

Review



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Sperm competition when transfer is dangerous

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Aggressive and cannibalistic female spiders can impose strong selection on male mating and fertilization strategies. Furthermore, the distinctive reproductive morphology of spiders is predicted to influence the outcome of sperm competition. Polyandry is common in spiders, leading to defensive male strategies that include guarding, plugging and self-sacrifice. Paternity patterns are highly variable and unlikely to be determined solely by mating order, but rather by relative copulation duration, deployment of plugs and cryptic female choice. The ability to strategically allocate sperm is limited, either by the need to refill pedipalps periodically or owing to permanent sperm depletion after mating. Further insights now rely on unravelling several proximate mechanisms such as the process of sperm activation and the role of seminal fluids.

This article is part of the theme issue ‘Fifty years of sperm competition’.

1. Introduction

Since its conception marked by Geoff Parker’s seminal paper on sperm competition [1], the fiftieth anniversary of which we honour with this review, sperm competition research has identified morphological, behavioural and ejaculate traits that convey selective advantages to males. It has, however, become increasingly clear that both sexes shape fertilization outcomes, with selection acting on female morphology or behaviour that biases fertilization towards preferred or compatible males. While cryptic female choice is often addressed separately or in opposition to sperm competition, recent research considers the reproductive interests of both sexes for a more comprehensive view of sperm competition.

Here, we argue that in spiders (48 692 described species; World Spider Catalogue [2]) the female role is central to the outcome of sperm competition. This may be why spiders have been key in the development of cryptic female choice in the first place [3]. While researchers have been charmed by some of their specialized behaviours, such as the ability to build silk webs, their distinctive reproductive biology poses a particularly costly challenge for males. Females can be aggressive, predatory, cannibalistic and often substantially larger, imposing strong selection on male mating and fertilization modes. Spider reproductive morphology, most importantly their paired genitalia, are advantageous for the study of post-mating selection as they afford the possibility to experimentally manipulate which male inseminates which spermatheca [4]. The presence of independent sperm storage sites, however, challenges general theoretical predictions because sperm from different males may not overlap spatially.

In this review, we provide an overview of post-copulatory sexual selection mechanisms in spiders with a focus on sperm competition. We first describe the reproductive biology of spiders, then we discuss female mating rates and male adaptations to competitive fertilization, before describing fertilization outcomes in spiders. We provide an overview of how copulation duration relates to sperm transfer and discuss strategic sperm allocation when sperm is limited.

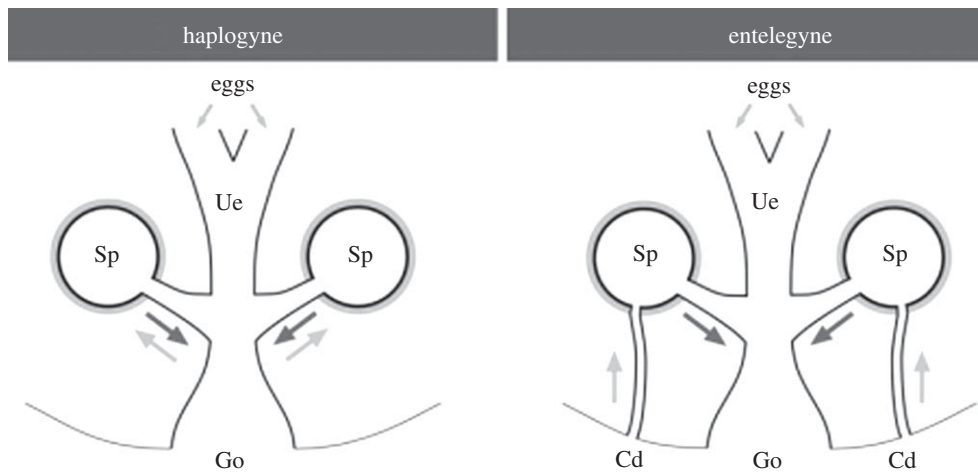


Figure 1. Schematic representation of the entelegyne and haplogyne female genital systems from Uhl *et al.* [5]. Arrows indicate sperm entering the spermathecae (grey) for storage and exiting for fertilizing the eggs (black); Cd, copulatory duct; Go, genital opening; Sp, spermathecae; Ue, uterus externus. Reprint with permission from Springer Nature.

