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Sperm form and function: what do we know about the role of sexual selection?

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1 Invited Review

2 **Sperm form and function:**
3 **what do we know about the role of sexual selection?**

4
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16 *Key words:* Sperm morphology, ejaculate evolution, sperm competition, cryptic female choice

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Goals of this review

Sexual selection is widely regarded as the principal agent underlying the astonishing diversification of sperm morphology. Consistent with this perspective, we recently provided evidence that the giant sperm flagella of some *Drosophila* species share attributes with sexual ornaments and in fact, at least using some indices, would qualify as one of the most exaggerated sexual traits in all of nature (Lüpold *et al.*, 2016; Box 1). Whereas we contend that over twenty years of experimental and comparative evolutionary studies of *Drosophila* sperm have conspired to support the claims made by Lüpold *et al.* (2016 and references therein), we note that evidence for a causative role of sexual selection in driving the evolution of sperm traits in the majority of taxa is thin. As such, any objective and rigorous review of the role of sexual selection in driving the evolution of sperm form and function would be anemic. Thus, before reviewing postcopulatory sexual selection theory as it applies to sperm traits, and assessing empirical evidence, we first address why, after over three centuries of interest, our understanding of the adaptive value of sperm form and function is in such a pathetic state.

A brief history of studying sperm diversity, or, why we know so little about the adaptive value of sperm form and function

Sperm are considered one of the most taxonomically diverse and rapidly evolving cell types (Pitnick *et al.*, 2009a; Fig. 1). In 1679, Antonie van Leeuwenhoek noted that sperm varied between taxa, and some 150 years later, Rudolf Wagner illustrated the marked differences in the sperm morphology of mammals, birds, reptiles, amphibians, fish, molluscs and insects (Birkhead and Montgomerie, 2009). Documentation of sperm diversity began in earnest in the late 19th and early 20th century, championed by Emil Ballowitz and Gustaf Retzius, the latter describing in spectacular detail the sperm of over 400 species (Afzelius, 1995; Birkhead and Montgomerie, 2009). The invention of the electron microscope and the development of ultrathin sectioning and staining techniques then sparked an explosion of comparative spermatology in the 1940s, revealing the sperm ultrastructure for thousands of species across several ensuing decades.

59 The fervent interest in sperm structure was predominantly driven by systematists. Retzius
60 realized that sperm structure increases in similarity with species relatedness and, critically, reflects its
61 own evolutionary history regardless of that of the overall animal bauplan (Afzelius, 1995; Birkhead
62 and Montgomerie, 2009). With the advent of transmission electron microscopy came the discovery of
63 the sperm axoneme and recognition that its structure was highly conserved across taxa (but see Dallai,
64 1979; Dallai *et al.*, 2006). The opportunity presented by the juxtaposition between this pattern and the
65 remarkable diversity in sperm size, shape and other aspects of gross morphology (Pitnick *et al.*,
66 2009a) did not escape systematists. As a prelude to modern molecular phylogenetic approaches,
67 examining sperm ultrastructure became a quick, easy and robust way of resolving relationships
68 between species and among higher-order taxa (e.g., Jamieson, 1987, 1991).

69 Although the endeavor of “spermiocladistics” (Jamieson, 1987) accumulated detailed
70 descriptions of sperm form for countless species across the tree of animal life, it generated shockingly
71 little knowledge about functional morphology or the selection pressures driving sperm diversification.
72 Cell biologists and biophysicists also capitalized on the discovery of the sperm axoneme, with myriad
73 investigations addressing the cellular and molecular mechanisms by which an axoneme drives
74 flagellar beating (e.g., Katz, 1991; Woolley, 2003). Such investigations have almost universally been
75 conducted on glass microscope slides with observations of sperm behavior in saline (often of varying
76 viscosities) beneath a glass coverslip and in planar view. Such a protocol is well-suited for addressing
77 questions of cellular biomechanics, although more so when capturing the complexity of sperm
78 motility in all three dimensions (Alvarez, 2017), but it is ill-suited to questions about actual sperm
79 “behavior” (see below) and the adaptive value of variation in sperm form.

80 In a series of papers published in the 1950s, Åke Franzén was the first to suggest that sperm form
81 should reflect the mode of fertilization and be shaped by details of the fertilization environment
82 (Birkhead and Montgomerie, 2009). No formal analyses were ever conducted, but Franzén correctly
83 recognized that the sperm of externally-fertilizing taxa not only tend to be shorter, but also simpler
84 and less evolutionarily divergent in form than those of internally-fertilizing taxa (see Fig. 2), in which
85 environmental selection on sperm is more complex. Sperm released into the water must swim to an

egg (usually involving chemotaxis; Evans and Sherman, 2013) and then fertilize it. In contrast, sperm of internally-fertilizing species must successfully perform numerous functions between insemination and fertilization, including (1) migration/transport to specialized sperm-storage organs (e.g., spermatheca(e) and/or seminal receptacle) or a site of quasi-specialized, short-term storage such as the “sperm reservoir” in mammals (Orr and Brennan, 2015), (2) survive and remain viable in storage for hours to decades (Birkhead and Møller, 1993; Orr and Brennan, 2015), (3) engage in molecular interactions with the female reproductive tract (FRT) epithelium and/or secretome (Pitnick *et al.*, 2009b; Holt and Fazeli, 2016), (4) undergo molecular/structural modifications required for survival, motility and/or fertilization competency (Pitnick *et al.*, 2009b; Stival *et al.*, 2016), (5) exit the storage site and migrate to the site of fertilization at the proper time (Schnakenberg *et al.*, 2012; Sasanami *et al.*, 2013), (6) successfully compete with competitor sperm for a position within the sperm-storage and/or fertilization site (Snook, 2005; Pizzari and Parker, 2009) and then (7) properly fertilize an egg (Karr *et al.*, 2009; Bianchi and Wright, 2016).

Sperm of most internally-fertilizing species are subject to selection at any of these reproductive stages. Nevertheless, the strength and type of selection on sperm adaptations related to these functions is expected to vary dramatically across species given variation in female reproductive ecology, remating behavior and FRT morphology, physiology and biochemistry, in addition to phylogenetic history. In most respects, however, the FRT and the nature of selection underlying its diversification remain largely unexplored despite increasing evidence that FRT morphology is rapidly divergent (e.g., Keller and Reeve, 1995; Eberhard, 1996; Pitnick *et al.*, 2009b; Puniamoorthy *et al.*, 2010; Higginson *et al.*, 2012).

To resolve structure-function relationships for sperm—a necessary first step in addressing the adaptive significance of sperm form—it is important to assay sperm function *in vivo* or under realistic simulated conditions. This is true for two reasons. First, the behavior of sperm may depend critically on the arena in which they are found (in terms of architecture, viscosity and association with other sperm), with misleading conclusions likely to result from studies of adaptation in the absence of the selective forces responsible for the origin and evolutionary maintenance of the traits in question. The

extent to which *in vitro* analyses of the relationship between sperm form and function (i.e., studying motility in planar view between a glass slide and coverslip) inform about the same relationship within the female reproductive tract is an open question (Katz, 1983; Alvarez, 2017). Longer sperm swim faster than shorter sperm in some taxa and more slowly in others (interspecific: e.g., Gomendio and Roldan, 2008; Fitzpatrick *et al.*, 2009; Lüpold *et al.*, 2009a; intraspecific: e.g., Lüpold *et al.*, 2012; Simpson *et al.*, 2014). Whilst relationships between flagellum length and beat frequency may hold across assays conducted in different environments, this is not necessarily true. Also, there is more to sperm behavior than beat frequency, and patterns of sperm motility may differ dramatically between a microscope slide and the more complex, three-dimensional environment of the FRT. For example, the sperm of *Drosophila melanogaster* exhibit sinusoidal flagellar beating when compressed on a glass slide, adopt a tight helical conformation (resembling the spring of a ball-point pen) and spin in place without forward progression in deeper saline, and show high, progressive mobility *in vivo* when making contact with one another and with the walls of the FRT (Manier *et al.*, 2010; S. Pitnick, personal observation). Further, at high density within the elongate seminal receptacle (SR) of females, sperm behave like snakes in a tube, with independent movements between individual sperm; at low-to-moderate density, however, they interact with one another and with the walls of the FRT to form vortexes and other complex, emergent behaviors (S. Pitnick, unpublished data). We contend that sperm form in *Drosophila* has evolved in the context of FRT morphology to execute such behaviors, none of which would be observable on a microscope slide. We are not aware of any studies examining sperm behavior both *in vitro* and *in vivo* to directly compare observed relationships, but such analyses would prove extremely valuable.

The second reason for studying sperm function *in vivo* is the growing recognition of the importance of molecular interactions between sperm and the FRT that are critical to sperm function and survival (Holt and Fazeli, 2016), including sperm behavior. For example, oviductal hormones of eutherian mammals, most notably progesterone, are known to qualitatively influence sperm behavior by mediating hyperactivation, with species-specific changes to flagellar amplitude, beat symmetry and swimming linearity (e.g., Suarez and Ho, 2003; Fujinoki *et al.*, 2016). Hyperactivation is associated

with capacitation, which is frequently posited as a mammalian-specific phenomenon (Gilbert and Barresi, 2016). However, because modifications to sperm within the FRT are taxonomically widespread, having been described for marsupial and prototherian mammals, reptiles, amphibians, tunicates, molluscs and a diversity of arthropods, such modifications likely represent the rule rather than exception for all internally-fertilizing species (Yoshida *et al.*, 2008; Pitnick *et al.*, 2009b). In fact, even in the diverse and widespread taxa with external fertilization (e.g., many species of cnidarians, echinoderms, molluscs, ascidians and fishes), ovarian (or egg-derived) fluid surrounding eggs has been shown to induce species-specific modifications to sperm motility and behavior, in addition to the chemotactic response (Evans and Sherman, 2013; Yeates *et al.*, 2013; Yoshida *et al.*, 2013), suggesting an ancient origin of molecular sperm \times female interactions.

