Sperm wars and the evolution of male fertility

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Abstract

Females frequently mate with several males, whose sperm then compete to fertilize available ova. Sperm competition represents a potent selective force that is expected to shape male expenditure on the ejaculate. Here, we review empirical data that illustrate the evolutionary consequences of sperm competition. Sperm competition favors the evolution of increased testes size and sperm production. In some species, males appear capable of adjusting the number of sperm ejaculated, depending on the perceived levels of sperm competition. Selection is also expected to act on sperm form and function, although the evidence for this remains equivocal. Comparative studies suggest that sperm length and swimming speed may increase in response to selection from sperm competition. However, the mechanisms driving this pattern remain unclear. Evidence that sperm length influences sperm swimming speed is mixed and fertilization trials performed across a broad range of species demonstrate inconsistent relationships between sperm form and function. This ambiguity may in part reflect the important role that seminal fluid proteins (sfps) play in affecting sperm function. There is good evidence that sfps are subject to selection from sperm competition, and recent work is pointing to an ability of males to adjust their seminal fluid chemistry in response to sperm competition from rival males. We argue that future research must consider sperm and seminal fluid components of the ejaculate as a functional unity. Research at the genomic level will identify the genes that ultimately control male fertility.

Introduction

Originally, it was thought that females required very few mating partners in order to realize their maximum reproductive potential (Bateman 1948). However, research over the last few decades has shown us how mating with multiple males (polyandry) is a taxonomically widespread female reproductive strategy (Simmons 2005). Females can increase their reproductive success by accepting copulations from multiple males, via resources provided by males, insurance against male infertility, or via the opportunity that multiple inseminations provide females for choosing the best sires for their offspring (Sheldon 1994, Arnqvist & Nilsson 2000, Simmons 2005, Slatyer *et al.* 2012). While females may gain benefits from mating with multiple males, the consequence of polyandry for males is that their sperm may face competition from the sperm of other males to fertilize a limited supply of ova.

Parker (1970) recognized that sperm competition would favor the evolution of male reproductive morphology, physiology, and behavior that promotes competitive fertilization success. In the ensuing 40 years since Parker's pioneering insights, numerous game theoretical models have been developed to predict how sperm competition influences evolutionary responses in male reproductive traits (reviewed by Parker & Pizzari (2010)). In particular, because it is the ejaculate that is at the frontline of competition, extensive attention has been devoted to modeling how sperm competition influences rates of sperm production, the quality of sperm, and male expenditure on the ejaculate under various competitive scenarios. However, sperm are, but one part of the ejaculate and recent theoretical attention has been devoted to understanding how sperm competition shapes male investment in the non-sperm component of the ejaculate, the seminal fluid (Hodgson & Hosken 2006, Cameron *et al.* 2007, Alonzo & Pizzari 2010, Fromhage 2012). The aim of this review is to provide a broad overview of adaptations in male ejaculate biology that are thought to have arisen as evolutionary consequences of the war between males for gaining fertilizations. We therefore look at sperm competition from the male perspective. We briefly describe the rich theoretical framework aimed at understanding how selection acts on the ejaculate (sperm and seminal fluid) and examine the evidence for sperm competition being a significant factor in the evolution of male fertility. We show how male investment in sperm production is indeed acutely sensitive to female mating behavior, both within and among species, and reveal

how selection acts not just on sperm production but also on sperm form and function, and the protein-rich seminal fluids within which sperm are transferred to the female.

Sperm: solders in the battle for fertilization

Testes size and sperm production

Game theoretical models have been developed to predict the evolutionary response of males to the probability that females will mate with more than one male (sperm competition risk) and the number of males that a female will typically accept (sperm competition intensity) (Parker & Pizzari 2010). These models assume that sperm production is costly insofar as resources invested in sperm production are unavailable for future survival and reproduction. Risk models are designed to make predictions for low levels of sperm competition where females have a given probability of mating with just two males. Intensity models are designed for high levels of sperm competition, where typically more than two males compete. Thus, in species where females are monogamous, males are predicted to invest minimally in their ejaculates, ensuring their functional fertility while maximizing their survival for future reproductive opportunities. As the levels of sperm competition increases across species, the males of a species should be selected to increase their expenditure on the ejaculate at the cost to future reproduction, a prediction for which there is much evidence.

Increases in testes size represent one of the most well-characterized evolutionary responses to sperm competition (Birkhead & Møller 1998, Simmons 2001). Typically, males that inseminate greater numbers of sperm achieve greater fertilization success (Martin *et al*. 1974, Parker 1982). As the size of a males' testes predicts the proportion of the testes made up of sperm producing seminiferous tissue and the rate of sperm production (Willett & Ohms 1957, Parker 1982, Møller 1989, Marconato & Shapiro 1996, Scharer *et al*. 2004, Lüpold *et al*. 2009c, Ramm & Stockley 2010, Rowe & Pruett-Jones 2011), males with larger testes are expected to be competitively advantaged when engaging in sperm competition. Consequently, increases in the level of sperm competition are expected to select for increased investment in testicular tissue (Parker & Pizzari 2010).

There is now taxonomically widespread evidence that sperm production increases with the level of sperm competition, both across and within species. This empirical evidence falls into four broad categories. First, comparative studies reveal that after controlling for body size, relative testes size increases with the level of sperm competition across species (Table 1). Specifically, those species with multi-male mating systems typically have larger testes than species with monogamous mating systems (Table 1). Secondly, within species comparisons among populations demonstrate that males have relatively larger testes in populations where the level of sperm competition is elevated (Gage 1995, Parker *et al*. 1997, Firman & Simmons 2008a, Dziminski *et al*. 2010a). Thirdly, relative testes size exhibits rapid evolutionary responses to the level of sperm competition in experimental evolution studies. For example, in the yellow dung fly *Scathopaga stercoraria* (Hosken & Ward 2001), *Drosophila melanogaster* (Pitnick *et al*. 2001), and the dung beetle *Onthophagus taurus* (Simmons & García-González 2008), experimental populations where monogamous mating conditions were enforced showed decreases in relative testes size over multiple generations compared with experimental populations that evolved under polyandrous mating conditions (but see Wigby & Chapman (2004), Crudgington *et al*. (2009) and Firman & Simmons (2010b) for experimental evolution studies where changes in relative testes size in response to sperm competition were not observed). Finally, clear within species evidence for the influence of sperm competition on testes size comes from studies in insects (Simmons *et al*. 2007b) and fishes (Montgomerie & Fitzpatrick 2009) with alternative reproductive tactics. In these species, males with different phenotypes use different mating behaviors to secure fertilizations: some males fight for and court females while others engage in sneak copulations. Sneaker males, who always experience sperm competition, typically have relatively, and in some cases absolutely, larger testes than courting males, who often mate in the absence of sperm competition (reviewed by Simmons (2001) and Montgomerie & Fitzpatrick (2009)).