2. Spider fertilization: an overview

Male spiders possess paired secondary sperm transfer organs (pedipalps) that are not connected to the testes. Consequently, prior to mating, males have to charge their pedipalps with sperm. During sperm induction, males commonly build a small sperm web, release sperm from their genital opening onto it and dip their pedipalps into the droplet to uptake sperm. Sperm are then transferred to the female sperm storage organs (the spermathecae) through pedipalp insertions into the female copulatory openings. The female genital system can be categorized crudely into two types (figure 1). The entelegyne spermathecal type consists of two bilaterally symmetrical copulatory openings, each leading to a distinct sperm storage organ through an insemination duct. From each spermatheca, a fertilization duct leads to the oviduct where fertilization occurs. Fertilized eggs are laid through an oviposition opening that is separate from the copulatory opening. Spiders with a haplogyne spermathecal type possess a single opening that functions as a copulatory and oviposition opening and leads directly to the oviduct (uterus externus). Pouches, paired or multiple sperm storage organs, are connected to the oviduct and the eggs released by the ovary are fertilized in the oviduct by sperm from one of the spermathecae. In all spider species, sperm are encapsulated in the testes and arrive at the spermathecae in this inactive form. In the spermathecae, sperm are activated before oviposition, turning into motile sperm [6,7].

These features articulate important differences between spiders and other arthropods, and warrant special consideration within the current sperm competition paradigm. For instance, paired sperm storage organs linked to separate copulatory ducts in entelegyne spermathecal types implies that sperm of different males may not necessarily mix in storage unless the same copulatory opening is used by multiple males. Fertilization outcomes may therefore differ dramatically based on whether sperm from the storage site is activated and released differentially, with females potentially playing an active role in the decapsulation and activation of sperm (see references in [6]). Another important feature is the indirect mode of insemination that separates the amount of sperm available at copulation from the production site. Thus, the insemination ability of a male depends not

only on the amount of sperm produced but also on the amount of sperm stored in its pedipalps. Moreover, the frequency and timing of sperm induction into the pedipalps, which varies across species, may have implications for male strategic sperm allocation and other reproductive decisions as described below.

3. Female multiple mating (polyandry)

Polyandry, which sets the stage for sperm competition, is taxonomically widespread [8], particularly in arthropods, for which monandry, mating with just one male, is the exception [9] and polyandry is probably the ancestral state [10]. Females that mate multiply are expected to collect direct and/or indirect benefits that exceed the costs of copulations beyond those needed to fertilize all eggs [9,11,12]. Spiders are no exception. Polyandrous females may gain indirect genetic benefits for their offspring (e.g. higher growth rates and offspring size [13]) via genetic bet-hedging (*Linyphia litigiosa*; [14,15]), inbreeding avoidance (*Oedothorax apicatus*; [16]) or by cryptically favouring sperm of unrelated partners (*Argiope lobata*; [17]). Alternatively, they may derive direct fecundity benefits such as increased egg-laying (*Pholcus phalangioides*; [13]), by either reducing the costs of rejections and/or foraging interference from males (*Li. litigiosa*; [18]). Resource and genetic benefits can also operate in concert. In the nuptial feeding spider *Pisaura mirabilis*, for instance, food donations from multiple mating partners lead to faster oviposition and multiple male ejaculates lead to higher hatching success [19]. Finally, polyandry may not necessarily be adaptive and females may mate multiply owing to sexual conflict, with males manipulating female re-mating behaviour (e.g. *Stegodyphus lineatus* [20,21]).

Accurate estimates of female mating rates not only ease interpretations on the adaptive value of polyandry, but address the evolutionary consequences of sperm competition, with implications for male mating strategies and patterns of sperm use. Unfortunately, the paucity of data available for spiders does not allow any general patterns to be drawn. In fact, only a handful of studies have investigated female mating frequencies in natural populations using laborious field observations of marked animals [22–25] or paternity

assessment from cocoons using allozyme [26–30] or microsatellite [31] markers. The latter might provide an underestimate of the degree of polyandry, given that females can employ post-copulatory choice to use sperm from particular males, reducing the number of sires.

Evidence for polyandry comes instead from experimental laboratory studies using mostly double matings. Double mating trials in quick succession might not represent natural mating frequencies or capture realistic re-mating intervals, both of which might strongly affect female receptivity and sperm dynamics. Indeed, experimental studies often reveal that mated females are particularly reluctant to re-mate (examples across families reviewed in [32]). They are aggressive [33] and unattractive to males [34–36]. While a decrease in female receptivity following mating could reflect increased choosiness in mated females, or male manipulations such as mechanical or physiological effects caused by sperm and/or seminal fluids (e.g. *Schizocosa malitiosa* [37]), decreased receptivity may not necessarily rule out female polyandry. Females may resume sexual receptivity at later stages, even after egg-laying [38]. Australian redback spiders (*Latrodectus hasselti*), for example, cease advertising by modifying the chemical composition of their webs immediately after mating, but resume pheromone production months later, after breeding [39]. If this is common across species, re-mating rates are likely to be underestimated. Monandry is seldomly female-driven [40,41], but results from large travelling costs for mate-searching males and female-biased sex ratios [23,40,42,43], or is largely under male control, as discussed below.

