

REVIEW

One size does not fit all: female–male interactions on the path to fertilization

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Abstract

In brief: Female–male interactions shape fertilization outcomes, with broad implications from evolutionary biology to applied studies of fertility. This review discusses our current knowledge of female–male interactions at each stage of the fertilization process across externally and internally fertilizing species.

Abstract: Extensive research indicates that fertilization outcomes are shaped by individual female and male traits that reflect their intrinsic quality. Yet, surprisingly little is known about the influence of interactions between the sexes and their adaptive significance in either externally or internally fertilizing species. Here, we review empirical evidence on how female–male interactions influence each stage of the fertilization process, including sperm transfer, transport, storage, chemoattraction and fertilization. We also address the challenges of examining female–male interaction effects within a realistic biological context and why research in this area lags behind understanding the role of individual sex-specific traits. While relatively little data are currently available to address the interactive effects between the sexes and their impact on the fertilization process, what is presently known suggests that these effects are likely to be more common across the animal tree of life than appreciated. Future research will help identify these interactions, and their understanding can also help to explain the maintenance of genetic variation and inform applied studies of fertility.

Keywords: genetic compatibility; genetic relatedness; female–male interactions; reproductive traits

Introduction

Fertilization is a complex, multistep process that culminates in the fusion of eggs and sperm. In sexually reproducing organisms, fertilization combines the genetic material of egg and sperm, enabling the transfer of genes from one generation to the next, and is essential to an individual's evolutionary fitness. On the path to fertilization, a number of processes can influence the probability that eggs and sperm will fuse, including aspects of sperm transfer, transport, storage, chemoattraction and molecular interactions between gametes during fertilization itself. During these processes, sperm contend with

physical and chemical obstacles that act to filter poor-quality sperm (Suarez & Pacey 2006, Sakkas *et al.* 2015, Holt & Fazeli 2016) while also being guided toward the site of fertilization (Eisenbach & Giojalas 2006). However, the path to fertilization can vary dramatically between species and is also influenced by whether eggs and sperm meet in the external environment (in external fertilizers) or in the female's reproductive tract (in internal fertilizers).

The path to fertilization becomes further complicated because females mate with multiple males during a

single reproductive episode in most animal species (Arnqvist & Rowe 2005, Taylor *et al.* 2014). When females mate multiply, sperm from rival males compete to fertilize the eggs (i.e. sperm competition, Parker 1970). Importantly, multiple mating provides females with an opportunity to bias paternity even after mating (i.e. cryptic female choice, Eberhard 1996, Thornhill 1983). These postmating processes impact ejaculate traits, influencing which sperm are more successful in reaching eggs and play an important role in shaping fertilization outcomes (e.g. Simmons & Fitzpatrick 2012). In addition, females are an active agent influencing fertilization outcomes through differential sperm storage or use (Snook & Hosken 2004, Dean *et al.* 2011, Lüpold *et al.* 2013), or simply by inciting competition that is most likely won by functionally superior sperm (Curtsinger 1991, Keller & Reeve 1995, Yasui 1997, Pitnick *et al.* 2020).

While the processes leading to fertilization necessarily involve consideration of both the female's and male's reproductive biology, our understanding of the associated female–male interactive effects is still developing. Reproductive outcomes might be mediated not only by individual female and/or male effects (i.e. sex-specific traits that define an individual's intrinsic quality) but also by interactive effects between the sexes (Trivers & Campbell 1972, Qvarnström & Forsgren 1998). For example, rather than choosing partners with intrinsically superior genes, individuals may choose mates with whom they, as individuals, are genetically more compatible. Under this paradigm, fertilization success and offspring fitness depend on the interaction between parental genotypes, so the most genetically suitable male for one female may not be the best for another. Differences in genetic incompatibilities between individuals can be created by multiple sources, including inbreeding (Thornhill 1983) or meiotic drivers (Zeh & Zeh 1996). Through nonadditive effects on fitness (alternatively called nontransitive effects), female–male interactions are expected to reduce the production of incompatible allele combinations in offspring (the genetic compatibility hypothesis, Trivers & Campbell 1972, Yasui 1997, Zeh & Zeh 1996) and generate nondirectional selection (Zeh & Zeh 1996, Tregenza & Wedell 2000). Such interactive effects complicate the study of ejaculates and fertilization because sperm that are successful in fertilizing the eggs of one female may be less effective, or even ineffective, in fertilizing the eggs of another female. In other words, fertilization and the steps leading to it are rarely 'one-size-fits-all' processes.