The accumulating evidence that relative testes size increases with the level of sperm competition has led to the widespread acceptance of the role of sperm competition in shaping testes size. So common is this view that many researchers, including the authors of this review (e.g. Fitzpatrick *et al*. (2012a, 2012b, 2012c)), commonly use relative testes size as a proxy measure for the strength of sperm competition in a given species, even in the absence of any information on a species mating behavior (Møller 1991, Briskie & Montgomerie 1992, Dunn *et al*. 2001, Pitcher *et al*. 2005, Calhim & Birkhead 2007). However, a degree of caution should be applied to accepting relative testes size as an absolute proxy for the level of sperm competition because testes can perform functions other than sperm production (Emerson 1997), and increased investment in testes mass could be favored in species where copulation occurs frequently (the male mating rate hypotheses) even in the absence of variation in the level of sperm competition (Crudgington *et al*. 2009, Vahed & Parker 2012). To clarify and quantify the role of sperm competition in shaping testes size, we feel that there would be great merit in a quantitative meta-analytical review of the relationship between relative testes size and level of sperm competition.

Table 1 Review of comparative analyses testing the prediction that increased selection from sperm competition should influence investment in testes size (correcting for body size) and commonly assessed sperm traits including sperm morphology (measured as sperm head length, midpiece length, and flagellum/total length), sperm viability, and sperm velocity. In the studies summarized, sperm competition risk is assessed using a variety of methods including data on mating systems, rates of extra-pair paternity, number of spermatophores recovered from female reproductive tracts (for some insect species), frequency of males adopting alternative reproductive tactics, female remating rates, reproductive behaviors, and sexual size dimorphism.

Taxa	Testes size	Sperm morphology			Sperm viability/velocity	References
		Head length	Mid-piece length	Flagellum/total length		
Mammals						
Mammals[a]	+, 0	+	+, 0	+, 0	+	Gage *et al.* (2002), Gage & Freckleton (2003), Anderson *et al.* (2005), Lemaître *et al.* (2009), Soulsbury (2010) and Tourmente *et al.* (2011*a*)
Bats	+	NA	NA	0	NA	Hosken (1997, 1998) and Lemaître *et al.* (2009)
Rodents	+	+	0	+, 0	+	Gomendio & Roldan (1991), Hosken (1997), Breed & Taylor (2000), Ramm *et al.* (2005), Bryja *et al.* (2008), Gomendio *et al.* (2011) and Montoto *et al.* (2011*a*, 2011*b*)
Ungulates	+	NA	NA	0	NA	Ginsberg & Rubenstein (1990), Lemaître *et al.* (2009) and Gomendio *et al.* (2011)
Carnivores	0	NA	NA	0	NA	Lemaître *et al.* (2009) and Gomendio *et al.* (2011)
Marsupials and monotremes	+	0	0	+	NA	Rose *et al.* (1997) and Tourmente *et al.* (2011*b*)
Primates	+	NA	NA	+, 0	+	Harcourt *et al.* (1981, 1995), Møller (1988), Gomendio & Roldan (1991), Kappeler (1997), Anderson & Dixon (2002), Nascimento *et al.* (2008), Lemaître *et al.* (2009) and Gomendio *et al.* (2011)
Voles	0	NA	NA	NA	NA	Heske & Ostfeld (1990)
Pinnipeds	−	NA	NA	NA	NA	Fitzpatrick *et al.* (2012*b*)
Birds						
Birds[a]	+	+	0	+, 0	NA	Møller (1991), Briskie & Montgomerie (1992), Møller & Briskie (1995), Stutchbury & Morton (1995), Garamszegi *et al.* (2005), Pitcher *et al.* (2005), Immler & Birkhead (2007) and Immler *et al.* (2011)
Finches	NA	0	+	+	NA	Immler & Birkhead (2007)
Warblers	NA	0	−	−	NA	Immler & Birkhead (2007)
Blackbirds	NA	0	+	+	+	Lüpold *et al.* (2009*a*, 2009*b*)
Wrens	+	NA	NA	NA	+	Rowe & Pruett-Jones (2011)
Sandpipers	+	NA	NA	NA	NA	Carter (1985)
Waterfowl	+	NA	NA	NA	NA	Coker *et al.* (2002)
Raptors	−	NA	NA	NA	NA	Olsen (1991)
Pheasants	NA	0	0	0	NA	Immler *et al.* (2007*b*)
Shorebirds	NA	0	0	+	NA	Johnson & Briskie (1999)
Fishes						
Fishes[a]	+	NA	NA	+, −	NA	Stockley *et al.* (1997), Petersen & Warner (1998) and Montgomerie & Fitzpatrick (2009)
Tanganyikan cichlids	+	NA	NA	+	+	Balshine *et al.* (2001) and Fitzpatrick *et al.* (2009)
Minnows	0	NA	NA	NA	NA	Pyron (2000)
Seahorses	0	NA	NA	NA	NA	Kvarnemo & Simmons (2004)
Sharks	+	NA	NA	NA	NA	Fitzpatrick *et al.* (2012*c*)
Reptiles and amphibians						
Frogs	+	+	NA	+	NA	Emerson (1997), Byrne *et al.* (2002, 2003) and Prado & Haddad (2003)
Snakes	NA	+	+	+	NA	Tourmente *et al.* (2009)
Invertebrates						
Insects[a]	NA	NA	NA	NA	+	Hunter & Birkhead (2002)
Butterflies	+	NA	NA	+	NA	Gage (1994) and Karlsson (1995, 1996)
Moths	NA	NA	NA	+	NA	Morrow & Gage (2000)
Eusocial ants and bees	+	NA	NA	0	NA	Baer & Boomsma (2004) and Fitzpatrick and Baer (2011)
Bush crickets	+	NA	NA	NA	NA	Vahed *et al.* (2011)
Beetles	+	NA	NA	NA	NA	Simmons *et al.* (2007*b*)
Fireflies	+	NA	NA	NA	NA	Demary & Lewis (2007)
Acanthocephalan worms	−	NA	NA	NA	NA	Poulin & Morand (2000)
Nematodes	NA	NA	NA	+	NA	LaMunyon and Ward (1999)
Drosophila	NA	NA	NA	+, 0	NA	Pitnick (1996), Pitcher *et al.* (2005), Holman *et al.* (2008), Joly *et al.* (2008) and Joly & Schiffer (2010)