On a related note, some of the post-insemination sperm modifications referred to above include dramatic alterations to gross sperm morphology in addition to beat frequency. For example, the sperm of the fungus gnat, *Sciara coprophila*, lose approximately one-half of their volume within the female spermathecae (Phillips, 1966). The sperm of spiders and most other chelicerates are quiescent, rolled into balls and encapsulated at insemination, with the capsules later lysed by the female to release motile, flagellated sperm (Alberti, 1990). In some ticks, immature “prospermia” are transferred to females, which, once inside the FRT, essentially turn inside-out and can nearly double in length (Oliver, 1982). Moreover, within the FRT of the Chinese soft-shelled turtle, *Pelodiscus sinensis*, the sperm midpiece loses its large cytoplasmic droplet containing lipid droplets and modifies its mitochondria, which is thought to be linked to endogenous energy production during several months of sperm storage (Zhang *et al.*, 2015). Finally, for all of the taxonomically diverse species with conjugated sperm, the sperm obviously must disassociate from one another within the FRT before fertilization (Higginson and Pitnick, 2011). Caution is thus warranted when assaying sperm form using sperm obtained from males. Whenever possible and biologically relevant, sperm should also be examined following protracted storage within the female.

Given the importance of examining sperm structure-function relationships within their selective environment, why have so few studies managed to do so? First, it is technically challenging, limiting

successful attempts primarily to transparent organisms (LaMunyon and Ward, 1998, 1999; Temkin and Bortolami, 2004; Schärer *et al.*, 2011; Ting *et al.*, 2014) and those for which sperm bearing fluorescent tags could be genetically engineered (see below; Civetta, 1999; Manier *et al.*, 2010, 2013b; Marie-Orleach *et al.*, 2014; Droge-Young *et al.*, 2016; but note that such investigations are also becoming increasingly tractable for other study organisms: e.g., Kim *et al.*, 2017). Second, evolutionary biologists interested in adaptation (as opposed to phylogenesis, see above) took little interest in sperm biology prior to the formal development of postmating sexual selection (post-MSS) theory by Parker (1970). Until then, investigators of sexual selection restricted their studies to ornaments, armaments and female preferences functioning in the competition for mates, overlooking sperm, seminal fluid, genitalia and FRTs as targets of sexual selection (Andersson, 1994). Third, throughout the first few decades of research into post-MSS, there was an overemphasis on pattern at the cost of investigating process, similar to the entire field of behavioral ecology (Birkhead and Monaghan, 2010). A plethora of investigations of diverse taxa quantified patterns of sperm precedence (e.g., the proportion of progeny sired by the last male to mate with a female). Although these investigations, importantly, served to stimulate hypotheses about mechanism giving rise to the observed patterns (e.g., Lessells and Birkhead, 1990), they tended to be uninformative about both the strength and targets of post-MSS. Meanwhile, relatively few studies directly investigated the mechanisms underlying variation in competitive fertilization success, such as genital, sperm and FRT traits and their interactions (e.g., Waage, 1979; Birkhead and Hunter, 1990; Gomendio and Roldan, 1993; Birkhead and Biggins, 1998; Simmons and Siva-Jothy, 1998; Hotzy *et al.*, 2012). Also, for a number of complex, interacting reasons (detailed in the following paragraphs), any variation in competitive fertilization success tended to be attributed to males (i.e., sperm competition *sensu stricto*; Parker, 1970) rather than to females (i.e., cryptic female choice [CFC]; Thornhill, 1983; Eberhard, 1996) and/or male \times female interactions. Further, male-mediated variation was largely ascribed exclusively to variation in sperm quantity rather than quality (i.e., sperm form).

In a series of influential theoretical papers, Geoff Parker developed post-MSS theory (Parker, 1970), widely-held models for the origin (Parker *et al.*, 1972) and maintenance of anisogamy (Parker,

1982), and sexual conflict theory (Parker, 1979). These topics interrelate, in that competition for gamete fusions underlies the origin of sexes, with the sex differences in gamete investment (and hence investment per zygote) also representing the “primordial sexual conflict” (Parker, 1979). Together with the related development of parental investment theory (Trivers, 1972), these contributions provide the foundations of modern sex difference theory (Parker, 2014). As with the genesis of any new field of thought, initial priorities were to explain the most prevalent patterns. In this case, the primary goal was to explain the causes and consequences of males typically producing vast numbers of tiny sperm. Over the intervening years, numerous theoretical models (mostly by Parker and colleagues) addressed conditions affecting relative investment by males in sperm production (reviewed by Parker and Pizzari, 2010), comparative studies examined the relationship between relative testis mass and the intensity of post-MSS (reviewed in Parker and Pizzari, 2010; Simmons and Fitzpatrick, 2012), and experimental studies explored relationships between sperm number and sperm competition success (e.g., Martin *et al.*, 1974). Given the conceptual goals and the amenability of “ejaculate investment” to game theory modeling, sperm size and other aspects of sperm form were not (until recently) considered (e.g., Parker *et al.*, 2010). Males were presumed to produce the smallest sperm possible, as competition was perceived as functioning in a manner equivalent to a raffle—either fair or loaded—with sperm equating to tickets (Parker and Pizzari, 2010). Because increases in sperm size were assumed to only be adaptive to the extent that they served as paternal investment enhancing zygote viability, one highly influential model found that even low levels of sperm competition would maintain the state of males producing the smallest size sperm possible. Simply put, even doubling the size of sperm would make only a negligible nutritional contribution to the zygote despite highly jeopardizing numbers-based sperm competition by halving the number of sperm produced (Parker, 1982).

These theoretical and empirical studies offered powerful explanations for the most widespread patterns and typical sex roles. Meanwhile, most of the astonishing variation in sperm size and other axes of sperm form were ignored (but see, e.g., Sivinski, 1984). This problem was compounded because, with few exceptions (e.g., Hellriegel and Ward, 1998; Ball and Parker, 2003), theoretical

models generally omitted any female role beyond providing the arena for competition, probably due to mathematical complexity. Modeling reproductive outcomes between varying numbers of sperm contributed by competing males is relatively straightforward. By contrast, female-mediated processes, such as biases in sperm storage or utilization, are inherently challenging to predict theoretically. The formulation of clear assumptions about possible trade-offs and constraints is often hampered in particular by missing information on the costs and benefits of any sperm-use biases, and on the mechanisms themselves (Parker, 2006).

An important shift in emphasis came in the 1990's that we credit to the coupled recognition that studies had been collectively too male-biased and insufficiently mechanistic. Birkhead *et al.* (1993a), Keller and Reeve (1995) and Eberhard (1996) all elegantly and convincingly made the case for FRT morphology, physiology and biochemistry as the wellspring of intense selection on male copulatory and ejaculatory traits, including sperm form. Snook (2005) considered the role of post-MSS in driving sperm form, and many subsequent reviews addressed sperm diversity, ejaculate biochemistry and ejaculate-female interactions (e.g., Poiani, 2006; Ravi Ram and Wolfner, 2007; Pitnick *et al.*, 2009a, b; Wolfner, 2011; Ah-King *et al.*, 2014; Fitzpatrick and Lüpold, 2014).

Some skepticism remains about the importance of CFC, possibly due to male bias (Ah-King *et al.*, 2014) and unwarranted incredulity reminiscent of criticisms of Darwin's (1871) consideration of female choice/epigamic selection in his (premating) sexual selection theory. An unjustified empirical primacy has also been afforded to male-male sperm competition over CFC, with the perspective that all variation in competitive fertilization success should be presumed attributable to sperm competition until female mediation has been demonstrated (see Birkhead, 1998; Eberhard, 2000; Pitnick and Brown, 2000). In addition to the methodological challenges of undeniably showing CFC (Pitnick and Brown, 2000), the role of female-imposed selection has also been deemed limited on theoretical grounds, based on the logic of asymmetric benefits. The contention is that selection on males to influence paternity be more intense than that on females, given that males risk losing offspring and females only risk producing offspring of lower quality (Parker, 1984). A larger consideration may be the extent of power asymmetry between the sexes. As discussed by Eberhard (1996) and McLeod and

Day (2017), females far outstrip males in diversity of mechanisms to influence sperm usage and in the likelihood that they will arise. Because the outcome is determined within their bodies, females also may physiologically and evolutionarily “get in the last word.” Indeed, Parker (1984, p. 25) agrees “it may be relatively easier for females, morphologically and behaviorally, to prevent males from achieving their objective.” Some of this logic, however, applies primarily to traits in conflict, and the extent of net sexual conflict over sperm traits influencing paternity is not apparent (McLeod and Day, 2017).

Having addressed the general methodological challenges and knowledge gaps in the study of sperm evolution, we will explore in the following sections the specific attributes of, and selection on, sperm and FRTs. We will adopt the traditional definitions of sexually selected traits as used in the context of premating sexual selection (pre-MSS), in order to draw attention to the interplay between male and female mediation in determining reproductive success and, ultimately, driving sexual trait evolution.

Pre- and postcopulatory sexual ornaments, armaments and preferences

In addition to gonads and gametes, which are essential for reproduction, reproductive characters also include the so-called ‘secondary’ sexual traits that mediate the likelihood of individuals *competitively* reproducing and propagating their genes (Darwin, 1871; Andersson, 1994; Leonard and Córdoba-Aguilar, 2010). As described by Darwin (1871), two mechanisms mediate pre-MSS: intrasexual (typically male) competition, and intersexual (typically female) choice. By definition, sexual armaments (e.g., horns or antlers) influence the outcome of (typically male–male) contest competition over mates, and ornaments provide information on their bearer’s breeding value that triggers nonrandom fitness biases resulting from ‘preferences’ of the opposite sex (usually females). For example, female preferences for brighter, more elaborate or otherwise exaggerated ornaments mediate male mating success (Darwin, 1871; Andersson, 1994). It is important to note, however, that many sexual traits (e.g., sexual calls or body size) may be under both intra- and intersexual selection, simultaneously or sequentially (Berglund *et al.*, 1996; Hunt *et al.*, 2009).

Due to the direct fitness effects of ornament or armament expression, males should increase their investment in these traits as sexual selection intensifies (Wade and Arnold, 1980), but this requires resources that could otherwise be allocated to somatic maintenance and survival, or even to paternal investment (Trivers, 1972). Consequently, sexually selected traits tend to be costly for males to grow and maintain (Emlen, 2001; Allen and Levinton, 2007). Any among-individual variation in energy reserves used to grow and maintain sexual traits can arise from underlying variation in somatic, genetic or epigenetic condition (i.e., “the capacity to withstand environmental challenges”: Hill, 2011). If so, males of superior condition should be able to invest more in such costly traits than others, rendering the phenotypic expression of sexual traits condition-dependent (Cotton *et al.*, 2004). Sexual selection will promote those female preferences that precisely target those condition-dependent male traits that honestly signal male genetic condition, as females would benefit by producing offspring that inherit those qualities (Andersson, 1994; Rowe and Houle, 1996). Condition-dependent sexual traits are therefore a central component of sexual selection theory, including ‘good genes’ models (Grafen, 1990; Iwasa *et al.*, 1991) and models of the maintenance of genetic variation in sexually selected traits (Rowe and Houle, 1996; Houle, 1998; Tomkins *et al.*, 2004). Sexual selection would further favor these female preferences to the extent that male ornaments reliably indicate any direct benefits accrued by females as a result of mating with well-ornamented males, such as better breeding territories, oviposition substrate, paternal care of young or even male fertility (Kirkpatrick and Ryan, 1991; Sheldon, 1994).