However, the mechanisms underlying postmating sexual selection (i.e. sperm competition and cryptic female choice) and trait diversification remain elusive (Howard *et al.* 2009, Lüpold & Pitnick 2018; see Box 1). This knowledge gap is partly attributable to the difficulties of discerning sperm between competitors and the fact that,

particularly in internally fertilizing species, postmating processes occur in the cryptic environment of the female reproductive tract (Manier *et al.* 2010, 2013a,b). More importantly, however, our understanding may be limited by the sheer complexity of how male traits (sperm and seminal fluids) and attributes of the female reproductive tract (morphology, physiology, neurology and biochemistry) interact across multiple reproductive stages to influence fertilization success (Snook 2005, Poiani 2006, Pitnick *et al.* 2009a,b, Carmel *et al.* 2016, Lüpold *et al.* 2020). Therefore, any female modulation of the selective environment may change the relative competitiveness of each male's sperm (Eberhard 1996, Firman *et al.* 2017, Lüpold & Pitnick 2018), for example, due to differential compatibility with the female reproductive tract. Competitive fertilization outcomes are thus rarely independent of female influences in both internal and external fertilizers, particularly as female reproductive fluids can influence the behavior and fertilizing capacity of sperm near the released ova (Gasparini *et al.* 2020). Consequently, the dynamic interactions between female and male reproductive traits highlight the challenges in isolating and interpreting the biological significance of individual sperm traits assayed outside the female context.

In this review, we summarize the current knowledge of how interactions between the sexes mediate the successive steps leading to fertilization, focusing on the processes from sperm release to egg–sperm interactions. More specifically, we discuss empirical studies that reveal statistically significant female–male interactive effects and describe how they contribute to variation in reproductive parameters (e.g. sperm transferred or stored). Where little or no data are currently available, we highlight the potential for female–male interaction effects to impact the fertilization process. Unsurprisingly, the scope and evolutionary potential for reproductive interactions between the sexes are shaped by the fertilization biology of the species in question (Kahrl *et al.* 2021, 2022). Therefore, we stress both the similarities and differences observed in female–male interaction effects between internally and externally fertilizing species throughout this review. Fertilization success is key to individual fitness, so our goal is to spur on future investigations determining the impact of nonadditive effects on reproductive outcomes.

Female–male interactions during sperm release/transfer

An early step on the path to fertilization (see Fig. 2) is the release or transfer of sperm. The number of sperm that are successfully released/transferred can be influenced by both differential male allocation of sperm and/or differential retention of sperm by females. Fertilization

Box 1 Methodological challenges in investigating female-male interaction effects.

The first challenge is to isolate the influence of sex-specific traits (additive effects) on fertilization success to avoid confounding variance in differential fertilization success with interindividual variation in quality. This can be overcome by implementing fully crossed experimental designs (see Fig. 1A and B). In externally fertilizing species, split-ejaculate and split-brood experimental designs that control for paternal and maternal effects (fertility or competitiveness between individuals) are usually employed, allowing to identify fertilization outcomes resulting specifically from sperm–egg genetic interactions (see Fig. 1B) (Evans *et al.* 2007, Pitcher & Neff 2007, Rodríguez-Muñoz & Tregenza 2009). In internally fertilizing species, there is a possibility to address this gap by quantifying female transcriptional responses after mating (i.e. postmating gene expression changes) with a male from the same isogenic strain vs a male from a different strain (see Fig. 1A) (McGraw *et al.* 2009). In addition, postmating transcription effects on reproductive success can be measured by physiological (e.g. fecundity/hatchability) and behavioral (e.g. female refractoriness to re-mating) responses (Delbare *et al.* 2017).

The second technical challenge is to properly define the mechanisms allowing cryptic mate choice through the complex anatomy of the female reproductive tract in internally fertilizing species. To tackle the time course of sperm transport within the female reproductive tract of mammals, studies typically involve the insemination of estrous females with known numbers of sperm from a single male (García-Vázquez *et al.* 2016). Females are sampled at intervals after insemination, whereupon the reproductive tracts are flushed and the different anatomical regions are examined for the presence of sperm, allowing researchers to directly observe sperm dynamics within the female reproductive tract.