+, −, and 0 Indicates positive, negative, or no relationship, respectively, with sperm competition risk. NA indicates that the traits have not been examined.
[a]Indicates studies with broad taxonomic coverage.

Strategic allocation of sperm

Ejaculates are costly for males to manufacture, and males can become depleted of sperm and seminal fluid reserves with successive copulations, limiting the numbers of females they can mate with (Dewsbury 1982, Nakatsuru & Kramer 1982, Birkhead & Fletcher 1995, Olsson *et al*. 1997, Sirot *et al*. 2009). Within a species, therefore, males are expected to adjust how they allocate sperm during a given mating, depending on the reproductive returns expected for their investment, which will depend on female quality and male perceptions of sperm competition levels (Wedell *et al*. 2002, Parker & Pizzari 2010). Specifically, males are expected to allocate more sperm when mating with high-quality females (to maximize genetic benefits) and previously mated females (to better contest sperm from rival males). However, within species predictions regarding male ejaculate expenditure under varying sperm competition levels are more complex. As the risk of sperm competition increases, males should increase ejaculate expenditure. However, the intensity of sperm competition is predicted to have an ameliorating effect on ejaculate expenditure, as the rate of fitness gain per unit investment in ejaculate production is expected to decline when more males are involved in the competition. Thus, male expenditure on their ejaculate is predicted to decline with increasing sperm competition intensity (Wedell *et al*. 2002, Parker & Pizzari 2010).

Extensive attention has been devoted to assessing patterns of strategic sperm allocation across a broad range of taxa (reviewed by Wedell *et al*. (2002), Montgomerie & Fitzpatrick (2009) and Parker & Pizzari (2010)), and two recent meta-analyses have synthesized this diverse literature to provide a quantitative review of the general hypothesis that males strategically allocate their ejaculates (delBarco-Trillo 2011, Kelly & Jennions 2011). These meta-analyses demonstrate that males consistently allocate more sperm to higher quality females (Kelly & Jennions 2011) and when mating in the presence of a single competitor male (when the risk of sperm competition is elevated; delBarco-Trillo 2011, Kelly & Jennions 2011). However, Kelly & Jennions (2011) found no statistical support for the less frequently studied intensity models of sperm competition and, contrary to expectations, found that males allocate greater numbers of sperm to matings with virgin females. This mismatch with theoretical expectation may lie in our ability to manipulate the appropriate cues to sperm competition intensity. For example, studies often alter the number of males in the environment and assume that they are manipulating intensity cues, but from a male's perspective the presence of multiple rivals may signal greater risk rather than greater number of males actually competing for fertilizations (Engqvist & Reinhold 2005). Studies that have manipulated a female's previous number of mating partners, and

thus the true intensity of sperm competition, provide good support for the intensity models (Thomas & Simmons 2008).

Sperm quality and male fertility

Sperm competition is also expected to influence the evolution of specific sperm traits, collectively referred to as sperm quality (Snook 2005), including sperm morphology (typically assessed using the length of the sperm head, midpiece, and flagellum), swimming speed, and viability (the proportion of live sperm in an ejaculate; Pizzari & Parker 2009). A common technique used to infer selection acting on these sperm traits is to determine how they influence fertilization success under either noncompetitive and/or competitive scenarios. Although there is mixed evidence for which sperm traits are important in determining fertilization success, we can draw some tentative conclusions from the available evidence (summarized in Table 2). First, sperm morphology exhibits an inconsistent relationship with fertilization success, with some studies demonstrating that longer sperm are competitively superior, others that shorter sperm yield greater fertilization rates, and still others that find no effect of sperm length on fertilization success (Table 2). Interestingly, in competitive fertilizations, the negative relationships between sperm length and fertilization success are more prevalent in studies using natural matings, as opposed to artificial inseminations where sperm numbers are typically controlled experimentally. This raises the possibility that in these cases, trade-offs between sperm number and size influence competitive fertilization success. Secondly, the majority of studies that have examined sperm velocity have reported a positive relationship with fertilization success under both noncompetitive and competitive conditions (Table 2). Finally, the relationship between sperm viability and fertilization success is the least well characterized of the sperm traits assessed in Table 2. However, as several studies have reported a positive relationship between sperm viability and fertilization success, particularly under competitive fertilization conditions, it seems highly likely that sperm viability is indeed an important factor influencing fertilization success.

The literature summarized in Table 2 also reveals some interesting similarities and differences between how selection might operate based on the level of sperm competition and the mode of fertilization. For example, in internal fertilizing species, sperm morphology often determines fertilization success, although the effects reported are neither consistently positive nor negative. In contrast, for externally fertilizing species, there is little evidence that sperm morphology impacts fertilization success under either noncompetitive or competitive fertilization conditions. Faster swimming sperm appear to be competitively superior under noncompetitive and

Table 2 The relationship between three commonly assessed sperm traits – sperm morphology, sperm velocity, and sperm viability – and fertilization success under i) noncompetitive and ii) competitive fertilizations. The method used in the fertilizations is classified as either natural matings (NM), where males and females mated without interference from the experimenter, or artificial inseminations (AI), which also includes IVFs, where sperm were experimentally introduced without matings taking place.

