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Review

Sperm competition, immunity, selfish genes and cancer

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Abstract. Sperm competition is widespread and has played an important role in shaping male reproductive characters such as testis size and numbers of sperm produced, and this is reflected in the rapid evolution of many reproductive genes. Additionally, sperm competition has been implicated in the rapid evolution of seminal fluids. However, our understanding of the molecular basis of many traits thought to be important in sperm competition is rudimentary. Furthermore, links between sperm competition and a range of issues not directly related to reproduction are only just beginning to be explored. These include associations between sperm competition and selfish genes, immunity and diseases such as cancer. We briefly review these topics and suggest areas we consider worthy of additional research.

Keywords. Reproductive proteins, accessory gland proteins, selfish genetic elements, immunity, intralocus conflict.

Introduction

When Darwin [1] first proposed his theory of evolution by natural selection he clearly distinguished between natural selection and sexual selection, selection arising through competition for mates. At the time, Darwin believed that sexual selection only occurred prior to copulation, via female choice and male-male competition. This was because he assumed that females were largely monogamous [2]. Observed incidences of extra-pair copulations in birds and other, more obvious examples of mixed parentage were discussed as anomalies, or were merely ignored [3]. However, in recent years the increased use of molecular techniques has overturned this long-standing view by revealing high levels of multiple paternity across a range of taxa [reviewed in 4]. This indicates that females typically mate with multiple males during the course of a single reproductive cycle.

One consequence of female multiple-mating is that sexual selection does not always end with copulation, but can continue through post-copulatory female-choice and sperm competition, and it is the latter that we focus on here. Sperm competition is defined as the "competition between the sperm from two or more males for the fertilisation of a given set of ova" [5]. This form of selection has profound evolutionary significance for almost all sexually reproducing animals [4, 6, 7] including humans [8], and is paralleled in plants, by pollen competition [9].

Under sperm competition, traits that increase both sperm offence and defence are likely to be favoured by selection. Characters that increase success in male

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fertilisation when a male mates with a female that has previously been inseminated by a rival are termed offensive, and include ejaculating large numbers of sperm in order to outcompete rival sperm numerically [5], removal of rival sperm [10], and the repositioning of rival sperm to locations within the female less favourable for fertilisation [11]. Defensive traits prevent or reduce the risk of future sperm competition when a male has mated with a female, and include compounds that switch off female receptivity to subsequent males [reviewed in 2,6]. These conflicting selection pressures have resulted in a huge array of adaptations in males, from the spoon-like penises in dragonflies, which scoop out rival males' sperm from the female reproductive tract [10], to the elaborate mating plugs of some butterfly species, that prevent females from remating with rivals [e.g. 13]. Sperm competition generally has been implicated in the divergent evolution of female sperm storage organs [14, 15], genital morphology [16, 17] and testis morphology [18–21]. For example, larger testes evolve through sperm competition because they produce more sperm, which is advantageous when sperm compete numerically, and this finding is so pervasive that relative testis size is frequently used as an index of sperm competition risk [22].

There are several excellent reviews available which deal with the morphological, physiological and behavioural adaptations that have arisen via selection under sperm competition, and the mechanisms that underlie this selection [e.g. 4, 6]. Here, we focus on recent and emerging areas of research that have resulted from advances in the fields of molecular biology and genetics, and discuss their impact on the study of sperm competition. We begin by examining the evidence for rapid evolution of various non-sperm ejaculate components that enhance male fertilisation success in a variety of ways. The available evidence for a genetic basis to variation in sperm competitive success between males is then explored, followed by some recent research highlighting the impact of selfish genes on male fertility, before we discuss the problem of conflicts between the haploid sperm genotype and the diploid genome. We end by discussing recent advances in the field of immunity and its implications for diseases, such as cancer, and how this is intimately linked to sperm competition.

Rapid evolution of reproductive proteins

The ejaculate of males contains components other than sperm. A large part of the ejaculate is made up of various reproductive proteins that are transferred at mating and have a range of effects on female reproductive physiology and behaviour. Many of these effects are directly aimed at enhancing male reproductive success in sperm competition. Hence, such proteins are intimately associated with male fitness and under strong selection.

