Selfish genetic elements and male fertility

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Selfish Genetic Elements (SGEs) are diverse and near ubiquitous in Eukaryotes and can be potent drivers of evolution. Here we discuss SGEs that specifically act on sperm to gain a transmission advantage to the next generation. The diverse SGEs that affect sperm often impose costs on carrier males, including damaging ejaculates, skewing offspring sex-ratios and in particular reducing sperm competitive success of SGE carrying males. How males and females tolerate and mitigate against these costs is a dynamic and expanding area of research. The intense intra-genomic conflict that these selfish elements generate could also have implications for male fertility and spermatogenesis more widely.

1. Introduction

For an allele securing a place in the next generation is critical. Achieving this success has traditionally been explained by the forces of natural and sexual selection. However, a third route to evolutionary success has been revealed, challenging the premise of 'fair' Mendelian inheritance. Here, Selfish Genetic Elements (SGEs) can increase their own frequency across generations without increasing the fitness of their carrier individuals, and often impose major costs on the rest of the genome (1). Several SGEs specifically act through sperm, from paternal genome eliminators to endosymbionts to toxic sperm killers (1–3). As a result, spermatogenesis through to fertilisation can be viewed as a series of arenas that are vulnerable to the activities of SGEs. The actions of SGEs in sperm have far reaching impacts, killing sperm and zygotes, changing the physiology and mating behaviour of males and females, and perhaps influenced the evolution of some of the deep structures of spermatogenesis (4,5,6; Figure 1).

2. SGEs and Sperm

SGEs affecting sperm were reported almost a century ago (7) and new SGEs are still being discovered (8,9). Here we explore three types of SGEs that affect sperm; those that kill or damage sperm, those that travel within sperm, and those that modify sperm to affect zygote formation.

Segregation distorters: sperm killers and disablers

Segregation distorters, sometimes referred to as killer meiotic drivers, are one of the best studied SGEs that manipulate sperm (3,10). As sperm are haploid, carrying only one allele from their diploid parent genome, one selfish haploid allele can gain a transmission advantage by sabotaging their opposite haploid allele during spermatogenesis. This directly benefits the selfish haploid sperm allele, as it then occurs in more than half of a male's sperm, despite its action generally being destructive to the ejaculate (6). Segregation distorters can kill all non-carrier sperm, transmitting the driver to 100% of offspring. However, this killing can reduce sperm number by up to 50%, and can even damage sperm that carry the segregation distorter (3). This reduction can directly reduce male fertility (4) and these costs can be exacerbated by contexts such as high polyandry, due to poor sperm-competitive ability (Table 1), or high temperatures (11). When the driver occurs on a sex-chromosome they also strongly bias offspring sex-ratios (10). This death of half a male's sperm, and potentially biased brood sex ratios, can impose major costs on the rest of the genome, causing strong intra-genomic conflict and promoting suppression of the SGE (12).

While our mechanistic understanding of how these SGEs kill sperm comes from only a few model systems, we know they act at different stages of spermatogenesis (13,14). There are also differences in the effect on non-carrier sperm, with some SGEs disabling sperm, for example the *t*-haplotype system in *Mus musculus* (15), while many others kill non-carrier sperm, for example the Paris sex-ratio drive system in *Drosophila simulans* (16). When and how non-carrier sperm are affected will likely have differing impacts on sperm quality and male fertility costs (Figure 1; Table 1). In general, the characterized drive systems share some commonalities in mechanism, frequently involving heterochromatin binding and small RNA pathways (13).

Sperm hitchhikers

Other SGEs appear to use sperm as a vehicle to hitchhike to the next generation. For example, viruses which have been found packaged within or on sperm can be paternally inherited (9,17). In *Diaphorina citri* psyllid insects, a retrovirus makes use of a virus-encoded non-structural protein for efficient vertical transmission (18) and remarkably, viruses can transmit through sperm without apparent costs,

like the rice gall dwarf arbovirus that interacts with proteoglycans on sperm heads (9). Supernumerary chromosomes, commonly known as B-chromosomes, can also transmit themselves through sperm (2,19), although paternal transmission is by no means universal with some B-chromosomes being excluded from sperm during spermatogenesis and instead showing biased transmission through the female germline (20). The *Paternal Sex-Ratio* (*PSR*) B-chromosome, which occurs in haplodiploid parasitic wasps, is a remarkable example (2). *PSR* travels within sperm and upon fertilization eliminates the paternal genome component in the zygote. This turns the offspring male, which means *PSR* always finds itself in the sex it uses for transmission. Given how recently many SGEs that travel within sperm have been characterized (particularly viruses), understanding their diversity, mechanisms and impacts on sperm is an emerging area of research.

