the first experiment is more important for his mating success than the nutritional quality of the gift when he encounters a virgin female. Our findings verified that male condition is essential for mating success (Shamble et al. 2009; Byers et al. 2010; Albo et al.

2012). Males in poor feeding condition (with or without gift) suffered a reduction in mating success which very probably leads to a reduction in reproductive success, as has been found in other species (Mappes et al. 1996; Andrade & Mason 2000; Ahtiainen et al. 2001; Kotiaho 2002; Hunt et al. 2004; Engels & Sauer 2006; Lomborg & Toft 2009). In contrast, males in good condition had the highest mating success, shorter latencies to mate and longer matings. Furthermore, males without a gift but in very good condition were able to mate longer and with a higher proportion of females than males with a gift but in poor condition.

Interestingly, these results on male body condition raise the question of how nuptial gifts are maintained in this mating system and below we give some arguments that can help in the discussion. We know from previous studies that when a female is nearby, the incentive to offer a gift is high, as males often attempt to collect inedible items (Albo & Costa 2010). Further, the gift-giving behaviour seems to be basically driven by female mating rejection, as rejected males (without gift) usually wrap worthless items. Female selectivity may arise due to age (Klein et al. 2012) and/or foraging history (Bilde et al. 2007; Prokop & Maxwell 2009), but our experimental design controlled for both variables and nevertheless documented female selectivity. The causes for the variation in selectivity among virgin females remain unknown, but here we show that offering a gift is especially significant when males encounter mated females. These females are more selective than virgins and do not accept matings without a gift even if males are in good condition. Consequently, if no prey is available males benefit from using worthless items found in the surroundings, obtaining similar mating success compared to males with genuine gifts. Therefore, during courtship females strongly favour males offering a gift regardless of the content.

Nuptial gifts can signal male attractiveness and/or quality, and females may select potential mates based on gift presence (Fisher 1930; Zahavi 1975). By selecting attractive gift-giving males, females would benefit having sons that probably be as attractive and successful as their fathers (Fisher 1930). Alternatively, as gifts can be condition dependent, they are reliable traits of some aspects of the male quality (Gwynne 1990; Engels & Sauer 2006; Vahed 2007; Immonen et al. 2009; Albo et al. 2011a). In *P. ornata* having a gift may represent males' ability to hunt or search for items to offer. This predicts that the nuptial gift trait has evolved losing what was probably its original function as a direct benefit and is maintained as a condition indicator of the potential partner. In this scenario, females can gain indirect benefits by selecting high quality males offering gifts, but this entails no reproductive costs for females that mate with males bearing non-nutritive gifts. However, preliminary data suggests that *P. ornata* females may actually suffer an important decrease in fecundity when they receive worthless gifts during mating (I. Pandulli & M.J. Albo unpublished data).

Consequently, differences in male investment in nuptial gift content ultimately create a co-evolutionary scenario in which females and males' interests diverge. If females benefit from males offering nutritive gifts, as they can increase their feeding rate, fecundity and the hatching success of their eggs (Arnqvist & Nilsson 2000), we should expect they counteract male deception during and/or after mating. Similar to other gift-giving spiders, *P. ornata* females can only recognize gift content when they start to eat it during mating. In *P. mirabilis*, mating duration is very long and females can reduce time in mating and potentially the amount of sperm stored with males offering worthless gifts (Albo et al. 2011b; Albo et al. 2013), however, this is not the case in *P. ornata* as they have very short matings. Thus, females may not have the time to assess gift content in such a short time, giving advantages to males for completing sperm transfer. Indeed, mating duration was not different between males offering genuine gifts and those offering worthless gifts. We know that sperm transferred is positively correlated to mating duration (M.J. Albo & A.V. Peretti in revision), as also occurs in other spiders and insect species (Parker & Simmons 1991; Simmons 2001; Engqvist & Sauer 2003; Herbestein et al. 2011), thus *P. ornata* males with genuine and worthless gifts will transfer similar amounts of sperm and will

have equal opportunity to compete for fertilizations inside the female genital tract. Thus, males hold the upper hand in the co-evolutionary cycle unless females counteract deception via some post-copulatory process (Eberhard 1996), as for instance by differential sperm storage (Albo et al. 2013).

Overall, our findings illustrate how ecological conditions play a central role in shaping evolution of gift-giving mating systems. Variations such as a decline in prey availability could affect male feeding regimes and body condition, consequently influencing nuptial gift nutritional quality. There are advantages for males to offer gifts regardless of the content, and so far, females do not seem to penalize matings with worthless gifts. The prevalence of genuine and worthless gifts creates a remarkable evolutionary scenario to study the strategies used by both sexes to favour their own reproductive interests.

Acknowledgments

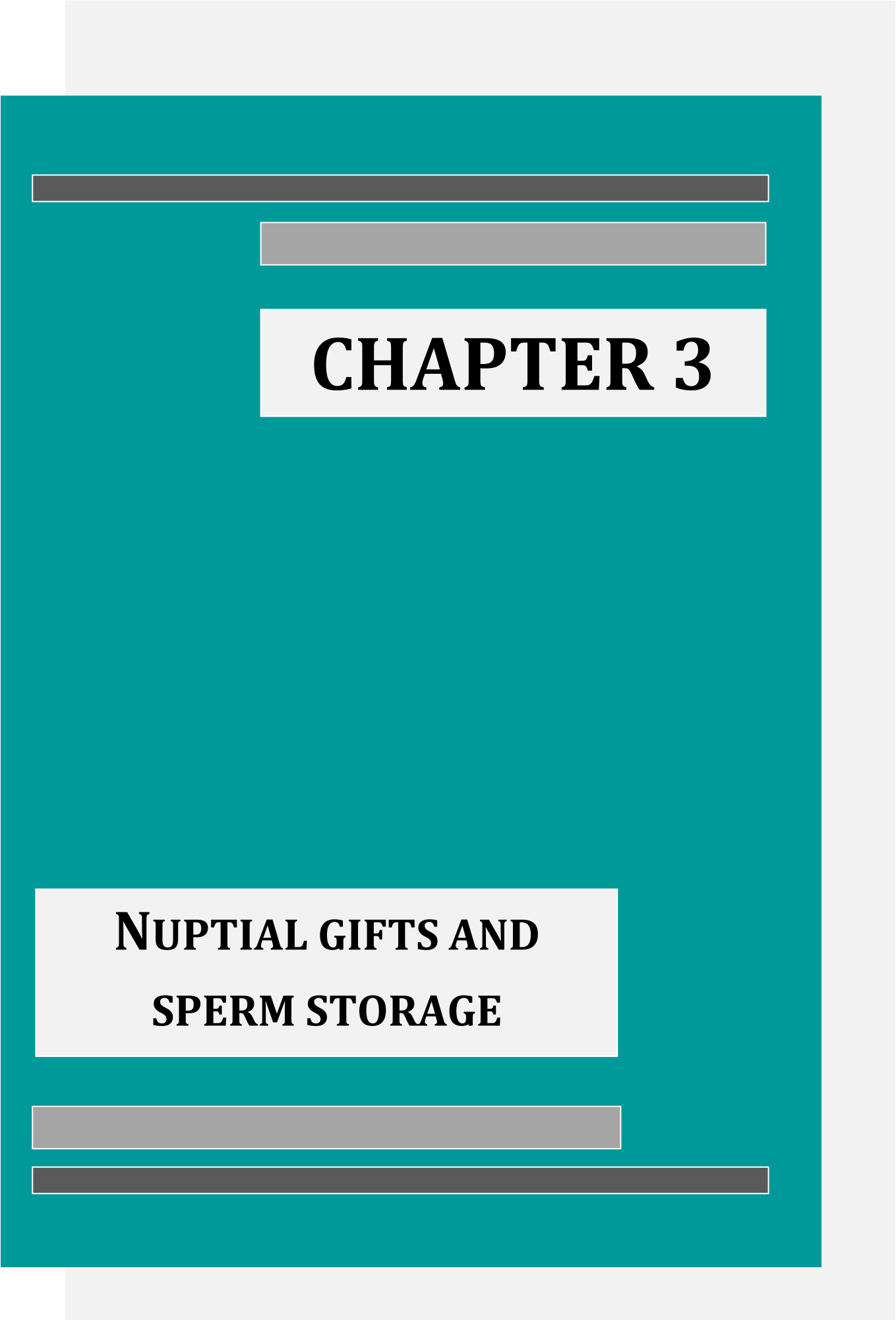
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CHAPTER 3

NUPTIAL GIFTS AND SPERM STORAGE

CAPÍTULO 3- REGALOS NUPCIALES Y ALMACENAMIENTO DE ESPERMA

ANTECEDENTES: Los procesos de selección sexual que ocurren durante o después de la cópula se producen internamente en hembras poliándricas y pueden incluir competencia espermática pero también elección críptica femenina del esperma y por lo tanto, sesgos en la fertilización de los huevos a favor de los machos preferidos. Las especies que tienen transferencia de regalos nupciales durante la cópula son particularmente prometedoras para el estudio de elección críptica de pareja debido a que los regalos que son nutritivos para las hembras pueden ser señales honestas de la calidad del macho. Se investigó la hipótesis de que la presencia o contenido del regalo están sujetos a elección críptica femenina en las arañas poliándricas *Pisaura mirabilis* y *Paratrechalea ornata*. En ambas especies los machos pueden copular sin regalo, pero aquellos que ofrecen regalo experimentan un mayor éxito de cópula y cópulas más prolongadas. Más aún, los machos de estas especies pueden variar el contenido del regalo de nutritivo a simbólico, una estrategia que va en contra de los beneficios nutricionales de las hembras. Las hembras de la araña *P. mirabilis* penalizan a los machos engañadores reduciendo la duración de la cópula y el esperma almacenado, pero este no es el caso en *P. ornata*.

DISEÑO EXPERIMENTAL: Para examinar si la presencia de regalo influye en el número de espermatozoides almacenado por la hembra se compararon cópulas con regalo y sin regalo fijando el mismo tiempo de cópula (cópulas interrumpidas). Para examinar la relación entre la duración de la cópula y el esperma almacenado, se determinó el número de espermatozoides en cópulas no-interrumpidas con machos que ofrecían regalo. Asimismo, como las hembras de *P. ornata* no reducen el tiempo de cópula cuando se aparean con machos que ofrecen regalos simbólicos, se examinó si el contenido del regalo influye en la cantidad de esperma almacenado en las espermatecas de dos grupos experimentales de hembras: copuladas con regalo nutritivo y con regalo simbólico (cópulas no-interrumpidas).

RESULTADOS PRINCIPALES: En *P. mirabilis* las hembras que copularon con machos que no ofrecían regalos almacenaron significativamente menos cantidad de espermatozoides que aquellas que recibieron un regalo en el mismo tiempo de cópula (cópulas interrumpidas). Además, se observó una correlación positiva entre el tiempo de cópula y el número de espermatozoides almacenado en las cópulas no-interrumpidas, y duraciones de apareamiento mayores resultaron en más espermatozoides almacenados en el tracto genital de la hembra. Por otro lado, la presencia de regalo no afectó el número de espermatozoides almacenado en las espermatecas femeninas en *P. ornata*. Sin embargo, también se observó una correlación positiva entre el tiempo de cópula y el número de espermatozoides almacenado en todos los grupos experimentales en esta especie. Curiosamente, la duración de la cópula fue más larga en machos que ofrecieron regalos simbólicos que en machos que ofrecieron regalos genuinos, pero las hembras que recibieron regalos simbólicos no almacenaron más esperma en la espermateca, como era de esperar de dicha correlación.

CONCLUSIONES: Permitiendo que machos donadores de alta calidad –con regalo o regalo más nutritivo – almacenen más esperma las hembras pueden ganar beneficios indirectos. Estos beneficios pueden ser a través de procesos Fisherianos (si éstas producen “hijos atractivos” que donaran mejores regalos), o mejorando la eficacia de sus descendientes (si los machos donadores pasan genes que son superiores para rasgos de supervivencia). Los machos de *P. mirabilis* y *P. ornata* que ofrecen regalos nupciales pueden reflejar buenas habilidades de caza que son heredadas por sus hijos. De hecho, en *P. mirabilis*,

los machos saciados y por lo tanto en buenas condiciones corporales, mostraron tener mayor éxito de cópula y paternidad que aquellos mal alimentados y en malas condiciones (Capítulos 1 y 2), lo que sugiere que las hembras pueden seleccionarlos en base a su calidad. Contrario a lo esperado, las hembras de *P. ornata* no almacenan más esperma de machos que ofrecen regalos comparado con machos que no ofrecen regalos. En esta especie, entonces, el regalo (más allá de su contenido) parece ser un rasgo seleccionado durante el cortejo pero no está bajo selección directa durante la cópula. Dichas diferencias entre estas dos arañas donadoras de regalos nupciales podrían atribuirse a condiciones ecológicas particulares u otros factores, pero nuevos estudios son necesarios para cualquier tipo de conclusión. Importante, al realizar selección post-copulatoria las hembras de *P. ornata* pueden parcialmente contrarrestar el engaño del macho al restringir la cantidad de esperma y potencialmente su paternidad. Desde el punto de vista del macho, el regalo nupcial funciona para aumentar la duración de la cópula y el almacenamiento de esperma en ambas especies.

CHAPTER 3- NUPTIAL GIFTS AND SPERM STORAGE

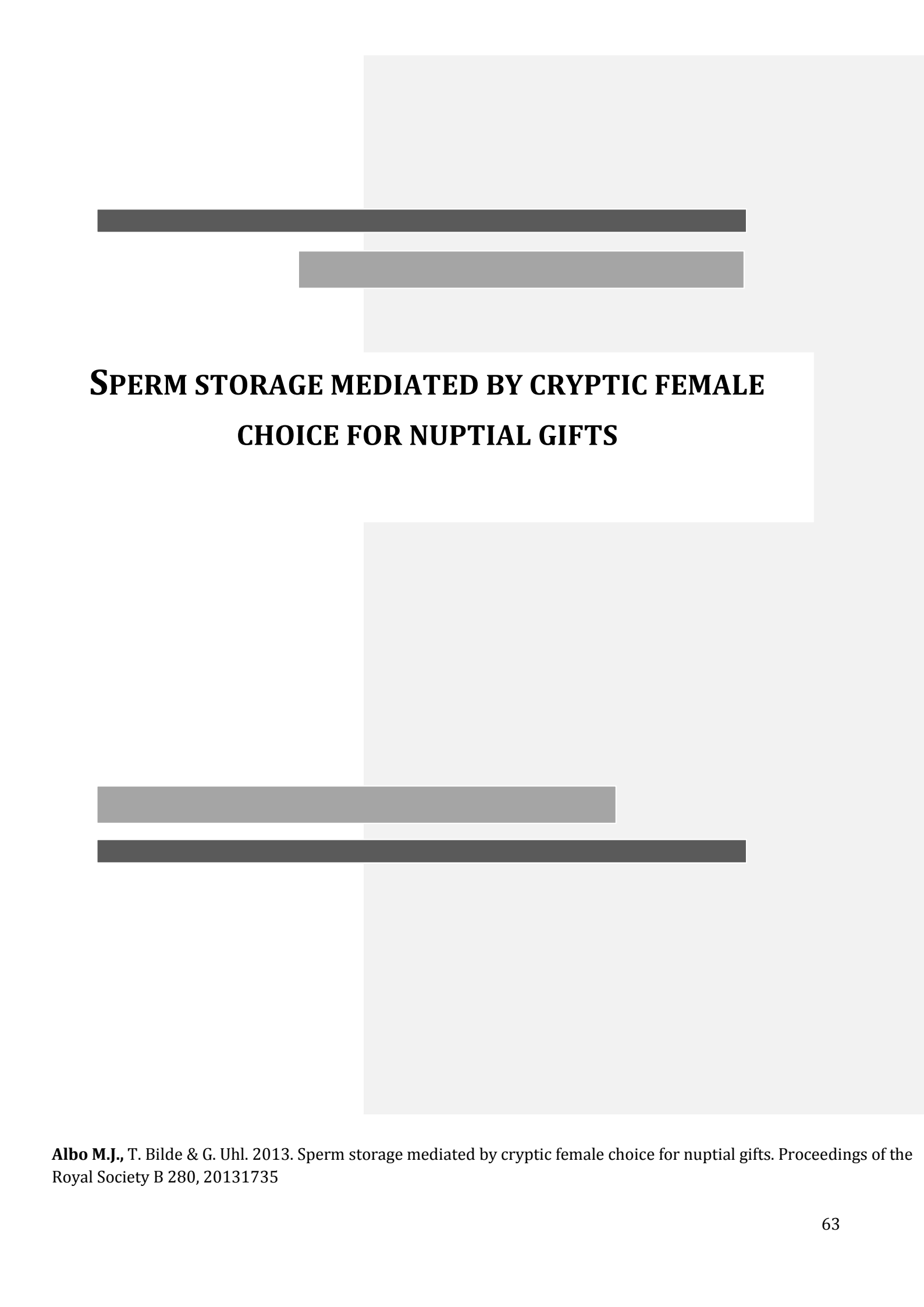
BACKGROUND: Cryptic syn or post-copulatory sexual selection occurs internally in polyandrous females and can include sperm competition but also cryptic female choice of sperm and therefore, bias in egg fertilization towards a preferred male. Species in which nuptial gifts are transferred during mating are particularly promising for studying cryptic mate choice, since gifts that are nutritious to females may be an honest signal of male quality. Here I tested the hypothesis that the nuptial gift presence and/or content are target of cryptic female choice in the polyandrous spiders *Pisaura mirabilis* and *Paratrechalea ornata*. In both species it is possible for males to copulate without a gift, but gift-giving males experience dramatically higher mating success and longer copulations. Further, males can vary gift content from nutritious to worthless, a strategy that is against female nutritional benefits. Females from *P. mirabilis* penalized deceiver males reducing copulation duration and the sperm stored, but this is not the case for *P. ornata*.

EXPERIMENTAL DESIGN: I tested the effect of gift presence on the number of sperm stored by the female, by experimentally terminating copulation duration in males with a gift to match the average duration of shorter copulations with males without a gift (interrupted matings). To examine the relationship between copulation duration and sperm storage, I also determined the number of sperm stored after uninterrupted matings with gift-giving males. Additionally, as females from *P. ornata* do not reduced copulation time with males offering worthless gifts I examined whether gift content influence the number of sperm stored in the female spermathecae. Thus, I analyzed the number of sperm from two mating groups: males offering genuine gifts and males offering worthless gifts (uninterrupted matings).