With pre-MSS, intrasexual competition and intersexual choice tend to be operationally discrete (but see Wiley and Poston, 1996), and hence so are designations of specific traits as either armaments and ornaments (but see Berglund *et al.*, 1996; Hunt *et al.*, 2009). In contrast, as described below, mechanisms of sperm competition and CFC tend to be far less discrete, blurring the lines between intra- and intersexual selection acting on post-MSS traits and, therefore, their function as armaments and ornaments following traditional terminology and definitions *sensu* pre-MSS traits. There are other conspicuous differences between pre- and postmating sexual traits, with important consequences for the evolvability and the evolutionary trajectory of traits under sexual selection. For example, pre-MSS

traits tend to be complex somatic traits controlled by multiple genes (Lande, 1980), whereas at least some ejaculate traits that are putative targets of post-MSS, such as seminal fluid proteins, are single active molecules expressed by single genes (Dorus and Karr, 2009; Pitnick *et al.*, 2009a; Avila *et al.*, 2011; but see Findlay *et al.*, 2014). Unfortunately, the genetic basis of flagellum length and of other putative sperm ornaments and/or armaments (e.g., hooks on the sperm heads of some rodents) is unknown. Another important difference is that female preferences for pre-MSS ornaments tend to be mediated by female sensory biology (e.g., vision, hearing, smell, touch) followed by cognitive processing (Ryan, 1990; Jennions and Petrie, 1997; Kirkpatrick *et al.*, 2006). In contrast, nonrandom paternity shares based on postmating female biases tend to result from interactions of ejaculates with the morphology, biochemistry and neurophysiology of the FRT (Pitnick *et al.*, 2009b) that do not require sensory organs and cognitive processing. Despite these differences, sperm, ejaculate, FRT and other sex-specific traits subject to post-MSS meet the definitional criteria of “ornament,” “armament” and “preference” as well as traditionally considered pre-MSS traits (Darwin, 1871; Andersson, 1994). Primary sexual traits therefore can bear secondary sexual traits (see *Theory of postcopulatory sexual selection* below).

To be clear, ejaculates may also share some of the fundamental attributes of pre-MSS traits, in that there can be heritable variation in their expression within populations (reviewed in Simmons and Moore, 2009) and such variation may influence competitive fertilization success (reviewed in Simmons and Fitzpatrick, 2012; Fitzpatrick and Lüpold, 2014). Additionally, there are significant costs associated with the production of ejaculates (Dewsbury, 1982; Pitnick, 1996; Olsson *et al.*, 1997; Thomsen *et al.*, 2006), and ejaculate quantity and/or quality can vary with the male’s nutritional state (Gage and Cook, 1994; Perry and Rowe, 2010; Rahman *et al.*, 2013; Kahrl and Cox, 2015; Kaldun and Otti, 2016). Ejaculate quality has further been linked to male quality as reflected by positive associations with well-characterized pre-MSS traits, although such links are usually weak (reviewed in Mautz *et al.*, 2013).

Keeping these similarities and differences between pre- and postcopulatory sexual traits in mind, we now review evidence of cryptic female choice and intermale sperm competition exerting selection

on ejaculate traits, as well as the extent to which such selection shares attributes with that on male premating ornaments and armaments.

Theory of postcopulatory sexual selection

As described above, early theoretical models analogized sperm competition to a lottery, with selection favoring males that produce the smallest possible sperm (Parker, 1982, 1993). The production of vast numbers of sperm as an adaptation to postcopulatory, intrasexual competition meets the definitional criteria of an armament. However, with growing empirical evidence from comparative studies for positive selection on sperm size in diverse taxa (Gage, 1994; Briskie *et al.*, 1997; Byrne *et al.*, 2003; Fitzpatrick *et al.*, 2009; Lüpold *et al.*, 2009b; Tourmente *et al.*, 2011), more recent sperm competition models have sought to predict when selection should indeed favor sperm number over sperm size and *vice versa* (Parker *et al.*, 2010). These models uniformly predicted that the overall gamete investment should increase with the intensity of post-MSS, which can arise from greater investments in both sperm size and number. However, when the overall investment in sperm production is maximized, the two traits necessarily trade off with one another (Pitnick, 1996; Lüpold *et al.*, 2009c, 2016). At this point, Parker *et al.* (2010) predicted selection to be stronger on sperm number than sperm size when sperm competition follows the principles of a raffle and the density of sperm at the fertilization site is relatively low (e.g., through sperm dilution within the FRT in relatively large-bodied species; Immler *et al.*, 2011; Lüpold and Fitzpatrick, 2015). If, however, sperm end up being densely packed within a small FRT (e.g., in insects and other invertebrates), physical interactions among sperm may be inevitable, in which case the quality of individual sperm may confer a competitive advantage. For example, sperm may compete to occupy limited space within the female's specialized sperm-storage organs through physical displacement, with excess sperm discarded by the female (Miller and Pitnick, 2002; Pattarini *et al.*, 2006; Manier *et al.*, 2010, 2013b; Lüpold *et al.*, 2012). Under such conditions, selection can favor sperm quality over quantity (Parker *et al.*, 2010; Immler *et al.*, 2011), with the quality trait (e.g., larger size) also meeting the definitional criteria of a sexually selected armament.

Yet, there is accumulating evidence for an active role of females in fertilization events (Firman *et al.*, 2017), with the most conspicuous example being females that eject sperm from their reproductive tract after copulation (e.g., Pizzari and Birkhead, 2000; Snook and Hosken, 2004; Wagner *et al.*, 2004; Peretti and Eberhard, 2010; Lüpold *et al.*, 2013; also see Schärer *et al.*, 2004 for active sperm removal in hermaphroditic flatworms). Further, when ejaculates compete within the FRT, variation in the morphology or biochemistry of this competitive environment is likely to influence the outcome through differential ejaculate–female compatibilities. For example, in both birds (Birkhead *et al.*, 1993b) and mammals (Suarez and Pacey, 2006), the selective environment of the FRT prevents the vast majority of inseminated sperm from reaching the egg(s). Even if such female selective processes may have initially evolved to fight pathogens invading the FRT during copulation, avoid polyspermy or reject fertilization-incompetent sperm rather than sexual selection (Birkhead *et al.*, 1993b; Eberhard, 1996; Pitnick *et al.*, 2009b), the same mechanisms will inevitably bias competitive fertilization whenever females mate with two or more males whose sperm differ in their ability to overcome the female barriers, thus giving rise to post-MSS (Curtsinger, 1991; Keller and Reeve, 1995; Yasui, 1997). Any variation in the FRT environment may change the conditions under which sperm compete, and therefore shift the relative competitive advantage between the same males (e.g., Lüpold *et al.*, 2013). Consequently, similar to premating sexual traits under both intra- and intersexual selection, post-MSS is largely mediated by male \times male \times female interactions (Bjork *et al.*, 2007), which do not necessarily favor the best sperm based purely on some intrinsic quality(ies), but rather those sperm that are best able to operate within the specific conditions set by the female and given the specific competitor male(s). Any sperm traits arising through selection generated by FRT-imposed fertilization biases meet the definitional criteria of sexually selected ornaments.

Postcopulatory male \times female interactions influencing reproductive outcomes, supportive of some female mediation in the process, have been documented in species with both internal fertilization (e.g., Lewis and Austad, 1990; Wilson *et al.*, 1997; Clark *et al.*, 1999; Miller and Pitnick, 2002; Nilsson *et al.*, 2003; Birkhead *et al.*, 2004) and external fertilization (e.g., Turner and Montgomerie, 2002; Evans and Marshall, 2005; Rosengrave *et al.*, 2008; Simmons *et al.*, 2009;

Alonzo *et al.*, 2016), thereby suggesting that the competitive fertilization process may rarely be independent of female effects. Experimental evidence suggests that sperm quantity and quality can both independently operate and interact to influence competitive fertilization success (Pattarini *et al.*, 2006; Parker *et al.*, 2010). Consequently, in a proximate sense, sperm competition and CFC represent a false dichotomy (Eberhard, 1996; Lüpold *et al.*, 2016), and any sperm traits arising through post-MSS are simultaneously ornament and armament (similar to certain traits under pre-MSS; Berglund *et al.*, 1996; Hunt *et al.*, 2009). Moreover, there may be developmental integration of sperm quantity and quality traits, given that they compete locally for resources within the testes, which in an ultimate sense may constrain the degree to which sperm competition and CFC operate as discrete processes of post-MSS (Parker *et al.*, 2010; Lüpold *et al.*, 2016; see below).

Despite the ubiquitous potential (Eberhard, 1996) and accumulating evidence (Firman *et al.*, 2017) for an active female role in reproductive outcomes, very little is known about the extent to which CFC ultimately influences the trajectory of ejaculate evolution. Current evidence is largely restricted to comparative studies showing co-diversification of sperm length and either female postmating behavior (Schärer *et al.*, 2011) or the length of some critical dimension of female sperm-storage structures, such as in birds (Briskie and Montgomerie, 1992; Briskie *et al.*, 1997), moths (Morrow and Gage, 2000), diopsid stalk-eyed flies (Presgraves *et al.*, 1999), *Drosophila* fruit flies (Pitnick *et al.*, 1999), *Scathophaga* dung flies (Minder *et al.*, 2005), *Bambara* featherwing beetles (Dybas and Dybas, 1981), bruchid seed beetles (Rugman-Jones and Eady, 2008), and dytiscid diving beetles (Higginson *et al.*, 2012). Higginson *et al.* (2012) importantly moved the paradigm of sperm-FRT co-diversification beyond the single axis of length (i.e., sperm flagellum and sperm-storage organ duct/capsule) with a multivariate, comparative analysis of 42 species of diving beetles. Evolutionary remodeling of several different FRT organs and structures were significantly associated with changes in sperm length, head shape, gains and losses of sperm conjugation and conjugation size. Moreover, analyses suggest that changes to FRTs occur first and then elicit changes in sperm form (Higginson *et al.*, 2012). Another interesting, and altogether different, example comes from hermaphroditic flatworms of the genus *Macrostomum*, in which sperm bear stiff, lateral bristles that

appear to have coevolved with thickened epithelium of the female’s sperm-receiving organ (Schärer *et al.*, 2011). Nevertheless, interspecific correlations themselves are no proof of causation. To date, Miller and Pitnick (2002) provide the only experimental evidence for the FRT as an agent of selection capable of driving the evolution of sperm form generally, and for the pattern of sperm and female sperm-storage organ length co-diversification in particular (also see Miller and Pitnick, 2003; Bjork and Pitnick, 2006; Pattarini *et al.*, 2006).