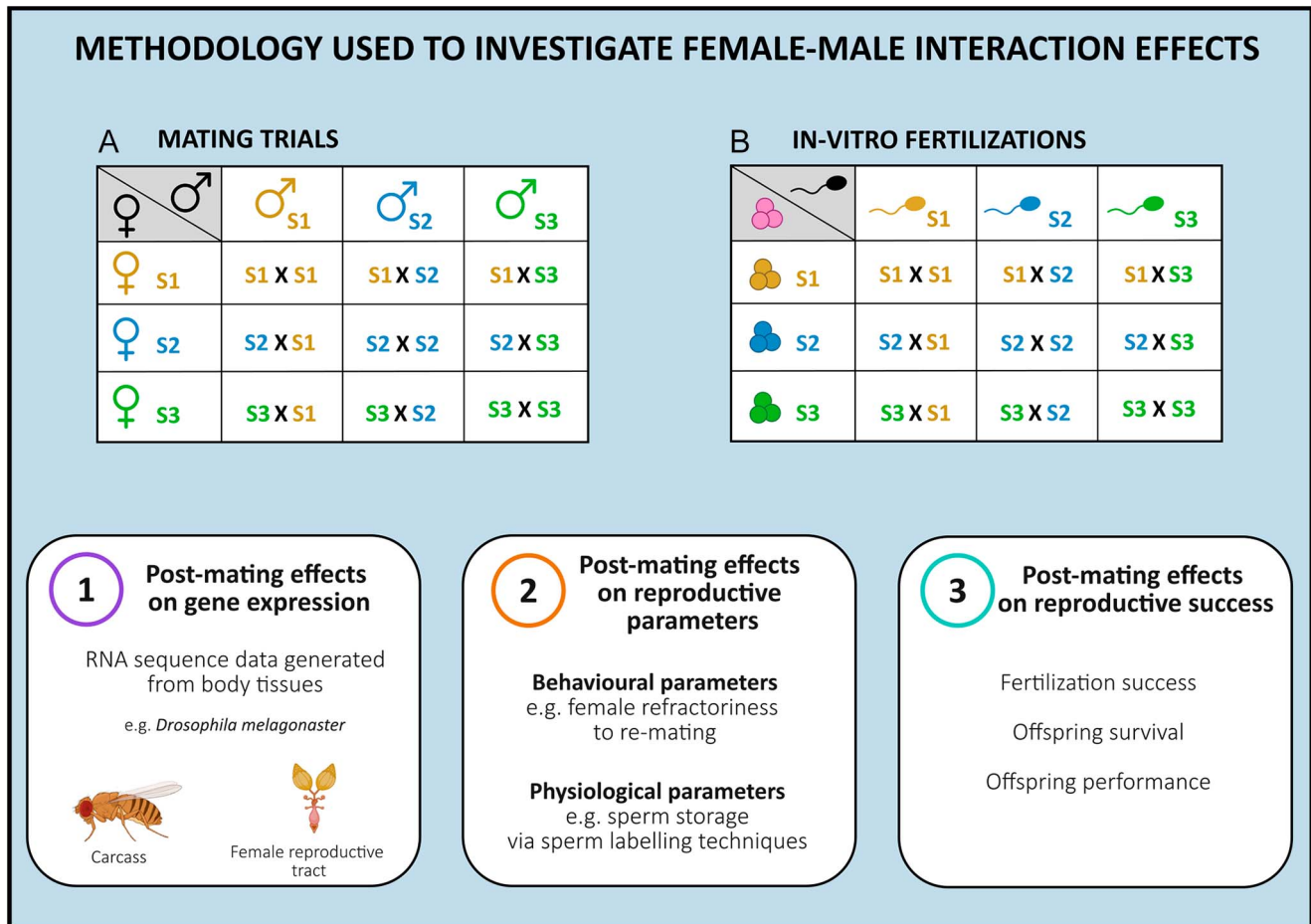
Understanding female–male interactive effects is even more complicated in a competitive context due to the limited ability to discriminate between sperm of different males within the female reproductive tract. Techniques based on genotyping markers (Bretman *et al.* 2009, Bussière *et al.* 2010) allow assessing the relative contribution of related and unrelated males to the sperm stores of double-mated females and ultimately investigating female–male interactions during sperm storage (Tuni *et al.* 2013). The challenge of discriminating competing sperm has recently been overcome in *D. melanogaster* by using genetically modified organisms that produce protamines labeled with green fluorescent protein or red fluorescent protein in sperm heads (Manier *et al.* 2010, Lüpold *et al.* 2012). Alternatively, competing male ejaculates can be tracked and quantified using a method of ejaculate staining with dyes (e.g. Hayashi & Kamimura 2002, Lymbery *et al.* 2018, Wylde *et al.* 2019). Such sperm labeling techniques offer researchers the possibility of observing and differentiate competing sperm within the female reproductive. Overall, recent technical advances enable a more detailed dissection of the complex and dynamic processes underlying female–male interactions, revealing the behavioral, physiological and molecular mechanisms relevant to postcopulatory selection.

After mating, postmating effects can be assessed on gene expression (1), specific reproductive parameters (2) and overall reproductive outcomes (3). The resulting offspring can also be tested on traits reflecting their performance (3). Mating trials are usually employed in internally fertilizing species, while *in vitro* fertilizations are usually employed in externally fertilizing species. Competitive fertilization trials could also be run using both (A) and (B) designs.

mode also influences processes that mediate sperm transfer. In external fertilizers, where sperm release is purely under male control, released sperm are passively lost in the external environment through abiotic mechanisms, such as wave action. The spatial and (in some cases) temporal separation between the sexes during reproduction in external fertilizers reduce the potential for female–male interactive effects to influence sperm allocation. In internal fertilizers, however, increasing evidence suggests that the number of sperm transferred to females is influenced by both sperm allocation during ejaculation and female sperm ejection following insemination, with ample potential for female–male interactive effects to influence sperm transfer.