MAIN RESULTS: In *P. mirabilis* females mated with males offering no gift obtained significantly lower sperm stored compared to females mated with males offering gifts at the same copulation time (interrupted matings). In addition, there was a positive effect of copulation duration on the number of sperm in the uninterrupted matings and longer copulations resulted in significantly more sperm stored in the female genital tract. On the other hand, in *P. ornata* gift presence did not affect the number of sperm stored in the female spermathecae. There was a positive effect of copulation duration on the number of sperm in all experimental groups. Curiously, copulation duration was longer for males offering worthless gifts compared to males offering genuine gifts, but females receiving worthless gifts did not stored more number of sperm in the spermathecae as expected from that correlation.

CONCLUSIONS: By allowing high quality donors -with gift or more nutritious gift- to store more sperm females can gain indirect benefits, either through Fisherian processes if females produce “sexy sons” that are more likely to provide nuptial gifts, or by elevated fitness of offspring if gift-giving males pass on genes that are superior for survivorship traits. Males from *P. mirabilis* and *P. ornata* that offer nuptial gifts may signal good hunting abilities that are inherited to their sons. Indeed, *P. mirabilis* males that are satiated and thus, in good condition were shown to achieve higher mating and paternity success compared to starved males in poor condition (Chapter 1 and 2), suggesting that females can select males based on their quality. Contrary to what was expected, in *P. ornata* females did not store more sperm from males having a gift compared to males having no gift. Thus, in *P. ornata* the gift is an important selected trait during courtship but it seems not under direct selection during mating. Such differences between these two gift-giving spiders may be attributed to particular ecological conditions or other factors, but further studies are needed for any conclusion. Importantly, by exercising post-

copulatory selection *P. ornata* females can partially counteract male deception and restrict the amount of sperm, potentially reducing his paternity. From the male perspective, nuptial feeding functions to enhance copulation duration and sperm stored in both species.



SPERM STORAGE MEDIATED BY CRYPTIC FEMALE CHOICE FOR NUPTIAL GIFTS

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Sperm storage mediated by cryptic female choice for nuptial gifts

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Polyandrous females are expected to discriminate among males through postcopulatory cryptic mate choice. Yet, there is surprisingly little unequivocal evidence for female-mediated cryptic sperm choice. In species in which nuptial gifts facilitate mating, females may gain indirect benefits through preferential storage of sperm from gift-giving males if the gift signals male quality. We tested this hypothesis in the spider *Pisaura mirabilis* by quantifying the number of sperm stored in response to copulation with males with or without a nuptial gift, while experimentally controlling copulation duration. We further assessed the effect of gift presence and copulation duration on egg-hatching success in matings with uninterrupted copulations with gift-giving males. We show that females mated to gift-giving males stored more sperm and experienced 17% higher egg-hatching success, compared with those mated to no-gift males, despite matched copulation durations. Uninterrupted copulations resulted in both increased sperm storage and egg-hatching success. Our study confirms the prediction that the nuptial gift as a male signal is under positive sexual selection by females through cryptic sperm storage. In addition, the gift facilitates longer copulations and increased sperm transfer providing two different types of advantage to gift-giving in males.

1. Introduction

Cryptic mate choice refers to sexual selection during mating (syncopulatory) or afterwards (postcopulatory) that leads to differences in sperm use among competing males [1–5]. Species in which nuptial gifts are transferred during mating [6] are particularly promising for studying cryptic mate choice, because gifts that are nutritious to females may be an honest signal of male quality [7]. If nuptial gifts are honest signals of good hunting ability and this trait is heritable, females would benefit from biasing paternity towards males that offer gifts [8]. The potential for acquiring benefits of cryptic choice is particularly high when there is variance in the quality of the nuptial gift. Gift-giving may, for example, be a target for sexually antagonistic coevolution, which favours the evolution of deceit or fake gifts, reducing the benefit of precopulatory female mate choice [9–12]. However, females may counteract deceit by favouring males that present genuine nuptial gifts during or after mating [12]. In nuptial gift-giving species, female preference for genuine gifts is thus expected to favour the evolution of cryptic female mate choice. Whereas the influence of the gift-giving trait on male mating success and the potential for males to exploit female preference for nuptial gifts have attracted much interest [9,11,12], remarkably little is known about cryptic female choice for nuptial gifts.

Internal processes that result in fertilization bias can be mediated by both sexes or by interactions between male and female traits [2,3,13]. There is a wealth of studies on syn- or postcopulatory processes focusing on male traits that confer advantages in sperm competition [2,4,5]. By contrast, there are few studies showing unequivocal evidence for cryptic sperm choice [14]. This is surprising, as cryptic female mate choice should be very common precisely because it is occurring in the genital tract, where females are expected to be

more in control of internal processes after copulation than males [3]. Empirical studies have suggested postcopulatory selection for males of high quality based on traits such as body condition, attractiveness, social status or relatedness [15–23]. However, few studies conclusively show that sperm selection is driven by female cryptic sperm choice [24–26]. This may be because it is intrinsically difficult to control for strategic male sperm allocation [27], and hence to disentangle male- and female-driven processes [28]. Unequivocal evidence of female sperm choice was shown in crickets [25,26], where males form a spermatophore before they encounter the female, and therefore are unable to strategically mediate sperm allocation in response to female phenotype. Preferential storage of sperm from unrelated males [25], or conspecific males [26], could therefore be attributed to cryptic female choice.

We tested the hypothesis that the nuptial gift is a target of cryptic female choice in the polyandrous spider *Pisaura mirabilis* (Pisauridae). In this species, males often offer an insect prey wrapped in white silk as a nuptial gift, which the female feeds on during copulation [29]. While it is possible for males to copulate without a gift, gift-giving males experience dramatically higher mating success [12,30,31]. Furthermore, males that offer a nuptial gift achieve longer copulations (100 min on average) than males without a gift (10 min on average), leading to a higher number of fertilized eggs for gift-giving males [12,30,32]. Females appear to have full control over both the initiation and termination of mating [12,30]. Spiders are excellent models for testing female cryptic sperm choice, because males use external sperm transfer by modified intromittent organs, the pedipalps, which are loaded with sperm prior to mate searching [33], thus limiting the scope for strategic sperm allocation. Females store sperm from multiple males in sperm storage organs, the spermatheca, for later fertilization of eggs when she produces an egg-sac.

We tested the effect of gift presence on the number of sperm stored by the female by experimentally terminating copulation duration in males with a gift (GT) to match the average duration of shorter copulations with males without a gift (NG). Following copulation, we determined the number of sperm in the female sperm storage organs. If females bias sperm storage towards males that offer a gift, females in the GT group were expected to store more sperm than females in the NG group. To examine the relationship between copulation duration and sperm storage, we also determined the number of sperm stored after uninterrupted copulations with gift-giving males (G). Finally, we examined the effect of gift presence and copulation duration on female fecundity and egg-hatching success in an additional set of females mated to NG, GT and G males to gain further insight into benefits of providing a nuptial gift.

2. Material and methods

We collected juveniles and subadults of *P. mirabilis* (Clerck, 1757) in April 2011 at the Mols Laboratory near Aarhus, Denmark. In the laboratory at Aarhus University, spiders were housed individually in vials (30 ml) containing moist moss (*Sphagnum* spp.). Water was provided regularly to maintain humidity. We raised individuals at room temperature ($23.4 \pm 0.1^\circ\text{C}$) and natural photoperiod and fed them with blowflies (*Calliphora* sp.) three times per week until maturation.

(a) Mating behaviour and experimental design

Males court females by offering the nuptial gift held in their chelicerae while waving their pedipalps (male intromittent organs). Once the female accepts and grasps the gift with her chelicerae, the male initiates sperm transfer by performing alternate insertions of the pedipalps into the female genital tract. During copulation, the male retains contact with the gift with the tarsal claws of his third pair of legs, while the female is consuming it. After each insertion, the male returns to a face-to-face position with the female, grabbing the gift with his chelicerae. No courtship in the form of pedipalp waving is performed before the second (and possibly subsequent) palp insertion. Copulation occurs in a similar way in matings with no gift, although these males experience a much reduced acceptance rate [29,34]. In staged mating trials, we registered the number of pedipalp insertions and copulation duration. Copulation duration was recorded from pedipalp insertion until pedipalp disengagement and was calculated as the sum of the duration of all insertions occurring within a trial. Total copulation duration thus represents the time of potential sperm transfer.

Our experimental design included three groups. NG males ($n = 53$) offered no gift and were allowed to copulate without interruption; copulation duration (mean $\pm$ s.e.): 10.4 ± 1.6 min, number of insertions (mean $\pm$ s.e.): 2.0 ± 0.2 . GT males ($n = 39$) offered a nuptial gift and had copulation experimentally terminated after 10 min to match the average copulation duration of NG males. G males ($n = 36$) offered a gift and were allowed to perform uninterrupted copulations; copulation duration (mean $\pm$ s.e.): 86.0 ± 6.0 min; number of insertions (mean $\pm$ s.e.): 4.2 ± 0.6 . Experimental manipulation of the GT group was based on the average copulation duration and number of insertions found in the NG group, thus allowing two pedipalp insertions. In the GT group, we terminated the first insertion after 5 min using a paintbrush. Subsequently, we allowed the male to resume copulation and perform the second insertion for another 5 min before the final separation. We analysed the number of sperm transferred in a subset of females, and fecundity and egg-hatching success in another subset of females from each of the three experimental groups (see details below).

Staged mating experiments were carried out in May 2011. A female was placed in a transparent plastic cage ($22 \times 17 \times 6$ cm) with paper-covered bottom at least an hour prior to the experiment, allowing her to deposit silk threads. We then removed the female and exposed the male to the female silk for 15 min. In the GT and G groups, a housefly (*Musca domestica*) was added to the cage which the male caught for gift construction. After 15 min, we gently reintroduced the female into the cage and mating trials started. NG males were exposed to the same procedure but with no prey available. Male spiders load their pedipalps (intromittent organs) with sperm after the final moult, and thus prior to mate search and production of nuptial gifts [34]. With this design, we aimed to minimize any effect of the presence or absence of the gift on differential loading of the pedipalps. Males were randomly allocated to the three treatment groups. Individuals used in the experiments were virgins and used only once.

(b) Sperm count

Females were frozen at -50°C between 3 and 6 h after mating (NG: $N = 39$; GT: $N = 20$; G: $N = 18$). For counting the number of sperm transferred, specimens were transferred to the University of Greifswald, where the female sperm storage organs were dissected out under a stereomicroscope (ZEISS) and treated by following a protocol established for *P. mirabilis* ([35], modified after [36]). The female spermathecae were transferred to $20 \mu\text{l}$ of saline solution (Casytone, Schärfe System). To homogenize samples, we ruptured the spermathecae with forceps and

applied ultrasonic treatment. We avoided sample loss by using indirect ultrasonic processing in a cup booster designed for small volumes (Bandelin UW 2070). Ultrasonication was done twice for 30 s at 50% power with a break of 30 s to avoid overheating of the sample. Afterwards, we centrifuged samples at 5000 g for 1 min and vortexed for 1 min. We placed 10 μ l on each counting chamber of the haematocytometer (1 mm Neubauer). The sperm were counted in 16 squares under a microscope 400 $\times$ (Olympus).

Our main aim was to test whether the number of sperm found in the female spermathecae after NG and GT matings differ. To reduce the variance in copulation duration in NG compared with the GT group, we reduced the dataset by selecting NG data with an average copulation duration of 9.6 min (± 0.9 s.e., range: 6–16 min, $N = 14$).

A copulation could be completed with a male using one or both pedipalps, therefore we tested the effect of the number of pedipalps used on the number of sperm stored. Whether one or more than one pedipalp insertion was performed in NG matings did not significantly affect the number of sperm found in the female genital tract (mean $\pm$ s.e. one insertion: 3020 ± 1079 ; more than one insertion: 4727 ± 946 ; $t = -1.18$, $p = 0.25$, $N_{\text{one insertion}} = 6$, $N_{\text{two or more insertions}} = 8$). Additionally, as males performing two or more insertions could have used the same or different pedipalps in the second or later insertions, we checked whether this affected the number of sperm stored by females in NG and GT matings. We found no significant differences in the number of sperm stored neither in the NG (mean $\pm$ s.e. same pedipalp: 2625 ± 892 , different pedipalp: 5885 ± 1736 ; $U = 19.5$, $p = 0.29$, $N_{\text{changing palps}} = 12$, $N_{\text{same palp}} = 5$) nor in the GT group (same pedipalp: 7098 ± 1001 , mean $\pm$ s.e. different pedipalp: 6197 ± 2332 ; $t = 0.92$, $p = 0.36$, $N_{\text{changing palps}} = 6$, $N_{\text{same palp}} = 14$). In two cases data points on pedipalp change are missing.

For analysing the relationship between copulation duration and sperm number, we used all data available from the NG group ($N = 39$) and pooled these with data from the G group ($N = 18$).

(c) Hatching success

To study the effect of gift presence and copulation duration on reproductive success, we determined the reproductive output of 14 females from the NG group, 19 females from the GT group and 18 females from the G group. After the mating trials, females were kept individually in 30 ml vials at room temperature ($23.4 \pm 0.1^\circ\text{C}$) and were fed one blowfly (*Calliphora* sp.) per day. After egg-sac construction, light bulbs were placed 20 cm above the vials to raise the temperature to 26.7°C (± 0.1 s.e.) during 3 h starting at noon in order to enhance hatching success of the eggs. As females carry the egg-sac in their chelicerae until the eggs hatch, feeding was stopped after oviposition. For the first egg-sac a female produced, we counted the total number of laid eggs (hatched + unhatched eggs = clutch-size). Hatching success was calculated as the proportion of hatched eggs.

(d) Statistical analyses

Statistical analyses were performed using JMP 7.0 software (SAS institute). Assumptions of parametric tests were examined using Shapiro–Wilk tests for normal distribution of residuals and Levene's test for homogeneity of variances. Generalized linear models (GLMs) were used for analysing sperm number (Poisson) and hatching success (binomial) among groups, and to examine the effect of copulation duration on hatching success (binomial). We used linear regression to analyse the effect of copulation duration on the number of sperm stored (log transformed).

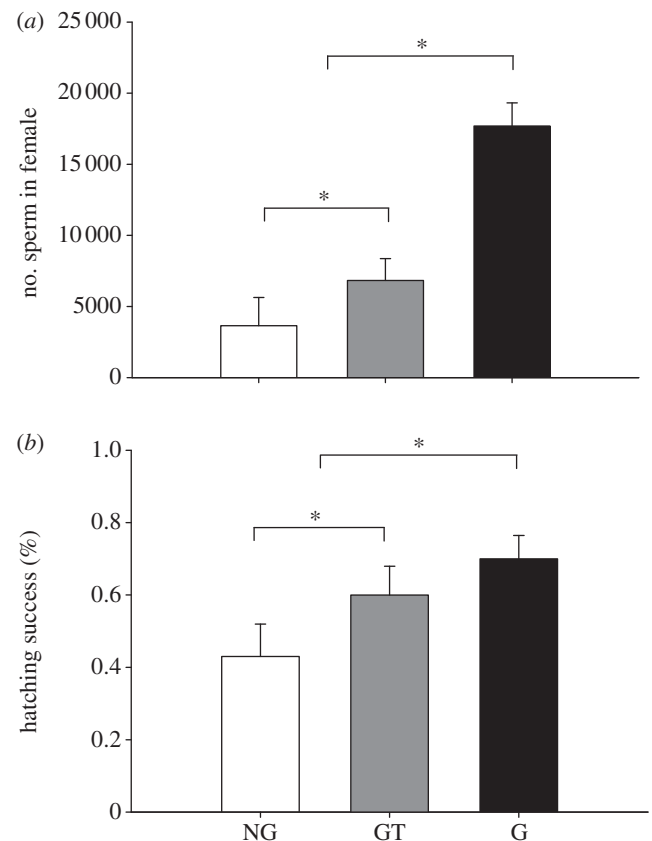


Figure 1. (a) Number of sperm in the female sperm storage organs and (b) hatching success, in NG (short copulations without gift), GT (short copulations with gift) and G matings (long copulations with gift). Asterisk (*) indicates significant differences ($p < 0.05$).

3. Results

(a) Sperm count

The number of sperm in the female spermatheca differed significantly among the three experimental groups (GLM, $\chi^2 = 174442.1$, $p < 0.0001$, d.f. = 2, $N = 52$; figure 1a), and was significantly lower in the NG group compared with the GT group ($\chi^2 = 12165.4$, $p < 0.0001$, d.f. = 1, $N_{\text{NG}} = 14$, $N_{\text{GT}} = 20$; figure 1a). Females in uninterrupted matings with gift-giving males G stored significantly higher numbers of sperm compared with females from the NG and GT groups ($\chi^2 = 162276.8$, $p < 0.0001$, d.f. = 1, $p < 0.0001$, $N_{\text{G}} = 18$, $N_{\text{NG+GT}} = 34$; figure 1a). In the G group, copulations resulted in significantly more sperm stored in the female genital tract (Linear regression, effect of copulation duration on log sperm number: $F = 11.6$, $p = 0.004$, $N_{\text{G}} = 18$; figure 2a). No parametric linear relationship was found in the group of males with no gift ($N = 39$, figure 2a).

(b) Hatching success

Generalized linear models showed that the hatching success differed significantly among groups ($\chi^2 = 235.9$, $p < 0.0001$, d.f. = 2; $N = 51$; figure 1b) and was lower in the NG compared with the GT group ($\chi^2 = 92.7$, $p < 0.0001$, d.f. = 1, $N_{\text{NG}} = 14$, $N_{\text{GT}} = 19$). Females in the G group experienced the highest hatching success compared with NG and GT females ($\chi^2 = 143.2$, $p < 0.0001$, d.f. = 1, $N_{\text{G}} = 18$, $N_{\text{NG+GT}} = 33$). We found a positive relationship between copulation duration

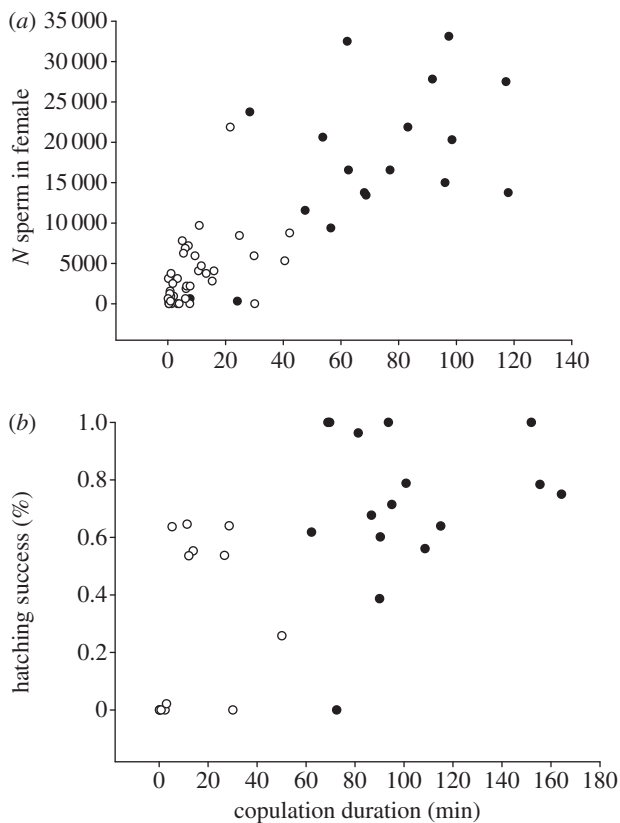


Figure 2. (a) The number of sperm stored in female sperm storage organs as a function of copulation duration in NG (short copulations without gift) and G (long copulations with gift); and (b) proportion of hatched eggs as a function of copulation duration in the NG and G groups. Statistics were performed using GLM with Poisson (sperm number) and binominal function (hatching success) and log link (see text). Filled circles, G group; open circles, NG group.

and hatching success (NG and G pooled, $N_{\text{NG+G}} = 32$, GLM binominal: $\chi^2 = 139.9$, $p < 0.0001$; figure 2b).