To more convincingly show a contribution of intersexual selection to the diversification of ejaculate traits, some understanding of the underlying mechanisms is necessary, thereby highlighting the critical need for investigations of post-MSS in the selective environment (Pitnick and Brown, 2000). Understanding the processes of post-MSS, particularly in internal fertilizers, requires overcoming important methodological challenges, such as reliably discriminating among sperm of different males and visualizing them within the FRT to follow their fate through the sequence of postmating reproductive events. Distinction of competing sperm itself has been achieved by use of discrete sperm sizes among males (LaMunyon and Ward, 1998; Hellriegel and Bernasconi, 2000; Pattarini *et al.*, 2006; Bennison *et al.*, 2015), application of fluorescent dyes (King *et al.*, 2002; Ting *et al.*, 2014; Lymbery *et al.*, 2016) or genetic engineering of males so their sperm express fluorescent protein (Civetta, 1999; Manier *et al.*, 2010; Marie-Orleach *et al.*, 2014; Droge-Young *et al.*, 2016). Fluorescently tagged sperm, so far successfully applied in several *Drosophila* species (Manier *et al.*, 2010, 2013a), the flatworm *Macrostomum lignano* (Marie-Orleach *et al.*, 2014) and the red flour beetle *Tribolium castaneum* (Droge-Young *et al.*, 2016), are particularly promising as, once transgenic populations with distinct sperm-tag colors are established, competing ejaculates are unambiguously identifiable and sperm behavior is visible in the selective environment following natural inseminations.

Such tools enable experimental studies addressing the functional significance of ejaculate traits in response to processes of female sperm selection, thereby filling an important gap by establishing the extent to which female biases contribute to variation in, and selection on, male ejaculate traits. Combining detailed examination of postcopulatory processes using transgenic flies with experimental

evolution and comparative studies has revealed a relatively good understanding of possible mechanisms through which female postcopulatory biases or ‘preferences’, interacting with male–male competition, may influence the evolution of sperm length in *Drosophila*, as outlined in Box 1. Yet, although these results strongly suggest that both sperm competition and CFC have been instrumental in driving the dramatic diversification in sperm length throughout this lineage, at least four important questions currently remain unanswered. First, it is unclear whether the parallel elongation of the female seminal receptacle and sperm, sharing characteristics of female preference and male preferred traits, is a case of true coevolution or whether sperm length simply tracks independent evolution of SR length (e.g., due in part to a genetic link between the two traits; Lüpold *et al.*, 2016). Second, we do not know what triggered the diversification of SR length in the first place, given the associated costs for females of developing and/or maintaining longer organs (Miller and Pitnick, 2003). Third, the extent to which different models of sexual selection (e.g., ‘good genes,’ Fisherian runaway, sensory exploitation, antagonistic coevolution) drive the evolution of female preferences (e.g., sperm choice) and hence selection on sperm is largely unknown (but see Lüpold *et al.*, 2016). Fourth, it also remains to be determined how the empirically-supported selective processes attributed to male- and female-mediated sperm evolution in drosophilid fruit flies apply to other organisms, for which co-diversification of sperm length and female sperm-storage structures has been reported (see above).

Where to go from here?

Recent theoretical and methodological advances have made substantial progress in uncovering the multifaceted patterns of ejaculate evolution. Yet, we would like to draw attention to several important gaps in our understanding for future investigation, pertaining in particular to (1) the role of different selective agents in ejaculate evolution, (2) the non-independence of different ejaculate traits and their phenotypic and genetic variation, and (3) the relative importance of intra- and intersexual selection, which we discuss in turn.

First, although there is evidence that sexual selection plays a pivotal role in ejaculate evolution, its importance relative to non-sexual selection generally remains unclear. Specifically, sexual

selection is a subset of natural selection, which shapes sperm form and function to maximize fertility in general (Tobias *et al.*, 2012). Thus, any change in the broad fertilization environment can select for modifications of ejaculate traits to ensure successful fertilization even in the absence of sexual selection. For post-MSS to operate, sperm of different males (at least potentially) must coincide within the FRT or the site of fertilization, and so its impact revolves around traits mediating *competitive* fertilization success. Thus, studies incorporating information on the overall intensity of selection, as well as on the relative importance of sexual and non-sexual selection, will provide a far more complete understanding of ejaculate evolution than those with an exclusive focus on post-MSS.

The relative importance of sexual and non-sexual selection may vary greatly with the mode of fertilization, duration of sperm storage, mating system (including the relative importance of pre-MSS and post-MSS), phylogenetic constraints, or general susceptibility of male fertility to environmental effects. For example, sperm length varies in response to female sperm storage duration (related to clutch size and egg laying frequency) but not to sperm competition levels across pheasant species (Immler *et al.*, 2007), whereas the opposite pattern seems to hold across a range of songbirds (Kleven *et al.*, 2009). Further, across fishes, sperm length differs between buccal and substrate spawners (Balshine *et al.*, 2001) and between internal and external fertilizers (Stockley *et al.*, 1996; also see Fig. 2). Sperm quantity and quality of externally-fertilizing species show interspecific associations with egg numbers and the risk of gamete dispersal by water currents (Stockley *et al.*, 1997; Liao *et al.*, 2018). Despite being associated with proxies of sperm competition in isolation (Balshine *et al.*, 2001; Fitzpatrick *et al.*, 2009; Liao *et al.*, 2018), simultaneous examination of different selective agents rendered non-sexual selection a more important predictor of macroevolutionary ejaculate variation than sexual selection (Liao *et al.*, 2018). Consequently, by focusing exclusively on sperm competition, we risk overlooking other, potentially important factors that can greatly influence the evolution of ejaculate traits either directly or by modifying the conditions under which sperm compete. It is thus critical to consider how the selective environment (e.g., spawning conditions or the FRT), as well as nutritional, health or environmental effects on the males themselves, influences

competitive fertilization success and selection on sperm form and function (also see Reinhardt *et al.*, 2015).

Second, individual ejaculate traits do not evolve in a univariate fashion. For example, according to Parker *et al.*'s (2010) models, evolutionarily stable patterns of investment between sperm size and number are expected to vary with the size of the fertilization site and associated mechanisms of post-MSS. Since the covariation between the two ejaculate traits is predicted to change from positive to negative as post-MSS intensifies and selection should be stronger on one or the other trait depending on the taxon (Parker *et al.*, 2010; Immler *et al.*, 2011), knowledge of different ejaculate traits is necessary to understand the evolutionary trajectory of each. In other words, examining multivariate selection on ejaculates and the resulting fitness landscape can be substantially more informative than single-trait studies (Pizzari and Parker, 2009; Fitzpatrick and Lüpold, 2014). This is particularly true if sperm quality and quantity exhibit trade-offs in time, space and resources during spermatogenesis. Such covariation exposes a false dichotomy between sperm competition and cryptic female choice, both of which may underlie the evolution of sperm number, size, shape and behavior (Lüpold *et al.*, 2016). In addition, to better predict the response to selection and the evolvability of ejaculate traits, we need detailed investigations of within-population relationships between sperm structures and measures of sperm performance, ideally even in a quantitative genetic framework (Birkhead *et al.*, 2005; Lüpold *et al.*, 2012). It would be particularly fruitful to conduct such studies with other putative sperm phenotypes believed to be subject to sexual selection, including but not limited to the hooked sperm heads of some murine rodents (in terms of their ability to form trains), the size and performance of sperm conjugates, the proportion of non-fertilizing sperm in polymorphic species, or of undulating membranes (Pitnick *et al.*, 2009a). Such intraspecific examination should then be integrated with broad macroevolutionary studies (including major changes in the general sperm bauplan) in response to differences in mating system and reproductive modes, or in reproductive physiology (e.g., Schärer *et al.*, 2011).

Third, processes of sperm competition and CFC are also challenging (if not impossible) to clearly delineate, particularly in internal fertilizers due to the intimate association between sperm and the

FRT (Pitnick *et al.*, 2009b). Whatever the primary agent of ejaculate evolution, changes in ejaculates may impact how they are processed by the female. Likewise, any modifications of the female reproductive tract or mode of sperm storage and utilization may select for adjustments in ejaculates to maximize their fertilization efficiency and competitiveness. When males and females differ in their optimal trait investment and each sex benefits by differentially influencing fertilization, a conflict between the sexes is likely to arise (Parker, 1979, 2006; Arnqvist and Rowe, 2005). Therefore, detailed information on the sex-specific costs and benefits, in addition to understanding the mechanisms of post-MSS, is necessary to disentangle the relative importance of intra- and intersexual selection and the potential conflict between the sexes in driving the evolution the sexual traits of interest. Until we achieve a more-than-cursory understanding of the functional design of the FRT and of the structure-function relationships underlying sperm behavior within the FRT, our understanding of the adaptive value of the staggering diversity in sperm form will be limited.