Species	Method	Sperm traits			References
		Sperm morphology	Sperm velocity	Sperm viability	
(i) Non-competitive fertilizations					
Internal fertilizers					
Domestic fowl (*Gallus domesticus*)	AI	NA	+	NA	Froman *et al.* (1999)
Red deer (*Cervus elaphus hispanicus*)	AI	+[a]	+	NA	Malo *et al.* (2005), Gomendio *et al.* (2007) and Gomendio & Roldan (2008)
Horse (*Equus ferus caballus*)	AI	+[a,b]	+	NA	Jasko *et al.* (1992) and Gravance *et al.* (1996)
Human (*Homo sapiens*)	AI	+[a]	+	NA	Marshburn *et al.* (1992), Barratt *et al.* (1993), Krause (1995), Donnelly *et al.* (1998), Shulman *et al.* (1998), Badawy *et al.* (2009) and Youn *et al.* (2011)
Rat (*Rattus* sp.)	AI	NA	+	NA	Moore & Akhondi (1996)
Boar (*Sus scrofa*)	AI	−, +[c,d]	+[c]	+[c]	Holt *et al.* (1997), Hirai *et al.* (2001) and Sutkeviciene *et al.* (2009)
Cricket (*Teleogryllus oceanicus*)	NM	NA	NA	0	García-González & Simmons (2005a)
External fertilizers					
Walleye (*Sander vitreus*)	AI	0	+	NA	Casselman *et al.* (2006)
Sea urchin (*Lytechinus variegatus*)	AI	NA	+	NA	Levitan (2000)
Sea urchin (*Anthocidaris crassispina*)	AI	NA	+	NA	Au *et al.* (2002)
Mussel (*Mytilus galloprovincialis*)	AI	−	−	NA	Fitzpatrick *et al.* (2012*d*)
Frog (*Crinia georgiana*)	AI	0	0	0	Dziminski *et al.* (2010*b*)
(ii) Competitive fertilizations					
Internal fertilizers					
Domestic fowl (*Gallus domesticus*)	AI	NA	+[e]	NA	Birkhead *et al.* (1999) and Pizzari *et al.* (2008)
Mallard (*Anas platyrhynchos*)	AI	0	+	NA	Denk *et al.* (2005)
Swordtail (*Xiphophorus helleri*)	AI	0	+	NA	Gasparini *et al.* (2010)
Swordtail (*Xiphophorus nigrensis*)	AI	NA	−[e]	+	Smith (2012)
Guppy (*Poecilia reticulata*)	AI	0	+	NA	Boschetto *et al.* (2011)
Mice (*Mus domesticus*)	NM	−	NA	NA	Firman & Simmons (2008*b*)
Dung beetle (*Onthophagus taurus*)	NM	−[f]	NA	NA	García-González & Simmons (2007*b*)
Fruit fly (*Drosophila melanogaster*)	NM	+[f]	−	NA	Miller & Pitnick (2002), Pattarini *et al.* (2006) and Lüpold *et al.* (2012)
Cricket (*Gryllus bimaculatus*)	NM	0, −	NA	NA	Morrow & Gage (2001) and Gage & Morrow (2003)
Cricket (*Teleogryllus oceanicus*)	NM	0	NA	+	Simmons *et al.* (2003) and García-González & Simmons (2005*b*)
Snail (*Viviparus ater*)	NM	+[g]	NA	NA	Oppliger *et al.* (2003)
Nematode (*Caenorhabditis elegans*)	NM	+[h]	+	NA	LaMunyon & Ward (1998)
Bulb mite (*Rhizoglyphus robini*)	NM	−[h]	NA	NA	Radwan (1996)
External fertilizers					
Atlantic salmon (*Salmo salar*)	AI	0	+	NA	Gage *et al.* (2004)
Arctic charr (*Salvelinus alpinus*)	AI	NA	+	NA	Liljedahl *et al.* (2008)
Cod (*Gadus morhua*)	AI	NA	+	NA	Skjaeraasen *et al.* (2009)
Frog (*Crinia georgiana*)	AI	0	−	0	Dziminski *et al.* (2009)

+, −, and 0 Indicates positive, negative, or no relationship, respectively, with fertilization success. NA indicates that the sperm trait has not been examined. Additional notes on the studies presented in the table include the following: [a]Demonstrated positive relationships between male fertility and the percentage of morphologically normal sperm. [b]Sperm morphology was compared between stallions known to be either fertile or subfertile. [c]Fertility measured as total litter size in multiparous females. [d]Assessed sperm head morphology, including area and length. [e]Effects are contingent on the time since insemination. [f]Female reproductive tract dimensions may influence the relationship between sperm size and competitive fertilization success. [g]Refers to length of non-fertilizing oligopyrene sperm, while fertilizing eupyrene sperm length did not influence competitive fertilization success. [h]Refers to sperm size as these species have aflagellate sperm.

competitive fertilizations in a number of species and in both internal and external fertilizing species, despite the fundamental differences in the fertilization environments (Table 2). While these results may be suggestive, they should be treated with caution, as there are few cases of the same species and sperm traits being assessed under both fertilization conditions for us to draw any firm conclusions. For example, in crickets, sperm viability was found to be a significant determinant of competitive fertilization success (García-González & Simmons 2005*b*) but not noncompetitive fertilization success (García-González & 2005*a*). Similarly, in frogs, sperm swimming speed affected competitive (Dziminski *et al.* 2009) but not noncompetitive fertilization success

(Dziminski *et al.* 2010*b*). Further investigations of sperm traits under both fertilization conditions in the same species may help to determine how selection acts on sperm traits for functional fertility (in noncompetitive fertilizations) compared with how selection operates on sperm traits during sperm competition.