4. Discussion

Our data show that females stored significantly more sperm from gift-giving males than from males without a gift when we kept copulation duration constant. By allowing gift donors to store more sperm females can gain indirect benefits, either through Fisherian processes if females produce ‘sexy sons’ that are more likely to provide nuptial gifts, or by elevated fitness of offspring if gift-giving males pass on genes that are superior for survivorship traits [7,8,13,19]. *Pisaura mirabilis* males that offer nuptial gifts may signal good hunting abilities that are inherited by their offspring and through which male offspring would further gain an advantage in attracting females. Indeed, males that were satiated, and therefore in good condition were shown to achieve higher mating and paternity success compared with starved males in poor condition, suggesting that females can select males based on their quality [37]. Our data suggest that sperm storage is at least partially under female control through cryptic sperm choice. Differential sperm storage may result from preferential sperm uptake during mating (syncopulatory sexual selection) [25,26], or through differential sperm selection or ejection immediately after mating (postcopulatory sexual selection) [24], processes that are proposed to occur widely [3].

Under the risk of sperm competition, males can increase fertilization success by increasing sperm transfer through longer copulations, by a higher rate of ejaculate transfer per unit time, by increasing sperm size, by producing different types of sperm within one ejaculate, thereby directly competing with the sperm of other males inside the female reproductive tract, or by a combination of these traits [5,38–42]. Strategic ejaculate transfer is unlikely to explain the pattern of differential sperm storage observed here. We used an experimental design where males were randomly allocated to treatment groups when their intromittent organs were already loaded with sperm. Loading takes place shortly after the maturation moult, precluding that males tailor the number of sperm in their pedipalps in response to the probability of being able to acquire a gift. Furthermore, as no-gift males experience shorter copulations ([12,30] and this study), males copulating without gifts are likely to be under selection pressure to increase sperm transfer rate if possible [5].

It is possible that the difference in number of sperm stored between GT and NG males may result from having the top performing males with the longest copulations excluded from the NG group. However, as males were randomly assigned to the experimental groups, we expect each group to contain males with similar variability in performance. Further, we selected a subset of NG males close to the mean copulation duration of 10 min for comparison with the GT males in order to minimize variance in copulation duration between groups. Consequently, it seems unlikely that the observed difference in number of stored sperm between NG and GT males can be explained by other factors than cryptic female choice.

From a male perspective, nuptial feeding functions to facilitate copulations and increases copulation duration and sperm transfer [5,43–46]. This is advantageous because *P. mirabilis* females are polyandrous and control copulation duration, which is positively correlated with nuptial feeding and gift quality [12,47,48]. Our data showed that both sperm storage and fertilization success increased linearly with copulation duration, suggesting that males that are able to offer gifts that take longer to consume would gain an advantage in sperm competition. Females do not only discriminate males on gift presence, they also accept males in good feeding condition with higher probability [37]. This supports a scenario in which male hunting ability, feeding condition and the gift-giving trait are associated. Males therefore gain two different types of advantage from the nuptial gift by preferential female storage of sperm from gift-giving males, and by prolonged copulation duration that correlates positively with sperm transfer.

Cryptic female choice may be expected if males extend copulation duration by offering non-nutritive items, ‘worthless gifts’ [12]. Females can only assess gift content and quality after having fed on the gift for some time during which males transfer significant numbers of sperm (C. Tunl & M. J. Albo 2011, personal observation). Whether cryptic female choice for genuine nuptial gifts evolves to counteract male deception should depend on the direct costs of accepting worthless gifts to females and indirect Fisherian benefits of son’s mating success when offering worthless gifts [32,48,49]. The combination of cryptic female sperm storage favouring gift-giving males, and advantages in sperm competition to males offering genuine gifts, probably acts to maintain selection for high-quality

gifts and may explain the prevalence of genuine nuptial gifts in natural populations [12]. Genuine gifts may also be favoured by direct nutritional benefits to females in the form of earlier oviposition [48].

We found that egg-hatching success was significantly higher in matings with gift-giving compared with gift-less males and correlated positively with the number of sperm stored. This effect can hardly be owing to sperm limitation, because gift-less males transferred several thousands of sperm that should be more than enough to fertilize a clutch of approximately 100 eggs. Nevertheless, hatching rates are in the range of 43–70%, suggesting that sperm storage, sperm activation and processes that lead to fertilization require large sperm numbers even in the absence of rival sperm. In spiders, sperm are transferred in an encapsulated and coiled state into the female sperm storage organs, and thus require being decapsulated and uncoiled latest when oviposition is due [50]. Whether internal processes that

mediate sperm activation and fertilization are targets for postcopulatory female choice requires further examination.

In conclusion, there seem to be two different types of advantage of nuptial gifts to *P. mirabilis* males. Firstly, nuptial gifts are favoured by cryptic female choice as females store more sperm from gift-giving males. Secondly, gift-giving males increase the number of sperm transferred by prolonging copulation. These processes in concert lead to a higher paternity success for gift-giving males and may confer indirect benefits to females.

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CRYPTIC FEMALE CHOICE MAY COUNTERACT MALE DECEPTION IN A GIFT-GIVING SPIDER

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Cryptic female choice may counteract male deception in a gift-giving spider

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Abstract

In nuptial gift-giving species females sometimes select their potential mates based on the presence and size of the gift. But in some species, such as the Neotropical polyandrous spider *Paratrechalea ornata* male gifts vary in quality, from nutritious to worthless, this male strategy can be in conflict with direct female nutritional benefits. Usually, males of this species which offer worthless gifts mate with similar frequencies and durations compared with those offering genuine gifts. The duration of copulation is apparently controlled by the female. If females benefit from mating with males with genuine gifts, there is scope for females to favour males with nutritious gifts by exercising cryptic choice (for instance, by differential sperm storage). We tested the hypothesis that females preferentially store sperm from males that offer the highest nutritive benefits by experimentally presenting females with males which offer either genuine or worthless gifts. We also examined whether females can select sperm based on gift presence by interrupting matings after the first pedipalp insertion, thus matching the mating duration for males that: offered and did not offer gift. The amount of sperm stored correlated positively with mating duration in all groups, hence, by using gifts and prolonging mating duration males can transfer more sperm. Gift presence during mating did not affect the number of sperm stored by females. Contrary to previous studies with this species, mating duration was longer in the worthless gift group compared to the genuine gift group, but females receiving worthless gifts did not store significantly higher numbers of sperm. We discuss whether females prefer genuine gifts and partially counteract male deception by limiting the number of sperm stored from males which offer worthless gifts.

Key-words: cryptic female choice; male deception; *Paratrechalea ornata*; sperm count; worthless donation; spiders

Introduction

Processes of inter-sexual and intra-sexual selection have enormous potential to shape behavioural, morphological and physiological traits involved in securing copulations and fertilization, and can act on male and female traits before (pre-copulatory), during (syn-copulatory) or after mating (post-copulatory) (1, 2, 3). Cryptic syn- or post-copulatory sexual selection occurs internally in polyandrous females from internally fertilizing taxa, and can include both sperm competition (3) and cryptic female choice of sperm (2, 4, 5, 6, 7, 8) that bias male paternity (9). These internal processes are complex to study because they can be shaped by either sex and by interactions between partners, but there is clear evidence that female decisions in fertilizing their eggs can be influenced by male copulatory courtship behaviours (2, 10, 11). In many species, it has been reported that males increase paternity success by rhythmically stimulating females (6, 12, 13, 14, 15, 16, 17). This indicates that by evaluating copulatory behaviours females can gain critical information about males and favour those with the highest inherent quality (2, 18). For instance, males of the cucumber beetle stroke females with their antennae, and fast-stroking males experience higher mating success. This trait is heritable, and sons of fast stroking males also stroke rapidly, and experience elevated mating success (19).

Males from gift-giving species that offer an immediate nutritive meal to females may influence female post-copulatory decisions by signalling high genetic quality (e.g. good gift donors). Since food is a central issue during the reproductive season (20, 21), females should favour paternity from males with the ability to produce a nutritive and/or large gift. Indeed, there are two main mechanisms reported by which females select males in gift-giving mating systems: 1) by preferring matings with males offering gifts during courtship, and 2) by modulating mating duration depending on gift size (22, 23, 24, 25). But, in some species males have evolved the ability to vary gift content from nutritious to worthless items, and offer both types of gift (26, 27, 28, 29). It appears that females only perceive gift content after accepting a mating when they consume the gift (29). Thus, by offering worthless gifts males inexpensively maintain the advantage of transferring sperm which may be against female's interests as they engage in multiple matings to some extent because the foraging benefits of the gift (30). In this scenario, we would expect female mate choice could be exercised during and after mating, for instance by exercising control over the number of sperm stored depending on gift quality and/or presence. Recently, it was reported that females of the spider *Pisaura mirabilis* cryptically select males based on gift presence (8). Even though in gift-giving species females have the potential to preferentially store sperm from males that offer the largest nutritive benefits, to our knowledge there are still limited studies testing for cryptic female processes in these mating systems.

We investigated whether cryptic female choice may occur in the Neotropical nuptial gift-giving spider *Paratrechalea ornata* (Trechaleidae). Females from this species are polyandrous (31) and prefer males offering nuptial gifts over males without gifts (32). Virgin females are less selective, and sometimes accept matings with males which do not offer gifts (32). However once mated, females radically become more reluctant to additional matings, even when a gift is offered (31). Indeed, mated females exert such strong selection on gift-giving behaviour that males that have been rejected and do not have prey available usually collect and wrap inedible items or "worthless gifts" to obtain a mating (29). In the field, 70% of the gifts carried by males are worthless (empty exoskeletons and/or plant parts) and 30% are genuine gifts (fresh prey) (29). These findings indicate a remarkable plasticity in gift-giving behaviour which in fact differ among spider species, as for instance, there are important differences in gift content compared with the Palearctic gift-giving spider *Pisaura mirabilis* (Pisauridae), in which genuine gifts are the most common (70%) (28). During pre-copulatory courtship, however, females from both species chose males based on gift presence and not content, as they accept matings with males offering worthless and genuine gifts at similar frequency (28, 29). This is not surprising, as females can only recognize gift content once they start to feed on it and mating has begun. Since in *P.*

mirabilis sperm stored in the female is positively correlated to mating duration (8), it is possible for females to counteract male deception by shortening matings with males offering worthless gifts (28), consequently diminishing the number of sperm stored (Tuni C & MJ Albo unpublished data). In contrast, mating duration in *P. ornata* is similar between males offering genuine and worthless gifts, suggesting that females either do not recognize gift content, or do not respond to it (29).

Therefore, two main hypotheses can be considered. Males offering worthless gifts are equally preferred as males with genuine gifts by *P. ornata* females, supporting the idea that gift presence per se correlates with male attractiveness and/or quality (33, 34). If so, we would expect females not biasing their preferences via post-copulatory processes, for instance storing similar amounts of sperm, in matings with genuine and worthless gifts. Alternatively, females prefer genuine gifts because these ones are food supply (contributing with female fecundity) and/or show male hunting ability. Under this hypothesis, we would expect females showing post-copulatory preference for genuine gifts or males that give them. One way in which this could occur is to limit sperm storage via cryptic processes that results in less sperm storage when the gift is worthless, potentially affecting male paternity success. We examined whether gift content influences the number of sperm stored in the female spermathecae in two mating groups: males offering genuine gifts and males offering worthless gifts. We also examined whether gift presence influences the number of sperm stored in the female spermathecae by standardizing mating duration in males that offered gifts and males that did not offer gifts.

Materials and Methods

Study species

We collected juveniles and subadults of *P. ornata* in August-September 2012 at Santa Lucía River (Paso del Molino, Arequita, Lavalleja, 34°16'40.10"S 55°14'00.80"W), Uruguay. In the laboratory, spiders were sexed and kept in plastic jars (8.5 cm inter diameter and 7.5 cm high) containing small pebbles. Water was provided regularly to maintain humidity. We raised individuals in a warm climate room 24.3 °C ($\pm$ 0.1 SE) to accelerate development, a procedure that is known to have no effects on spiders' behaviours. Moults were checked daily and spiders were fed with fruitflies (*Drosophila melanogaster*) three times per week until maturation. Once females (N = 82) and males (N = 82) reached adulthood, we placed them in a room with an average temperature of 22.7 °C ($\pm$ 1.7 SE), and fed them with fruitflies twice per week. For all mating experiments we used virgin females and males and we did not reuse them. We also used mated females to induce silk wrapping behaviour (see below). We measured adult size in all individuals used in the experimental groups (N = 164). Adult male's averaged size (mm) was: 3.7 $\pm$ 0.1 in FG; 4.0 $\pm$ 0.1 WG; 3.9 $\pm$ 0.1 FG-1; 3.9 $\pm$ 0.1 NG-1, with no statistical differences among groups (ANOVA: $F_{3,78} = 1.22$, $p = 0.31$). Adult female's averaged size (mm) was: 3.8 $\pm$ 0.1 in FG and WG; 4.0 $\pm$ 0.1 FG-1; 3.7 $\pm$ 0.1 NG-1, with no statistical differences among groups (ANOVA: $F_{3,78} = 2.20$, $p = 0.10$). Neither male nor female size affected sperm stored in the female spermathecae (Two-way ANOVA: $F_{3,78} = 0.53$, $p = 0.66$).

Mating behaviours and experimental design

Typically, the male wraps the gift in silk and offers it to the female in a particular posture called "hyperflexion" (35). Female mating acceptance occurs when she grasps the gift with her chelicerae in a face-to-face position, allowing the male to mount and initiate sperm transfer via pedipalp insertions into her genitalia. Males can perform up to four insertions during a mating. Between two insertions the male returns to a face-to-face position and again grasps the gift (32, 35). Mating behaviours are similar when no gift is present, including the time in the face-to-face position after pedipalp insertion (32).

Gift content and sperm stored in the female spermathecae

To analyze whether gift content influences the number of sperm stored by females, we created two experimental groups (uninterrupted matings). FG males (N = 22) offered a fly gift (*Musca domestica*), WG males (N = 17) offered a worthless gift (a dry exoskeleton from *Tenebrio molitor* larvae, following the protocol from (29, see below). After mating all females retained the gift and continued manipulating it.

Gift presence and sperm stored in the female spermathecae

To examine whether the presence of a gift confers advantages to males in the number of sperm stored by females, we conducted mating trials with two experimental groups where we interrupted the mating after one pedipalp insertion to match mating duration in trials with and without a gift. We interrupted matings by using a paintbrush, when the male returned to the face-to-face position after the first insertion. FG-1 males (N = 19) offering a fly gift were allowed to perform one pedipalp insertion for an average of 0.38 ± 0.04 min (mean $\pm$ SE). NG-1 males (N = 24) offering no gift were allowed to perform one pedipalp insertion for an average of 0.39 ± 0.07 min (mean $\pm$ SE). As expected, mating duration was similar between the two interrupted matings, FG-1 and NG-1 (Student t-test: $t_{1,28} = 0.50$, $p = 0.61$, $N_{FG-1} = 17$, $N_{NG-1} = 13$).

Behavioural data

All mating experiments were carried out during October-December 2012. A virgin female was placed in a transparent plastic cage (30 cm diameter and 10 cm height) with pebbles covering the bottom one day before the experiment, allowing her to deposit silk threads which are important stimuli for male courtship (36). We then exposed each female to one male carrying a wrapped housefly (FG, FG-1), or a wrapped exoskeleton (WG), or no gift (NG-1). As we wanted all males with gifts to wrap them (FG, FG-1 and WG), we elicited gift wrapping by exposing the experimental males with a housefly or an exoskeleton to a mated female 30 min before the experiment started. These females were mated with another male in a different experiment. Mated females reject males more often than virgin ones (29, 31), and males usually wrap the item in silk after rejection (32). We only allowed males to physically contact females once as we simulated female rejection by pushing her away with a paintbrush. Males without gift experienced the same procedure.

We registered the number of pedipalp insertions, whether the insertions were long or short and mating duration. We classified pedipalp insertions depending on their duration following (37). 'Long insertions' lasted for at least 0.1 min while 'short insertions' were less than 0.1 min. Expansions of the hematochoae - blood inflatable structure that allows injection of the sperm into the female genitalia- (38), were observed for both long and short insertions. Short insertions were too brief for their duration to be measured accurately and were not included in the total mating duration. We registered the occurrence (yes/no) and number of long and short pedipalp insertions per mating and compared their frequencies between groups. Males can perform only long insertions, only short insertions or both types of insertions during a single mating. However, we wanted to know if females stored sperm from males that perform only short insertions or contrary, females stored sperm only from males performing long insertions. In FG and WG groups, sperm number was similar whether males performed only long insertions (N = 15) or both long and short insertions (N = 17) during a mating (Student t-test: $t_{1,30} = 0.60$, $p = 0.54$). Thus, if a mating contained both long and short insertions, we only counted as with long insertions. On the other hand, in FG-1 and NG-1 we allowed males to perform only a single long insertion (interrupted) or to naturally perform short insertions, but these last data were only used to compare the frequencies and the amount of sperm stored during both types of insertions. Mating duration was calculated as the sum of the duration of all long insertions occurring within a trial, from

pedipalp insertion until pedipalp disengagement; because duration of short insertions was impossible to be measured these ones were not included in mating duration. Total mating duration thus represents the actual time of potential sperm transfer.