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Declaration of interest

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541 **References**

- 542 **Afzelius BA** (1995) Gustaf Retzius and spermatology. *International Journal of Developmental*
543 *Biology* **39** 675–685.
- 544 **Ah-King M, Barron AB and Herberstein ME** (2014) Genital evolution: Why are females still
545 understudied? *PLoS Biology* **12** 1–7.
- 546 **Alberti G** (1990) Comparative spermatology of Araneae. *Acta Zoologica Fennica* **190** 17–34.
- 547 **Allen BJ and Levinton JS** (2007) Costs of bearing a sexually selected ornamental weapon in a
548 fiddler crab. *Functional Ecology* **21** 154–161.
- 549 **Alonzo SH, Stiver KA and Marsh-Rollo SE** (2016) Ovarian fluid allows directional cryptic female
550 choice despite external fertilization. *Nature Communications* **7** 12452.
- 551 **Alvarez L** (2017) The tailored sperm cell. *Journal of Plant Research* **130** 455–464.
- 552 **Andersson M** (1994) *Sexual Selection*. Princeton, New Jersey: Princeton University Press.
- 553 **Arnqvist G and Rowe L** (2005) *Sexual Conflict*. Princeton, NJ: Princeton University Press.
- 554 **Avila FW, Sirot LK, LaFlamme BA, Rubinstein CD and Wolfner MF** (2011) Insect seminal fluid
555 proteins: Identification and function. *Annual Review of Entomology* **56** 21–40.
- 556 **Ball MA and Parker GA** (2003) Sperm competition games: Sperm selection by females. *Journal of*
557 *Theoretical Biology* **224** 27–42.
- 558 **Balshine S, Leach BJ, Neat F, Werner NY and Montgomerie R** (2001) Sperm size of African
559 cichlids in relation to sperm competition. *Behavioral Ecology* **12** 726–731.
- 560 **Bennison C, Hemmings N, Slate J, Birkhead T and Bennison C** (2015) Long sperm fertilize more
561 eggs in a bird. *Proceedings of the Royal Society B* **282** 20141897.
- 562 **Berglund A, Bisazza A and Pilastro A** (1996) Armaments and ornaments: an evolutionary
563 explanation of traits of dual utility. *Biological Journal of the Linnean Society* **58** 385–399.
- 564 **Bianchi E and Wright GJ** (2016) Sperm meets egg: The genetics of mammalian fertilization. *Annual*

- 565 *Review of Genetics* **50** 93–111.
- 566 **Birkhead TR** (1998) Cryptic female choice: criteria for establishing female sperm choice. *Evolution*
- 567 **52** 1212–1218.
- 568 **Birkhead TR and Biggins JD** (1998) Sperm competition mechanisms in birds: models and data.
- 569 *Behavioral Ecology* **9** 253–260.
- 570 **Birkhead TR and Hunter FM** (1990) Mechanisms of sperm competition. *Trends in Ecology &*
- 571 *Evolution* **5** 48–52.
- 572 **Birkhead TR and Møller AP** (1993) Sexual selection and the temporal separation of reproductive
- 573 events: sperm storage data from reptiles, birds and mammals. *Biological Journal of the Linnean*
- 574 *Society* **50** 295–311.
- 575 **Birkhead TR and Monaghan P** (2010) Ingenious Ideas: The history of behavioral ecology. In
- 576 *Evolutionary Behavioral Ecology*, pp 3–15. Eds DF Westneat and CW Fox. New York: Oxford
- 577 University Press.
- 578 **Birkhead TR and Montgomerie R** (2009) Three centuries of sperm research. In *Sperm Biology: An*
- 579 *Evolutionary Perspective*, pp 1–42. Eds TR Birkhead, DJ Hosken and S Pitnick. San Diego:
- 580 Academic Press.
- 581 **Birkhead TR, Pellatt EJ and Fletcher F** (1993a) Selection and utilization of spermatozoa in the
- 582 reproductive tract of the female zebra finch *Taeniopygia guttata*. *Journal of Reproduction and*
- 583 *Fertility* **99** 593–600.
- 584 **Birkhead TR, Møller AP and Sutherland WJ** (1993b) Why do females make it so difficult for
- 585 males to fertilize their eggs? *Journal of Theoretical Biology* **161** 51–60.
- 586 **Birkhead TR, Chaline N, Biggins JD, Burke T and Pizzari T** (2004) Nontransitivity of paternity in
- 587 a bird. *Evolution* **58** 416–420.
- 588 **Birkhead TR, Pellatt EJ, Brekke P, Yeates R and Castillo-Juarez H** (2005) Genetic effects on
- 589 sperm design in the zebra finch. *Nature* **434** 383–387.

- 590 **Bjork A and Pitnick S** (2006) Intensity of sexual selection along the anisogamy-isogamy continuum.
 591 *Nature* **441** 742–745.
- 592 **Bjork A, Starmer WT, Higginson DM, Rhodes CJ and Pitnick S** (2007) Complex interactions
 593 with females and rival males limit the evolution of sperm offence and defence. *Proceedings of*
 594 *the Royal Society B* **274** 1779–1788.
- 595 **Briskie J V and Montgomerie R** (1992) Sperm size and sperm competition in birds. *Proceedings of*
 596 *the Royal Society B* **247** 89–95.
- 597 **Briskie J V, Montgomerie R and Birkhead TR** (1997) The evolution of sperm size in birds.
 598 *Evolution* **51** 937–945.
- 599 **Byrne PG, Simmons LW and Roberts JD** (2003) Sperm competition and the evolution of gamete
 600 morphology in frogs. *Proceedings of the Royal Society B* **270** 2079–2086.
- 601 **Civetta A** (1999) Direct visualization of sperm competition and sperm storage in *Drosophila*. *Current*
 602 *Biology* **9** 841–844.
- 603 **Clark AG, Begun DJ and Prout T** (1999) Female \times male interactions in *Drosophila* sperm
 604 competition. *Science* **389** 217–220.
- 605 **Cotton S, Fowler K and Pomiankowski A** (2004) Do sexual ornaments demonstrate heightened
 606 condition-dependent expression as predicted by the handicap hypothesis? *Proceedings of the*
 607 *Royal Society B* **271** 771–783.
- 608 **Curtsinger JW** (1991) Sperm competition and the evolution of multiple mating. *American Naturalist*
 609 **138** 93–102.
- 610 **Dallai R** (1979) An overview of atypical spermatozoa in insects. In *The Spermatozoon*, pp 253–265.
 611 Eds DW Fawcett and JM Bedford. Baltimore: Urban and Schwarzenberg.
- 612 **Dallai R, Lupetti P and Mencarelli C** (2006) Unusual axonemes of hexapod spermatozoa.
 613 *International Review of Cytology* **254** 45–99.
- 614 **Darwin C** (1871) *The Descent of Man and Selection in Relation to Sex*. London: John Murray.

- 615 **Dewsbury DA** (1982) Ejaculate cost and male choice. *American Naturalist* **119** 601–610.
- 616 **Dorus S and Karr TL** (2009) Sperm proteomics and genomics. In *Sperm Biology: An Evolutionary*
 617 *Perspective*, pp 435–469. Eds TR Birkhead, DJ Hosken and S Pitnick. San Diego: Academic
 618 Press.
- 619 **Droge-Young EM, Belote JM, Perez GS and Pitnick S** (2016) Resolving mechanisms of short-term
 620 competitive fertilization success in the red flour beetle. *Journal of Insect Physiology* **93–94** 1–
 621 10.
- 622 **Dybas LK and Dybas HS** (1981) Coadaptation and taxonomic differentiation of sperm and
 623 spermathecae in featherwing beetles. *Evolution* **35** 168–174.
- 624 **Eberhard WG** (1996) *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton, New
 625 Jersey: Princeton University Press.
- 626 **Eberhard WG** (2000) Criteria for demonstrating postcopulatory female choice. *Evolution* **54** 1047–
 627 1050.
- 628 **Emlen DJ** (2001) Costs and the diversification of exaggerated animal structures. *Science* **291** 1534–
 629 1536.
- 630 **Evans JP and Marshall DJ** (2005) Male-by-female interactions influence fertilization success and
 631 mediate the benefits of polyandry in the sea urchin *Heliocidaris erythrogramma*. *Evolution* **59**
 632 106–112.
- 633 **Evans JP and Sherman CDH** (2013) Sexual selection and the evolution of egg-sperm interactions in
 634 broadcast-spawning invertebrates. *Biological Bulletin* **224** 166–183.
- 635 **Findlay GD, Sitnik JL, Wang W, Aquadro CF, Clark NL and Wolfner MF** (2014) Evolutionary
 636 rate covariation identifies new members of a protein network required for *Drosophila*
 637 *melanogaster* female post-mating responses. *PLoS Genetics* **10** e1004108.
- 638 **Firman RC, Gasparini C, Manier MK and Pizzari T** (2017) Postmating female control: 20 years of
 639 cryptic female choice. *Trends in Ecology and Evolution* **32** 368–382.

- 640 **Fitzpatrick JL and Lüpold S** (2014) Sexual selection and the evolution of sperm quality. *Molecular*
 641 *Human Reproduction* **20** 1180–1189.
- 642 **Fitzpatrick JL, Montgomerie R, Desjardins JK, Stiver KA, Kolm N and Balshine S** (2009)
 643 Female promiscuity promotes the evolution of faster sperm in cichlid fishes. *Proceedings of the*
 644 *National Academy of Sciences of the United States of America* **106** 1128–1132.
- 645 **Fujinoki M, Takei GL and Kon H** (2016) Non-genomic regulation and disruption of spermatozoal
 646 in vitro hyperactivation by oviductal hormones. *Journal of Physiological Sciences* **66** 207–212.
- 647 **Gage MJG** (1994) Associations between body size, mating pattern, testis size and sperm lengths
 648 across butterflies. *Proceedings of the Royal Society B* **258** 247–254.
- 649 **Gage MJG and Cook PA** (1994) Sperm size or numbers? Effects of nutritional stress upon eupyrene
 650 and apyrene sperm production strategies in the moth *Plodia interpunctella* (Lepidoptera:
 651 Pyralidae). *Functional Ecology* **8** 594–599.
- 652 **Gilbert SF and Barresi MJ** (2016) *Developmental Biology*. Sunderland, MA: Sinauer Associates.
- 653 **Gomendio M and Roldan ERS** (1993) Mechanisms of sperm competition: linking physiology and
 654 behavioural ecology. *Trends in Ecology & Evolution* **8** 95–100.
- 655 **Gomendio M and Roldan ERS** (2008) Implications of diversity in sperm size and function for sperm
 656 competition and fertility. *International Journal of Developmental Biology* **52** 439–447.
- 657 **Grafen A** (1990) Sexual selection unhandicapped by the Fisher process. *Journal of Theoretical*
 658 *Biology* **144** 517–546.
- 659 **Hellriegel B and Bernasconi G** (2000) Female-mediated differential sperm storage in a fly with
 660 complex spermathecae, *Scatophaga stercoraria*. *Animal Behaviour* **59** 311–317.
- 661 **Hellriegel B and Ward PI** (1998) Complex female reproductive tract morphology: Its possible use in
 662 postcopulatory female choice. *Journal of Theoretical Biology* **190** 179–186.
- 663 **Higginson DM and Pitnick S** (2011) Evolution of intra-ejaculate sperm interactions: Do sperm
 664 cooperate? *Biological Reviews* **86** 249–270.