Evolution of sperm size and speed

The available literature reviewed in Table 2 provides little evidence for a general pattern of selection acting on sperm size within species. Not surprisingly, therefore, extensive debate remains regarding how sperm competition influences the evolution of sperm size and the underlying relationship between sperm form and function. Sperm competition has alternatively been argued to influence sperm size in one of two ways. Under conditions where males gain a reproductive advantage by inseminating large numbers of sperm, and assuming that sperm number trade-offs against sperm size, increases in the levels of sperm competition are thought to favor the production of smaller sperm (Parker 1970, 1982). Recent comparative evidence suggests that such trade-offs between sperm size and number are more prevalent in species where fertilization follows a raffle mechanism rather than a sperm displacement mechanism (Immler *et al.* 2011). Alternatively, sperm competition has been argued to favor the evolution of larger sperm (Gomendio & Roldan 1991), provided there is a positive relationship between sperm size and speed and faster sperm are competitively superior to slower sperm. While there is some evidence demonstrating that faster sperm are competitively superior (see Table 2), resolving the empirical and theoretical relationship between sperm size and speed has proven problematic (Humphries *et al.* 2008).

There is growing evidence of a relationship between sperm size and swimming speed across species (Table 1). For example, in a series of comparative studies, Gomendio & Roldan *et al.* (1991, 2008), have repeatedly demonstrated that longer sperm swim faster among mammals (Montoto *et al.* 2011*a*, Tourmente *et al.* 2011*a*). Similarly, comparative studies in fishes (Fitzpatrick *et al.* 2009) and birds (Lüpold *et al.* 2009*a* but see Kleven *et al.* (2008)) have also demonstrated that larger sperm swim faster than smaller sperm. However, in contrast to the results from comparative studies, few within species studies have revealed a link between sperm size and speed (Humphries *et al.* 2008). Recently, Fitzpatrick *et al.* (2010) argued that a functional relationship between sperm size and speed may be masked by extensive within male variance in sperm traits and that a clear relationship between sperm size and speed was evident when assessing the covariance between these sperm traits at the level of the individual sperm cell. While further validation of Fitzpatrick *et al.*'s (2010) techniques accounting for within male variance

in sperm traits across a broad range of species is certainly required, these preliminary findings suggest that a relationship between sperm size and speed may be more prevalent than currently thought and may offer an explanation for how sperm competition operates on sperm traits.

Despite the debate regarding how selection may act on sperm traits, a growing body of evidence suggests that sperm competition plays a major role in shaping the coevolution of sperm size and speed. Comparative studies across a broad range of taxa offer three compelling lines of evidence that sperm competition influences the coevolution of sperm size and speed (reviewed in Table 1). First, comparative studies in mammals, fishes, and birds have found that species experiencing greater levels of sperm competition have faster swimming and more motile sperm than species where sperm competition is relaxed or absent (Table 1). Secondly, phylogenetic reconstructions of sperm trait evolution in a group of well-studied cichlid fishes provided solid support for the role of sperm competition in shaping ejaculate traits, by revealing that evolutionary changes in mating behavior (and thus the level of sperm competition) occurred before evolutionary changes in sperm size and speed (Fitzpatrick *et al.* 2009). Finally, the majority of comparative studies performed over the past 20 years have reported positive relationships between the level of sperm competition and both flagellum and total sperm length (Table 1). However, studies reporting either negative relationships or no influence of sperm competition on these measures have prevented a clear picture from emerging (Gomendio & Roldan 2008, Montgomerie & Fitzpatrick 2009, Pitnick *et al.* 2009*a*). Similarly, no clear patterns are evident regarding how sperm competition influences the evolution of sperm head and midpiece lengths (Table 1). Recently, theoretical and empirical work posited a potential explanation for the ambiguity surrounding evolutionary responses in sperm size by suggesting that the relationships between sperm size and sperm competition may stem from trade-offs between how selection acts on sperm size and number in response to variation in the mechanisms regulating sperm competition (Parker *et al.* 2010, Immler *et al.* 2011).

Within species studies have also revealed complex and oftentimes contradictory evolutionary responses of sperm size and speed to sperm competition. Using experimentally evolving populations to contrast sperm traits in selection lines experiencing either high or low levels of sperm competition, studies in insects have demonstrated that sperm size is not influenced by selection history (Hosken & Ward 2001, Pitnick *et al.* 2001). In mice, *Mus musculus*, sperm velocity and competitive fertilization success were increased in high sperm competition selection lines while sperm length remained unchanged (Firman & Simmons

2010*a*, 2010*b*). Similarly, in species with alternative reproductive tactics, relatively few studies have reported differences in sperm morphology between or among tactics while most studies report that sneaker males have faster swimming sperm than guarding males (Montgomerie & Fitzpatrick 2009, Pitnick *et al*. 2009*a*). Finally, intriguing evidence that sperm competition influences sperm performance and morphology comes from studies demonstrating gamete plasticity in response to changing selective environments. For example, in the Arctic charr, *Salvelinus alpinus*, and fowl, *Gallus gallus* (Rudolfsen *et al*. 2006, Cornwallis & Birkhead 2007), changes in social status resulted in changes in sperm velocity, with socially dominant males, who experience reduced levels of sperm competition, producing ejaculates in which sperm have reduced swimming speeds compared with socially subordinate males. In the swordtail *Xiphophorus nigrensis*, a species with alternative reproductive tactics, small sneaker males increased their sperm swimming speed when paired with other sneaker males (Smith & Ryan 2011). These changes in sperm velocity occurred rapidly: over 3 days in Arctic charr, 7 days in *X. nigrensis*, and 2 weeks in fowl. Over a slightly longer time frame (∼1 month), following experimental manipulation of adult densities in the broadcast spawning ascidian *Styela plicata*, males experimentally allocated to high-density treatment groups, where sperm competition is more likely, produced longer and more motile sperm that swam for longer periods of time than did males in low-density treatment groups (Crean & Marshall 2008). Finally, in the Gouldian finch, *Erythrura gouldiae*, changes in social environment that reflect changes in the levels of sperm competition led to changes in sperm midpiece and flagellum lengths (Immler *et al*. 2010).