Sperm count

Females from all groups were frozen 4 hours after mating at -80 °C, giving them time to consume the gift in the case of the FG and FG-1 groups. Male spiders transfer inactive, encapsulated sperm that females store in the spermathecae, and later activate for egg fertilization (39, 40). We did not find decapsulated sperm in our samples, so decapsulation evidently takes place several days after mating, and our sperms counts were based on encapsulated sperm (Fig. 1). For counting the number of sperm stored, the female spermathecae was dissected out under a stereomicroscope (Olympus, SZH) and treated following a protocol established for spiders (41). We transferred the female spermathecae and ruptured it with forceps into 75 µl of a sperm counting solution. We first created a solution with 10ml spider saline (3.26g NaCl, 0.13g KCl, 0.30g CaCl₂ + 2H₂O, 0.26g MgCl₂ + 6H₂O and 250ml Distilled Water) and 10 µl of Triton X detergent (solution A). Afterwards, we obtained the sperm counting solution by adding 10ml of spider saline to 150 µl of solution A. We vortexed each sample for 30 s and centrifuged it at 4 x 1000 RPM for 10 min. After repeating this process three times, we placed 10 µl of the supernatant in a counting chamber of a hemacytometer (1mm Neubauer improved). The number of sperm was counted in 16 squares from the 4 corner squares under a microscope 400 x (Olympus VANOX), and the total number was calculated based on the total volume.

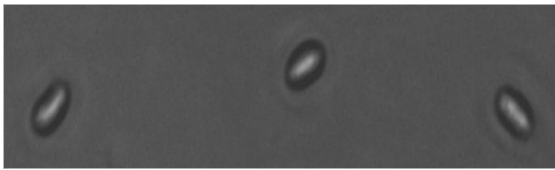


Figure 1. Picture of the capsulated sperm cells from *P. ornata* captured under microscope (40x). Photo: MJ Albo.

Statistical analyses

Statistical analyses were performed using JMP 7.0 software (SAS institute). Assumptions of parametric tests were examined using Shapiro-Wilk tests for normal distribution of residuals, and Levene's test for homogeneity of variances. Data on mating duration and number of sperm stored were log transformed to meet parametric assumptions. Student t-tests were used for analysing sperm numbers between groups. Number of sperm scored in female spermathecae was calculated for both long and short pedipalp insertions. One-way ANOVA was used to analyze effect of insertions on sperm numbers. Mating duration was analyzed using only data from long insertions, reducing sample size in some analyses (FG, N = 17, WG, N = 16; FG-1, N = 17; NG-1, N = 13). Occurrences of long and short pedipalp insertions in each group were analysed with Chi-square test. Number of insertions per mating was analyzed using Mann-Whitney test. We used two-way ANOVAs to analyse the effect on the amount of sperm stored in the female spermathecae by mating duration, group and their interaction and by type of insertion, group and their interaction. All tests were two-tailed. Raw data is given as supplementary material.

Results

Gift content and sperm stored in the female spermathecae

Mating duration averaged 0.72 ± 0.09 min (mean $\pm$ SE) in the FG group, and 0.99 ± 0.09 min (mean $\pm$ SE) in the WG group. In contrast to previous studies (29) mating duration was significantly longer in the WG group compared to the FG group (Student t-test: $t_{1,31} = 2.38$, $p = 0.02$, $N_{FG} = 17$, $N_{WG} = 16$). Despite this difference, females from WG did not store higher numbers of sperm in their spermathecae (Student t-test: $t_{1,37} = 0.35$, $p = 0.73$, $N_{FG} = 22$, $N_{WG} = 17$; Fig. 2). We found a positive effect of mating duration on the number of sperm in both FG and WG groups (Table 1; Fig. 3A). It appears that sperm number increased at a lower rate per unit of time in the WG compared to the FG group (Fig. 3A), but this difference was not statistically significant (Table 1).

In matings from FG and WG males often performed long pedipalp insertions while few males performed only short pedipalp insertions (Table 2). The occurrence of matings with long or short pedipalp insertions did not differ significantly between FG and WG (Chi-square: $X^2 = 1.41$, $p = 0.23$). Both types of insertions transferred sperm, but in both groups we found more sperm stored in the female spermathecae when males performed long insertions than when males performed only short insertions (Table 1; Fig. 4A). The number of long insertions per mating was similar between FG and WG (Table 2; Mann-Whitney: $U = 90.5$, $P = 0.19$, $N_{FG} = 16$, $N_{WG} = 15$). Similarly, the number of short insertions per mating did not differ statistically between FG and WG (Table 2; Mann-Whitney: $U = 4$, $P = 0.62$, $N_{FG} = 6$, $N_{WG} = 2$).

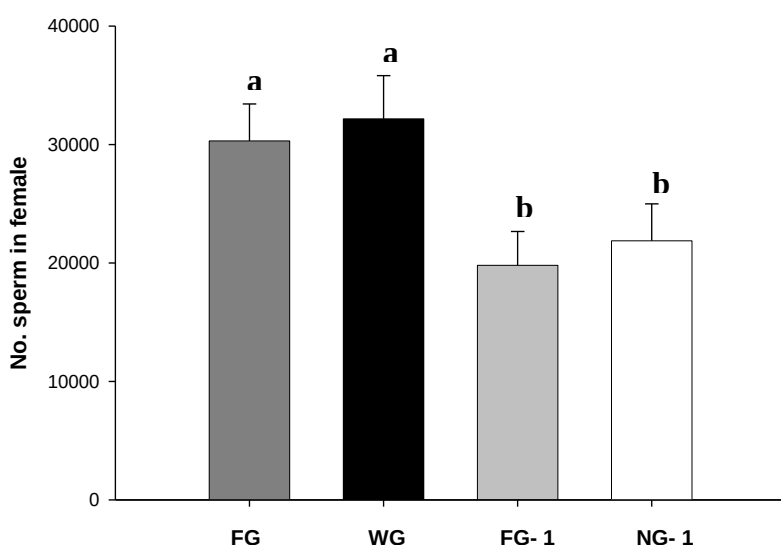


Figure 2. Number of sperm in female spermathecae in uninterrupted matings: FG, males offering a fly gift and WG, males offering a worthless gift; and in interrupted matings: FG-1, males offering a fly gift and performing one pedipalp insertion, NG-1, males offering no gift and performing one pedipalp insertion. Different letters indicate significant differences.

Gift presence and sperm stored in the female spermathecae

Gift presence did not affect the number of sperm stored in the female spermathecae (Student t-test: $t_{1,41} = 0.48$, $p = 0.63$, $N_{FG-1} = 19$, $N_{NG-1} = 24$) (Fig. 2). As expected, females that received one pedipalp insertion (FG-1 and NG-1) stored significantly lower number of sperm compared to females from uninterrupted matings (FG and WG) (ANOVA: $F_{3,59} = 12.95$, $p < 0.0001$). We found a positive effect of mating duration on the number of sperm in both groups (Table 1; Fig. 3B). From Figure 3B it seems that the number of sperm increases at a higher rate per unit of time in NG-1 compared to FG-1, but the difference was not statistically significant (Table 1).

In matings from FG-1, males often performed long pedipalp insertions (Table 2); these frequencies differed compared to matings in NG-1 as higher number of males performed short pedipalp insertions (Table 2; Chi-square: $X^2 = 5.11$, $p = 0.02$). Females stored more sperm when males performed long insertions but showed no significant differences within and between groups (Table 1; Fig. 4B). In the

NG-1 group, the number of short insertions ranged between 1- 4 (Table 2). In this group, whether the male performed one or more short insertions did not affect the number of sperm in female spermathecae (ANOVA: $F_{3,6} = 1.10$, $p = 0.41$).

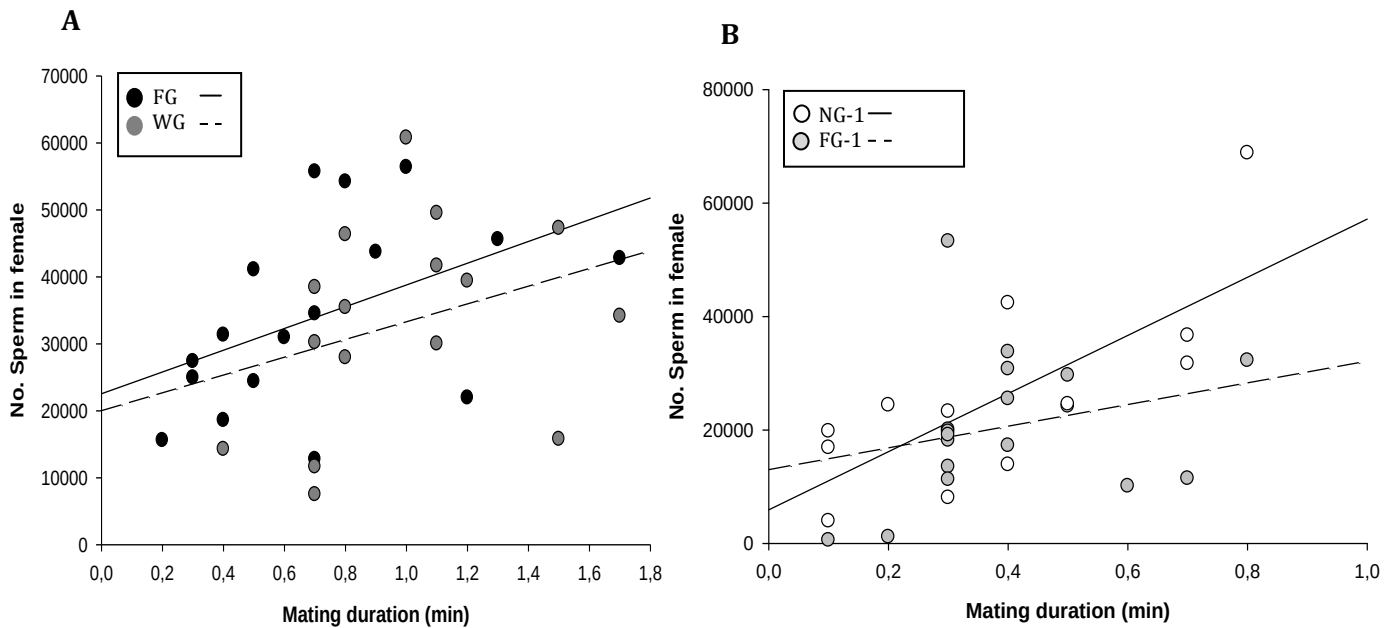


Figure 3. Linear relation between mating duration (min) and number of sperm in female spermathecae in: A) uninterrupted matings: FG, males offering a fly gift and WG, males offering a worthless gift; B) interrupted matings: FG-1, males offering a fly gift and performing one pedipalp insertion, NG-1, males offering no gift and performing one pedipalp insertion.

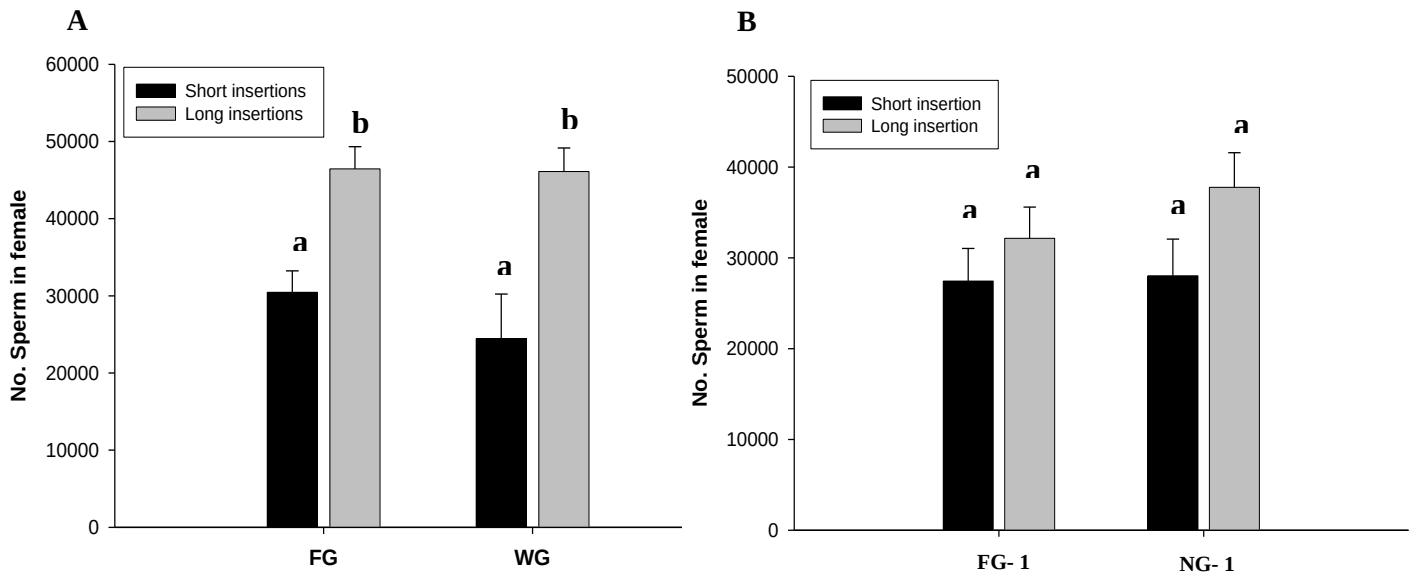


Figure 4. Number of sperm in female spermathecae resulting from long insertions and short insertions in A) uninterrupted matings: FG, males offering a fly gift and WG, males offering a worthless gift; B) interrupted matings: FG-1, males offering a fly gift and performing one pedipalp insertion, NG-1, males offering no gift and performing one pedipalp insertion. Different letters indicate significant differences.

Table 1. Effects on the number of sperm stored by females in FG, WG, FG-1, NG-1 groups. Left: model including group, mating duration and their interaction; right: model including group, type of insertion and their interaction.

	Group (df = 1)	Mating duration (df = 1)	Group*Mating duration (df = 1)	Group (df = 1)	Type of insertion (df = 1)	Group*Type of insertion (df = 1)
Number of sperm in female FG/WG	F = 2.14 p = 0.15	F = 7.59 p = 0.01	F = 0.17 p = 0.68	F = 0.65 p = 0.52	F = 3.83 p = 0.001	F = 0.58 p = 0.57
Number of sperm in female FG-1/NG-1	F = 2.55 p = 0.13	F = 12.46 p = 0.001	F = 0.06 p = 0.82	F = 0.28 p = 0.59	F = 1.51 p = 0.22	F = 1.18 p = 0.67

Table 2. Occurrence and average number of long and short pedipalp insertions in FG, WG, FG-1 and NG-1. Data are shown as Means $\pm$ SE.

	FG (n = 22)	WG (n = 17)	FG-1 (n = 19)	NG-1 (n = 24)
Long pedipalp insertions				
Occurrence per group	16	15	17	14
Number per mating	1.75 $\pm$ 0.15	2.10 $\pm$ 0.18	1	1
Short pedipalp insertions				
Occurrence per group	6	2	2	10
Number per mating	7.16 $\pm$ 2.48	12.5 $\pm$ 4.29	3 $\pm$ 0	2.3 $\pm$ 0.42

Discussion

From the results it can be argue that females from *P. ornata* do not discriminate between males offering genuine and worthless gifts as they stored similar amount of sperm, potentially having similar paternity success. Under this scenario, females would benefit from having sons that will enjoy higher reproductive success since they will inherit the attractiveness and/or quality from their gift-giving fathers (33, 34). However, even if males offering worthless gifts were able to mate significantly longer than males offering genuine gifts, females did not store significantly more sperm in the spermathecae as would have been expected from the positive correlation between sperm number and mating duration. Hence, our findings seems to better support the idea that females prefer genuine gifts and limit the number of sperm stored from males offering worthless gifts. Further, since these results arise from experiments with virgin females, we would actually expect mated females to exert higher control on sperm number and egg fertilization depending on whether they received worthless or genuine gifts, as well as depending on the number of mates during the reproductive season.

Two facts are important in understanding the evolution of post-copulatory cryptic female choice in this species. Females are apparently unable to recognize gift content during pre-copulatory courtship interactions before they grab the gift and start eating it as mating begins (29). In addition, mating is

extremely short (1 min average) and females may not have enough time to evaluate the gift content during copulation. Spiders have external digestion and they need to release digestive fluids necessary to absorb the prey. These fluids are sucked in and released into the prey while the tissue is gradually sucked out and the process of consuming the whole prey may take 1 hour or more (38). Females from other gift-giving spiders modify mating time from 1h with males offering genuine gifts to 30 min in matings with worthless gifts (28), reducing the amount of sperm that is stored (Tuni C & MJ Albo unpublished data). Usually these females leave the male and the worthless gift, something that never occurs with genuine gifts as females always engage in important struggles with males over gift possession (28). In our experiments, females always ran away with the gift, even when it was worthless, indicating they did not recognize gift content during mating. Thus, probably *P. ornata* females recognize gift content some time after mating when most of the sperm have been transferred, and by exercising post-copulatory selection they can partially counteract male deception and restrict the amount of sperm, potentially reducing his paternity. In fact, females can suffer a reduction in fecundity when mating with males offering worthless gifts (Pandulli I & MJ Albo unpublished data) which may explain the preference for genuine gifts (presented here) and its maintenance in the field (30%) (29). The mechanism used by females to bias sperm stored is unknown. However, they could differentially choose sperm or eject some after mating, as it happens in other species (2, 4, 15, 42).

Female post-copulatory selection for genuine gifts suggests that they can gain indirect benefits, as their gift-giving and honest sons would be more attractive to females and/or represent higher genetic quality, e.g. good hunters (33, 34, 43, 44). Not surprisingly adult male condition (body weight/cephalothorax width regression) and gift content are linked, as in the field males in better condition usually carry heavier fresh prey, while males in lower condition carry lighter empty exoskeletons and/or plant parts (29). Males in poor condition are likely to consume the prey to gain energy for reproduction (45), while simultaneously reducing gift quality, consequently gift content is a mirror of some aspects of male quality. Our findings suggest that most probably females can select males based on gift content via post-copulatory mechanisms.

Mating duration with genuine gifts was particularly short (less than 1 min) in this study compared to previous studies (more than 1 min) (29, 31, 32). Consequently, matings with worthless gifts were longer than those with genuine gifts, which contradicts previous findings where mating durations were similar between these groups (29). It is unknown what caused this difference, but it may be related to silk wrapping of the prey. In the other gift-giving spider *P. mirabilis*, several functions are suggested for silk wrapping, including male control of the gift (46), prolonging mating duration (47) and hiding gift content (28). We did not control for the amount of silk covering the gifts either with the genuine or worthless items, but it could be possible that males offering genuine gifts have added less silk to the prey while those offering worthless gifts added more silk. Investing heavily in silk wrapping may be a possible strategy for males offering worthless gifts, as according to the suggested functions they would better hide the content and potentially prolong mating duration. In addition, silk -highly rich in proteins- may also be a type of gift in itself. Food limitation has been shown to affect silk production in both quality and/or quantity, in web building and also wandering spiders (48, 49). Further research is needed on this topic.