Female–male interaction effects on sperm allocation

Males can adjust the quantity and/or quality of their semen based on the perceived reproductive quality of the female they mate with to limit the energetic costs of spermatogenesis (Wedell *et al.* 2002, Parker & Pizzari 2010). However, female–male interactions can also lead to strategic ejaculation in males. For example, in both the red junglefowl (*Gallus gallus*) and horse (*Equus caballus*), males transfer more sperm to females whose major histocompatibility complex (MHC) alleles are more dissimilar from their own (Gillingham *et al.* 2009, Jeannerat *et al.* 2018). These findings indicate that males can strategically adjust their ejaculate

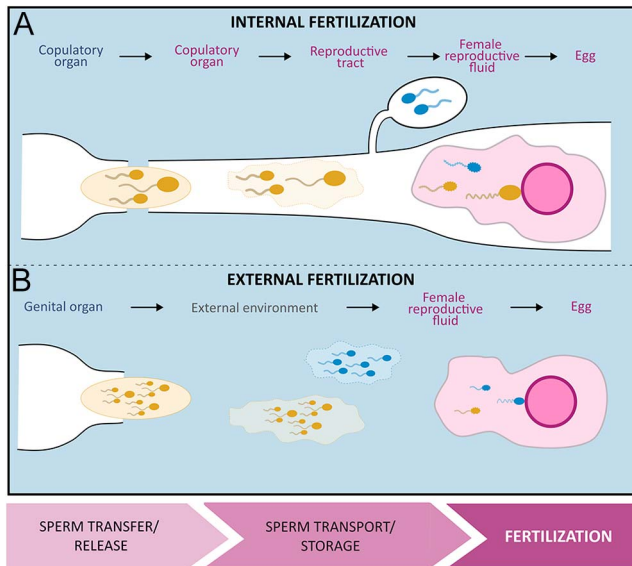
**Figure 1**

Fully crossed experimental designs usually employed in studies investigating the effects of female–male interactions on reproductive outcomes. These designs separate the sources of variance in fertilization success attributable to females, males and the interaction between the sexes. Here, we show two 3×3 factorial crossed designs. (A) Females are singly mated to males of three different strains (S1, S2 and S3) for a total of nine combinations. (B) Split-ejaculate and split-brood experimental designs using females' and males' gametes of three different strains (S1, S2 and S3). Reproductive outcomes (fertilization success, hatching success and offspring performance) result specifically from sperm–egg genetic interactions.

investment based on female genetic relatedness. Similarly, males of the moth *Plodia interpunctella* transfer fewer sperm when mating with their sisters than with unrelated females, likely due to lower fitness returns through inbreeding (Lewis & Wedell 2009). Interestingly, male fowl show an opposite tendency, inseminating more sperm when exposed to sisters (Pizzari *et al.* 2004). Although this finding appears counterintuitive, because female fowl preferentially fertilize their eggs with sperm from unrelated males, the increased allocation of sperm by related males may be an attempt to counteract this female preference (Pizzari *et al.* 2004). Overall, there is emerging evidence that female–male interaction effects on sperm allocation are influenced by genetic compatibility/relatedness or similarity between mating partners.

Female–male interaction effects on sperm ejection

Not all sperm that are transferred to females are part of the fertilization set, that is, the sperm that are available for fertilization. For example, sperm stored within the female reproductive tract can be physically removed by competing males (e.g. male damselflies remove sperm from rival males using their intromittent organ, Waage 1979). However, perhaps the most studied sperm loss mechanism in internally fertilizing animals is sperm ejection (or dumping), the expulsion of sperm (and, if present, the mating plug) from the female reproductive tract in a single post-insemination event (Pizzari & Birkhead 2000, Simmons 2002, Snook & Hosken 2004, Firman *et al.* 2017). Sperm ejection is observed in a diversity of taxa, such as insects, birds and mammals,

**Figure 2**

Female–male interaction effects occur on the successive steps of the fertilization process in species with both internal (A) and external (B) fertilization. Sperm are transferred to the female (left side, A) or released in the external environment (left side, B) and transported toward the site of sperm storage and/or fertilization (right side). The mechanisms by which female–male interaction occurs can influence sperm competition between males (here, blue and yellow sperm represent sperm from two different males and are released/transferred along with seminal fluid). For instance, the light pink color around the egg represents the female reproductive fluid, including ovarian, follicular fluid or egg chemoattractants, that may differentially influence sperm performance from the two males depending on the genetic compatibility/relatedness or similarity.

including primates (Dean *et al.* 2011). The timing of sperm ejection can alter the relative number of sperm from competing males that are present in the female reproductive tract and thereby affect paternity (Dean *et al.* 2011, Lüpold *et al.* 2013).

Sperm ejection can be influenced by male behavior (e.g. Davies 1983) or by physiological attributes of the ejaculate (e.g. Dixson & Anderson 2002). Females can also differentially bias sperm ejection depending on the quality of the male they are paired with (Firman *et al.* 2017). For example, sperm ejection may be influenced by male dominance, with females more likely to eject sperm from subdominant males (Pizzari & Birkhead 2000), and by male nutritional status, with females ejecting more sperm from protein-deprived males (Abraham *et al.* 2023). However, female–male interactions can also lead to strategic ejection in females. The extent of sperm dumping in *Drosophila melanogaster* exhibits high variance between pairs of males and females, suggesting that this behavior may vary based on the interaction between male and female compatibility (Snook & Hosken 2004). Recently, Mahdjoub *et al.* (2023) have substantiated this suggestion by showing

that both male quality (additive) and female–male compatibility (nonadditive effects) influence sperm ejection timing in *D. melanogaster*. Similarly, female–male interactive effects on sperm ejection have been observed in hermaphroditic flatworms that exhibit a postcopulatory suck behavior, during which the sperm recipient places its pharynx over its own female genital opening (Schärer *et al.* 2004, Vizoso *et al.* 2010, Marie-Orleach *et al.* 2013, 2017). The number of suck behaviors performed depends on both the focal and partner genotypes (Marie-Orleach *et al.* 2017).