Reproductive proteins and the genes which encode them generally evolve at a faster rate than proteins expressed in non-reproductive tissues [23–25]. In many cases, reproductive tissues exhibit a higher than expected frequency of non-synonymous substitutions, base changes that result in a different amino acid being incorporated into the protein product [25, 26]. This suggests that the high rate of evolution in reproductive proteins is the result of positive selection, the spread of novel alleles that increase fitness [27]. Perhaps the best known example comes from the seminal fluid proteins of *Drosophila* spp. Many genes encoding seminal fluid proteins have been shown to have extremely high rates of divergence, at both the population level and the species level [28–31]. Some have evolved so rapidly that the gene appears to be unique to a single species [32, 33]. Similarly in humans and chimpanzees there is evidence that the rates of change in gene expression levels are three times higher for genes active in the testes than those in the brain, heart, kidneys or liver [34, 35]. In addition to *Drosophila* [28, 36] and primates [35–39], rapidly evolving reproductive proteins have been found in a wide variety of other taxa including crickets [40], rodents [41, 42], sea urchins [43], abalone [44], and gastropods [45].

Selection through sperm competition is thought to be important in the rapid evolution of reproductive proteins [23], and comparative studies support this idea. For example, a number of studies have found that the rate of evolution of reproductive proteins is correlated with the mating system of the species, or with a similar proxy measure of sperm competition risk [30, 46–50]. In muroid rodents, for example, rates of evolution of the *Svs2* gene are significantly higher in species with a high risk of sperm competition compared to species exhibiting a low risk of sperm competition [51, but see 52]. The *Svs2* gene is involved in the production of the seminal or mating plug, a gelatinous structure produced by males of many invertebrate and vertebrate species, to physically block the distal part of the female reproductive tract after copulation [reviewed in 4, 6, 7]. Similarly across primates, the rate of evolution of the seminal fluid protein SEMG2, is positively correlated with levels of female promiscuity and sperm competition [53]. SEMG2 is a major ejaculatory protein that has been implicated in plugging the female reproductive tract and increasing the number of sperm that make it to the site of insemination by preventing loss

from the female and protecting sperm from damage [54].

Not all evidence points to a role for sperm competition in the rapid evolution of reproductive proteins, and there is considerable evidence that female genes and proteins also evolve rapidly in many species, although usually not as rapidly as male reproductive genes [23]. The rapid evolution of female reproductive genes may be the result of evolutionary conflict between males and females over reproduction [55]. Sexual conflict occurs because the reproductive optima of males and females frequently differ [reviewed in 56, 57]. This can potentially result in cycles of antagonistic co-evolution. Imagine a novel trait that increases male fertilisation success under sperm competition, but at a cost to female fitness. This would select on females and possibly result in the evolution of female characters that counter the original male adaptation, resulting in selection on males again, and so on [58, 59]. These cycles can cause rapid co-evolution of reproductive characters, although this may not occur frequently [60, 61]. Co-evolutionary cycles of male-female conflict are unlikely to be the main explanation for rapid evolution in male reproductive proteins, because if they were, then male and female reproductive proteins would be expected to show similar rates of evolution. As discussed above, male reproductive proteins usually evolve more rapidly than female reproductive proteins. At present the best direct evidence for rapid evolution of reproductive genes through sperm competition and sexual conflict probably comes from abalone (*Haliotis* spp.), a free-spawning marine invertebrate [62]. In this group it has been suggested that males and females are currently locked in an antagonistic co-evolutionary battle over the speed at which fertilisation occurs. In abalone, the strength of binding between egg and sperm proteins influences the rate of fertilisations [62]. It has been suggested that in males, alleles that increase the speed with which sperm binds to an egg are under positive selection through sperm competition [63]. However, this may be costly for females, as increased binding speed may raise the risk of polyspermy. This is when a single egg is fertilised by several sperm, and results in developmental failure and therefore, lower female reproductive success. Males are therefore being selected to increase the fertilisation speed of their sperm to succeed during sperm competition, while females may be selected to slow down sperm-egg binding to reduce polyspermy [63]. Supporting this hypothesis, in *Haliotis rufescens*, both the male seminal protein, lysine, that mediates egg penetration at fertilisation, and the female egg protein, VERL, which slows fertilisation, are currently under strong selection [64, 65]. However, it is possible that female choice (more classically) is also involved [4].