Post-segregation distorters

Some SGEs modify sperm to cause serious downstream consequences during zygote formation (Figure 1), and so are often referred to as post-segregation distorters. These SGEs include maternally inherited endosymbionts (e.g. *Wolbachia*, *Rickettsia*, *Cardinium* bacteria), that are transmitted in the cytoplasm of eggs from mother to offspring. Many SGEs from these groups gain a transmission advantage by killing males, or turning genetic males into females, which favours the transmitting sex and hence the SGEs (21,22). However, many endosymbionts modify sperm into weapons that poison the eggs they fertilise if the egg lacks the same endosymbiont. The resulting reproductive incompatibility (cytoplasmic incompatibility, CI) can dramatically reduce offspring production of uninfected females compared to infected females, allowing the endosymbiont to spread. These 'toxin' and 'rescue-factor' systems favour the offspring production of SGE-carrying females that translates into a large transmission advantage favouring the spread of the selfish endosymbiont through a population (23; Figure 1). This sperm manipulation can also negatively impact male reproductive success by damaging the weaponised sperm, for example *Wolbachia* may cause reduced fertility in infected males by affecting expression of immune genes that result in oxidative damage and cell death in the males' testes (24). However, the impact of *Wolbachia* on male fertility can vary in both magnitude and direction (4,25–29) and can be context dependent, for example frequently reducing sperm competitive ability (Table 1). Such sperm-modifying endosymbionts have been shown to occur in numerous arthropods including spiders, mites and filarial nematodes, and have been particularly well characterised in insects where they are predicted to be present in ~65% of all species (30).

Sperm competitive ability

There is strong evidence that sperm killing meiotic drive substantially reduces sperm competitive ability in insects and mice, and single studies find similar effects of endosymbionts and B chromosomes (Table 1). At present the vast array of other SGEs (1) have not yet been evaluated for their impact on sperm competitive success. Given how ubiquitous SGEs are in animals, it is likely that SGEs are affecting fertility, mating behaviour, and co-evolution in a far broader range of taxa. In particular, given how easy it is to PCR screen for common endosymbionts in insects, it is surprising how few studies have investigated their effect on sperm competitive success.

3. Mitigation strategies

An important impact of many SGEs in sperm is that they impose costs to their carrier. This results in a fascinating intersection between sexual selection and SGEs where males and females may adapt to mitigate against harm from SGEs.

Mitigation by males

Seeing that males of several species suffer reduced ejaculate quality due to harbouring SGEs, how can they maximize their fitness? In flies infected with CI-inducing *Wolbachia* endosymbionts, repeated male mating may lessen the severity of CI, possibly due to reduced exposure time to the *Wolbachia* toxin during sperm development (31). In support of this suggestion, increased mating rate observed by *Wolbachia*-infected *D. simulans* males is shown to restore their reproductive compatibility with uninfected females resulting in increased male reproductive success (25,26). It is also suggested that SGE-carrying males may benefit by dispersing to a low-density population with reduced risk of sperm competition, which appears to be the case in house mice where *t*-carrying individuals show increased dispersal, especially at higher densities (32). Sperm competition models predict that disfavoured males (i.e. SGE-carrying males) consistently mating in a disfavoured role (e.g. after a non-carrying male, 33) should increase their ejaculate expenditure, but that this will depend on the likelihood of mating in a disfavoured role (34). To date, there is insufficient data to evaluate these predictions, and what we know relates to the outcome of sperm competition rather than males' ejaculate allocation strategies. The predictions will also depend on the severity of sperm limitation experienced by SGE carrying males and females.

We also expect an evolutionary response in males to compensate the cost of reduced fertility. For example, in *Teleopsis dalmanni* stalk-eyed flies, males carrying a sperm killing segregation distorter transfer the same number of sperm as non-carrying males (35). They are able to maintain high fertility by preferentially investing in testes size at the expense of accessory gland size (35, 36). However, this trade-off could come at a cost of reduced mating rate, which is determined by accessory gland size (37). Similar evolutionary compensation in sperm production may also be present in other taxa (e.g. 38; Figure 1), but it is currently unknown how widespread this is and likely to be shaped by the cost of sperm production (e.g. sperm and ejaculate size). Nonetheless, it is clear there are male mitigation strategies that reduce the cost imposed by SGEs.