Female–male interactions during sperm transport and storage

The next step on the path to fertilization (see Fig. 2) is the transport of sperm from the site of deposition to the site of sperm storage or fertilization. Here, interactive effects between the sexes can influence sperm motility, viability, the probability of sperm stored by females and patterns of sperm accumulation around the egg. In external fertilizers, the potential for female–male interactions during sperm transport increases as the physical distance between gametes decreases. In contrast, interactive effects during sperm transport act from the moment of insemination to gamete contact for internal fertilizers.

Female–male interaction effects on sperm performance

Numerous studies examining metrics of sperm performance, such as sperm swimming speed and viability, demonstrate that sperm quality cannot be thought of as only a male-specific trait. Instead, sperm performance often depends on the female-derived environment that sperm are operating in (Zadmajid *et al.* 2019). Moreover, sperm performance may depend on the specific female–male combination examined. For example, among external fertilizers, such as the chinook salmon (*Oncorhynchus tshawytscha*, Rosengrave *et al.* 2008), the marine mussel (*Mytilus galloprovincialis*, Oliver & Evans 2014) and the zebrafish (*Danio rerio*, Poli *et al.* 2019), sperm swimming performance depends on the female from which the ovarian fluid/egg water is derived in.

Similarly, the potential of female reproductive fluids to influence sperm performance in internal fertilizers is becoming increasingly clear, thanks to a series of recent studies in humans. Jokiniemi *et al.* (2020) documented how cervical mucus, a viscoelastic fluid secreted by cervical glands, from nine women differentially influenced sperm swimming speed, hyperactivation or plasma membrane integrity across eight men. In other words, sperm quality depended on female–male combination, which could affect sperm

movement through the cervix. Sperm viability also exhibited strong female–male interactive effects following exposure to cervical mucus, explained by the (dis)similarity of the human leukocyte antigen class I immunoglobulin between pairs (Magris 2021).

The cervix, similar to other regions of the female reproductive tract, mounts an immune response following insemination (Suarez & Pacey 2006). In humans, Sharkey *et al.* (2007) reported individual variation in immune responses, following interactions between the seminal plasma and ectocervical epithelia cells. Specifically, the authors examined the inflammatory cytokine gene expression in immortalized ectocervical epithelia (Ect1) cells from three different women when exposed to seminal plasma obtained from ten different fertile men. Comparisons of the seminal plasma-induced expression in Ect1 cells revealed that the seminal fluid from some men triggered greater inflammatory cytokine-inducing activity than that of others. Whether cytokine activity also varies among female–male combinations was not examined in the study, although this would be an interesting avenue for future investigation.

Female–male interactive effects on sperm performance are also evident in non-human internally fertilizing animals. For example, sperm performance is enhanced when swimming in reproductive fluids from unrelated females as compared to related females in guppies (*Poecilia reticulata*, Gasparini & Pilastro 2011) and house mice (*Mus domesticus*, Firman & Simmons 2015), although sperm performance was unaffected when swimming in reproductive fluids of females fed high or restricted diets in the pygmy halfbeak (*Dermogenys collettei*, Fernlund Isaksson & Fitzpatrick 2023). These results reinforce the idea that female–male interactive effects can act as a postmating filter against inbreeding, if not a female condition. The female reproductive tract presents spermatozoa with anatomical and physiological barriers that control their progress. There is some evidence that this tract can block passage of genetically incompatible sperm through self-recognition markers between sperm and its own tissues. In the colonial ascidian (*Diplosoma listerianum*), for example, sperm–female reproductive tract interactions block the passage of genetically incompatible sperm in the anterior portion of the oviduct. Sperm sharing self-recognition markers are removed from the female reproductive tract via phagocytic mechanisms to avoid incompatible genetic combinations, resulting in embryonic inviability (Bishop 1996).

Female–male interactions effects on sperm storage

Among internal fertilizers, sperm can be stored for relatively short (i.e. days) to long (i.e. years) durations

before being used for fertilization (Orr & Brennan 2015, Holt & Fazeli 2016). Consequently, there is ample potential for female–male interactions during sperm storage. For example, in some insects, both sperm length and the morphology of the female sperm storage organ interact and influence paternity (Otronen *et al.* 1997, Miller & Pitnick 2002, Laugen *et al.* 2022). Overall, the anatomy of female sperm storage organs (Orr & Brennan 2015) has an important impact on the scope for female–male interactions during sperm storage. For example, the presence of multiple sperm storage organs could facilitate cryptic female choice and magnify the potential for female–male interactions (Ward 1993, Pitnick *et al.* 1999, Snow & Andrade 2005).