The role of seminal fluid and accessory gland proteins in sperm competition

As noted above, the ejaculates of most animals contain not only sperm, but also other compounds that may play a role in sperm competition [4, 6, 12, and reviewed in 66]. In many species of Lepidoptera, for example, males transfer volatile chemical compounds known as "anti-aphrodisiacs" to the female during copulation, which reduce female attractiveness to other males [67]. These compounds are emitted by the female after mating, and discourage other males from copulating [68, 69]. Additionally, human seminal fluid contains peptides such as calcitonin and glutathione which, in high concentration, are associated with enhanced sperm motility [70, 71], and greater motility may influence siring success under sperm competition [reviewed in 72]. Mating plugs are also frequently composed of seminal fluid components [e.g. 54], and in both vertebrates and invertebrates there are numerous examples of seminal fluid-mediated suppression of female receptivity, as a mechanism of sperm competition avoidance by males [e.g. 68; reviewed in 56]. Much of our current knowledge with regard to the function of seminal fluid molecules comes from fertility studies of mammals, in particular humans [66]. There is, however, less information available relating to the function of these compounds in sperm competition, and the best studied system in this regard is probably *Drosophila melanogaster.*

The *D. melanogaster* ejaculate contains a cocktail of molecules from the paired accessory glands, the ejaculatory duct, and the ejaculatory bulb [reviewed in 12, 73]. The bulk of the seminal fluid consists of the accessory gland proteins (Acps), of which 112 have been identified (to date) in *D. melanogaster* alone [reviewed in 52]. Acps are very diverse in size and structure, ranging from small peptides to prohormone-like polypeptides of between 200 and 400 amino acids, and large glycoproteins [reviewed in 73]. The functions of many of these molecules are still poorly understood, but there is an increasing body of data showing their importance in reproduction.

Several Acps have a marked effect on the mated female, for example increasing rates of oogenesis, ovulation and oviposition [73–77]. In addition, Acps cause a behavioural switch which reduces mated females' receptivity to remating with other males [reviewed in 77]. By reducing female receptivity, males thereby avoid sperm competition with rivals. In *D. melanogaster*, this response is thought to be elicited

by the sex peptide (SP), Acp70A, a small, 36 amino acid molecule which binds to specific receptors in the female central nervous system [77]. This post-mating response lasts approximately one week. However, in mutant "XO" males that transfer seminal fluid but no sperm, the response only lasts a day. This suggests that sperm is necessary for the full effect of SP on female post-mating behaviour, a phenomenon dubbed "the sperm effect" [78]. SP does bind to sperm, but sperm only act as a carrier; on entering the female reproductive tract, the sperm releases the SP which then enters the female haemolymph and moves to the sites of action. The female receptor for SP has only recently been identified and, as expected, receptors were found in specific parts of the brain and many nerves [79], consistent with histochemical studies [80]. The "short-term" effect is probably elicited by the direct transfer of SP into the female haemolymph at mating, while the "long-term" effect is probably the result of continual release of SP from sperm whilst it is stored in the female sperm storage organs [77].

Another Acp, the egg-laying hormone ovulin (Acp26Aa), directly stimulates ovulation in female *D. melanogaster* (as does SP), but only for a period of up to six hours post-mating [81]. Ovulin is among the most rapidly evolving proteins in the *Drosophila* genome [82]. Stimulating immediate oviposition is beneficial for the male, as it increases the number of eggs sired before the female remates. In addition, it has recently been suggested that ovulin also affect males' sperm competitive success when mating with already inseminated females [83]. However, there has been no firm demonstration of this to date.

In *D. melanogaster*, males also construct seminal mating plugs that reduce female remating and/or facilitate sperm storage. A large part of this mating plug is composed of the ejaculatory bulb protein, PEB-me [84]. The PEB-me protein has amino acid repeat motifs similar to those of spider silk proteins and mussel byssal threads that form homopolymers, suggesting that this portion of the mating plug is formed via the coagulation of PEB-me. The smaller, anterior portion of the mating plug is composed largely of Acps, and is thought to concentrate sperm closer to the site of sperm storage within the female reproductive tract [reviewed in 73].

Female *D. melanogaster* store approximately 20% of the 4000 sperm they receive during a single mating. Sperm can be stored for up to two weeks in the two sperm storage organs: the single seminal receptacle and the paired spermathecae [reviewed in 52]. Acps are essential for sperm storage to occur; females mated to males that produce no Acps store 90% fewer sperm than females mated to wild-type males [85]. At least one Acp has been characterised that is essential for sperm storage, the glycoprotein Acp36DE, which is thought to modulate the muscular contractions of the female reproductive tract which transport sperm closer to the sperm storage organs [reviewed in 52]. Interestingly, females mated to males that produce no Acps are infertile, despite storing small quantities of sperm [86, but see 87]. This suggests that Acps also function in the utilisation of sperm from the sperm storage organs, although the mechanism by which this might occur is, as yet, unknown.