Variation in sperm morphology

Persistent directional selection is expected to erode variance in sperm morphology, generating a single optimal sperm phenotype that maximizes competitive fertility (Parker 1993, Parker & Begon 1993, Birkhead *et al*. 2005). Indeed, several comparative studies in birds have found that sperm are morphologically less variable in species where the level of sperm competition is greater (Calhim *et al*. 2007, Immler *et al*. 2008, Kleven *et al*. 2008, Lüpold *et al*. 2009*b*). These studies provide support for the notion that sperm morphology is under strong selection from sperm competition. However, reductions in sperm variance in response to sperm competition may be due to either selection for improved sperm production efficiency, leading to a reduction in developmental errors (Hunter & Birkhead 2002), or a reduction in genomic conflict between the evolutionary interests of the haploid sperm and the diploid male (Parker 1993, Parker & Begon 1993, Immler *et al*. 2008, Pizzari & Foster 2008). Unfortunately, distinguishing

between these two, though not mutually exclusive, hypotheses can be challenging. To overcome this challenge, Fitzpatrick & Baer (2011) assessed how sperm competition influences sperm length variation in eusocial ants and bees, where haploid males produce sperm that do not experience haploid–diploid genomic conflict. They found that variation in sperm length was negatively associated with the level of sperm competition. Because variation in sperm morphology cannot be explained by genomic conflict in these eusocial insects, these results suggest that selection is acting on sperm production machinery to improve sperm quality in response to increased sperm competition.

Despite the apparent evolution of sperm toward a single optimal phenotype, variation in morphology can persist. Some level of variation might always be present within an ejaculate simply because random developmental errors will occur during sperm production. However, Calhim *et al*. (2011) recently offered an alternative explanation for how sperm variance might be maintained in an interesting study of a natural population of the highly promiscuous superb fairy-wren (*Malurus cyaneus*). Calhim *et al*. (2011) found that males with sperm composed of a short flagellum and relatively long head had greater reproductive success when mating in the disfavored role of the cuckold, while males with sperm composed of a long flagellum and relatively short head secured more paternity with their social partner by preventing cuckoldry. Such opposing patterns of selection on sperm morphology depending on male mating roles may help to explain the maintenance of variation in sperm morphology despite selection aimed at eroding this variance.

In addition to maintaining variation in sperm length, selection for different sperm phenotypes under different competitive situations could ultimately favor the evolution of distinct sperm morphologies. Indeed, there are many examples of species that produce heteromorphic sperm, which in animals typically results in the production of both fertile and non-fertile sperm morphs (Till-Bottraud *et al*. 2005). There is evidence from insects that non-fertilizing sperm morphs can control female remating rates (Cook & Wedell 1999) and protect fertilizing sperm from immunological attack from the female's reproductive tract (Holman & Snook 2008). However, by and large, the adaptive significance of sperm heteromorphism is poorly understood and deserves further study.

Sperm cooperation

Cooperation among sperm within a given male's ejaculate is another exciting area of research in sperm competition that deserves greater attention (Pizzari & Foster 2008, Higginson & Pitnick 2010). In a variety of species, sperm can form conjugates of two or more individuals, who collectively swim faster than can

individual sperm (Hayashi 1998, Moore *et al.* 2002, Fisher & Hoekstra 2010). Among carabid ground beetles, the size of sperm bundles increases with traits known to be subject to selection from sperm competition, such as postcopulatory mate guarding and genital complexity (Takami & Sota 2007). In muroid rodents, the characteristic hooked morphology of the sperm head can be involved in the production of sperm conjugates, and the curvature of the hook increases with testes size, a widely used proxy for the strength of selection from sperm competition, suggesting that sperm conjugation may be an evolutionary response to selection via sperm competition in this group (Immler *et al.* 2007*a*). Nonetheless, experimental evolution failed to find divergence in the curvature of the sperm hook among populations of house mice experiencing variation in the strength of selection from sperm competition (Firman & Simmons 2009, Firman *et al.* 2011). The evolution of cooperation among sperm in the race to fertilize ova will depend strongly on whether sperm are under individual haploid control or whether they are controlled by the diploid male producing them (Immler 2008, Pizzari & Foster 2008, Higginson & Pitnick 2010).

The evolution of sperm viability

Owing to the relatively paucity of studies, it is currently not possible to draw broad conclusions regarding how sperm viability is influenced by variation in the level of sperm competition. However, there is some evidence that sperm viability influences competitive fertilization success (see Table 2). Importantly, the comparative studies reviewed in Table 1 reveal that polyandrous insect species have more viable sperm than closely related monandrous species (Hunter & Birkhead 2002) and that sperm viability is positively associated with sperm competition among Australian wrens (Rowe & Pruett-Jones 2011). More studies that explore responses to selection on sperm viability are required.

Seminal fluid proteins: the neglected weaponary

Despite the considerable volume of research that has focused on how selection acts on testes size and sperm production, and sperm form and function, researchers often neglect the critical role that seminal fluids play in imparting motility and fertilization capacity to sperm. Sperm are delivered from the male, bathed in a complex soup of proteins that are derived from the male accessory glands. Seminal fluid proteins (sfps) play a role in the nourishment, protection, capacitation, and motility of sperm within the female reproductive tract and should therefore be expected to contribute to a male's fertilization success (Poiani 2006). In humans, for example, seminogelin I and seminogelin II inhibit sperm motility, while seminal fluid proteases cleave seminogelins after ejaculation, promoting the rapid onset of motility in the female tract (Veveris-Lowe *et al.* 2007). Fertilization-promoting peptide stimulates sperm penetration abilities and thus promotes fertilization (Fraser & Osiguwa 2004), and the glutathione peroxidase family protect sperm from oxidative damage by reactive oxygen species (Drevet 2006). The function of sfps has been the subject of considerable research focus in the *Drosophila* model system. Here, protein secretions from the male accessory glands contribute to the seminal fluid and have been found to play major roles in the movement of sperm through and storage in the female reproductive tract, the competitive fertilization success of a male's sperm, the future receptivity of females to remating, and female investment in oviposition (Ram & Wolfner 2007). Given its critical influence, both on sperm performance and on the interactions between sperm and egg at the time of fertilization (Clark *et al.* 2006), seminal fluid productivity and function are expected to evolve in response to sperm competition.