Female–male interactions in sperm storage could particularly arise from cryptic female choice for partners that are genetically compatible. Females have been shown to store more sperm from unrelated males compared to related males and bias paternity toward genetically dissimilar males in the red junglefowl (Pizzari *et al.* 2004), the orb-web spider *Argiope lobata* (Welke & Schneider 2009) and the cricket *Teleogryllus oceanicus* (Bretman *et al.* 2009, Tuni *et al.* 2013). This cryptic female choice has subsequently been shown to bias fertilization toward MHC dissimilar males (Løvlie *et al.* 2013). Hence, female–male interactions could arise from females preferentially storing sperm from genetically dissimilar males to avoid inbreeding or to increase MHC diversity in their offspring.

From a mechanistic perspective, female–male interactions during sperm storage seem likely. Multiple genes expressed in the female reproductive tract, as well as proteins in sperm or more often seminal fluid, are affected by sperm storage (Orr & Brennan 2015). For example, in *D. melanogaster*, ejaculate proteins mediate female sperm storage (Peng *et al.* 2005, Adams & Wolfner 2007), while females produce compounds that are essential for sperm maintenance during storage (Wolfner 2011). Importantly, there can be genetic variation in males for female sperm storage, as demonstrated in *D. melanogaster* (Lüpold *et al.* 2012). In stored *D. melanogaster* sperm, ejaculate proteins and female proteins are associated with sperm after transfer, but following long-term storage, female proteins can constitute 20% of the composition of sperm (McCullough *et al.* 2022). Such direct interactions between male and female proteins on the sperm surface may affect successful sperm function through genetic interactions between the sexes, although this potential remains largely unexplored.

Another potential hotspot for female–male interactions during sperm storage could arise from immunity and sperm aging. The female immune system must be fine-tuned to avoid attacking sperm, as they are cells foreign to the female organism (Wigby *et al.* 2019).

This could be particularly relevant during sperm storage. Indeed, activation of the female immune system has been shown to trigger changes in sperm-storage patterns (Radhakrishnan & Fedorka 2012), and both the female sperm-storage organs and sperm can produce proteins that suppress the immune responses during storage (Das *et al.* 2006). In addition, sperm face specific metabolic requirements when stored. Limiting the production rate of oxygen radicals by reducing the metabolic rate during sperm storage has been shown to be essential for maintaining fertility (Ribou & Reinhardt 2012, Reinhardt & Ribou 2013). Both in immunity and sperm aging, the female environment during sperm storage and the individual properties of sperm likely interact. Nevertheless, this remains underexplored and the extent to which female–male interactions affect fertility due to these processes is currently unknown.

Female–male interaction effects on sperm chemoattraction

As sperm approach eggs, chemical communication between the gametes becomes increasingly important in both external and internal fertilizers. Ova, or the cells surrounding them, often release chemicals/substances that attract sperm (Eisenbach & Giojalas 2006). Traditionally, egg-derived chemoattractants were thought to increase the target area of the egg and maintain species boundaries (Riffell *et al.* 2004, Howard *et al.* 2009). However, recent evidence demonstrates that chemoattractants can also mediate female–male interactions (Evans *et al.* 2012). First described in the marine mussel, chemoattractants released from eggs attract sperm from some males more than others (Evans *et al.* 2012). These interactive effects have important fitness consequences, as fertilization rates and embryo survival were higher in cases where sperm were more responsive to egg chemoattractants (Evans *et al.* 2012, Oliver & Evans 2014).

Chemoattractants can also play a role in female–male interactions in internally fertilizing species, although this potential has rarely been explored. In a recent study in humans, Fitzpatrick *et al.* (2020) demonstrated that sperm accumulation in female follicular fluid was dependent on the combination of men and women being considered. Sperm chemoattraction (measured by quantifying sperm accumulation in follicular fluid) was assessed in two distinct experiments, exposing sperm to follicular fluid from two different women either simultaneously or nonsimultaneously. Under both experimental conditions, there was strong evidence of female–male interactive effects in patterns of sperm accumulation, with follicular fluid attracting up to 40% more sperm from one male over another.

Female–male interactions during fertilization

Eggs and sperm come into contact in the final steps along the path to fertilization (see Fig. 2), initiating gamete-level protein interactions and differential fertilization biases. Here, too, female–male interactions abound, which has been particularly well studied in external fertilizers. For example, in sea urchins, the probability of fertilization depends on the allelic combinations of the gamete recognition protein binding between the surface of eggs and sperm (Palumbi 1999). Such biochemical interactions between gametes are likely widespread in external fertilizers and are commonly used to explain nontransitivity in experimental crosses of eggs and sperm across different female–male combinations (Marshall *et al.* 2004, Evans 2012, Oliver & Evans 2014, Kekäläinen & Evans 2016). In externally fertilizing fishes, genetic similarity (likely mediated by MHC alleles) between females and males can shape fertilization outcomes (e.g. Liljedal *et al.* 2008, Geßner *et al.* 2017, Lenz *et al.* 2018).