Contrary to our second prediction, in the experiments in which mating duration was similar females did not store more sperm from males having a gift compared to males having no gift. Thus, in *P. ornata* the gift is an important selected trait during pre-copulatory courtship but it seems not under direct selection after mating. It could be possible that males without gift invest differentially in copulatory courtship persuading the female to store sperm, or that the bias in sperm stored is done by females after the first insertion. Alternatively, females may exercise cryptic choice only when they are deceived with respect to the gift, as when the male offers a worthless gift. Contrary, in the case of

matings without gift, which are significantly shorter than those with gifts (32), females can simply restrict the number of sperm by shortening the mating. Mated females are more reluctant to accept additional matings, and reject matings with males lacking a gift (29); but virgin females benefit from accepting even short matings without a gift since they can secure sperm that are needed for successful fertilization (32). Our findings contrast with those reported for *P. mirabilis* in which females stored more sperm from males offering gifts than from males without gifts (8). Such differences between these two gift-giving spiders may be attributed to particular ecological conditions or other factors, but any conclusion may be precipitated.

Under the risk of sperm competition males from polyandrous species usually have enormous pressures to increase sperm stored and thus, increase chances of gaining in the competition for egg-fertilization (1, 3, 50, 51). Whether sperm precedence is biased to the first or the last male in *P. ornata* is unknown. But preliminary studies have shown that males strongly compete for access to virgin females (Melo-Gonzalez V & C Ferrer unpublished data), suggesting a relatively low or intermediate P2 (last male precedence) as in other entelegyne spiders (52). On the other hand, as have been reported for other gift-giving species (8, 53, 54, 55, 56) by using gifts males prolong mating duration (32) and therefore, as it is shown here, they increase the amount of sperm stored by females, potentially increasing paternity success. Further, the gift presence allows males to perform better during mating (probably securing mating position) as they achieve larger number of long insertions compared to males without gift. As we show here, these last males significantly engage in several short insertions that ultimately transfer fewer sperm when the mating is completed.

In conclusion, by using nuptial gifts *P. ornata* males prolong mating time and consequently stored more sperm. Males often offer worthless gifts to prolong mating duration, but females seem to partially counteract deception by exercising control on the sperm stored depending on the gift quality. Important implications of post-copulatory female control over paternity arise in this mating system where worthless gifts are the rule in the field. Further research is necessary to understand the potential for sexual conflict, including the balance between benefits and costs associated to matings with worthless gifts for both sexes.

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Author contributions

MJA and AVP contributed with the conceptual development of the work and the writing of the manuscript. MJA carried out the experiments and performed data analyses. Both authors read and approved the final version of the manuscript.

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CHAPTER 4

TRECHALEIDAE AND PISAURIDAE PHYLOGENY

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CAPÍTULO 4- FILOGENIA DE TRECHALEIDAE Y PISAURIDAE

ANTECEDENTES: De las más de 44.000 especies de arañas descritas en el mundo, sólo unas pocas presentan el carácter regalo nupcial en forma de presa envuelta en seda. Estas especies pertenecen exclusivamente a las familias Pisauridae y Trechaleidae. Ambas familias junto con Lycosidae, Ctenidae y ocho familias más integran la superfamilia Lycosoidea, que incluye arañas vagabundas distribuidas en todo el mundo. Trechaleidae es una pequeña familia que contiene 16 géneros y 119 especies que están asociadas a cursos de agua y distribuidas en las Américas. Al momento, hay descritas siete especies de cuatro géneros con machos que ofrecen regalos nupciales. Por su parte Pisauridae contiene 48 géneros y 333 especies que se distribuyen por todo el mundo, de hábitos tanto terrestres como acuáticos. Se conocen ocho especies de cuatro géneros que poseen regalo nupcial. La clasificación y filogenia de las especies que pertenecen a Pisauridae y Trechaleidae ha sido muy discutida considerando datos morfológicos y comportamentales. Algunas filogenias morfológicas sugieren que Trechaleidae es grupo hermano de Lycosidae, pero otras han indicado que Lycosidae es grupo hermano de Pisauridae. Dado que no existe evidencia sobre la presencia de regalo nupcial en Lycosidae, la clasificación actual basada en caracteres morfológicos sugiere que el regalo nupcial es un carácter que apareció de forma independiente en Pisauridae y Trechaleidae. Análisis moleculares usando ADN mitocondrial sustentan la relación Lycosidae-Pisauridae pero no hay datos para Trechaleidae.

DISEÑO EXPERIMENTAL: Se investigó las relaciones filogenéticas entre estas familias, usando secuencias mitocondriales y nucleares de 16 especies pertenecientes a Trechaleidae, Pisauridae y Lycosidae. Se analizaron las secuencias usando 3 aproximaciones: Máxima Verosimilitud (ML), Inferencia Bayesiana (BI) y Máxima Parsimonia (MP). Una vez obtenido el árbol con mejor topología se mapearon los datos comportamentales y se discutieron las posibles hipótesis sobre el origen y evolución del regalo nupcial en forma de presa envuelta en seda en arañas.

RESULTADOS PRINCIPALES: Los análisis de ML y BI produjeron la misma topología y similar soporte, resultando Trechaleidae junto con Lycosidae, y éstos junto con Pisauridae. Los análisis de MP produjeron una topología un poco diferente que la revelada por ML y BI pero resultando en la misma resolución de las relaciones entre familias (Trechaleidae, Lycosidae) Pisauridae)). Todos los métodos recobraron la monofilia de Lycosidae y Trechaleidae pero no la de Pisauridae. Las relaciones entre especies de Trechaleidae y Lycosidae no presentaron conflicto en los nodos, mientras que los soportes variaron más en Pisauridae.

CONCLUSIONES: Los resultados son consistentes con algunas de las filogenias morfológicas, dado que los datos combinados muestran que Lycosidae y Trechaleidae son grupos hermanos, mientras que Pisauridae queda como grupo hermano de ambos. Estos datos aportan implicancias significativas para la discusión sobre el origen de las presas envueltas en seda. Estas pudieron haber aparecido en el nodo que une Lycosidae, Trechaleidae y Pisauridae, con una posterior reversión en la base de Lycosidae y reversiones en algunos géneros de Pisauridae, hipótesis que asume tres cambios en la filogenia encontrada. Sin embargo, considerando datos comportamentales disponibles, son posibles otras explicaciones. Por ejemplo, bajo la hipótesis de explotación sensorial, las preferencias de las hembras evolucionan primero que el carácter del macho. Considerando la presente filogenia, la información comportamental indica que no existen preferencias por los regalos nupciales en hembras de licósidos

basales, y que sí existen preferencias por los regalos nupciales en hembras de pisáuridos basales. Por lo tanto, las presas envueltas en seda pudieron haber aparecido por lo menos tres veces de forma independiente, una vez en el nodo basal de Trechaleidae y varias veces en Pisauridae. Se mantiene la incógnita de por qué en algunas especies el regalo nupcial evolucionó y en otras no. Evidentemente, factores ecológicos, aparte de la historia filogenética, han jugado un factor central en el modelado de los comportamientos reproductivos.

CHAPTER 4 - TRECHALEIDAE AND PISAURIDAE PHYLOGENY

BACKGROUND: From about 44.000 spider species described in the world, the wrapped prey gift character is only known in few species belonging to two families: Pisauridae and Trechaleidae. Both families together with Lycosidae, Ctenidae and 8 more families integrate superfamily Lycosoidea that enclose wandering spiders distributed along the world. Trechaleidae is a small family with 16 genera and 119 species distributed in Americas, contains species associated to water courses and at the moment seven species from four genera are known to have males giving nuptial gifts. Pisauridae has 48 genera and 333 species with a world distribution, contains terrestrial and semi-aquatic species, and the presence of nuptial gifts is known for eight species from four genera. The classification and phylogeny of the species belonged to Pisauridae and Trechaleidae have been extensively discussed, considering morphological and behavioral data. Some morphological phylogenies suggested Trechaleidae as sister group of Lycosidae, but other had suggested that Lycosidae and Pisauridae are sister groups. Since, there are no records on presence of nuptial gifts in Lycosidae the current classification based in morphological data suggests nuptial gifts are an independent trait (convergence) in Pisauridae and Trechaleidae. Molecular analyses using mitochondrial DNA support the relationship between Lycosidae and Pisauridae, but this study is lacking Trechaleidae data.

EXPERIMENTAL DESIGN: I investigated these families' relationship by analyzing mitochondrial and nuclear sequences from 16 species belonging to Trechaleidae, Pisauridae, and Lycosidae. I analyzed the sequences using three approaches: Maximum Likelihood (ML), Bayesian Inference (BI) and Maximum Parsimony (MP). Afterwards, I mapped the behavioral data to the presented phylogeny and discussed plausible hypotheses of the origin and evolution of wrapped gifts in spiders.

MAIN RESULTS: Analyses with ML and BI produced the same topology and similar support, resulting Trechaleidae clustered with Lycosidae, and this clade clustered with Pisauridae. Analyses with MP produced a slightly different topology than the one revealed by ML and BI but resulting in the same resolution for the families' relationships (Trechaleidae Lycosidae) Pisauridae)). All methods recovered the monophyly of Lycosidae and Trechaleidae but not the monophyly of Pisauridae. Trechaleidae and Lycosidae did not present conflict nodes among species, while supports varied more in Pisauridae.

CONCLUSIONS: The results are consistent with some of the existing morphological phylogenies, as the combined molecular data shows that Lycosidae and Trechaleidae are sister groups, while Pisauridae is sister group of this clade. These findings bring significant implications for the discussion on the origin of wrapped prey gifts. The wrapped prey may have appeared in the node clustering Lycosidae, Trechaleidae and Pisauridae, posterior suffering a reversion in the base of Lycosidae and a reversion in some Pisauridae genera. This hypothesis assumes three changes in the presented phylogeny. However, considering some behavioral data available other plausible explanations exist. For instance, under the sensory exploitation hypothesis female preferences evolve first than male traits. Considering the phylogeny presented here, the behavioral information indicates no preferences for nuptial gifts in females from basal lycosids and preference for nuptial gifts in females from basal pisaurids. Thus, it may be probable that the origin of wrapped gifts may have implied at least three independent origins, one in the basal node of Trechaleidae and several in Pisauridae. It remains unknown why some species evolve nuptial gifts and why others not. Evidently, ecological constraints, aside from the phylogenetic history, have played a central role shaping reproductive behaviors.

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MOLECULAR PHYLOGENETIC ANALYSES OF TRECHALEIDAE, PISAURIDAE AND LYCOSIDAE: IMPLICATIONS FOR THE ORIGIN OF WRAPPED NUPTIAL GIFTS IN SPIDERS

Albo M.J., Bidegaray-Batista L., Bechsgaard J., Bilde T. & F. Perez-Miles (in preparation). Molecular phylogenetic analyses of Trechaleidae, Pisauridae and Lycosidae: implications for the origin of wrapped nuptial gifts in spiders

Molecular phylogenetic analyses of Trechaleidae, Pisauridae and Lycosidae: implications for the origin of wrapped nuptial gifts in spiders

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Abstract

Nuptial gifts of wrapped prey that are offered by males to females during courtship are rare in spiders, and only known in a few species of Pisauridae and Trechaleidae. Both families are part of the Lycosoidea clade, which also includes, among others the families Lycosidae and Ctenidae, and is comprised of a diverse range of wandering spider species that inhabit varied environments from terrestrial to semi-aquatic. Phylogenetic analyses of these families based on morphological and some behavioral data have yielded contradictory results, while molecular information is limited. This lack of clarity puzzles the discussion on the origin and evolution of wrapped prey gifts in spiders. We investigated these families' relationships by analyzing five mitochondrial genes (*cox1*, *12S*, *16S-L1* and *nad1*) and three nuclear genes (*18S*, *28S* and *h3*) from 16 species belonging to Trechaleidae, Pisauridae and Lycosidae. We used three approaches: Maximum Likelihood (ML), Bayesian Inference (BI) and Maximum Parsimony (MP) and mapped the presence/absence of nuptial gifts on the preferred phylogeny. Our results are consistent with some existing morphological phylogenies, recovering Lycosidae and Trechaleidae as sister groups, which are in turn sister to Pisauridae. There are several plausible hypotheses for the origin of wrapped prey gifts and our discussion based upon behavioral data suggest either multiple origins or losses for this trait within these two families.

Introduction

Nuptial gifts as wrapped prey offered by males to females during courtship are rare and unique behavioral character in spiders (Vahed 1998, 2007). Of the 44.032 spider species described in the world (Platnick 2013), this character is only known in few species belonging to the families Pisauridae and Trechaleidae (Bristowe 1958, Costa-Schmidt et al. 2008). Both families were described by Simon in 1890, and together with Lycosidae, Ctenidae and eight more families constitute the superfamily Lycosoidea (Griswold 1993, Silva Davila 2003, Coddington 2005), which include wandering spiders distributed throughout the world.

Trechaleidae is a small family with 16 genera and 119 species (Platnick 2013). Species of this family are associated to water courses (Carico 2005), and distributed in the Americas from Argentina to United States (Platnick 2013). Seven trechaleids species from four genera in the Neotropical region are known to have gift-giving males: *Paratrechalea azul*, *P. galianoae*, *P. ornata*, *Trechalea bucculenta* (Costa-Schmidt et al. 2008), *Trechalea amazonica* (Silva & Lise 2009), *Paradossenus longipes* (Costa-Schmidt LE personal communication) and *Trechaleoides keyserlingi* (Trillo MC & Albo MJ unpublished data). Pisauridae has 48 genera and 333 species (Platnick 2013). Species in this family are both terrestrial and semi-aquatic (Sierwald 1990), and distributed throughout the world (Platnick 2013). Nuptial gifts have been documented in eight species from four genera from this family, including *Pisaura mirabilis* (Bristowe and Locket 1926), *Pisaura lama* (Itakura 1993), *Pisaura quadrilineata* (Toft S & Albo MJ unpublished data), *Perenethis fascigera* (Itakura 1998), *Thaumasia argenteonotata* (Nitzsche 1988); *Thaumasia heterogyna* (Bastos-Pereira 2009), *Thaumasia velox* (Albo MJ unpublished data) and *Tinus peregrinus* (cf. Nitzsche 2011).

The classification and phylogeny of Pisauridae and Trechaleidae have been extensively discussed, on the basis of morphological and some behavioral data (Dondale 1986, Sierwald 1990, Griswold 1993, Carico 2005). It was first suggested by Carico (1986) that a certain group of genera included in Pisauridae should be recognized as a different family, Trechaleidae. In the same year, Dondale referred to the genus *Trechalea*, as a peculiar group having lycosid behaviors (Dondale 1986). Afterwards, a morphological study supported the “*Trechalea* genus group” as an independent family from Pisauridae and Lycosidae (Sierwald 1990). Subsequent cladistic analyses of the superfamily Lycosoidea, based on morphological characters and, including the genera *Trechalea* (Trechaleidae), *Dolomedes* (Pisauridae) and *Lycosa* (Lycosidae), suggested Trechaleidae as sister group of Lycosidae (Griswold 1993). This relationship is supported by a unique palpal bulb having the tegulum notched probasally, a lobate spermathecal base, and the behaviour of carrying the eggsac on the spinnerets, which is carried on the quelicerae by pisaurid females. These groupings were also recovered in a phylogeny that included more species in each family (Cruz da Silva unpublished data). Controversially, other morphological analysis including *Neoctenus* and *Trechalea* (Trechaleidae), *Dolomedes* and *Thalassius* (Pisauridae) and *Lycosa* and *Aglaoctenus* (Lycosidae) recovered Lycosidae and Pisauridae as sister groups (Silva Davila 2003). This relationship is supported by the endite shape and, the ventral tibial processes shared by both families. Phylogenetic studies based on molecular data are scarce in lycosoids and the only study based on mitochondrial genes (partial 12S and 16S) revealed that Lycosidae and Pisauridae are closely related (Fang et al. 2000). Specimens from Trechaleidae were not included in this last analysis, however. Therefore, until now there is no molecular information concerning the relationship between Trechaleidae, Pisauridae and Lycosidae.

The species of Lycosidae commonly called “wolf spiders”, are very diverse and, widely distributed throughout the world, and their reproductive behaviour has been extensively studied (Huber 2005). To our knowledge there are no records of nuptial gifts in Lycosidae. Thus, the current classification based in morphological data (Dondale 1986, Sierwald 1990, Griswold 1993), suggests that nuptial gifts are

convergent in Pisauridae and Trechaleidae. However, many similarities have been reported between these families, including nuptial gift construction and mating systems (Costa-Schmidt et al. 2008, Albo et al. 2014). These behavioral similarities, together with the historical discussion of the classification of these families puzzle the families' relationships and the discussion of the origin of wrapped prey gifts in spiders. Consequently, studies based on molecular data can help to clarify relationships among these families and help in the discussion on the evolution of nuptial gifts. Here we analyze mitochondrial and nuclear sequences from 16 species -with known reproductive behaviour- belonging to Trechaleidae, Pisauridae and Lycosidae. We then map the behavioral trait onto the resulting phylogeny and discuss plausible hypotheses of the origin and evolution of wrapped gifts in spiders.

Material and Methods

Taxonomic sampling

To establish a molecular phylogeny of Trechaleidae, Pisauridae and Lycosidae and discuss plausible hypotheses on the origin of wrapped prey items, we included representatives of some genera that are known to have wrapped gifts and others that are known to not have the trait. In total we used 13 genera and 16 species belonging to Trechaleidae, Pisauridae and Lycosidae (Table 1). Since the family Ctenidae is closely related to the subject families (Griswold 1993, Silva Davila 2003, Coddington 2005) we used two species from two genera of Ctenidae as outgroups (Table 1). Specimens were collected by authors and some were kindly provided by colleagues; specimens were preserved in 95% ethanol and later stored at -20°C in the Department of Biosciences, Aarhus University, Denmark.