- 665 **Higginson DM, Miller KB, Segraves KA and Pitnick S** (2012) Female reproductive tract form
 666 drives the evolution of complex sperm morphology. *Proceedings of the National Academy of*
 667 *Sciences of the United States of America* **109** 4538–4543.
- 668 **Hill GE** (2011) Condition-dependent traits as signals of the functionality of vital cellular processes.
 669 *Ecology Letters* **14** 625–634.
- 670 **Holt W V and Fazeli A** (2016) Sperm selection in the female mammalian reproductive tract. Focus
 671 on the oviduct: Hypotheses, mechanisms, and new opportunities. *Theriogenology* **85** 105–112.
- 672 **Hotzy C, Polak M, Rönn JL and Arnqvist G** (2012) Phenotypic engineering unveils the function of
 673 genital morphology. *Current Biology* **22** 2258–2261.
- 674 **Houle D** (1998) How should we explain variation in the genetic variance of traits? *Genetica* **102/103**
 675 241–253.
- 676 **Hunt J, Breuker CJ, Sadowski JA and Moore AJ** (2009) Male-male competition, female mate
 677 choice and their interaction: Determining total sexual selection. *Journal of Evolutionary Biology*
 678 **22** 13–26.
- 679 **Immler S, Saint-Jalme M, Lesobre L, Sorci G, Roman Y and Birkhead TR** (2007) The evolution
 680 of sperm morphometry in pheasants. *Journal of Evolutionary Biology* **20** 1008–1014.
- 681 **Immler S, Pitnick S, Parker GA, Durrant KL, Lüpold S, Calhim S and Birkhead TR** (2011)
 682 Resolving variation in the reproductive tradeoff between sperm size and number. *Proceedings of*
 683 *the National Academy of Sciences of the United States of America* **108** 5325–5330.
- 684 **Iwasa Y, Pomiankowski A and Nee S** (1991) The evolution of costly mate preferences. II. The
 685 handicap principle. *Evolution* **45** 1431–1442.
- 686 **Jamieson BGM** (1987) *The Ultrastructure and Phylogeny of Insect Spermatozoa*. Cambridge:
 687 Cambridge University Press.
- 688 **Jamieson BGM** (1991) *Fish Evolution and Systematics: Evidence from Spermatozoa*. Cambridge:
 689 Cambridge University Press.

- 690 **Jennions MD and Petrie M** (1997) Variation in mate choice and mating preferences: a review of
 691 causes and consequences. *Biological Reviews* **72** 283–327.
- 692 **Kahl AF and Cox RM** (2015) Diet affects ejaculate traits in a lizard with condition-dependent
 693 fertilization success. *Behavioral Biology* **26** 1502–1511.
- 694 **Kaldun B and Otti O** (2016) Condition-dependent ejaculate production affects male mating behavior
 695 in the common bedbug *Cimex lectularius*. *Ecology and Evolution* **6** 2548–2558.
- 696 **Karr TL, Swanson WJ and Snook RR** (2009) The evolutionary significance of variation in sperm–
 697 egg interactions. In *Sperm Biology: An Evolutionary Perspective*, pp 305–365. San Diego:
 698 Academic Press.
- 699 **Katz DF** (1983) The evolution of mammalian sperm motility in the male and female reproductive. In
 700 *The Sperm Cell*, pp 340–344. Ed J André. The Hague: Marinus Nijhoff Publishers.
- 701 **Katz DF** (1991) Characteristics of sperm motility. *Annals of the New York Academy of Sciences* **637**
 702 409–423.
- 703 **Keller L and Reeve HK** (1995) Why do females mate with multiple males? The sexually selected
 704 sperm hypothesis. *Advances in the Study of Behavior* **24** 291–315.
- 705 **Kim K-W, Bennison C, Hemmings N, Brookes L, Hurley LL, Griffith SC, Burke T, Birkhead**
 706 **TR and Slate J** (2017) A sex-linked supergene controls sperm morphology and swimming
 707 speed in a songbird. *Nature Ecology & Evolution* **1** 1168–1176.
- 708 **King LM, Brillard JP, Garrett WM, Bakst MR and Donoghue AM** (2002) Segregation of
 709 spermatozoa within sperm storage tubules of fowl and turkey hens. *Reproduction* **123** 79–86.
- 710 **Kirkpatrick M and Ryan MJ** (1991) The evolution of mating preferences and the paradox of the
 711 lek. *Nature* **350** 33–38.
- 712 **Kirkpatrick M, Rand AS and Ryan MJ** (2006) Mate choice rules in animals. *Animal Behaviour* **71**
 713 1215–1225.
- 714 **Kleven O, Fossøy F, Laskemoen T, Robertson RJ, Rudolfson G and Lifjeld JT** (2009)

- 715 Comparative evidence for the evolution of sperm swimming speed by sperm competition and
716 female sperm storage duration in passerine birds. *Evolution* **63** 2466–2473.
- 717 **LaMunyon CW and Ward S** (1998) Larger sperm outcompete smaller sperm in the nematode
718 *Caenorhabditis elegans*. *Proceedings of the Royal Society B* **265** 1997–2002.
- 719 **LaMunyon CW and Ward S** (1999) Evolution of sperm size in nematodes: sperm competition
720 favours larger sperm. *Proceedings of the Royal Society B* **266** 263–267.
- 721 **Lande R** (1980) Sexual dimorphism, sexual selection, and adaptation in polygenic characters.
722 *Evolution* **34** 292–305.
- 723 **Leonard JL and Córdoba-Aguilar A** (2010) *The Evolution of Primary Sexual Characters in*
724 *Animals*. Princeton, NJ: Oxford University Press.
- 725 **Lessells CM and Birkhead TR** (1990) Mechanisms of sperm competition in birds: mathematical
726 models. *Behavioral Ecology and Sociobiology* **27** 325–337.
- 727 **Lewis SM and Austad SN** (1990) Sources of intraspecific variation in sperm precedence in red flour
728 beetles. *American Naturalist* **135** 351–359.
- 729 **Liao WB, Huang Y, Zeng Y, Zhong MJ, Luo Y and Lüpold S** (2018) Ejaculate evolution in
730 external fertilizers: Influenced by sperm competition or sperm limitation? *Evolution* **72** 4–17.
- 731 **Lüpold S and Fitzpatrick JL** (2015) Sperm number trumps sperm size in mammalian ejaculate
732 evolution. *Proceedings of the Royal Society B* **282** 20152122.
- 733 **Lüpold S, Calhim S, Immler S and Birkhead TR** (2009a) Sperm morphology and sperm velocity in
734 passerine birds. *Proceedings of the Royal Society B* **276** 1175–1181.
- 735 **Lüpold S, Linz GM and Birkhead TR** (2009b) Sperm design and variation in the New World
736 blackbirds (Icteridae). *Behavioral Ecology and Sociobiology* **63** 899–909.
- 737 **Lüpold S, Linz GM, Rivers JW, Westneat DF and Birkhead TR** (2009c) Sperm competition
738 selects beyond relative testes size in birds. *Evolution* **63** 391–402.
- 739 **Lüpold S, Manier MK, Berben KS, Smith KJ, Daley BD, Buckley SH, Belote JM and Pitnick S**

- 740 (2012) How multivariate ejaculate traits determine competitive fertilization success in
 741 *Drosophila melanogaster*. *Current Biology* **22** 1667–1672.
- 742 **Lüpold S, Pitnick S, Berben KS, Blengini CS, Belote JM and Manier MK** (2013) Female
 743 mediation of competitive fertilization success in *Drosophila melanogaster*. *Proceedings of the*
 744 *National Academy of Sciences of the United States of America* **110** 10693–10698.
- 745 **Lüpold S, Manier MK, Puniamoorthy N, Schoff C, Starmer WT, Luepold SHB, Belote JM and**
 746 **Pitnick S** (2016) How sexual selection can drive the evolution of costly sperm ornamentation.
 747 *Nature* **533** 535–538.
- 748 **Lymbery RA, Kennington WJ and Evans JP** (2016) Fluorescent sperm offer a method for tracking
 749 the real-time success of ejaculates when they compete to fertilise eggs. *Scientific Reports* **6** 1–8.
- 750 **Manier MK, Belote JM, Berben KS, Novikov D, Stuart WT and Pitnick S** (2010) Resolving
 751 mechanisms of competitive fertilization success in *Drosophila melanogaster*. *Science* **328** 354–
 752 357.
- 753 **Manier MK, Belote JM, Berben KS, Lüpold S, Ala-Honkola O, Collins WF and Pitnick S**
 754 (2013a) Rapid diversification of sperm precedence traits and processes among three sibling
 755 *Drosophila* species. *Evolution* **67** 2348–2362.
- 756 **Manier MK, Lüpold S, Belote JM, Starmer WT, Berben KS, Ala-Honkola O, Collins WF and**
 757 **Pitnick S** (2013b) Postcopulatory sexual selection generates speciation phenotypes in
 758 *Drosophila*. *Current Biology* **23** 1853–1862.
- 759 **Marie-Orleach L, Janicke T, Vizoso DB, Eichmann M and Schärer L** (2014) Fluorescent sperm in
 760 a transparent worm: validation of a GFP marker to study sexual selection. *BMC Evolutionary*
 761 *Biology* **14** 148.
- 762 **Martin PA, Reimers TJ, Lodge JR and Dziuk PJ** (1974) Effect of ratios and numbers of
 763 spermatozoa mixed from two males on proportions of offspring. *Journal of Reproduction and*
 764 *Fertility* **39** 251–258.