The evolution of male reproductive accessory glands

The male reproductive accessory glands are the principle sites for the manufacture and secretion of sfps. As with testes, there is now good evidence that male expenditure on accessory glands does evolve in response to sperm competition. A comparative analysis of male reproductive anatomy among rodent species found a positive association between the strength of selection from sperm competition and the size of the seminal vesicles and the anterior prostate, two male reproductive accessory glands that contribute proteins to the seminal fluid (Ramm *et al.* 2005). In *Drosophila melanogaster*, laboratory evolution studies have shown that accessory gland productivity responds to variation in selection from sperm competition (Linklater *et al.* 2007). Thus, Linklater *et al.* established populations of flies with either a male-biased or female-biased sex ratio. The probability that females will encounter and mate with multiple males and thus the strength of selection from sperm competition was assumed to be greater in populations with a male biased sex ratio. After more than 60 generations of laboratory evolution, accessory glands evolved greater productivity in male-biased populations compared with populations with a female-biased sex ratio (Linklater *et al.* 2007). Similar findings were obtained in a study of *Drosophila pseudoobscura* in which the strength of selection from sperm competition was manipulated by either enforcing monogamy or controlling the degree to which females could mate polyandrously (Crudgington *et al.* 2009). Interestingly, testes size did not respond to variation in selection from sperm competition in either of these experiments, suggesting that in *Drosophila* seminal fluids may play an even more important role in

determining competitive fertilization success than does the number of sperm a male can produce. Molecular research is now revealing how individual sfps evolve in response to sperm competition.

The evolution of sfps

At the molecular level, the signature of positive selection can be recognized from a comparison of the rate of non-synonymous to synonymous (dN/dS) nucleotide substitutions in the DNA that encodes a given protein (Goldman & Yang 1994). Non-synonymous substitutions change an amino acid while synonymous substitutions do not. Because dS provides an estimate of the neutral rate of nucleotide substitution, a ratio of dN/dS >1 indicates a selective advantage to amino acid substitutions in a protein that results in adaptive divergence. Reproductive proteins facilitate three fundamental stages of reproduction; the transit of sperm through and capacitation within the female genital tract, the storage and survival of sperm before egg release, and the interactions between sperm and egg that culminate in successful fertilization (Clark *et al*. 2006). Genes that encode reproductive proteins involved at each of these stages appear to have evolved more rapidly than other genes. Thus, genome screens from taxa ranging from gastropods (Metz *et al*. 1998) to insects (Haerty *et al*. 2007) and mammals (Clark & Swanson 2005) show that reproductive proteins have substitution ratios >1 and are among the most evolutionarily divergent proteins known.

There is good evidence that sexual selection via sperm competition has been instrumental in the evolutionary divergence of sfps. In house mice, the seminal vesicle protein gene *Pate4* (*Svs7*) is evolving particularly rapidly (Karn *et al*. 2008), as is *Svs2* (Ramm *et al*. 2009). Moreover, the ratio dN/dS for *Svs2* is positively associated with the strength of selection from sperm competition across rodent species. Seminal vesicle proteins are involved in the formation of the mating plug, the size of which is also associated with the strength of selection from sperm competition (Ramm *et al*. 2005). Mating plugs are thought to play a role in the prevention of mating by, and thus sperm competition from, rival males (Simmons 2001). In primates, the *SEMG2* gene product is a major structural component of the semen coagulum and also shows positive selection as indicated by a high ratio of dN/dS. The rate of evolution of *SEMG2* also correlates with the levels of female promiscuity, as does the firmness of the semen coagulum (Dorus *et al*. 2004). These studies strongly suggest that in primates too, sfps involved in the formation of mating plugs are under positive selection from sperm competition. Evidence for a role of sperm competition in protein evolution also comes from studies of *Drosophila*. Here too accessory gland genes show evidence of strong positive selection (Haerty *et al*. 2007),

and accessory gland protein divergence is greater in lineages of *Drosophila* with greater postmating sexual selection (Wagstaff & Begun 2007).

It seems we know much about sfps and their function. However, to place these studies in context, for *Drosophila*, the number of newly discovered sfps is rising almost exponentially. At last count, 133 sfps had been detected, with just a handful of these having known function (Chapman 2008, Findlay *et al*. 2008). In mice, 69 sfps have been identified from the female reproductive tract following mating (Dean *et al*. 2011), while in humans, 923 sfps have been found (Pilch & Mann 2006), but again only in a very limited number of cases are the functions of individual proteins known. The exploration of sfps and their functional significance is in its infancy but promises to shed light on the functional mechanisms underlying sperm competitiveness.

Strategic adjustments in seminal fluid composition

Much research has explored male responses to sperm competition in terms of the numbers of sperm ejaculated (reviewed earlier). However, there are a growing number of studies that suggest the quality of those sperm may also be adjusted in an adaptive manner. Work with field crickets, *Teleogryllus oceanicus*, has shown how the viability of sperm in the ejaculate varies with a male's perceptions of sperm competition (Simmons *et al*. 2007*a*, Thomas & Simmons 2007). In these insects, males appear able to detect not just the mating status of a female, be she mated or unmated, but also the number of males she has accepted, based on chemical cues left by males during copulation. Thus, when the perceived risk of sperm competition is elevated, males will produce ejaculates containing sperm of higher viability, but as the number of males competing for fertilizations is increased beyond two males, so that the payoff from a given male's investment in his ejaculate declines, males produce ejaculates containing sperm of decreasing viability (Simmons *et al*. 2007*a*, Thomas & Simmons 2007). Research on humans (Kilgallon & Simmons 2005), feral fowl (Cornwallis & Birkhead 2007), and fish (Smith & Ryan 2011) point to strategic adjustments in sperm quality with risk of sperm competition; in these cases, males ejaculate sperm with faster swimming speeds when they perceive a threat from rival males, or with females of greater reproductive value. However, these studies beg the question of how males adjust the quality of their sperm? The most likely candidate would appear to be adjustments in seminal fluid. Because gene transcription is apparently absent in sperm cells, their functionality is largely dependent on post-translational modifications to their protein compliment that are brought about by sfps. Sfps are known to influence the viability of sperm (den Boer *et al*. 2008, Holman 2009, Simmons & Beveridge 2011) and their motility (Lindholmer 1974, Poiani 2006). In some insect species,

seminal fluid has been implicated in the killing of sperm present from rival males (Fry & Wilkinson 2004, den Boer *et al*. 2010), and it has even been suggested that males might exploit the expenditure on sfps made by their rivals, for example, if sfps buffered the female reproductive tract against spermicidal secretions (Hodgson & Hosken 2006). Seminal fluid may be more costly to produce than sperm themselves (Simmons 2001), so that males might be expected to allocate these costly secretions to their ejaculates, depending on the levels of sperm competition.