Table 1. Species and genes used to produce the molecular phylogeny. (P) indicates wrapped gifts presence; (A) indicates wrapped gifts absence

	Wrapped gifts Presence/Absence		Mitochondrial genes			Nuclear genes		
Trechaleidae								
<i>Paratrechalea ornata</i>	P	-	12S	16S	Nad1	18S	28S	h3
<i>Trechaleoides keyserlingi</i>	P	Cox1	12S	16S	-	18S	-	h3
<i>Trechalea buccullenta</i>	P	Cox1	12S	16S	Nad1	18S	28S	h3
<i>Paradossenus longipes</i>	P	Cox1	12S	16S	-	-	28S	h3
Pisauridae								
<i>Pisaura mirabilis</i>	P	Cox1	-	16S	Nad1	18S	28S	h3
<i>Pisaura lama</i>	P	Cox1	12S	16S	Nad1	18S	28S	-
<i>Pisaura quadrilineata</i>	P	Cox1	-	16S	Nad1	18S	28S	h3
<i>Perenethis fascigera</i>	P	Cox1	12S	16S	Nad1	18S	28S	h3
<i>Thaumasia heterogyna</i>	P	Cox1	12S	16S	Nad1	18S	28S	h3
<i>Thaumasia velox</i>	P	-	12S	16S	Nad1	18S	28S	h3
<i>Dolomedes fimbriatus</i>	A	Cox1	12S	16S	-	18S	-	h3
<i>Cladycnis insignis</i>	A	-	12S	16S	-	18S	28S	-
Lycosidae								
<i>Schizocosa malitiosa</i>	A	Cox1	-	16S	Nad1	-	-	h3
<i>Allocosa brasiliensis</i>	A	Cox1	12S	16S	Nad1	18S	28S	h3
<i>Aglaoctenus lagotis</i>	A	Cox1	12S	16S	Nad1	18S	28S	h3
<i>Pardosa prativaga</i>	A	Cox1	12S	16S	Nad1	18S	-	h3
Ctenidae								
<i>Asthenoctenus borelli</i>	A	Cox1	12S	16S	Nad1	-	28S	h3
<i>Ctenus longipes</i>	A	Cox1	12S	16S	Nad1	18S	28S	h3

DNA extraction, amplification and sequencing

We extracted DNA from the entire body of small species or a leg of large species using the DNeasy Blood and tissue kit, following the manufactures' protocol for animal tissues. We amplified seven gene regions (Table 1). Four partial fragments of the mitochondrial genes, cytochrome c oxidase subunit 1 (*cox1*), the 12S small ribosomal subunit (*12S*), the 16S large ribosomal subunit (*16S*), plus the complete Leucine transfer (*L1*) and the NADH dehydrogenase subunit 1 (*nad1*). Three partial fragments of the nuclear genes, the ribosomal units (*18S* and *28S*) and the Histone H3 (*h3*). We carried out PCR reactions with universal primers and the annealing temperatures specified in Table 2; and we sequenced in one direction and directly the PCR products. All the sequences used here are new and will be deposited in Genbank.

Table 2. Annealing temperatures, primer names and sequences used for each locus.

Locus	Annealing temperature	Primer	Sequence	Reference
Cox1	48-50 °C	LCO1490 C1-N-2776	GGT CAA CAA ATC ATA AAG ATA TTG G GGA TAA TCA GAA TAT CGT CGA GG	Folmer et al., 1994 Hedin and Maddison (2001)
12S	48-53 °C	12S-ai 12S-bi	AAA CTA GGA TTA GAT ACC CTA TTA T AAGAGCGACGGGCGATGTGT	Simon <i>et al.</i> (1994) Simon <i>et al.</i> (1994)
16S-L1	48 °C	16SF 16SR	CGC CCT GTT TAA CAA AAA CAT CCT TTA ACG AAT TTG AAT ATA	Kocher et al. (1989) Hedin and Maddison (2001)
Nad1	48 °C	LR-N N1-J	CGA CCT CGA TGT TGA ATT AA TCG TAA GAA ATT ATT TGA GC	Hedin 1997 Hedin 1997
18S	48 °C	18S-4F 9r	ATT AAA GTT GTT GCG GTT A GAT CCT TCC GCA GGT TCA CCT AC	Giribet et al. (1996) Giribet et al. (1996)
28S	50 °C	28SO 28SC	GAA ACT GCT CAA AGG TAA ACG G GGT TCG ATT AGT CTT TCG CC	Whiting et al. (1997) Whiting et al. (1997)
h3	56 °C	H3aF H3aR	ATG GCT CGT ACC AAG CAG ACV GC ATA TCC TTR GGC ATR ATR GTG AC	Colgan et al. (1998) Colgan et al. (1998)

Alignment and phylogenetic analysis

We edited raw sequences using BioEdit (Hall 2007). We aligned the sequences from ribosomal gene fragments (*12S*, *18S*, *28S* and *16S-L1*) applying the Q i-INS algorithm from the online version of MAFFT (Katoh et al. 2005). We aligned the protein coding sequences (*H3*, *COI* and *ND1*) using Clustal X program (Thompson et al. 1997). Afterwards, we translated them to amino acids to carefully check and corroborate the absence of stop codons. We used jModeltest v. 2.1.4 (Darriba et al. 2012) to estimate the evolutionary model for each gene, under the Akaike Information Criterion (AIC) (Akaike 1974).

For the phylogenetic analysis we concatenated sequences from all genes using Winclada (Nixon 2002) and used three approaches: Maximum Likelihood (ML), Bayesian Inference (BI) and Maximum Parsimony (MP). For the ML analyses we used RAX-ML from the online page CIPRES sciences gateway portal (Miller et al. 2010). In this analysis we included the evolutionary model and its parameters for each independent gene. We selected the best likelihood tree from 100 iterations of the random addition of taxa and non-parametric bootstrap support values were drawn from 1000 resample matrices. We investigated the tree topology with BI using MrBayes v. 3.2.2 (Ronquist & Huelsenbeck 2003). We carried out simultaneously two independent runs of 10 million generations, sampling each 1000 generations, with eight simultaneous MCMC chains each starting from random trees. We used the program TRACER v.1.5 (Rambaut & Drummond 2007) to ensure the Markov chains had reached stationary, run convergence and correct mixing of each run. In this program we also checked for the correct number of generations to discard as a burn-in for the analyses (10%). We performed the MP analyses using TNT program (Goloboff et al. 2003). We selected a traditional search (Heuristic search)

using TBR branch swapping and TBR collapsing rule, we ran 1000 replications with 25 addition sequences. We used implied weighting varying K between 3 and 100. Gaps were codified as missing data. We estimated the Bremer support for each node.

Results

Analyses with ML and BI produced the same topology, in which Trechaleidae and Lycosidae were sister groups, and Pisauridae was the sister group of Trechaleidae + Lycosidae (Figure 1). Both methods recovered Lycosidae and Trechaleidae as monophyletic but the monophyly of Pisauridae was not supported since *Dolomedes fimbriatus* was excluded from this family. The families' relationships were very well supported by Bayesian posterior probability, pp = 1, but slightly supported by ML bootstrap support, ML = 68. All relationships among species from Trechaleidae family were well supported by BI and ML, pp = 1 and ML range = 72-100. Similarly, relationships among species from Lycosidae were also supported by BI and ML, pp range = 0.87-1 and ML range = 70-100. In contrast, the supports varied more in Pisauridae, the monophyly of *Pisaura* and *Thaumasia* were both strongly supported by BI and ML, pp = 1 and ML range = 98-100. BI supported well the node linking these two genera, pp = 1, but this was not the case for ML, with ML = 64. The relationship of the genus *Cladycnis* as sister group of *Perenethis* and *Pisaura* was also well supported by both methods pp = 1, ML = 96, while the node *Perenethis* + *Pisaura* was not supported either by BI or by ML, pp = 0.7, ML = 62.

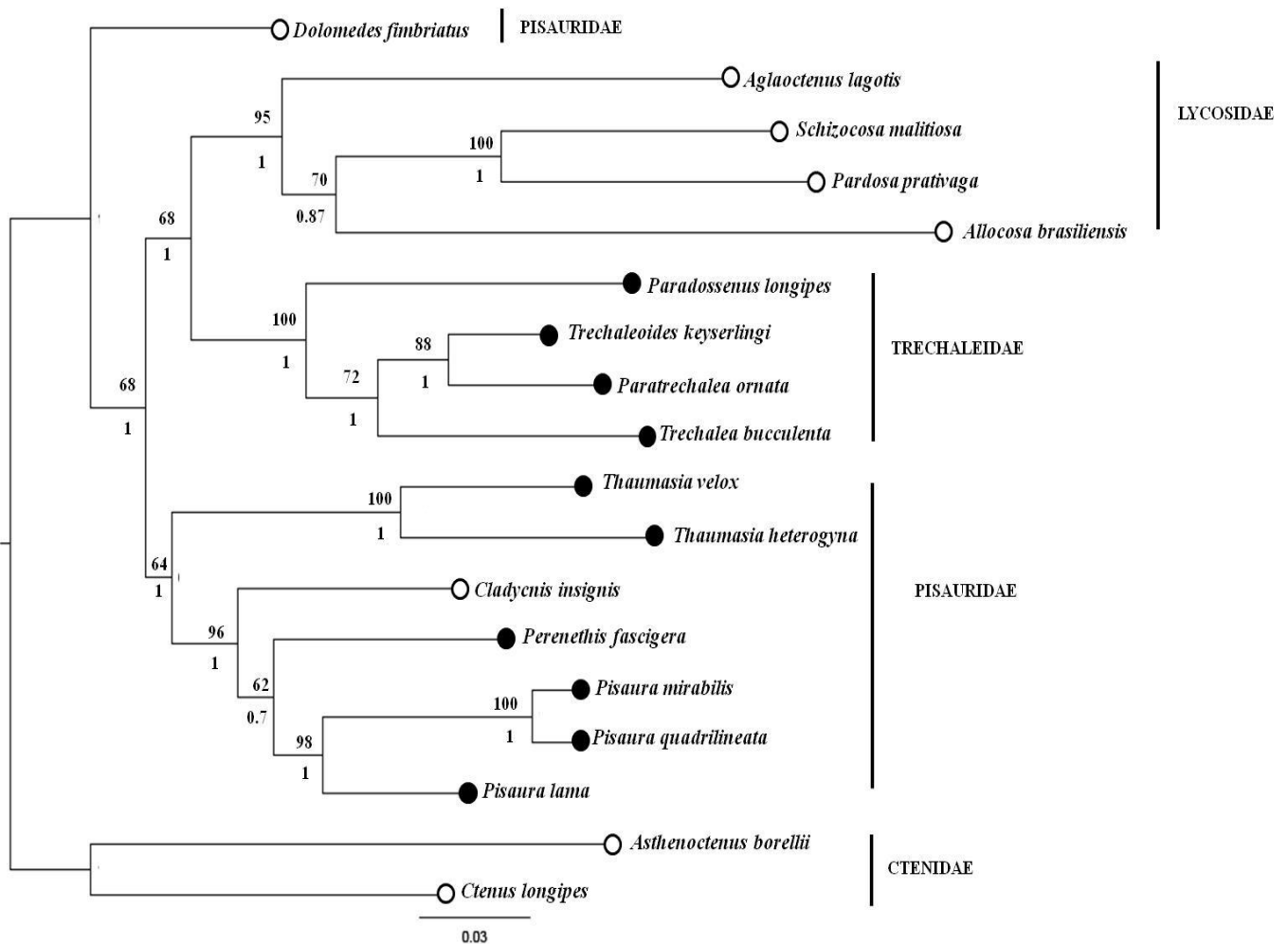


Figure 1. Topology from Maximum Likelihood analysis using the concatenated genes (*cox1*, *12S*, *16S-L1*, *nad1*, *18S*, *28S* and *h3*). Numbers above branches represent non-parametric bootstrap support from Maximum Likelihood (ML); numbers below branches represent posterior probability values from the Bayesian inference (BI). Symbols for gift presence and absence are: ● presence, ○ absence.

ML analysis with all mitochondrial genes concatenated (results shown in Appendix I) produced a single tree with similar topology to the tree obtained with all genes concatenated (Figure 1). Independent ML analyses of nuclear genes produced trees with different topologies (results shown in Appendix II) but the conflicting nodes had low non-parametric bootstrap values (ML< 70). Consequently, there was no major conflict in the topology to concatenate all genes. The models selected for each gene by jmodeltest and used in both ML and BI are shown in Table 3.

Table 3. Evolutionary model selected by jmodeltest

Gene fragment	Alignment length	Model selected*
Cox1	891	GTR+I+G
12S	336	TIM2+G
16S-L1	617	GTR+I+G
Nad1	372	GTR+I+G
18S	1112	TrNef+I+G
28S	816	GTR+I+G
h3	292	TIM2+I+G

The MP analysis resulted in only one tree of fit 15.11; with a slightly different topology than the one revealed by ML and BI but resulting in the same resolution for the families' relationships (Trechaleidae Lycosidae) Pisauridae)) (Figure 2); the topology was invariable from K = 3 to K = 100.

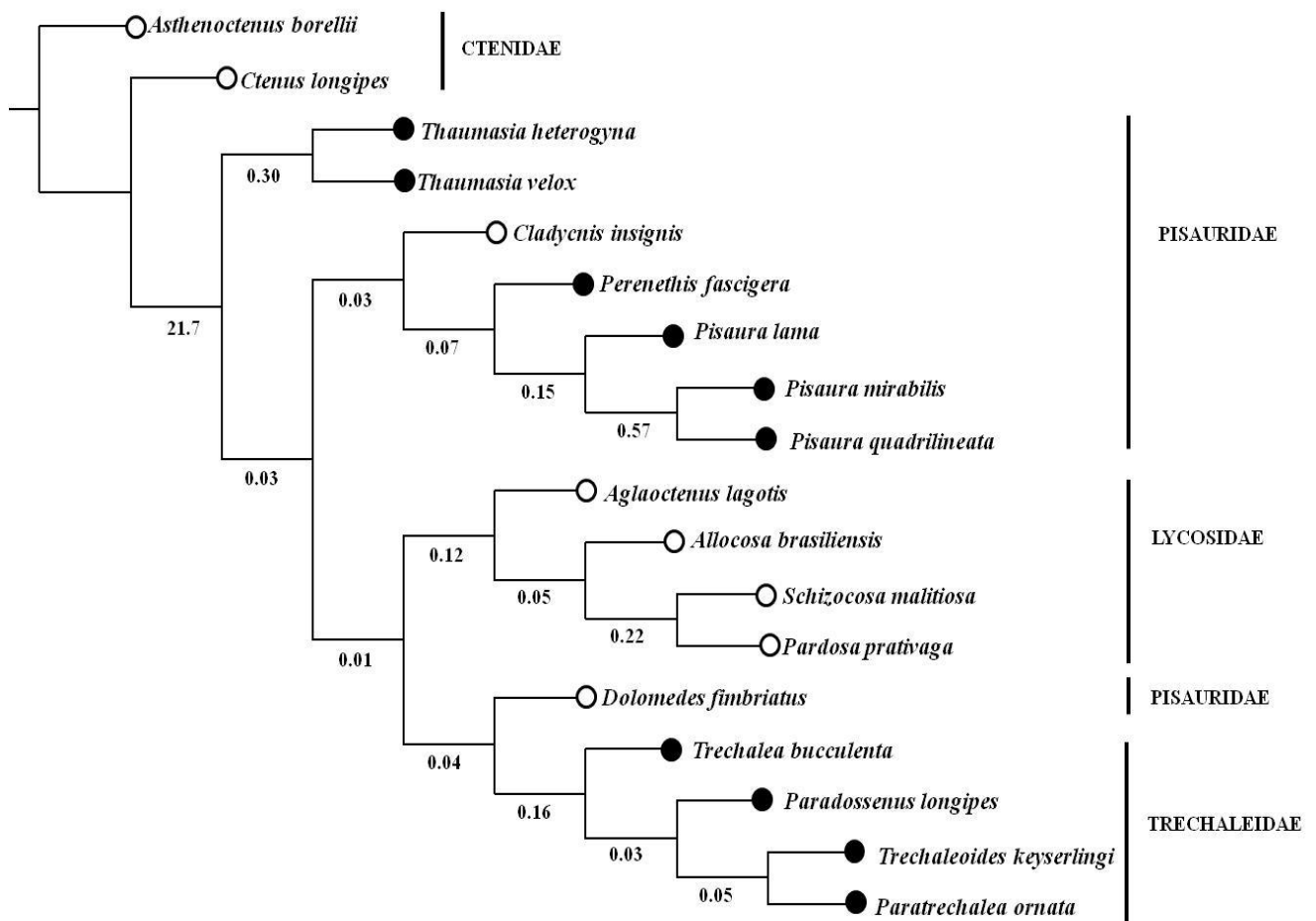


Figure 2. Topology from Maximum Parsimony analysis using the concatenated genes (*cox1*, *12S*, *16S-L1*, *nad1*, *18S*, *28S* and *h3*). Numbers below branches represent Bremer support indices. Symbols for gift presence and absence are: ●presence○absence.

The tree length was 3465 with K= 3 to 10, and 3461 from K= 10 to 100; showing a Consistency index of -CI = 55 and a Retention index of -RI = 40. Similarly, this method also recovered Lycosidae and Trechaleidae as monophyletic but not Pisauridae, both *Dolomedes fimbriatus* and *Thaumasia* genus were excluded from this family. In this case, *Dolomedes fimbriatus* was placed basally in Trechaleidae, while *Thaumasia* genus was excluded from all the three families. Another difference in relation to the ML and BI analyses was the positions established in Trechaleidae for *Trechalea bucculenta* and *Paradosenus longipes*, which were switched. Bremer support indices ranged between 0.01 and 0.57 and most commonly had stronger support for relations between species than among families.

Discussion

Our results are consistent with the morphological phylogeny proposed by Griswold (1993) and the recent morphological phylogeny obtained by Cruz da Silva (unpublished data). The combined molecular data supports a sister group relation for Lycosidae and Trechaleidae, while Pisauridae is sister group of this clade. On the other hand, the exclusion of *Dolomedes fimbriatus* from the three families deserves a special discussion, since it has consistently been placed in Pisauridae (Griswold 1993; Cruz da Silva unpublished data). It may not be surprising that this genus appears conflictive here, as Lehtinen (1967) placed it in a different family “Dolomedidae”. However, Griswold (1993) was unable to validate this family, but highlighted the fact that *Dolomedes* differs from *Pisaura* largely in the retention of plesiomorphic characters. Further analyses including other species from the genus are necessary to corroborate this result.

As a general picture, these findings bring significant implications for the discussion on the origin of wrapped prey gifts. It is important to note that we will use the topology resulting from ML and BI in the following discussion due to the congruence arisen from both methods in contrast to ML analyses. One possibility is that wrapped gifts appeared at the node clustering Lycosidae, Trechaleidae and Pisauridae, subsequently suffering a reversion in the base of Lycosidae and a reversion in the pisaurid genus *Cladycnis*. This hypothesis assumes three changes in the presented phylogeny (stars symbols in Fig. 3A), with only one acquisition of new behavioral patterns. However, other plausible explanations exist. Evolution of male traits and female preferences may co-evolve as it is proposed by both the good genes and the Fisherian runaway hypotheses (Fisher 1930; Zahavi 1975). But sexual traits can also evolve separately with the preferences evolving prior to the trait (Basolo 1990), and males may use and exploits those female pre-existing preferences, which is known as “sensory exploitation” hypothesis (Basolo 1990; Ryan et al. 1990; Christy 1995). Thus, valuable information for the discussion may arise if females have the preference for wrapped prey gifts in species where the trait is absent or even if females do not have the preference at all. For instance, in the lycosid *Aglaoctenus lagotis* males do not wrap prey as gifts (González M and Albo MJ unpublished data). Lab experiments showed that males may court with a recently captured prey but females seems to be not attracted to the prey, as they do not attempt to grab it, and males usually leave the prey to copulate (González M and Albo MJ unpublished data). This indicates that females from this basal lycosid probably do not have preferences for prey gifts. In contrast, female preferences for wrapped prey gifts are known to exist in the basal and non gift-giving pisaurid species *Cladycnis insignis* (Albo MJ, Macías-Hernández N and Toft S unpublished data). Females from this species are attracted from a distance to wrapped gifts offered by heterospecific males from *Pisaura mirabilis*, grasping the gift and accepting the mating while they feed on the gift (Albo MJ, Macías-Hernández N and Toft S unpublished data). Considering this information, it is improbable to think that the prey gift was lost when female preference is still present and thus, a most probable hypothesis is that the gift never appeared in *C. insignis*. When considering the phylogeny presented here, these behavioral data showed that female preferences exist in the base of Pisauridae but not in Lycosidae. Under this scenario the origin of wrapped gifts may have implied at least three independent

origins, one in the basal node of Trechaleidae and two in Pisauridae, one in the node clustering *Thaumasia* genus and one in the node clustering *Perenethis* and *Pisaura* genera (star symbols in Fig. 3B).