The fact that Acps are necessary for the storage and utilisation of sperm within the female reproductive tract suggests they are important in sperm competition: an Acp which provides a direct storage advantage to a male's sperm is likely to be selectively favoured, as is any Acp which does so indirectly by affecting the storage of competitors sperm by displacement or incapacitation for example. In *D. melanogaster*, the second male to mate with a female sires the majority of her offspring, on average around 80% [88]. It has been proposed that this second male sperm precedence is the result of displacement and incapacitation of the first male's sperm by Acps [89–91]. Initial studies showed that when females mated to a normal male, followed by an XO male, the second male was still able to reduce the fertilisation success of the first male, despite ejaculating only seminal fluid [89]. Visualisation of the movement of the first male's sperm using green fluorescent protein expression appeared to confirm that this reduction in fertilisation was the result of displacement and incapacitation of the first male's sperm by the seminal fluid of the second male, rather than sperm loss from the female sperm storage organs [91]. The idea of sperm incapacitation is still controversial, as it is unclear how a male could damage the sperm of his rival, without affecting his own. Instead, observed examples of incapacitation seem to be the result of sperm ageing during storage or sperm dumping by females [87].

When interpreting results from studies on *Drosophila melanogaster*, however, it is important to acknowledge that almost all studies in this area have been conducted on long-term laboratory populations, and it is unclear exactly how this work relates to free-living populations. Females in laboratory populations may display very specific preferences for males because they are consistently surrounded by large numbers of high quality potential mates and costs of choosiness may be low. In contrast, wild females may be less likely to show complete infertility when mating to males that do not transfer Acps [86], because in wild populations females may encounter fewer males, and these males are more likely to be lower quality and have limited Acp production and reserves. Studies comparing these

traits in laboratory and wild populations would be interesting.

The genetics of sperm competition

Given the large effect that fertilisation success has on overall male fitness, it is expected that traits related to sperm competition will be under strong positive selection [4, 6]. However, despite the evolutionary importance of sperm competition, there are relatively few quantitative genetic studies of traits related to sperm competitiveness, even though genetic variation is needed for these characters to evolve. Nonetheless, testis weight and ejaculate volume exhibit high additive variation in the dung beetle, *Onthophagus taurus* [92], and testis weight has a genetic component in tettigonids [93]. Furthermore, experimental removal of sperm competition through enforced monogamy leads to reductions in testis size and therefore sperm production, suggesting a genetic basis to these traits in two species of fly [20,21]. Several studies have also reported significant heritabilities for specific sperm characteristics that are likely to be under selection through sperm competition, for example sperm length and sperm viability [94–96]. Researchers have also examined sperm competitive ability as a whole. Results are mixed, with some studies finding fertilisation success evolves or has significant heritability, while others find sperm competitiveness exhibits relatively low levels of additive genetic variation [e.g. 97–104 and see 105]. There are a number of factors that could contribute to the low heritabilities reported in some studies and thereby constrain the response of sperm competitive ability to positive selection. Firstly, the outcome of sperm competition may not only be a function of the male's genetic background; it can also relate to the genetic background of the female he mates with, and the genetic background of the male he is competing against [101, 102, 106–108]. Sperm competitiveness can therefore be non-transitive, where every genotype wins against some but loses against others [102, 106–108]. In addition, negative genetic correlations may exist between the individual traits underlying sperm competitive ability, as a result of pleiotropy or linkage disequilibrium, which could limit the extent to which sperm competitiveness responds to positive selection [96].

Investigating potential genetic associations is important because several hypotheses explaining the evolution of female multiple mating are predicated on the existence of additive genetic variation in sperm competitiveness and genetic correlations between this and other characters. For example, the good sperm hypothesis is based on positive genetic correlations between male sperm competitiveness and the quality of his offspring [109], while the sexy sperm hypothesis requires polyandrous females to produce sons that are superior sperm competitors and daughters that mate more often [103, 110, 111]. However, as the evidence presented above makes clear, at present we only have a rudimentary understanding of the precise mechanisms driving the evolution of sperm competitiveness and how broadly applicable various mechanisms are. For example, studies of *D. melanogaster* find sperm competition success is not heritable, while studies in the closely related species *D. simulans* do [102, 103]. Furthermore, the studies cited above demonstrate that there can be quantitative genetic variation to aspects of sperm competition success, but do not provide a molecular basis for the observed variation. However, increasingly, advances in molecular genetics and genomics allow researchers to characterise in detail the genes involved and their effects [e.g. 83, 112, 113]. For example, Clark et al. [113] found significant associations between male sperm defence capabilities and polymorphism at four seminal fluid protein loci. Similarly, a recent study also showed there is allelic variation in male reproductive genes affecting female post-mating receptivity [114]. This is an area that warrants more work.