Among internal fertilizers, interactive effects between the sexes can also determine fertilization outcomes. Once again, genetic relatedness (e.g. Fitzpatrick & Evans 2014) and specific combinations of MHC alleles (either their similarity or dissimilarity) predict fertilization success in a wide range of taxa (e.g. salamanders, Bos *et al.* 2009; kestrels, Alcaide *et al.* 2012; fowl, Løvlie *et al.* 2013; and guppies, Gasparini *et al.* 2015). Moreover, experimental crosses in both insects and birds indicate that fertilization success often depends on the female–male combination rather than being attributed to male or female effects alone (Clark *et al.* 1999, 2000, Birkhead *et al.* 2004, Zhang *et al.* 2013, Reinhart *et al.* 2015, Lüpold *et al.* 2020). However, because interactive effects occur throughout the path to fertilization (as shown above), it can be difficult to determine whether female–male interactive effects on fertility are specifically attributable to egg–sperm interactions in internal fertilizers.

Female–male–male interactions on the path to fertilization

The prevalence of female multiple mating in animals means that considering only female–male interactive effects may not sufficiently capture the reality of most fertilizations. Instead, incorporating sperm competition into an interactive framework requires that we consider female–male–male interactions. Such three-way interactions are multivariate and can constrain or dilute the fitness contributions of any single trait (Lüpold *et al.* 2020), and any interactive effects on fitness may limit the intensity of directional selection on these traits (Clark 2002). However, empirical

evidence of these predictions is largely lacking. One recent study in *D. melanogaster* has aimed to disentangle the sex-specific and interactive contributions of multiple male and female traits that may underlie the reported interactive effects in reproductive outcomes in this species (Clark *et al.* 1999, 2000, Zhang *et al.* 2013, Reinhardt *et al.* 2015a,b). By leveraging males with fluorescently labeled sperm to track sperm competition within the female reproductive tract (Manier *et al.* 2010; Lüpold *et al.* 2012), Lüpold *et al.* (2020) revealed two- or three-way interactions between sex-specific phenotypes during critical reproductive events, from male sperm transfer to female sperm ejection and storage. Importantly, however, the same study also indicated that these complex interactions by no means prevent multivariate systems from responding to directional sexual selection. Rather, some degree of interactive effects may, in fact, maintain genetic variation for selection to act upon, facilitating the evolution of sex-specific reproductive traits. The increased potential for intricate postmating interactions might also explain two observations: the significantly higher variation in sperm phenotypes observed in internal fertilizers compared to external fertilizers (Kahrl *et al.* 2021) and the coevolution of sperm and female sperm storage organs to sometimes remarkable dimensions (Pitnick *et al.* 1999, Lüpold *et al.* 2016).

Conclusions and perspectives

The path to fertilization is a complex interplay between female and male reproductive traits, where numerous interactions shape the ultimate success of egg-sperm fusion. This review reinforces the view that fertilization is not merely a straightforward race of sperm to the egg defined by separate female and male reproductive traits. Rather, it is becoming increasingly evident across both externally and internally fertilizing animals that female-male interactive effects have the potential to influence every aspect of the multistep process of fertilization; in other words, one size does not fit all during fertilization. A recurring explanation highlighted in this review is that female-male interactions along the path to fertilization ensure that more compatible genetic combinations reach the next generation.

While significant progress has been made in understanding female-male interactive effects, much remains to be explored. Future research should aim to uncover in more detail the molecular, genetic and physiological mechanisms underpinning these interactions. For instance, there is much scope for female-male interactions to impact fertilization success via female control of the amount of sperm transferred and stored (Firman *et al.* 2017). In species where the sperm are transferred gradually, research showed that

females can alter the number of sperm transferred by manipulating the duration of copulation in favor of what they perceive to be attractive males (e.g. in guppies (Pilaastro *et al.* 2007), in damselflies (Andrés & Cordero Rivera 2000) and in spiders (Andrade 1996, Herberstein *et al.* 2011)). Although female control over copulation duration appears to be an effective strategy for influencing whose sperm reach the fertilization pool, the few studies that have investigated whether this mechanism relies on the interaction between focal and partner genotypes have found no evidence of such interactive effects (Krebs 1991, Edward *et al.* 2014, Tennant *et al.* 2014). Given the diversity of taxa exhibiting mechanisms of cryptic female choice affecting either sperm transfer or sperm loss, future research is necessary to fully understand the extent of female-male interactions on these aspects of fertilization success. There also seems to be great potential for female-male interactions during female sperm storage, often likely mediated by cryptic female choice, but direct tests for this remain scarce. In addition, we encourage expanding studies to a broader range of species, particularly those with unique reproductive biology that do not clearly fit into broad definitions of fertilization mode. For instance, in species that exhibit internal fertilization without copulation, where sperm is transferred externally via spermatophores (e.g. in some insects, arachnids and amphibians), female-male interactions may contribute to variation in reproductive parameters (e.g. sperm allocation) in ways that differ from those observed in traditional internal fertilizers. Investigating such systems would provide deeper insights into the evolutionary dynamics of fertilization. Although we have restricted our review to animals, female-male interactive effects are also likely to be common in plants, where the potential for self-fertilization has driven the evolution of self-incompatibility systems (Matton *et al.* 1994, Sage *et al.* 1994). Such systems promote outcrossing by reducing self-fertilization (Waser 1993) and avoid inbreeding by leading to incompatibility with other individuals carrying the same incompatibility alleles (Gigord *et al.* 1998). Further work should also investigate the extent of complex female-male genetic interactions in natural matings compared to artificial inseminations and the context of continuous population genetic variation vs interactions among genetically clonal populations.