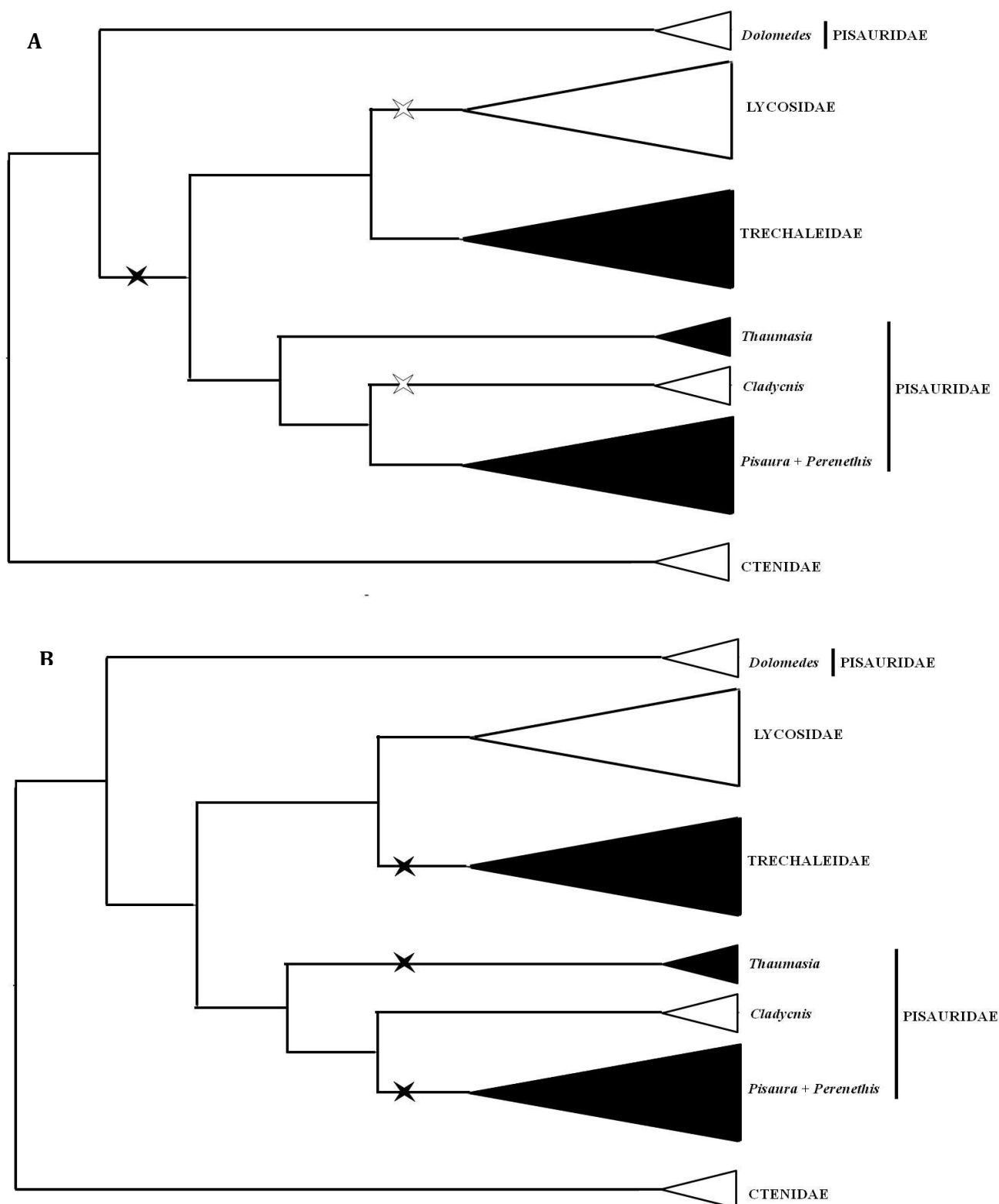


Figure 3. Schematic representation of two plausible hypotheses of the origin of wrapped prey gifts in Trechaleidae and Pisauridae based on the obtained phylogeny. Symbols for gift presence and absence are: presence, absence. Black star symbols represent possible origins and white star symbols represent possible losses of wrapped prey gifts. A) One origin in the node ((Trechaleidae Lycosidae) Pisauridae) and two reversions (one in Lycosidae node and one in *Cladycnis* genus). B) Three independent origins one in Trechaleidae node, and two in Pisauridae node (one in *Thaumasia* genus and one in *Perenethis* + *Pisaura* node)

Nevertheless, it remains unknown why some species evolve nuptial gifts (preference and trait), and why others not. Ecological constraints, aside from the phylogenetic history, may have played roles shaping reproductive behaviors. The addition of new species and a more complete phylogeny will bring novel information to the discussion of the origin of wrapped prey gifts in spiders. It seems that Trechaleidae is a good candidate to have most of the genera with males wrapping gifts, and more behavioral information regarding the presence/absence of wrapped gifts is needed for the remaining genera. Contrary, Pisauridae appears to be a more complicated family, potentially not monophyletic and much more work on the genera relationships is required to argue about the origin of wrapped prey gifts.

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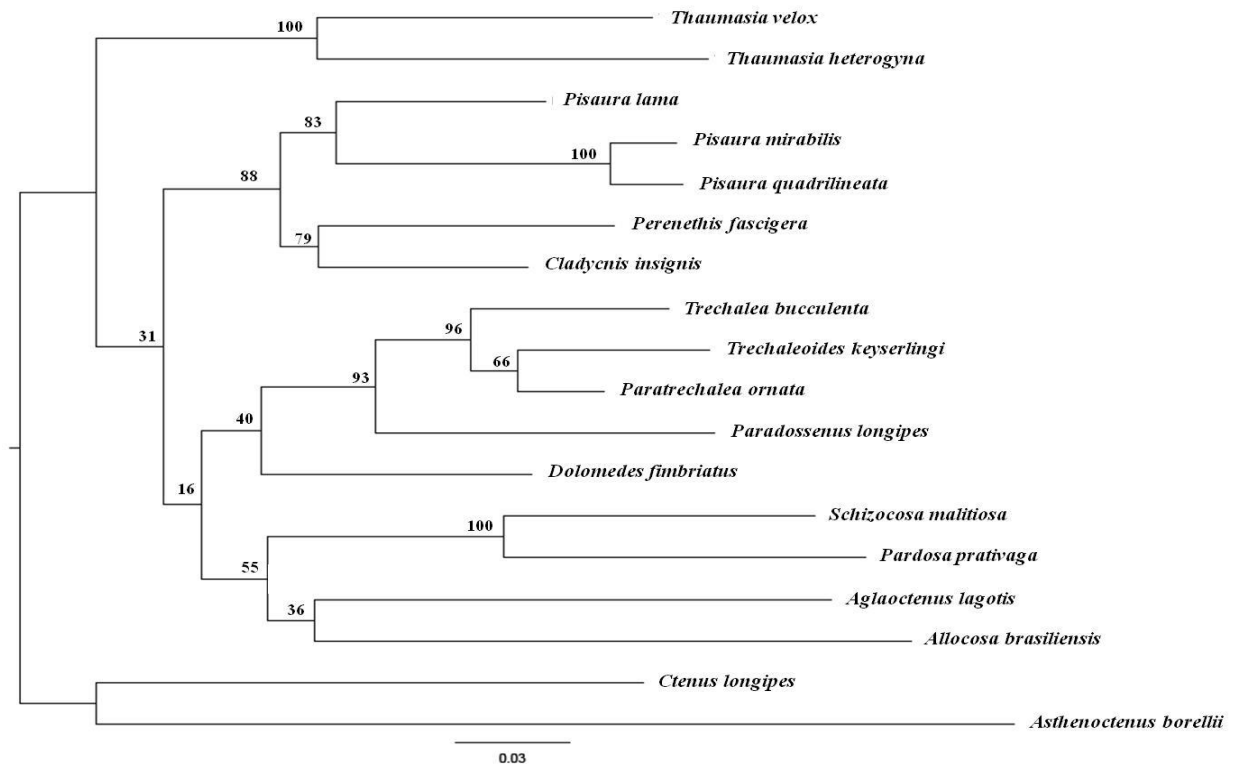
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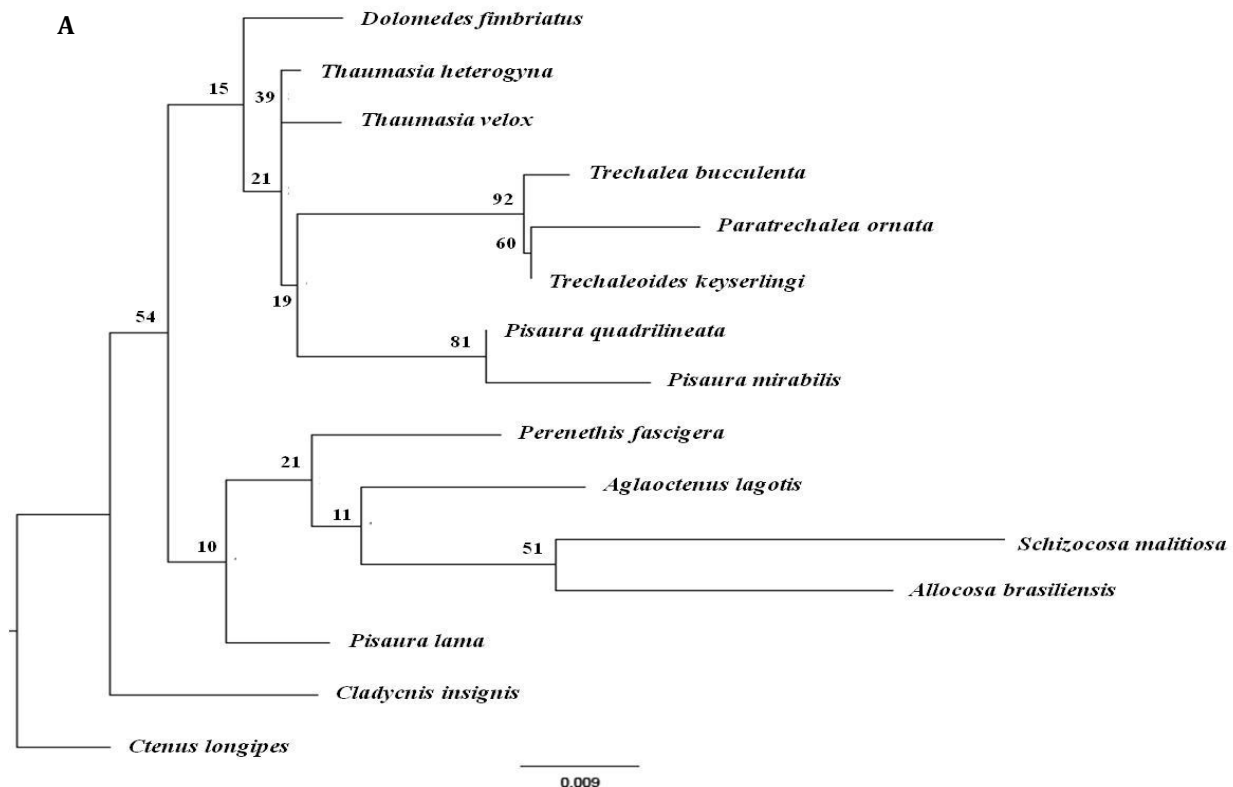
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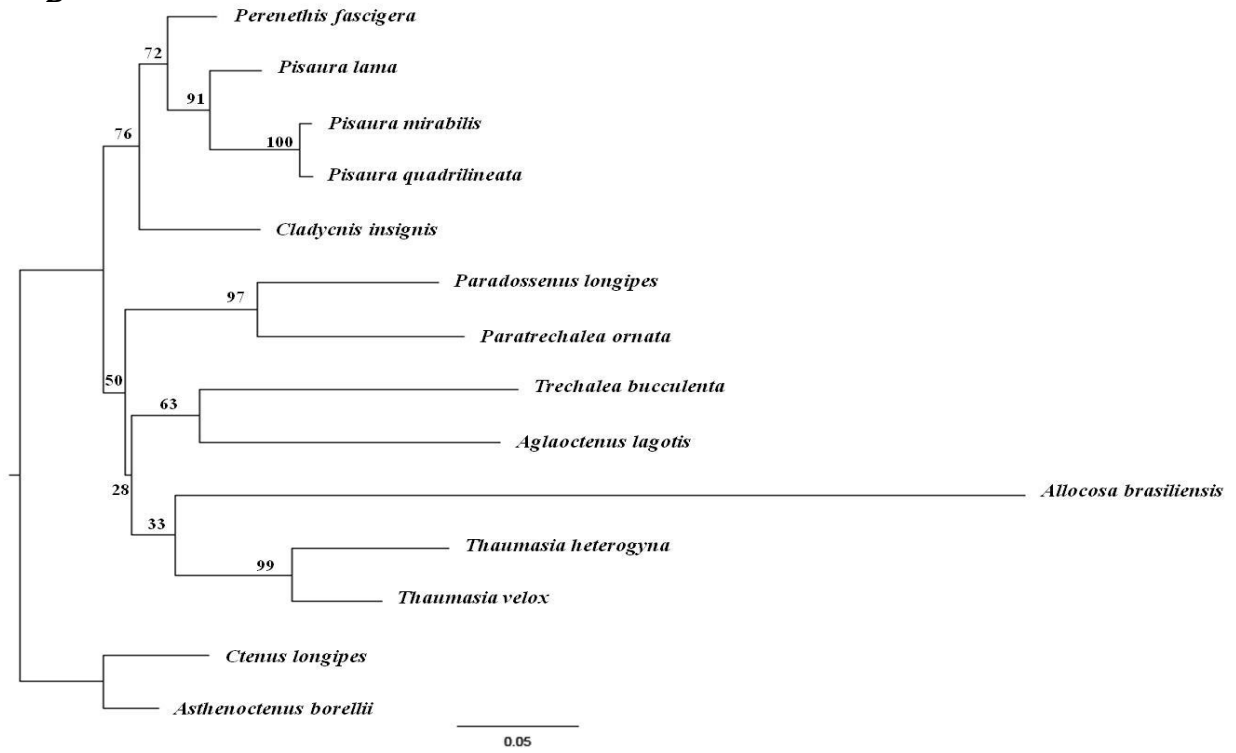
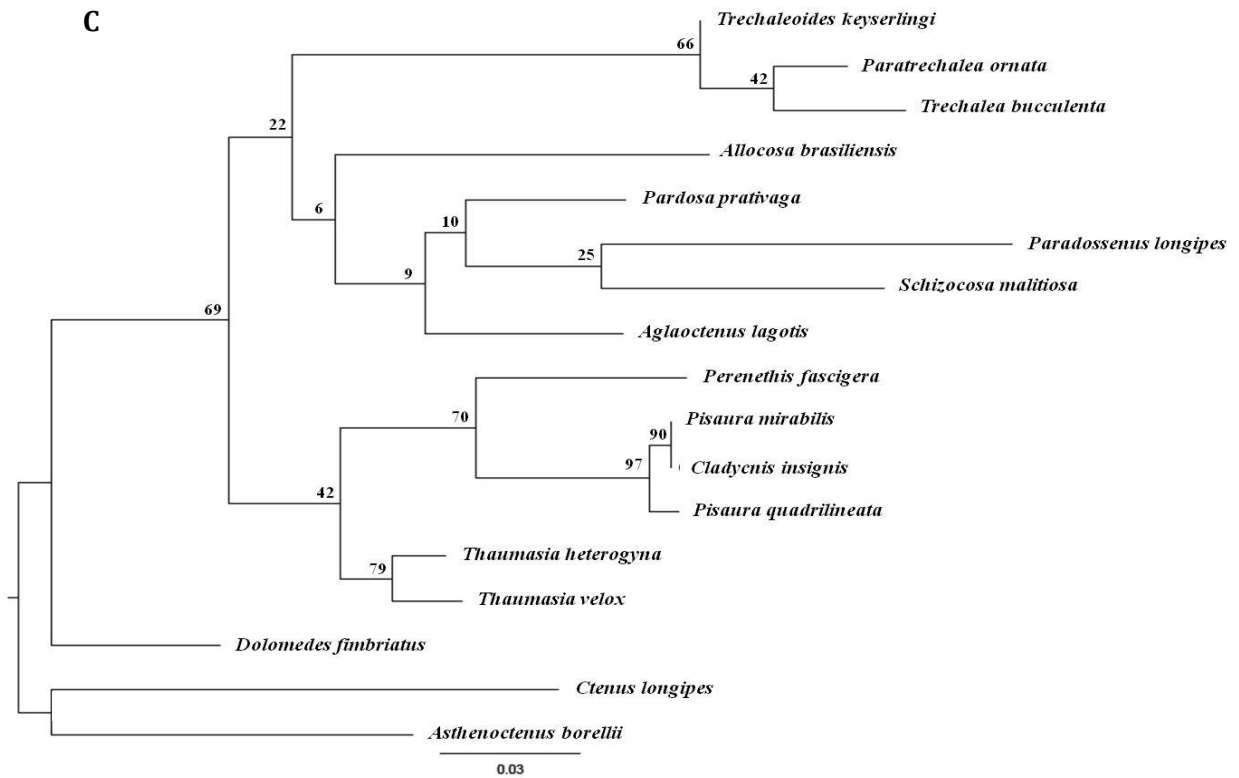
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Appendix I. Topology from Maximum Likelihood analysis using the concatenated mitochondrial genes (*cox1*, *12S*, *16S-L1*, *nad1*). Numbers above branches represent non-parametric bootstrap support from Maximum Likelihood (ML).