Mitigation by females

SGEs involving sperm manipulation confer direct fitness costs to males that carry them, therefore we expect females to mitigate against mating to such 'inferior' males (4–6). A simple strategy is pre-copulatory mate choice to avoid mating with SGE males entirely. However, evidence for direct mate choice is remarkably scarce; with only a few good examples of discrimination against SGE carrying males whereas most studies find no such evidence (for review see 5,39; Figure 1). However, SGEs can be costly to a male's fitness in a variety of ways and, as a result, any female-choice for high fitness males might generally select against SGE carrying low-fitness males (40).

Post-copulatory mechanisms offer another mitigation route for females. The importance of polyandry, when females mate with multiple males, when at risk of mating with an SGE-carrying male has received much attention (5,39). Polyandry is favoured because SGE-carrying males can be at a disadvantage when competing against other males' undamaged ejaculates due to the production of fewer sperm or sperm with lower vigour (41) and multiple studies across taxa have demonstrated SGE-carrying males to be inferior sperm-competitors (e.g. 33,42,43; Table 1). It is worth noting that studies are heavily biased towards SGEs that kill sperm (Table 1). There is also evidence suggesting polyandry could influence SGE frequency in the wild (44,45). The relationship between polyandry and SGEs is dynamic (39). While polyandry can regulate the frequency of SGEs in populations (46) and maintain population viability when at risk from SGEs (47), the presence of SGEs can also in turn directly affect the level of polyandry in a population (48) in part due to female sperm limitation promoting increased remating frequency (49). Apart from promoting sperm competition through polyandry, females at risk of mating with SGE-carrying males may also bias against such males' ejaculates post-mating by selective sperm dumping and/or sperm storage. However, these possibilities are yet to be examined

more widely (50). In summary, there is a growing body of evidence to suggest that due to the reduced sperm competitive ability of SGE-carrying males, polyandry is an effective female mitigation strategy (Table 1).

4. Evolutionary consequences

The intra-genomic conflict caused by SGEs has implications for male fertility and spermatogenesis. First, the genome can evolve to counteract the costly effects of SGEs and this could disrupt male fertility between populations or between related species harbouring different SGEs. Secondly, intra-genomic conflict stemming from SGEs could contribute to the complexity of spermatogenesis.

Suppressed SGEs and male fertility

The genome can respond to intra-genomic conflict by evolving to suppress the SGEs (12,51). This means many segregation distorters may exist but are fully suppressed. Evidence from *Drosophila* supports this prediction, with several drive systems only being revealed when closely related species and subspecies hybridize, creating offspring that carry the driver but not its suppressors (52,53). In mice, there is evidence that *Sly*, a multi-copy Y-linked gene is involved in a co-evolutionary arms-race with the X chromosome resulting in skewed offspring sex-ratios and disrupted gene expression (54). These cryptic *Drosophila* and mouse drive systems result in abnormal spermatogenesis and damage male fertility when expressed. Evidence from inter-population crosses involving a non-suppressed sperm-killer in *D. subobscura* also show hybrid males suffer severe fertility costs (55). These studies are consistent with sperm-killing SGEs and their suppressors playing a role in reducing male fertility in crosses between populations (or species) that harbour SGEs and those that do not. However, an open question remains about how widespread a force this genetic conflict is in creating male fertility barriers between populations and potentially contributing to reproductive isolation (56,57).

SGEs and spermatogenesis

The dissection of spermatogenesis at the cellular and molecular level has revealed some intricacies that could be attributed to SGE-fuelled intra-genomic conflict. SGEs could contribute to complexity in spermatogenesis in several ways. First, spermatogenesis-genes may be particularly vulnerable to harbouring SGEs themselves, because suppression of SGEs may come at a cost to male fertility. For example there are three different sperm-killing SGEs in *D. simulans*, some that are unsuppressed (16,58). Second, if specific SGEs become suppressed or co-evolve with suppressors, over time, these genes could become integral to achieving successful sperm production, with male fertility being compromised if either the SGE or the suppression genes are lost (54,59,60). The co-evolution between SGEs and their suppressors could thus lead to increasing number of genes being required for successful spermatogenesis, as has been observed in the *Winters* meiotic drive system in *D. simulans* (60). A third related explanation for increasing complexity is the evolution of a general defence against SGEs, whereby genes critical to guarding spermatogenesis against SGEs accumulate (61). The proliferation of certain testes-specific gene families, for example the argonautes in *Drosophila*, is suggested to have evolved to suppress the activity of transposable elements during spermatogenesis (60,61). The impact of SGE-fuelled genomic conflict could therefore contribute new testes specific genes and promote diversification of gene-families associated with generally suppressing a variety of SGEs during spermatogenesis (62,63). Haploid silencing of many genes during spermatogenesis has been implicated as management by the diploid genome to avoid such intra-genomic conflict, (see Sutter et al 2020 in this issue). As our understanding of spermatogenesis deepens, some of its intricacies may turn out to be the result of SGE-fuelled intra-genomic conflict.