Sperm competition and selfish genetic elements

Genes are generally expected to produce effects that enhance the success of the entire organism because all genes benefit from the organism's success [115]. However, selfish genetic elements (SGEs) are genes which promote their own success, often at a cost to the whole organism [116]. These genes are thought to be very common, although in most cases they are hard to detect due to the evolution of modifiers that suppress their action [116–118]. If unchecked by modifiers, SGEs can spread to fixation very rapidly [119, 120]. SGEs increase their success through many different mechanisms (see reviews in [116, 119]), but these frequently involve the manipulation of sperm [121], and sperm competition is often used by SGEs to enhance their transmission [122]. Although sperm competition is usually thought of as occurring between sperm from more than one male (inter-male sperm competition), it can also occur when different sperm genotypes compete within the ejaculate of a single male (intra-male sperm competition) [122]. This requires a degree of haploid (sperm) control and there is evidence for this generally – Joseph and Kirkpatrick [123] estimate that approximately 1.3 % of mouse genes are expressed in the haploid phase as

sperm – and for SGEs in particular. Basically, if a SGE can damage sperm that carry the allelic alternative, then in heterozygotes, the SGE will gain a transmission advantage over the non-selfish allele. For example, the *t*-complex in mice is an inversion series on an autosome, comprising 1 % of the mouse genome [124]. Having two copies of the *t*-complex is almost always lethal, and so carriers are nearly always heterozygotes [125]. When a *t*-carrying mouse mates, over 90 % of offspring will be fertilised by sperm carrying the *t*-complex [126]. This occurs because spermatids not carrying the complex are damaged during spermatogenesis [127]. This transmission advantage allows the *t*-complex to persist in populations despite the high evolutionary cost of homozygote lethality [128]. Many other SGEs also damage sperm that do not carry a copy of the SGE itself, thereby using intra-individual sperm competition to increase their share of fertilisations [129]. Many of the specialised mechanisms of spermatogenesis, such as the regulatory action of Sertoli cells and cytoplasmic bridges that are maintained during spermatogenesis, are believed to have evolved in order to facilitate diploid control of sperm production [130].

Sperm competition – here standard inter-male sperm competition – can also play a role in preventing the spread of SGEs through populations [131]. The means by which SGEs manipulate sperm to enhance their success within a male are also likely to reduce the success of SGE-carriers in sperm competition [122, 131]. For example, in male *Drosophila pseudoobscura* that carry the meiotic driving X chromosome "SR" (Sex ratio) all sperm that carry the Y chromosome of the male fail early in spermatogenesis [132]. This loss of 50 % of sperm production directly reduces the number of sperm that SR-carrying males transfer to females, and this in turn severely reduces their success in sperm competition with normal males [133]. The sperm competitive ability of SR carriers is lower than expected due to the loss of 50 % of sperm production, suggesting that even the sperm that carries a copy of the SGE is impaired during spermatogenesis [133, 134]. In fact, every species that carries a sperm-manipulating SGE and that has been tested appropriately, shows a significant reduction in inter-male sperm competitive ability for the SGE carrier [135–138]. These reductions are particularly common in male gametes that carry heterozygous sex chromosomes [134], but have also been found in autosomal SGEs (e.g. SD in *Drosophila melanogaster* [129], *t*-complex in mice [125]). The available evidence suggests that SGEs generally reduce fertility in male carriers, and this – in addition to the evidence above – further suggests that poor inter-male sperm competitive ability is likely to be a widespread consequence of SGEs [139]. However, this conclusion is based on a small number of studies, and many types of SGEs have not been tested for fertility effects at all [139]. A broad survey of SGEs and their impacts on fertility would be very useful.