Recent attempts have been made to model the evolution of male allocation to sfps within Parker's sperm competition game framework. Cameron *et al*. (2007) considered the case where sfps act to increase the competitive ability or fertilization capacity of the sperm contained within the ejaculate. Their game theoretic analyses did indeed predict that males should invest more on sfps than sperm under these conditions. Moreover, in some species, sfps can contribute to female fitness through their contributions to egg production and/or offspring development (Simmons 2001, García-González & Simmons 2007a). Theoretical models predict that male fitness could be enhanced by withholding these beneficial sfps from females with whom the risk of lost paternity through sperm competition is high (Cameron *et al*. 2007, Alonzo & Pizzari 2010). Despite these first prospective attempts to model the evolutionary dynamics of seminal fluid investments made by males, few studies have attempted to examine whether males can vary the composition of their seminal fluid in an adaptive manner.

In bank voles, *Myodes glareolus*, males exposed to the odors of rival males during their development invested more in the growth of their seminal vesicles, the glands responsible for sfps that contribute to the mating plug, than did males with only limited exposure to rivals (Lemaître *et al*. 2011). In fowl, changes in seminal fluid quality have been implicated in the elevated motility of sperm when males copulate with high-quality females (Cornwallis & O'Conner 2009). The seminal fluid of *D. melanogaster* contains a cocktail of more than 130 proteins that are gradually being isolated and characterized (Findlay *et al*. 2008). One protein known to be important in sperm competition is sex peptide, which suppresses female sexual receptivity following mating so that males avoid sperm competition from future rivals (Liu & Kubli 2003). Seminal fluid also contains ovulin, a protein that stimulates an immediate elevation in egg production and oviposition by the mated female (Herndon & Wolfner 1995). As noted earlier, experimental evolution under elevated levels of sperm competition results in responses to accessory gland size and productivity (Linklater *et al*. 2007), and in particular productivity of sex peptide (Wigby *et al*. 2009). Male flies exposed to potential rivals during copulation will copulate for longer and transfer larger

quantities of both sex peptide and ovulin (Wigby *et al*. 2009). What is even more remarkable is that males appear able to tailor their seminal fluid composition to a particular mating event. Thus, when mating with a virgin female, males will transfer relatively more ovulin than when mating with a previously mated female, presumably because oviposition has already been induced by the female's previous mating partner (Sirot *et al*. 2011). Such strategic adjustments in fecundity enhancing sfps are consistent with the predictions arising from game theoretic modeling of male ejaculate expenditure (Cameron *et al*. 2007, Alonzo & Pizzari 2010).

Future directions

There is now overwhelming evidence that sperm competition has been an influential agent in the evolution of male reproductive biology. Comparative studies across species of a wide range of taxa, from parasitic worms to primates, consistently support the expectation that sperm competition should favor increased male investment in testicular tissue and sperm production; multiple mating by females drives the evolution of increased competitive fertility. However, previous research has focused predominantly on sperm numbers. We are only now beginning to examine features of the sperm themselves, and our findings are often equivocal. Sperm morphology is sometimes associated with swimming performance and sometimes not. We feel the problem here is a lack of consideration of the ejaculate as an integrated unit. All else being equal, perhaps sperm length should increase swimming speed. However, all else is rarely equal. Sperm motility is influenced by the selective environment in which it operates (i.e. internal vs external fertilization, sensu Humphries *et al*. (2008)) and is critically dependent on sfps contained within the ejaculate. We have discussed studies that show increased swimming performance in response to sperm competition, in the absence of increased sperm length, and it is possible that changes in competitive male fertility can be achieved by alternative routes, either via changes in sperm morphology, seminal fluid chemistry, or both. Such a scenario would add considerable noise to any attempt to find general patterns of evolutionary change in sperm morphology across taxa. We argue that it is time to begin to consider the ejaculate as an integrated functional unit and to assess the fitness of whole ejaculates in a multivariate analytical framework (Fitzpatrick *et al*. 2012*d*). Only then will we reveal the relative importance of individual traits (sperm number, morphology, and function, seminal fluid composition, etc.) to male fertility and to identify trade-offs that might occur between these individual ejaculate traits. Importantly, in this review, we have focused on sperm wars between males. Females can have a considerable selective impact on fertilization success, via the mechanisms of cryptic

female choice that determine the number of sperm in competition, their motility, and fertilization capacity (Eberhard 1996, Simmons 2001, Ball & Parker 2003, Humphries *et al.* 2008, Pitnick *et al.* 2009*b*). Incorporating female effects into multivariate approaches will also be necessary.

We also envisage exciting future developments in sperm competition research. It is not sufficient for sperm simply to arrive at the ova before those of other males. We expect that selection will act on the mechanisms that make sperm fertilization-competent. Indeed, recent work on four closely related rodent species has found that the males of species with higher levels of polyandry produce ejaculates containing a greater proportion of sperm that undergo capacitation and a greater proportion of sperm that respond to progesterone, an ovum-associated signal (Gomendio *et al.* 2006). Sperm competition thus appears to favor the evolution of fertilization competency. Future studies, both within and between species, that focus on the fertilization process itself will be of great interest. Research on sfps illustrate the power of studies at the genomic level, and future work on the expression of sperm genes is likely also to be revealing. For example, studies of genes in the genus *Mus* suggest that high divergence in promoters of the gene protamine 2 is associated with selection from sperm competition (Martin-Coello *et al.* 2009). These genes control the packaging of DNA in the sperm head, subsequent head dimensions, and sperm swimming speed. We believe that modern genomic approaches such as these will not only enhance our understanding of the evolutionary consequences of sperm competition for ejaculate form and function but will also provide us with the knowledge of what makes a male fertile, knowledge that can be brought to bare on real-world problems of male fertility and infertility.

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

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