- 765 **Mautz BS, Møller AP and Jennions MD** (2013) Do male secondary sexual characters signal
 766 ejaculate quality? A meta-analysis. *Biological Reviews* **88** 669–682.
- 767 **McLeod D V and Day T** (2017) Female plasticity tends to reduce sexual conflict. *Nature Ecology &*
 768 *Evolution* **1** 54.
- 769 **Miller GT and Pitnick S** (2002) Sperm-female coevolution in *Drosophila*. *Science* **298** 1230–1233.
- 770 **Miller GT and Pitnick S** (2003) Functional significance of seminal receptacle length in *Drosophila*
 771 *melanogaster*. *Journal of Evolutionary Biology* **16** 114–126.
- 772 **Minder AM, Hosken DJ and Ward PI** (2005) Co-evolution of male and female reproductive
 773 characters across the Scathophagidae (Diptera). *Journal of Evolutionary Biology* **18** 60–69.
- 774 **Morrow EH and Gage MJG** (2000) The evolution of sperm length in moths. *Proceedings of the*
 775 *Royal Society B* **267** 307–313.
- 776 **Nilsson T, Fricke C and Arnqvist G** (2003) The effects of male and female genotype on variance in
 777 male fertilization success in the red flour beetle (*Tribolium castaneum*). *Behavioral Ecology and*
 778 *Sociobiology* **53** 227–233.
- 779 **Oliver JHJ** (1982) Tick reproduction: Sperm development and cytogenetics. In *Physiology of Ticks*,
 780 pp 245–275. Eds FD Obenchain and R Galun. Oxford: Pergamon Press.
- 781 **Olsson M, Madsen T and Shine R** (1997) Is sperm really so cheap? Costs of reproduction in male
 782 adders, *Vipera berus*. *Proceedings of the Royal Society B* **264** 455–459.
- 783 **Orr TJ and Brennan PLR** (2015) Sperm storage: Distinguishing selective processes and evaluating
 784 criteria. *Trends in Ecology and Evolution* **30** 261–272.
- 785 **Parker GA** (1970) Sperm competition and its evolutionary consequences in the insects. *Biological*
 786 *Reviews* **45** 526–567.
- 787 **Parker GA** (1979) Sexual selection and sexual conflict. In *Sexual Selection and Reproductive*
 788 *Competition in Insects*, pp 123–166. Eds MS Blum and NA Blum. New York: Academic Press.
- 789 **Parker GA** (1982) Why are there so many tiny sperm? Sperm competition and the maintenance of

- 790 two sexes. *Journal of Theoretical Biology* **96** 281–294.
- 791 **Parker GA** (1984) Sperm competition and the evolution of animal mating systems. In *Sperm*
 792 *Competition and the Evolution of Animal Mating Systems*, pp 1–60. Ed RL Smith. San Diego:
 793 Academic Press.
- 794 **Parker GA** (1993) Sperm competition games: sperm size and sperm number under adult control.
 795 *Proceedings of the Royal Society B* **253** 245–254.
- 796 **Parker GA** (2006) Sexual conflict over mating and fertilization: an overview. *Philosophical*
 797 *Transactions of the Royal Society of London B* **361** 235–259.
- 798 **Parker GA** (2014) The sexual cascade and the rise of pre-ejaculatory (Darwinian) sexual selection,
 799 sex roles, and sexual conflict. *Cold Spring Harbor Perspectives in Biology* **6** a017509.
- 800 **Parker GA and Pizzari T** (2010) Sperm competition and ejaculate economics. *Biological Reviews*
 801 **85** 897–934.
- 802 **Parker GA, Baker RR and Smith VG** (1972) The origin and evolution of gamete dimorphism and
 803 the male-female phenomenon. *Journal of Theoretical Biology* **36** 529–553.
- 804 **Parker GA, Immler S, Pitnick S and Birkhead TR** (2010) Sperm competition games: Sperm size
 805 (mass) and number under raffle and displacement, and the evolution of P_2 . *Journal of*
 806 *Theoretical Biology* **264** 1003–1023.
- 807 **Partridge L, Hoffmann A and Jones JS** (1987) Male size and mating success in *Drosophila*
 808 *melanogaster* and *D. pseudoobscura* under field conditions. *Animal Behaviour* **35** 468–476.
- 809 **Pattarini JM, Starmer WT, Bjork A and Pitnick S** (2006) Mechanisms underlying the sperm
 810 quality advantage in *Drosophila melanogaster*. *Evolution* **60** 2064–2080.
- 811 **Peretti A V and Eberhard WG** (2010) Cryptic female choice via sperm dumping favours male
 812 copulatory courtship in a spider. *Journal of Evolutionary Biology* **23** 271–2781.
- 813 **Perry JC and Rowe L** (2010) Condition-dependent ejaculate size and composition in a ladybird
 814 beetle. *Proceedings of the Royal Society B* **277** 3639–3647.

- 815 **Phillips DM** (1966) Fine structure of *Sciara coprophila* sperm. *Journal of Cell Biology* **30** 499–517.
- 816 **Pitnick S** (1996) Investment in testes and the cost of making long sperm in *Drosophila*. *American*
817 *Naturalist* **148** 57–80.
- 818 **Pitnick S and Brown WD** (2000) Criteria for demonstrating female sperm choice. *Evolution* **54**
819 1052–1056.
- 820 **Pitnick S, Spicer GS and Markow TA** (1995) How long is a giant sperm? *Nature* **375** 109.
- 821 **Pitnick SS, Markow TA and Spicer GS** (1999) Evolution of multiple kinds of female sperm-storage
822 organs in *Drosophila*. *Evolution* **53** 1804–1822.
- 823 **Pitnick S, Hosken DJ and Birkhead TR** (2009a) Sperm morphological diversity. In *Sperm Biology:*
824 *An Evolutionary Perspective*, pp 69–149. Eds TR Birkhead, DJ Hosken and S Pitnick. San
825 Diego: Academic Press.
- 826 **Pitnick S, Wolfner MF and Suarez SS** (2009b) Ejaculate-female and sperm-female interactions. In
827 *Sperm Biology: An Evolutionary Perspective*, pp 247–304. Eds TR Birkhead, DJ Hosken and S
828 Pitnick. San Diego: Academic Press.
- 829 **Pizzari T and Birkhead TR** (2000) Female feral fowl eject sperm of subdominant males. *Nature* **405**
830 787–789.
- 831 **Pizzari T and Parker GA** (2009) Sperm competition and sperm phenotype. In *Sperm Biology: An*
832 *Evolutionary Perspective*, pp 207–245. Eds TR Birkhead, DJ Hosken and S Pitnick. San Diego:
833 Academic Press.
- 834 **Poiani A** (2006) Complexity of seminal fluid: a review. *Behavioral Ecology and Sociobiology* **60**
835 289–310.
- 836 **Presgraves DC, Baker RH and Wilkinson GS** (1999) Coevolution of sperm and female
837 reproductive tract morphology in stalk-eyed flies. *Proceedings of the Royal Society B* **266** 1041–
838 1047.
- 839 **Puniamoorthy N, Kotrba M and Meier R** (2010) Unlocking the ‘Black box’: internal female

- 840 genitalia in Sepsidae (Diptera) evolve fast and are species-specific. *BMC Evolutionary Biology*
 841 **10** 275.
- 842 **Rahman MM, Kelley JL and Evans JP** (2013) Condition-dependent expression of pre- and
 843 postcopulatory sexual traits in guppies. *Ecology and Evolution* **3** 2197–2213.
- 844 **Ravi Ram K and Wolfner MF** (2007) Seminal influences: *Drosophila* Acps and the molecular
 845 interplay between males and females during reproduction. *Integrative and Comparative Biology*
 846 **47** 427–445.
- 847 **Reinhardt K, Dobler R and Abbott J** (2015) An ecology of sperm: sperm diversification by natural
 848 selection. *Annual Review of Ecology, Evolution, and Systematics* **46** 435–459.
- 849 **Rosengrave P, Gemmell NJ, Metcalf V, McBride K and Montgomerie R** (2008) A mechanism for
 850 cryptic female choice in chinook salmon. *Behavioral Ecology* **19** 1179–1185.
- 851 **Rowe L and Houle D** (1996) The lek paradox and the capture of genetic variance by condition
 852 dependent traits. *Proceedings of the Royal Society B* **263** 1415–1421.
- 853 **Rugman-Jones PF and Eady PE** (2008) Co-evolution of male and female reproductive traits across
 854 the Bruchidae (Coleoptera). *Functional Ecology* **22** 880–886.
- 855 **Ryan MJ** (1990) Sexual selection, sensory systems and sensory exploitation. *Oxford Surveys in*
 856 *Evolutionary Biology* **7** 157–195.
- 857 **Sasanami T, Matsuzaki M, Mizushima S and Hiyama G** (2013) Sperm storage in the female
 858 reproductive tract in birds. *Journal of Reproduction and Development* **59** 334–338.
- 859 **Schärer L, Joss G and Sandner P** (2004) Mating behaviour of the marine turbellarian *Macrostomum*
 860 *sp.*: these worms suck. *Marine Biology* **145** 373–380.
- 861 **Schärer L, Littlewood DTJ, Waeschenbach A, Yoshida W and Vizoso DB** (2011) Mating
 862 behavior and the evolution of sperm design. *Proceedings of the National Academy of Sciences*
 863 **108** 1490–1495.
- 864 **Schnakenberg SL, Siegal ML and Bloch Qazi MC** (2012) Oh, the places they'll go: Female sperm

- 865 storage and sperm precedence in *Drosophila melanogaster*. *Spermatogenesis* **2** 224–235.
- 866 **Sheldon BC** (1994) Male phenotype, fertility, and the pursuit of extra-pair copulations by female
867 birds. *Proceedings of the Royal Society B* **257** 25–30.
- 868 **Simmons LW and Fitzpatrick JL** (2012) Sperm wars and the evolution of male fertility.
869 *Reproduction* **144** 519–534.
- 870 **Simmons LW and Moore AJ** (2009) Evolutionary quantitative genetics of sperm. In *Sperm Biology:*
871 *An Evolutionary Perspective*, pp 405–434. Eds TR Birkhead, DJ Hosken and S Pitnick. San
872 Diego: Academic Press.
- 873 **Simmons LW and Siva-Jothy MT** (1998) Sperm competition in insects: Mechanisms and the
874 potential for selection. In *Sperm Competition and Sexual Selection*, pp 341–434. Eds TR
875 Birkhead and AP Møller. London: Academic Press.
- 876 **Simmons LW, Roberts JD and Dziminski MA** (2009) Egg jelly influences sperm motility in the
877 externally fertilizing frog, *Crinia georgiana*. *Journal of Evolutionary Biology* **22** 225–229.
- 878 **Simpson JL, Humphries S, Evans JP, Simmons LW and Fitzpatrick JL** (2014) Relationships
879 between sperm length and speed differ among three internally and three externally fertilizing
880 species. *Evolution* **68** 92–104.
- 881 **Sivinski J** (1984) Sperm in competition. In *Sperm Competition and the Evolution of Animal Mating*
882 *Systems*, pp 85–115. Ed RL Smith. New York: Academic Press.
- 883 **Snook RR** (2005) Sperm in competition: not playing by the numbers. *Trends in Ecology & Evolution*
884 **20** 46–53.
- 885 **Snook RR and Hosken DJ** (2004) Sperm death and dumping in *Drosophila*. *Nature* **428** 939–941.
- 886 **Stival, Puga Molina LC, Paudel B, Buffone MG, Visconti PE and Krapf D** (2016) Sperm
887 capacitation and acrosome reaction in mammalian sperm. *Advances in Anatomy, Embryology*
888 *and Cell Biology* **220** 93–106.
- 889 **Stockley P, Gage MJG, Parker GA and Møller AP** (1996) Female reproductive biology and the