Similar reductions in sperm competition success have also been found in male *Drosophila simulans* carrying the intracellular bacterium *Wolbachia* [140]. *Wolbachia* commonly induces Cytoplasmic Incompatibility (CI) – the production of inviable offspring when an infected male mates with an uninfected female [141]. CI is thought to occur via parasite-induced sperm modifications that occur during spermatogenesis [142]. It appears that as a consequence of this modification, infected males have lower fertility than uninfected males [143], which compromises their sperm competition success. This reduced sperm competitive ability of SGE-carriers is predicted to be able to stabilise the spread of a SGE if the degree of polyandry is sufficiently high [135,144]. The reduced sperm competitive ability of SGE carrying males may also allow females to decrease the likelihood of their offspring being fathered by SGE carrying males through mating with multiple males [122, 131]. In most well-studied SGE systems, females are unable to distinguish between SGE carriers and normal males before mating [121, 139] (but see [145–147]). However, if a female copulates with several males this will increase the chance of sperm competition between the sperm of SGE carrying and normal males, which normal males are expected to win, thereby decreasing the expected proportion of offspring that will carry the SGE [122, 131]. This could provide a selective advantage for polyandry (which is very common [4, 6]) despite the costs to females associated with mating frequently [56, 148]. However, this scenario does not apply to CI-inducing *Wolbachia.* This is because the level of incompatible matings is inversely related to the frequency of *Wolbachia*-infection in the population. When most individuals are infected, matings result in viable *Wolbachia*-infected offspring [149]. It is worth noting that the frequency of many types of SGE remains unknown, and the frequency of modifiers more so [135]. Is it possible that sperm competition is a general mechanism that limits the spread of many SGEs? At present we simply do not know.

Conflict between sperm (haploid) and male (diploid) phases

In previous sections, different evolutionary conflicts and their interactions with sperm competition have been discussed. Evolutionary conflicts occur when a shared trait has different optima for the different

interacting parties such that both optima cannot be simultaneously achieved. For example, intralocus sexual conflict occurs when selection favours different values of a trait depending on whether it occurs in males or females [150]. One such example is human hip width [150]. Wider hips are beneficial in women because they facilitate childbirth, whereas narrower hips that are better for load bearing are selectively favoured in men [150]. Work, largely with *Drosophila*, suggests that such intralocus sexual conflict occurs widely throughout the genome [151–156]. Although some intralocus sexual conflict can be resolved by sex specific expression and genomic imprinting [56], a growing body of work suggests that intralocus sexual conflict is common and occurs at all stages of an organisms development [154], and can have implications for many areas of sexual selection [155, 156], including sperm competition.

In essence, when a gene performs several jobs – either in different sexes or different tissues – each with different optimal alleles, intralocus conflict may occur, and there is no reason to assume that intralocus conflict is only limited to differences between sexes [157]. For example, there are reasons to suggest that the performance optima for spermatogenic and somatic cells in the same male may differ – approximately 4% of mouse genes are expressed only in spermatogenesis and never in somatic cells [158]. Many other genes are expressed at unusual levels in spermatogenesis or produce transcripts that are very different to the somatic cell products [159, 160]. Clearly, the genes in these cases will not show sperm-adult intralocus conflict because the modifiers that alter their expression in sperm allow them to have specific expressions for each situation. However, these genes do illustrate the enormous differences between the genetic optima for sperm production and that for somatic male cell production. If Prasad and co-workers [154] are correct in their argument that not all genes that have several optima will have modifiers or differential expression, then there are likely to be many genes that have different optima when expressed in somatic and in spermatogenic cells, but which are nevertheless expressed at similar levels in both. Additionally, and as reported above, Joseph and Kirkpatrick [123] estimate that about 1.3% of mouse genes are expressed in the haploid phase (i.e. in sperm) and will be selected for haploid as well as diploid optima. It is therefore likely that some of these genes will be the source of intralocus conflict and limit the optimisation of sperm performance. Furthermore, the outcome of intralocus conflict should be influenced by the risk of sperm competition. For example, in a purely monogamous species, because all ova will eventually be fertilised by sperm from a single male, the genes expressed in both sperm and somatic cells should be close to the somatic optima. Alternatively, in species with very high levels of sperm competition, allele frequencies at these genes should be much closer to the sperm optima. This is an area that has been subject to very little research and almost any contribution would be valuable.

Sperm competition and immunity

There are alternative explanations for the rapid rate of evolution seen in reproductive tissues. These include female choice, self versus non-self recognition, meiotic drive and immune defence [24]. Unfortunately, distinguishing between these different processes is often very difficult, as many of the explanatory hypotheses make similar predictions. One of the strongest alternative explanations for rapidly evolving reproductive genes is the influence of immune function genes [161, 162]. Genes related to immune function often show extremely high rates of evolution [e.g. 163, 164]. Many immune function genes are expressed in reproductive tissues, possibly as a means of defence against sexually transmitted diseases [165, 166], and this could produce an apparent increase in the average rate of evolution of reproductive genes. It is possible that all these explanations play at least some role in the rapid evolution of reproductive proteins [23, 25].