4. Paternity protection and fertilization outcome

Spiders offer a spectacular array of male adaptations that protect paternity and exclude rivals [44,45], suggesting a high risk of sperm competition. Behavioural adaptations include mate guarding of the female against rivals either before or after copulation [33,46–51]. Cohabiting males respond agonistically to other males that enter the females' web, with aggression levels depending on male future reproductive prospects [52] or on the degree of paternity certainty [53,54]. Web manipulation (reducing the female's web, wrapping it up in their own silk and sometimes discarding it) is also common. This behaviour may reduce female attractiveness through reduced pheromone dissemination, or release male pheromones that deter other males and decrease female receptivity [55–57]. A rather extreme paternity protection strategy evolved independently in *Larinia jeskovi* and *Cyclosa argentoalba*. Here, males remove a female genital structure required for genital coupling [58–60], rendering re-mating impossible even though females remain receptive and attract males.

The males of many species defend their paternity by plugging the female genitalia [5]. Mating plugs may consist of amorphous masses visible on the female genital openings. These may not necessarily be male-derived however; both mating partners may jointly produce the plug [61]. Plugs can be extremely durable and last until oviposition [62], or can be removed partly or entirely by subsequent males, suggesting that female control and male quality affect plug efficacy [63,64]. Although the production of amorphous plugs can be costly [61], it does not prevent males from mating with several females [65]. However, mating plugs

are formed from broken male genitalia left inside the female genital opening, which may come at the expense of the male's future reproduction. Depending on the species, either the entire pedipalp or the tip of the intromittent organ are detached [66], rendering the pedipalp dysfunctional after a single use [5]. One-shot genitalia that limit males to a maximum of two copulations are generally associated with a monogynous mating system, a male-biased sex ratio and extreme sexual dimorphism that have evolved several times independently in spiders [67–69]. Under conditions when more males than females mate, monogynous (or rarely bigynous) males that succeed in monopolizing paternity with a single female will gain above-average paternity [70]. Indeed, genital mating plugs can reduce re-mating probability of females considerably [5,71–73]. In some species sterile males survive and guard the female, in others they die during copulating with or without female intervention [66,74,75].

One of the most frequent adaptations to enhance fertilization success is transferring high numbers of sperm (e.g. [76,77]). If ejaculates differ in size, fertilization may become a function of the relative number of sperm transferred by each male, similar to a fair raffle [78]. Accordingly, P_2 (the proportion of offspring sired by the second male) often relates to the relative copulation duration of two or more males (e.g. *Latrodectus*; [79], *Argiope bruennichi*; [80]; *Pardosa agrestis* [81]).

Ejaculate characteristics may interact with other important processes, such as storage modalities (e.g. stratification, displacement, sperm loss) to determine paternity outcomes. Mating order is often associated with skewed paternity in other invertebrates [76], with fertilizations biased towards the first or last male to mate. An early attempt to explain patterns of sperm precedence in spiders relied exclusively on female reproductive anatomy, suggesting the two separate ducts (one for insemination and one for fertilization) of entelegynes favours first-male sperm priority, in a first-in first-out fashion, and the single duct of haplogynes favours last-male sperm priority (last-in-first-out) [82]. Despite abundant empirical tests measuring P_2 in double matings (electronic supplementary material, table S1), this hypothesis has been largely confuted. Spider spermathecal morphology is far more diverse [83] and the considerable variation in P_2 reported in spiders (values range from 0% to 100%) argues against rigid first- or last-male priority patterns.

Strict first-male precedence can occur when sperm becomes a physical impediment for additional sperm to enter the storage organ or by plugging the female genital opening [5]. However, this pattern may vary with the effectiveness of the mating plug [84,85]. Pronounced last-male precedence can occur owing to sperm removal as in haplogyne pholcids (*Ph. phalangoides* and *Holocnemus pluchei*) in which males use pedipalp movements to reach the sperm storage site with shovel-like structures that seem to remove rival sperm [86,87].

Finally, female decisions during mating are pivotal for determining fertilization outcomes and interact inextricably with the processes described above. Females may for instance delay sexual cannibalism at mating, allowing preferred partners to copulate for longer and therefore transfer more sperm [78]. They can selectively store sperm from preferred males [17,88–90], or dump sperm from their spermathecae [91] to bias paternity outcomes.

5. Copulation duration and sperm transfer

Copulation duration among spiders can vary considerably between species, and in some cases within individuals. For example, certain cave-dwelling linyphiids copulate for 18 h while some orb-web spiders (e.g. *Argiope* spp.) only copulate for a few seconds [92]. Moreover, a male's copulation duration can change substantially between his first and second mating [93].