Appendix II. Topology from Maximum Likelihood analysis using nuclear genes: A) *18S*, B) *28S*, C) *h3*. Numbers above branches represent non-parametric bootstrap support from Maximum Likelihood (ML).



B**C**

**GENERAL
DISCUSSION
AND
FINAL
CONCLUSIONS**

GENERAL DISCUSSION AND FINAL CONCLUSIONS

WRAPPED NUPTIAL GIFTS IN AN ECOLOGICAL AND EVOLUTIONARY CONTEXT

The origin of wrapped nuptial gifts may have implied several independent origins in Trechaleidae and Pisauridae. Trechaleidae is a relatively small family with 16 genera (Platnick 2013) and already four of them are reported to have males wrapping gifts while little is known about the natural history of the rest of the genera. In this family, the wrapped gift may potentially be an ancestral trait (Chapter 4). Pisauridae is a big family with 48 genera (Platnick 2013), with some genera presenting wrapped prey gifts while others not, thus several origins or losses may have occurred in this family (Chapter 4). Alternatively, wrapped gifts could have appeared in the node linking Trechaleidae, Lycosidae and Pisauridae and suffered several reversions, at family and genera levels (Chapter 4). It is unknown why some species evolve nuptial gifts, while others not. Probably, ecological constraints, aside from the phylogenetic history, have played a central role shaping reproductive behaviors.

Sexual conflict over mating rate may occur when the evolutionary interests diverge between the sexes (Arnqvist & Rowe 2002, 2005). Most commonly, males increase reproductive success by increasing the number of mating partners and by being successful in sperm competition, whereas increased mating rates may not benefit females. This can lead to an evolutionary arms race in which males evolve traits to lure females into mating, while females evolve resistance to these traits. In such cases, adaptations in one sex are sometimes disadvantageous for the other sex, and thus may sometimes drive selection towards counter-adaptations to minimize these costs. Differences in male investment in nuptial gifts ultimately may create a co-evolutionary scenario in which females' and males' interests diverge. Although males of many species benefit from gift-giving by luring females into mating, they may also experience costs of gift production (Gwynne 1990; Engqvist & Sauer 2001; Engels & Sauer 2006; Immonen et al. 2009). There is evidence for condition-dependence in the production of nuptial gifts, which therefore may function as honest indicators of male quality for female choice (Zahavi 1975; Zahavi & Zahavi 1997).

In both *P. mirabilis* and *P. ornata* gift construction is condition-dependent since males in poor feeding condition perform silk wrapping ineffectively and present badly wrapped gifts (Chapter 1). This means that the amount of silk deposited, may potentially function as an honest indicator of some aspects of male quality. Contrary to this prediction, *P. mirabilis* females seem to ignore this information and instead evaluate males directly by their body condition (Chapter 1). This makes sense as silk itself seems to facilitate male interests, and not female interests, for example by disguising the gift or by making it more difficult to consume. Even if *P. ornata* females also evaluated males based on their body condition (Chapter 2), a different scenario appears for this species, as females are attracted to males with white color on their chelicerae. This suggests a significant role of visual cues during courtship and mate choice and future research is needed to analyze how silk wrapping is evaluated by females. In contrast to what is known in *P. mirabilis* there is no evidence that silk wrapping functions against female interests in *P. ornata*. Recent researches indicate the existence of chemical substances (pheromones) associated to the silk wrapping which are also responsible of female attraction during courtship (Brum et al. 2012). Nothing is known about the role of the silk providing phagostimulants or being a protein resource.

Under food limited conditions, male gift-giving behavior may evolve in the direction of reducing the costs by decreasing the time of production and therefore gift quality. This becomes evident when males offer exogenous prey gifts, as they may try to avoid the costs of searching and capturing prey by re-using gifts after mating, or by using inedible items (Thornhill 1976; Preston-Mafham 1999; LeBas &

Hockham 2005). Indeed, worthless gifts have been suggested as an alternative mating tactic used by males in some insect species (Preston-Mafham 1999; LeBas & Hockham 2005). Males from gift-giving spider species may also offer worthless gifts (Chapter 2). In these species, it is likely that gift content – i.e. whether the male presents a genuine or a worthless gift – is influenced by prey availability, male condition, or their interaction. When males particularly need food, they must decide between eating and wrapping the prey, and when food is scarce they may be tempted to produce worthless gifts by eating it first.

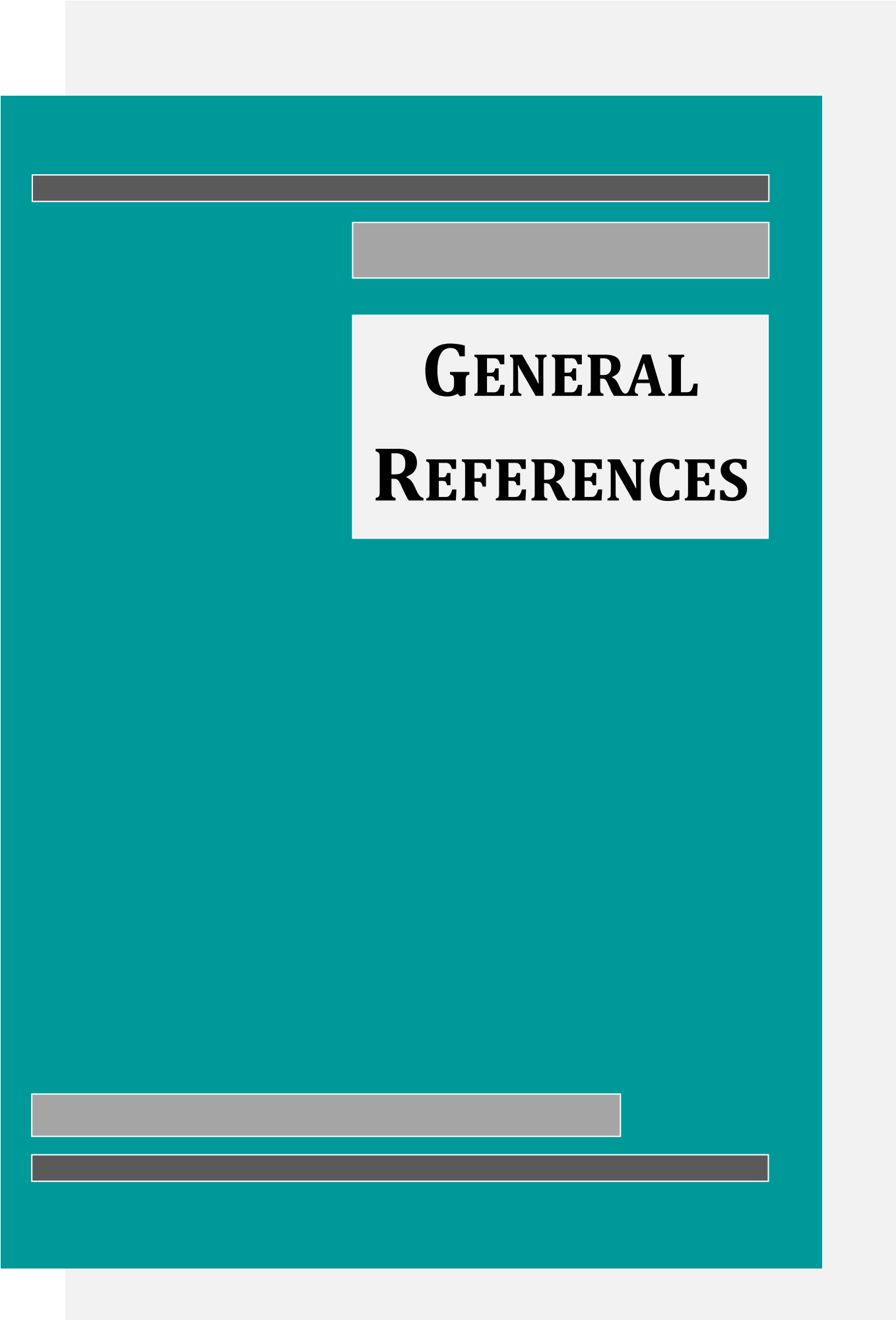
On the other hand, females would prefer genuine nutritive gifts, and favor encounters and matings with males offering such gifts. In *P. mirabilis*, males may wrap prey leftovers or parts of plants, which have no nutritive value for females (Chapter 2). At least two facts indicate strong female preference for nutritive gifts: first, females penalize males that present a worthless gift by interrupting matings earlier compared to those with genuine gifts; second, worthless donations are a minority in the field (about one third) supporting the idea that they are in fact an alternative male mating tactic. Alternatively, in conditions of high food abundance, females may be more tolerant of worthless gifts, imposing less selection on males, which seems to be the case in *P. ornata*. Worthless donations also occur in this species (Chapter 2). Contrary to *P. mirabilis* and to the alternative male tactic hypothesis, field studies suggest that *P. ornata* males use prey that have already been sucked dry and occasionally parts of plants more often than genuine prey (about two thirds). Furthermore, females appear not to penalize males offering worthless gifts in terms of reduced mating success or copulation duration (Chapter 2).

By offering gifts and prolonging matings males from both species increase the number of sperm transferred (Chapter 3) and potentially the number of fertilized eggs (Drengsgaard & Toft 1999; Stålhandske 2001). Females are likely to favor selection on male's ability to transfer a gift during courtship and mating (Chapter 1 and 2) but they can also select males by influencing sperm storage via syn- or post-copulatory decisions depending on gift presence and content (Chapter 3). Males from *P. mirabilis* and *P. ornata* that offer nuptial gifts may signal good hunting abilities that are inherited to their sons. Aside from copulation duration, *P. mirabilis* females select to store more sperm when mating with males offering gifts than when mating with males offering no gifts, evidencing major roles of cryptic syn or post copulatory processes (Chapter 3). This is not the case in *P. ornata*, consequently in this species the gift is an important selected trait during courtship but it seems not under direct selection during mating. In contrast, *P. ornata* females seem to partially counteract males' deception and restrict the amount of sperm from males offering worthless gifts, potentially reducing their paternity.

Thus, in *P. mirabilis*, males seem to hold the upper hand in the co-evolutionary cycle, since despite female preference for genuine gifts, they are only able to discover male deception after copulation is initiated, and some sperm is transferred. Cryptic female choice for gifts allows gift donors to potentially increase paternity. Whether cryptic female choice for genuine gifts counteracts male deception would depend on the direct costs of accepting worthless gifts. Interestingly, the balance between female and male interests is even more biased towards males in *P. ornata*, since females seem to not penalize male deception by shorting the mating. Apparently, females from this species do not have enough time to evaluate the gift content during mating, although they can exercise cryptic choice limiting but not reducing the sperm from males offering worthless gifts.

SEXUAL SELECTION: COOPERATION AND CONFLICT

Reproduction is an outcome of cooperation between females and males, and encompasses common interests for encountering and recognizing potential mates. However, differences in gamete investment, through interactions during courtship, mating, and parental investment, arising from diverse selective pressures, and ultimately may shape the differences in sex roles (Bateman 1948; Trivers 1972; Arnqvist & Rowe 2005). As it is discussed in this thesis, ecological fluctuations may be important factors influencing whether cooperation or conflict dominates, and may lead to co-evolutionary responses that affect mating systems. Direct benefits leading to reproductive advantages for females arise when males offer nutritive gifts, while males benefit by potentially increasing their share of paternity. However, conflicts of interest over mating rate may lead males to exploit the female foraging motivation to gain additional copulations. Fluctuations in food availability could, for example, favor female multiple mating and polyandrous mating systems if females are under strong selection to accept a nutritious gift. If prey availability is low, males may be selected to reduce the cost of producing nuptial gifts, and thus favor the evolution of worthless gifts. Females may counteract male deception by restricting the male's paternity when the costs of receiving inedible items are high. If food availability and costs and benefits of mating changes over the course of the mating season, this may ultimately explain the occurrence of polymorphism (genuine and worthless gifts) in the nuptial gift giving trait. Differences in food resource availability among seasons, habitats and regions might explain differences in sexually selected traits between *P. ornata* and *P. mirabilis*, and focused studies are needed to verify this idea.



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Dedico las siguientes y últimas páginas a un cuento corto, digno de ser contado, porque es de esas historias que como biólogos estamos acostumbrados a vivir pero rara vez escribimos.

CRÓNICAS DE UN FIN DE SEMANA POR ALGÚN LADO DE URUGUAY

Desde el sábado a las 8.30 am sentía una inmensa expectativa del gran viaje. Nos preparábamos para atravesar el país durante 5 o 6 horas hasta llegar al lugar donde colectaríamos cientos de arañas y empachados volveríamos a Montevideo, planeando a la vez las experiencias en el laboratorio. Nos encontramos en el punto acordado, nos abastecemos de alimentos y bebidas varias y arrancamos camino hacia el noroeste del país, próximo destino Río Queguay. Éramos 4, nos conocíamos de trabajar juntos, aunque Irene recién empezaba a trabajar con nosotros. Charlamos, paramos para ir al baño, fumamos, comimos, volvimos a charlar, a parar, a fumar, a comer y así hasta llegar al lugar. Había llovido mucho durante los días pasados, por eso un día antes de partir llamamos al Municipio. El comentario de la gente de Guichón fue “el puente da paso, el agua está 3 metros por debajo del puente”, eso nos había dado la idea de que no estaría tan inundado y por eso tomamos la decisión de emprender el viaje. Atravesando el puente del río negro riendo me imaginé donde estábamos en el mapa, pero al atravesar otros puentes de otros ríos y arroyos me dio la sensación que estaban bastante inundados y la risa se me borró un poco. A esa altura pensar que podía estar todo inundado no era buena señal.

La predicción fue acertada cuando llegamos al lugar cerca de las 5.30 pm, lo encontramos inundado. En segundos uno piensa mucho y concluye poco, desesperada corrí hacia el lugar para verificar minutos después que el agua rodeaba todo, ahogando los árboles, el puente y todas las piedras en las que nuestras amigas arañas viven. Necesitábamos un plan B. Manejamos hasta el puente al final de la ruta 4, con certeza el agua estaba a 3 metros del gigantesco puente, pero el río enfurecido y caudaloso se llevaba toda la orilla. Al pasar el puente un grupo de hombres descansaba al costado de una camioneta, 5 o 6 cazadores. Me vino una especie de nerviosismo y decidimos que debíamos irnos del lugar, no era bueno quedarse en la noche cerca de armas y hombres rabiosos de caza. Discutiendo qué hacer, Irene oriunda del lugar, propuso ir hacia Paysandú, exactamente a un lugar donde hay un arroyo y además la gente acampa, curiosamente llamado “Esperanza”. Perdido por perdido nos pareció buena idea y arrancamos. A esa altura mis horas de manejo estaban cerca de las 12, por lo que propuse que pasara lo que pasara, en el siguiente lugar deberíamos descansar un rato.

A Esperanza se llega por la ruta 90 y se entra por el km 11, pasamos y estábamos en el 10, volvimos para atrás y estábamos en el 12... finalmente lo encontramos, entramos por el camino de tierra, manejando cansados y despacio, estaba sereno, y el cielo lleno de estrellas, de esas que solo se ven en el hemisferio sur. El arroyo estaba inundado también pero era un lugar maravilloso para pasar la noche, abrimos un vino, brindamos por la vida, nosotros y el universo, nos relajamos. Al pasar un rato Seba e Irene se fueron a explorar los alrededores del río alumbrándose con linternas de minero, mientras Vale y yo charlábamos de quien sabe qué. De repente se siente una voz, diciendo se ven ojos, ojos! Eran los ojos de las arañas que al iluminarlos reflejan una luz azulada, algo que usamos mucho para encontrarlas. En minutos teníamos las botas de goma puestas y toda la

indumentaria necesaria para agarrar a las preciadas arañas semiacuáticas. La alegría se transformó en euforia al descubrir cada vez más y más arañas, andábamos en esa cuando a lo lejos escuchamos el ladrido de un perro. Los cuatro nos miramos y comentamos “un perro” pero seguimos con nuestro trabajo. No recuerdo cuánto tiempo pasó pero de repente veo dos ojos amarillos, bien cercanos al suelo, caminando despacito, me pareció que era un animal pequeño y comento, “miren un zorrito”. El supuesto zorrito nos pasó a unos metros por el costado y husmeó nuestros rastros cerca del auto, siempre mirándonos cada tanto, luego desapareció. Apareció de nuevo y con él aparecieron cuatro ojos más del otro lado del puente, es decir dos zorritos más (raro), nos juntamos para entender de qué se trataba. Hasta ese momento pensar en que un grupo de zorritos estaba en la vuelta no nos causaba ningún tipo temor, estos bichos son tranquilos y usualmente merodean en busca de alguna comida fácil. De repente el que estaba solo comenzó a avanzar nuevamente por el costado, se le veían los ojos pero no el cuerpo, y solo cuando estuvo exactamente enfrente nuestro pudimos observar que se trataba de un enorme perro cimarrón, que caminaba agazapado, cazador y temeroso, tal cual una película de suspenso. La imagen tierna de los zorritos se desvaneció rápidamente y se transformó en miedo. Pensar en que tres perros están cazando, en la noche pasa a producir cierto pánico y de hecho el terror se apoderó de nosotros. Intranquilos alguien dijo caminen hacia el auto pero no corran. Fue lo único que se nos ocurrió. Los perros se fueron pero ninguno quería volver en busca de arañas y mucho menos acampar. Permanecemos un buen rato en el auto, vale y yo adelante tratando de ver nuevamente a los perros, mientras seba relataba historias de documentales y perros salvajes cazando venados en África! Irene vaya a saber en qué pensaba pero expectante a lo que ocurría afuera decide súbitamente comer papas chips, al mejor estilo cine. Explosión de risas y comentarios, el miedo se había ido.

Como toda ciudad pensamos que Paysandú podía tener algún camping. Allí emprendimos viaje, ya era más de medianoche y estábamos bastante cansados. Un buen hombre que estaba trabajando en la estación de servicio nos indicó donde quedaba el camping; contentos de que íbamos por fin a dormir nos pusimos en marcha. Como ese día nada podía salir exactamente como lo queríamos, encontramos que el camping no era camping, sino que simplemente era un parque con mesitas y asientos usado por algunos habitantes de la ciudad para fiestas nocturnas y por otros para mateadas diurnas. Ya sin importarnos mucho decidimos armar la carpa en el paseo público y finalmente dormimos allí. La noche estuvo agitada, música, risas, gritos, pero sobrevivimos. Al otro día viajamos nuevamente horas, riendo de las anécdotas, lo que cada uno había sentido y pensado. Aunque con menos frecuencia (por el cansancio), nuevamente charlamos, paramos para ir al baño, fumamos, comimos, volvimos a charlar, a parar, a fumar, a comer y así hasta llegar a Montevideo.

Maria Jose Albo
10 de setiembre, 2012
Montevideo, Uruguay

100%

80%

60%

40%