All organisms are resource limited and, as a result, they face complicated trade-offs as they attempt to maximise fitness. Recent work suggests that one such trade-off is between immune function and reproductive success, including sperm competitiveness [167–172]. More generally, mating itself may alter immune system activity [173]. In insects, the immune response is a complex (mostly) innate response, consisting of humoral and temporal immunity, which can respond to a variety of pathogens and parasites. For experimental purposes, the immune response in this taxon is usually measured in terms of encapsulation ability and phenoloxidase (PO) activity [reviewed in 174]. The former is the encapsulation of multicellular pathogens and artificial foreign bodies by a wall of haemocytes; the encapsulated material is then destroyed through starvation, and the active release of cytotoxic molecules into the capsule by the host tissues. Encapsulation rate is measured in terms of the optical density or volume of capsules: larger or thicker capsules are usually deemed to be more efficient. PO is an enzyme released in response to the detection of a foreign body or when a wound is formed. PO converts tyrosine-precursor molecules to melanin, usually in conjunction with the action of the haemocyte capsule.

Melanisation reduces the permeability of the capsule, thereby suffocating the foreign body. More rapid rates of PO activity – the slope of the reaction curve (absorbance v. time) during the linear phase of the reaction – are generally taken to represent a greater immune response [e.g. 175].

Several studies have shown a trade-off between male reproductive success and indicators of immune function. For example in *D. melanogaster*, males were selected under high levels of sexual selection, induced by biasing the sex ratio towards males [169]. After 58 generations, males from the male-biased lines became more sexually competitive compared to control lines evolving under an equal sex ratio, but were also more susceptible to infection by *Escherichia coli* D21 bacteria. Similarly, in polyandrous (i.e. with high levels of sperm competition) lines of yellow dung flies (*Scathophaga stercoraria*), individuals had lower PO reaction rates compared to flies reared in monandrous lines [170], and males from the polyandrous lines were also superior sperm competitors [104]. This indicates there are micro-evolutionary trade-offs between immunity and sperm competitiveness. However, there is no evidence that copulation itself alters immune function in yellow dung flies [175]. This contrasts with the damselfly *Matrona basilaris japonica*, where males show a rapid and significant decrease in encapsulation ability following copulation [168, also see 174].

It has been suggested that trade-offs between immune function and ejaculate quality also occur in vertebrates [176]. Haploid sperm cells are not recognised as "self" by the diploid immune system, therefore immune activity within the testes should be suppressed as much as possible during spermatogenesis, in order to maximise the size and quality of the ejaculate [although see 177]. This immune suppression is thought to be mediated by the action of cytokines and other humoral mediators such as testosterone. Similar to the situation discussed above in insects, if trade-offs between immune activity and sperm production occur, males that are forced to fight off an infection by upregulating their immune system may, as a consequence, produce poor quality ejaculates. There is some support for this. In the Arctic charr (*Salvelinus alpinus*), several measures of heightened immune function, including increased spleen mass and high intensity of infection by nematode worms, were negatively associated with ejaculate quality [178]. This is likely to have important consequences for sperm competitive ability as the size and quality of a male's ejaculate are often important determinants of paternity success [5, 6]. Similarly, a recent meta-analysis in humans showed that males treated for infertility with corticosteroids – which induce an immunosuppressive effect – exhibited an increase in ejaculate size and sperm motility [179]. The current level of sperm competition in humans is hotly debated. However, the frequency of extra-pair paternity is thought to be approximately 10%, and comparison with other primates suggests that size of testes and sperm numbers, relative to body size, are similar to those which would be predicted in a system with intermediate levels of sperm competition [180 and see 8]. This suggests that sperm competition has influenced the evolution of human male reproductive physiology.

Much of the evidence suggesting a trade-off between immune function and reproduction stems from phenotypic studies. However, the evolutionary relevance of such relationships depends on their underlying genetic architecture [181], and researchers are increasingly adopting techniques such as quantitative genetic analyses and the study of quantitative trait loci to dissect the relationship between reproduction and immunity [e.g. 182]. In addition, many studies possibly underestimate the complexity of the immune system, for example by using PO activity or encapsulation ability as measures of immune function in insects [174]. For example, a recent quantitative genetic analysis study showed positive phenotypic and genetic correlations between sperm viability, and both encapsulation ability and the number of circulating haematocytes in the cricket *Teleogryllus oceanicus* [96]. However the same study reported both phenotypic and genetic trade-offs, between sperm viability and lysozome activity. In crickets, lysozome activity is the main defence against bacterial infection and this is therefore likely to represent a substantial trade-off. Studies such as this highlight the importance of considering the genetics underlying apparent phenotypic trade-offs.