5. Summary and future perspectives

We have discussed the widespread and diverse impact of SGEs on sperm and male fertility and their consequences for mating behaviour and spermatogenesis. There is no doubt that SGEs have profound impact on males' sperm production and reproductive success under polyandry, but that the impacts on male fertility are diverse, ranging from extreme to undetectable costs. Males carrying SGEs will suffer variable fitness consequences depending on the species' mating system biology. However, our current knowledge is limited to a few well studied taxa, and we anticipate that the impact of SGEs are far more widespread than discussed here.

While we have focused on vertically transmitted SGEs there are links to other SGEs that are predominantly horizontally transmitted. The ejaculate contains not only sperm, but also a cocktail of seminal proteins with diverse roles in reproduction (64). One speculation is that SGEs are associated with accessory gland proteins (Acps) in the ejaculate. Is it possible that SGEs may be indirectly associated with sperm if bound to Acps that in turn are bound to sperm (65) and/or are present in the ejaculate at mating? The La Crosse virus and Zika virus in mosquitoes can be transmitted by male accessory sex gland fluid rather than by sperm (66,67). However, such SGEs while adversely affecting male fertility are less likely to be transmitted vertically and hence may have a different dynamic.

Another expanding area of research is the role of the microbiota in reproduction, and although we know little about the male reproductive microbiome it can contain microorganisms from diverse taxa (68–70) that can be transferred to the female at mating (71,72). There is evidence that the microbiota within the male reproductive tract can adversely affect sperm performance. For example, in humans, there is an association between the microbial community and sperm quality (73). However, it remains unclear how these microbes influence sperm parameters or if this promotes their transmission (see 68 for review). Nonetheless, these impacts on male fertility have clear parallels with impacts of vertically transmitted SGEs.

The next 50 years of sperm competition research promises to elucidate the prevalence and impact of SGEs on the outcome of sperm competition, and their potency for shaping male mating and ejaculate strategies. SGEs are likely to affect a multitude of areas where the conflict between SGEs and the rest of the genome has not yet been identified. There are also aspects of male reproductive biology where the presence of SGEs have not been extensively considered. No doubt SGEs, and other selfish agents present in the male reproductive tract, have the potential to illuminate some unexplained aspects of male fertility and spermatogenesis and may even be harnessed to suppress harmful vector and pest populations (74).

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Table and Figure

Table 1

Effects of selfish genetic elements (SGE) on sperm competitive ability.

SGE	Host	P1[1]	P2[2]	Overall paternity[3]	Male mated status	Reference
SR (X-linked driver)	*Teleopsis* whitei*	0.099	0.101	**0.1**	Virgin	(43)
SR (X-linked driver)	*T. whitei*	0.125 [5]	NA	**NA**	Virgin	(75)
SR (X linked driver)	*T. dalmanni*	NA	0.25	**NA**	Non-virgin	(76)
SR (X linked driver)	*Drosophila pseudoobscura*	0.02	0.83	**0.42**[5]	Virgin	(77)
SR (X linked driver)	*D. pseudoobscura*	0.38	0.32	**0.35**[5]	Non-virgin	(77)
SR (X linked driver)	*D. pseudoobscura*	0.35	0.14	**0.25**	Non-virgin	(33)
SR (X linked driver)	*D. recens*	NA	NA	**0.30**[5]	Non-virgin	(78)
SR (X linked driver)	*D. simulans*	0.10	0.50	**0.30**	Virgin	(79)
SR (X linked driver)	*D. simulans*	0.12	0.34	**0.22**	Virgin	(80)
Wolbachia (Riverside strain)	*D. simulans*	0.15	0.72	**0.44**	Non-virgin	(81)
PSR (B chromosome)	*Nasonia vitripennis*	0.08	0.58	**0.25**	Non-virgin	(82)
t-haplotype (autosomal driver)	*Mus musculus*	0.22	0.05	**0.13**	Virgin	(83)

t-haplotype (autosomal driver)	*M. musculus*	NA	NA	**0.24**	Virgin	(84)

Sperm-competitive success of SGE males where a female is mated to 2 males unless otherwise stated. [1]P1 is the percentage of offspring fathered by the first of two males to mate with the same female. [2]P2 is the percentage of offspring fathered by the second of two males to mate with the same female. [3]Overall paternity is the mean of P1 and P2, and is the overall paternity expected under sperm competition when SGE status does not affect mating order. [4] 2nd male only transferred seminal fluids and not sperm, an estimate of 0.125 is therefore extrapolated from SR males producing 25% as many offspring as ST males when exposed to the seminal fluid of a 2nd male. [5]Paternity estimated from competition of Sex-Ratio and standard males against an inferior tester mutant strain, potentially causing an underestimated P1 and overestimated P2. Table modified from (4). *The *Cyrtodiopsis* genus was synonymized with *Teleopsis* in 2001.