Our review suggests that female-male interactions are evolutionarily significant, finely tuned to increase individual fitness by maximizing genetic compatibility, while also contributing to population-level benefits by promoting phenotypic diversity. Female preference for intrinsically superior males is traditionally expected to decrease genetic variation through constant directional sexual selection (Kirkpatrick & Ryan 1991). Female-male interactions, on the contrary, likely contribute to the maintenance of genetic variation within populations, providing a substrate for evolutionary processes.

Indeed, increased genetic variation within traits induced by female–male interactions could play a crucial role in maintaining population viability by inducing adaptive evolutionary responses to environmental changes. Growing evidence suggests that intraspecific diversity strengthens population stability and persistence by dispersion of risks due to predation and extinction under fluctuating environmental conditions (Wennersten & Forsman 2012, Karpestam *et al.* 2016, Takahashi *et al.* 2018). Moreover, postmating mechanisms that enhance preferences for compatible combinations have the potential to cause reproductive isolation by promoting interpopulation divergence of cues/molecules required for reproduction to occur. In *Drosophila*, experiments using spermless males revealed that male seminal fluid is a crucial factor in controlling conspecific sperm precedence (i.e. the ability of same-species sperm to fertilize eggs more efficiently than those of a different species) (Price 1997, Price *et al.* 2000). In hybridizing *Allonemobius* crickets, Gregory & Howard (1994) also showed that fewer heterospecific sperm (i.e. sperm from a different species) exhibited motility within the female reproductive tract and sperm storage organs. The effectiveness of conspecific sperm precedence at limiting gene exchange between closely related species suggests that female–male interactions at the postcopulatory stage may play a role in reproductive isolation during early stages of divergence, potentially promoting speciation (e.g. Devigili *et al.* 2016, Garlovsky *et al.* 2023).

Our review aimed to emphasize the role of genotypic interactions between the sexes in processes contributing to fertilization success. However, we acknowledge that female–male interaction effects are not necessarily ubiquitous or the primary drivers of fertilization outcomes across all animal species (e.g. Wedekind *et al.* 2004, Skarstein *et al.* 2005, Bjork *et al.* 2007, Ala-Honkola *et al.* 2011, Reinhardt *et al.* 2015a,b). It is also important to acknowledge that directional selection for sexual traits that indicate mate quality and preference for genetically compatible mates can coexist (Neff & Pitcher 2005), as has been empirically shown in a few species, including the sedge warblers *Acrocephalus schoenobaenus* (Marshall *et al.* 2003), the house finches *Haemorrhous mexicanus* (Oh & Badyaev 2006) and *D. melanogaster* (Lüpold *et al.* 2020, Mahdjoub *et al.* 2023). Disentangling the relative importance of main (i.e. female and male) and interactive (i.e. female × male) effects is crucial for a clearer understanding of the evolutionary implications of postmating mechanisms, and future meta-analyses addressing this would help to clarify the prevalence of female–male interactive effects during fertilization.

Understanding female–male interactions in greater detail will not only enhance our knowledge of reproductive and evolutionary biology but also offer perspectives for addressing fertility issues in humans. Large-scale studies have shown that about 20–30% of infertility cases are

caused by male reproductive issues, 30–50% by female reproductive issues and 30–40% by both male and female reproductive issues or by unknown factors (Hull *et al.* 1985, Thonneau *et al.* 1991). Clarifying the importance of female–male interactions during fertilization and uncovering the molecular mechanisms that affect varying sperm responses could provide insights into the unexplained infertility variance observed in humans. Such knowledge could ultimately enhance the efficiency of the current assisted reproductive methods employed to treat infertility in humans.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the work reported.

Funding

LD, JLF, RRS and MQRP-S were funded by the Swedish Research Council grants (Vetenskapsrådet 2018-04598 awarded to RRS and 2021-04615 awarded to JLF), and LW was supported by a Wenner-Gren Postdoctoral Stipend (UPD-2023-0113).

Author contribution statement

All authors contributed to the writing and editing of the text.

Acknowledgments

We thank the members of the Biology of Spermatozoa meeting for helping to inform our ideas about reproductive evolution over the past many years.

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