- 890 coevolution of ejaculate characteristics in fish. *Proceedings of the Royal Society of London B*
 891 **263** 451–458.
- 892 **Stockley P, Gage MJG, Parker GA and Møller AP** (1997) Sperm competition in fishes: the
 893 evolution of testis size and ejaculate characteristics. *American Naturalist* **149** 933–954.
- 894 **Suarez SS and Ho HC** (2003) Hyperactivation of mammalian sperm. *Cellular and Molecular*
 895 *Biology* **49** 351–356.
- 896 **Suarez SS and Pacey AA** (2006) Sperm transport in the female reproductive tract. *Human*
 897 *Reproduction Update* **12** 23–37.
- 898 **Temkin MH and Bortolami SB** (2004) Waveform dynamics of spermatzeugmata during the
 899 transfer from paternal to maternal individuals of *Membranipora membranacea*. *Biological*
 900 *Bulletin* **206** 35–45.
- 901 **Thomsen R, Soltis J, Matsubara M, Matsubayashi K, Onuma M and Takenaka O** (2006) How
 902 costly are ejaculates for Japanese macaques? *Primates* **47** 272–274.
- 903 **Thornhill R** (1983) Cryptic female choice and its implications in the scorpionfly *Harpobittacus*
 904 *nigriceps*. *American Naturalist* **122** 765–788.
- 905 **Ting JJ, Woodruff GC, Leung G, Shin N-R, Cutter AD and Haag ES** (2014) Intense sperm-
 906 mediated sexual conflict promotes reproductive isolation in *Caenorhabditis* nematodes. *PLoS*
 907 *bBiology* **12** e1001915.
- 908 **Tobias JA, Montgomerie R and Lyon BE** (2012) The evolution of female ornaments and weaponry:
 909 social selection, sexual selection and ecological competition. *Philosophical Transactions of the*
 910 *Royal Society of London B* **367** 2274–2293.
- 911 **Tomkins JL, Radwan J, Kotiaho JS and Tregenza T** (2004) Genic capture and resolving the lek
 912 paradox. *Trends in Ecology & Evolution* **19** 323–328.
- 913 **Tourmente M, Gomendio M and Roldan ERS** (2011) Mass-specific metabolic rate and sperm
 914 competition determine sperm size in marsupial mammals. *PLoS ONE* **6** e21244.

- 915 **Trivers RL** (1972) Parental investment and sexual selection. In *Sexual Selection and the Descent of*
 916 *Man 1871-1971*, pp 136–179. Ed B Campbell. Chicago, IL: Aldine-Atherton.
- 917 **Turner E and Montgomerie R** (2002) Ovarian fluid enhances sperm movement in Arctic charr.
 918 *Journal of Fish Biology* **60** 1570–1579.
- 919 **Waage JK** (1979) Dual function of the damselfly penis: sperm removal and transfer. *Science* **203**
 920 916–918.
- 921 **Wade MJ and Arnold SJ** (1980) The intensity of sexual selection in relation to male sexual
 922 behaviour, female choice, and sperm precedence. *Animal Behaviour* **28** 446–461.
- 923 **Wagner RH, Helfenstein F and Danchin E** (2004) Female choice of young sperm in a genetically
 924 monogamous bird. *Proceedings of the Royal Society B* **271** S134–S137.
- 925 **Wiley RH and Poston J** (1996) Indirect mate choice, competition for mates, and coevolution of the
 926 sexes. *Evolution* **50** 1371–1381.
- 927 **Wilson N, Tubman SC, Eady PE and Robertson GW** (1997) Female genotype affects male success
 928 in sperm competition. *Proceedings of the Royal Society B* **264** 1491–1495.
- 929 **Wolfner MF** (2011) Precious essences: female secretions promote sperm storage in *Drosophila*. *PLoS*
 930 *Biology* **9** e1001191.
- 931 **Woolley DM** (2003) Motility of spermatozoa at surfaces. *Reproduction* **126** 259–270.
- 932 **Yasui Y** (1997) A ‘good-sperm’ model can explain the evolution of multiple mating by females.
 933 *American Naturalist* **149** 573–584.
- 934 **Yeates SE, Diamond SE, Einum S, Emerson BC, Holt W V and Gage MJG** (2013) Cryptic choice
 935 of conspecific sperm controlled by the impact of ovarian fluid on sperm swimming behavior.
 936 *Evolution* **67** 3523–3536.
- 937 **Yoshida M, Kawano N and Yoshida K** (2008) Control of sperm motility and fertility: Diverse
 938 factors and common mechanisms. *Cellular and Molecular Life Sciences* **65** 3446–3457.
- 939 **Yoshida M, Hiradate Y, Sensui N, Cosson J and Morisawa M** (2013) Species-specificity of sperm

940 motility activation and chemotaxis: A study on ascidian species. *Biological Bulletin* **224** 156–
941 165.

942 **Zhang L, Yang P, Bian X, Zhang Q, Ullah S, Waqas Y, Chen X, Liu Y, Chen W, Le Y *et al.***
943 (2015) Modification of sperm morphology during long-term sperm storage in the reproductive
944 tract of the Chinese soft-shelled turtle, *Pelodiscus sinensis*. *Scientific Reports* **5** 16096.

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Box 1: Evolution of sperm length in *Drosophila* fruit flies

An example of how the interplay between sperm competition and cryptic female choice may drive the evolution of exaggerated sperm phenotypes comes from *Drosophila* fruit flies (Fig. 3), with *D. bifurca* producing by far the longest sperm in any species examined to date (58.3 mm or approximately 20-fold body length; Pitnick *et al.*, 1995; Figs. 2 and 3). In *D. melanogaster*, the length of the primary female sperm-storage organ, the seminal receptacle (SR), has been shown to play an important role in biasing competitive fertilization: longer SRs enhance the advantage for relatively long sperm in the process of sperm displacement, when sperm of the last male enter the SR and displace resident sperm from it (Miller and Pitnick, 2002; Lüpold *et al.*, 2012). The female effect on sperm storage is mediated primarily by variation in the timing of ejecting a mass containing displaced resident sperm and excess last-male sperm, a process that itself is genetically correlated with SR length (Lüpold *et al.*, 2013, 2016). Comparative studies, however, have revealed that selection for longer sperm enhances the evolutionary trade-off between sperm size and number (Pitnick, 1996; Immler *et al.*, 2011), thereby reducing the number of sperm available to fertilize eggs (Bjork and Pitnick, 2006). Consequently, as sperm length evolutionarily increases, females remate faster to replenish sperm reserves. In fact, SR length again is genetically correlated with both sperm length and faster female remating (Lüpold *et al.*, 2016). Each mating event creates an opportunity for sexual selection both before and after mating. Larger males have a higher mating success (e.g., Partridge *et al.*, 1987), and females gain genetic benefits for their offspring by mating with larger, healthier males. Since body size but not sperm length is condition-dependent, larger males also pay a relatively lower price per sperm, which allows them to produce more sperm and become less susceptible to sperm depletion than smaller males (Lüpold *et al.*, 2016). Consequently, these larger males are best able to capitalize on the heightened mating opportunities, and the genes associated with their longer sperm are more likely to spread in the population, thus feeding the cycle of gradual sperm length exaggeration.

(Insert Figure 3 here)

Figure legends

Figure 1: Sperm morphological diversity. Scanning electron micrographs of: A *Macrobiotus cf. hufelandi* (Tardigrada: Macrobiotidae; L. Rebecchi, U. of Modena e Reggio Emilia), B *Caenorhabditis elegans* (Nematoda: Rhabditida; T. Roberts, Florida State U.), C *Mytilocypris mytiloides* (Crustacea: Ostracoda – posterior end of long, filiform sperm; R. Matzke-Karasz, Ludwig Maximilian U. of Munich), D *Drosophila bifurca* (Insecta: Drosophilidae; R. Dallai, U. of Siena), E *Patinopecten yessoensis* (Mollusca: Ostreoida; from Li *et al.*, 2000), F *Iporangaia pustulosa* (Arachnida: Opiliones; from Moya *et al.*, 2007), G *Trialeurodes vaporariorum* (Insecta: Aleyrodidae; R. Dallai), H *Allacma fusca* (Hexapoda: Sminthuridae; from Dallai *et al.*, 2009), I *Colostethus marchesianus* (Anura: Aromobatidae; from Veiga-Menoncello *et al.*, 2007), J *Paralichthys olivaceus* (Actinopterygii: Paralichthyidae; from Zhang *et al.*, 2003), K *Gopherus agassizii* (Reptilia: Testudinata; L. Liaw, Beckman Laser Institute at U. California Irvine), L *Passer domesticus* (Aves: Passeridae; R. Dallai), M *Phataginus tricuspi* (Pholidota: Manidae; L. Liaw), N *Uromys caudimaculatus* (Rodentia: Muridae; W. Breed, U. of Adelaide). All published photos reprinted with permission from Elsevier; all unpublished photos courtesy of authors in parentheses.

Figure 2: Variation in sperm length across the animal kingdom (in μm on a logarithmic scale). Each horizontal line spans the range of sperm lengths reported in the literature for each taxon. Line colors depict different fertilization modes (light gray: external fertilization, from broadcast spawning in marine invertebrates to female-directed sperm release in frogs; dark gray: spermcasting; black: internal fertilization). Dotted lines indicate considerable extension of the sperm length range by inclusion of an extreme outlier (e.g., *Neoceratodus forsteri* in the externally fertilizing bony fishes and *Discoglossus pictus* in the frogs). Data were retrieved from Pitnick *et al.* (2009a).

Figure 3: Schematic illustration of a likely process of postcopulatory sexual selection on sperm length in *Drosophila*, involving aspect so of both sperm competition and cryptic female choice. SS = sexual selection at both pre- and postmating stages.