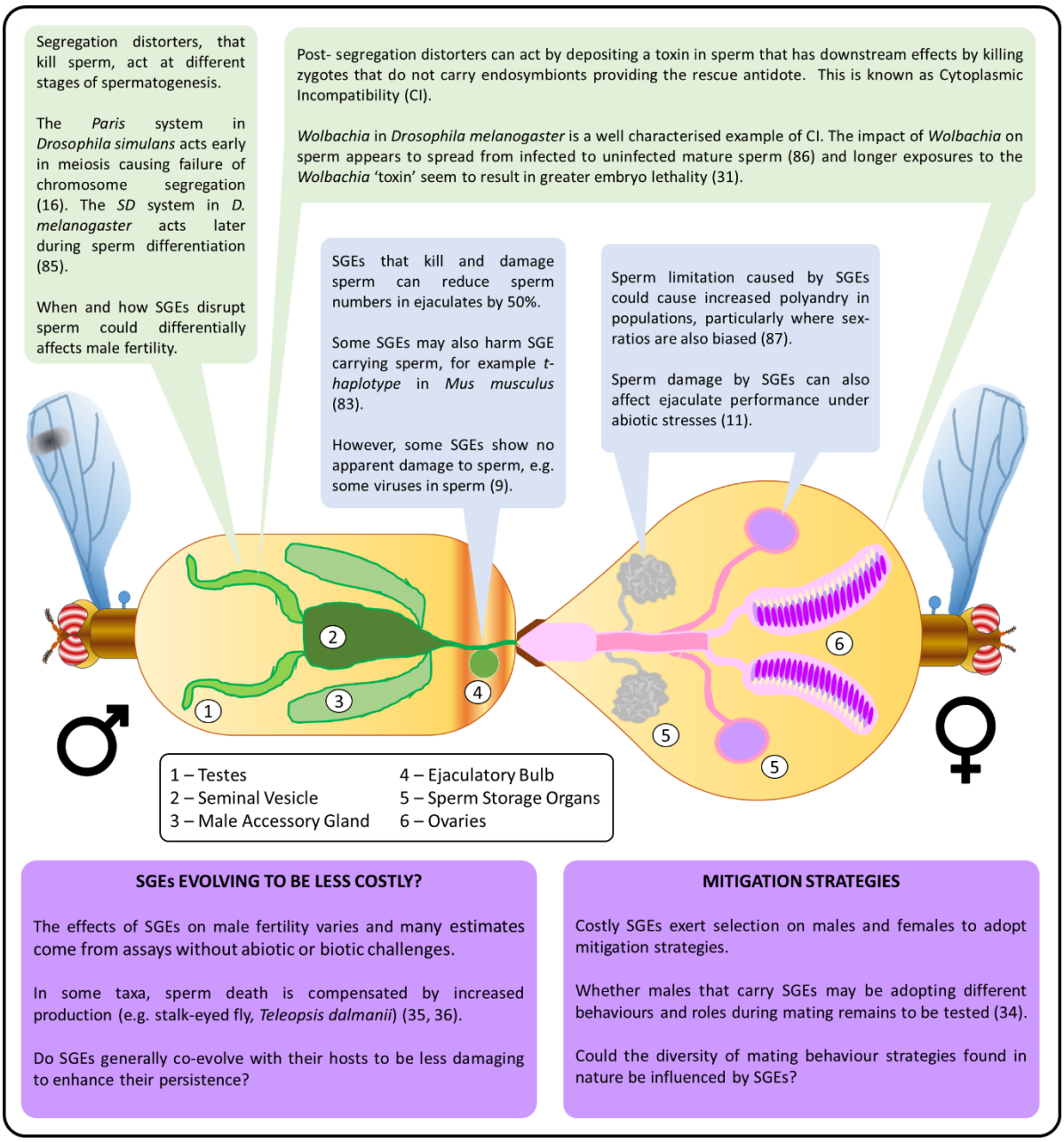

Figure 1. The main arenas where SGEs are known to act from spermatogenesis to fertilisation (green), pictured in insects. In blue, highlights costs of carrying SGEs to sperm production and sperm competitive ability. In purple, examples of evolutionary impacts of SGEs on sperm and mitigation strategies.

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