Sperm competition and cancer

The trade-off between immune function and ejaculate quality also has implication for sperm competitive ability and disease. This is because the demand for high quality sperm may directly make males more vulnerable to disease. In addition, there are also other dangers to male health associated with the demand for high sperm producing capacities. As previously discussed, intense sperm competition selects for traits that increase sperm competitiveness, particularly the production of large numbers of sperm and the intense selection for rapid spermatogenesis has been suggested to increase the risk of cancer [130, 183]. Firstly, it is likely that selection for high rates of cell replication reduces the ability of the genome to "police" itself

[184] in spermatogenic tissues [185, 186]. Many genes that promote cell growth are also frequently over-expressed or atypically expressed in spermatogenic cells and have been implicated in the progression towards cancer [131]. Furthermore, several mammalian genes are expressed at very high levels only in rapidly dividing embryonic cells, spermatogenic cells and malignant cancer cells [186], and there are at least 88 genes expressed only in testes and malignant cancer cells [187]. Secondly, alleles that pleiotropically cause cancer can spread to fixation if they also promote a strongly selected trait [188]. For example, some breeds of dogs selected for large size suffer high rates of cancer due to the spread of alleles that promote both large size and cancer [189]. The co-evolutionary nature of sperm competition means that evolution should be rapid and non-equilibrial [190]. This is likely to produce a pattern of rapid spreads of alleles beneficial in sperm competition, even if they have moderately harmful pleiotropic effects [188, 191]. For example, Apert syndrome is a craniofacial/limb abnormality in humans, usually caused by the inheritance of a mutant FGF receptor from the father. The mutation responsible also increases the rate of replication of spermatogonial cells, causing a single mutant spermatogonial cell to spread through the testicle [192].

This work is at an early stage and our understanding of putative associations between sperm competition and cancer is still in its infancy. So far, most work has progressed by taking genes implicated in cancer and then determining if they play a role in sperm competition. Another avenue yet to be pursued is to correlate the risk of sperm competition and rates of cancer, particularly testicular cancer, across species. It might also be possible to investigate rates of abnormal development or cancer in populations that have been selected long term under conditions of high sperm competition or monogamy. This may be possible in some wildlife and their domestic relatives. In depth studies of growth factor genes implicated in sperm competition and cancer would also be beneficial.

Concluding remarks and future directions

In this review we have focused on what we consider to be emerging areas of study that are directly or indirectly related to sperm competition. For example, we now have a much clearer understanding of the non-sperm components that make up a large part of a male's ejaculate. With the sequencing of *Drosophila* genomes, detailed genomic analyses have now begun to reveal that subtle differences at the gene expression level can have dramatic effects on female reproductive physiology and therefore influence male reproductive success. With the recent discovery of the elusive female receptor for one of the most intensively studied Acps, sex peptide, we can now begin to unravel the hypothesized co-evolutionary path between male and female reproductive molecules that lies at the heart of male sperm competitive success in *D. melanogaster*. We have also highlighted other areas that we find particularly fascinating and where recent research has revealed hitherto unrecognized links with sperm competition. For example, the impact of SGEs, which are ubiquitous in living organisms, are likely to have a dramatic effect on male fertility and may also be important in shaping both male ejaculates and female mating patterns. We have also discussed the trade-offs between immunity, ejaculate quality and disease, which to date has largely been examined at the phenotypic level. It is now clear there can be a direct link between genes regulating immunity and male ejaculate quality, and there appears to be a link between the demand for the production of many sperm to succeed in sperm competition and the risk of cancer, and possibly other diseases. Recent research into the impact of senescence has also discovered links with male fertility. It is now clear that both male and sperm age can directly influence the outcome of sperm competition, with the ejaculate of older males performing badly, and this may even be an additional reason for female multiple mating. Similarly, there is evidence that females preferentially use sperm from more recent inseminations to ensure high fertility levels [193]. However, the direct impact of male versus sperm ageing on female mating patterns and sperm competitive success is only now beginning to be examined [194]. There are clearly several areas that will see a dramatic increase in research activity in the near future and these are likely to provide more detailed information about what makes a male's sperm successful and may, along the way, provide useful information regarding other aspects of infertility and health.

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