Intuitively, copulation duration should reflect sperm transfer resulting in a linear relationship between the amount of time in copulation and the amount of sperm stored. However, even if males have ceased sperm transfer, prolonged copulation duration may have a positive impact on fertilization success if it prevents females from ejecting sperm, mating with other males [94], if males remove the sperm from previous males [87] or perform copulatory courtship [86].

The relationship between copulation duration, sperm released by the male, sperm storage by the female and consequently fertilization success is complex. A male that copulates for longer may fertilize more eggs [78,89] because he transferred more sperm or because the female stored more of his sperm. Unravelling this complexity requires information on how much sperm the male released and how much of this sperm was stored by the female. Because the male pedipalps are paired, and most entelegyne species insert one pedipalp at a time, the amount of sperm released from a given pedipalp can be estimated by counting the amount of sperm in the unused pedipalp and the amount of sperm left in the used pedipalp [95].

To date, only a few studies have related copulation duration to sperm release and/or sperm storage in spiders (electronic supplementary material, table S2). While some studies report a linear increase of sperm release/storage with copulation duration, the majority reports no relationship (electronic supplementary material, table S2). It stands to reason that in species with very short copulations the main function of copulation is sperm transfer, in which case, a linear relationship is predicted. Species where copulation duration is much longer however, other functions, such as sperm removal, plugging and copulatory courtship are more likely to generate nonlinear relationships between copulation duration and sperm release/storage. More in-depth studies are needed to comprehensively test these predictions.

6. Strategic sperm allocation, when sperm limitation is at play

Theory predicts that males can maximize their fitness returns by strategically allocating sperm to females depending on the risk of sperm competition, their reproductive prospects (male age, female availability) or partner quality (female fecundity, age) [77,96]. Male spiders can distinguish female reproductive status and mating history [34,97], with males commonly investing more towards unmated females, performing enhanced copulatory behaviour [98,99], releasing more sperm [100] or discriminating against already inseminated genital openings [101] (but see [102]). Given its correlation with fecundity, female size is also an indicator of individual quality. Male *Ph. phalangioides*, for instance perform more pedipalp movements, which relate to sperm transfer, when mating with larger females [103]. When responding to sperm competition risk (presence of rivals), males are reported either to

not adjust (*Trichonephila senegalensis*) [104] or to reduce (*Pi. mirabilis*) [105] sperm allocation.

Whether male spiders strategically tailor their sperm investment in response to the above-mentioned factors may depend largely on permanent or temporary sperm limitations, and whether males face physical danger during mating. In nephilids, araneids and theridiids, there is a phylogenetic signal for independently evolved severe sperm limitation [106,107]. In these spider families, the testes shut down sperm production after the male matures as an adult [93,107,108]. Consequently, the males of these species have no opportunity to refill their palps. Permanent sperm depletion appears to have coevolved with mono- or bigyny, genital mutilation and plugging of the female genitalia with male body parts [73]. As a result, males maximize their fertilizations by transferring as much of their sperm during a single copulation with their 'one-shot genitalia'. Transferring as much sperm as possible during a single mating may represent a terminal investment strategy, especially when facing cannibalistic females. However, strategic sperm allocation is still predicted when trading off current versus future mating opportunities. For example, in *T. senegalensis*, a species with sperm depletion but no genital mutilation and cannibalism, males can mate up to four times by partitioning sperm among females [108].

In most spider species, however, testes actively produce sperm throughout the male's lifetime. While males in some species can successfully sire broods from two consecutive matings without reloading their palps [109,110], others deplete their entire sperm load after a single mating [94]. The degree of sperm depletion from the pedipalps (examples in the electronic supplementary material, table S2), and the timing and frequency of sperm induction is likely to determine strategic sperm allocation. In some species, sperm induction occurs during the mating sequence itself. In linyphiids, for example, initial copulation without transfer (pseudocopulation) is followed by a sequence of transfers and inductions [54,111]. In other spiders, induction occurs shortly after copulation [112] or at some point between matings with different females [65,113]. The associated costs of recharging the pedipalps may also vary. Cost may include the ability for males to build sperm webs [112,114,115], the risks of losing the mating partner or risk of predation associated with interrupting the mating sequence to reload palps.

7. Outlook

The last decades of sperm competition research in spiders has progressed our understanding of the evolutionary implications of male adaptations to competitive fertilization success tremendously, but the proximate mechanisms involved remain poorly understood. The most pressing outstanding questions include: how intense is sperm competition in natural populations? How does sperm storage (together or separately) affect sperm precedence patterns? What is the site and timing of sperm activation and how does it affect fertilization outcomes? What is the degree of sperm depletion in male pedipalps and what role does seminal fluid play? Integrating these functional processes with the fitness consequences of male traits will fill important gaps in our understanding of spider reproduction and evolution.

Data accessibility. This article has no additional data.

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