1 **Selfish genetic elements and male fertility**

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

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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,

58 like the rice gall dwarf arbovirus that interacts with proteoglycans on sperm heads (9). Supernumerary
59 chromosomes, commonly known as B-chromosomes, can also transmit themselves through sperm
60 (2,19), although paternal transmission is by no means universal with some B-chromosomes being
61 excluded from sperm during spermatogenesis and instead showing biased transmission through the
62 female germline (20). The *Paternal Sex-Ratio* (*PSR*) B-chromosome, which occurs in haplodiploid
63 parasitic wasps, is a remarkable example (2). *PSR* travels within sperm and upon fertilization eliminates
64 the paternal genome component in the zygote. This turns the offspring male, which means *PSR* always
65 finds itself in the sex it uses for transmission. Given how recently many SGEs that travel within sperm
66 have been characterized (particularly viruses), understanding their diversity, mechanisms and impacts
67 on sperm is an emerging area of research.

68 *Post-segregation distorters*

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

88 *Sperm competitive ability*

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

96 **3.   Mitigation strategies**

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

100 *Mitigation by males*

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

116 We also expect an evolutionary response in males to compensate the cost of reduced fertility. For
117 example, in *Teleopsis dalmanni* stalk-eyed flies, males carrying a sperm killing segregation distorter
118 transfer the same number of sperm as non-carrying males (35). They are able to maintain high fertility
119 by preferentially investing in testes size at the expense of accessory gland size (35, 36). However, this
120 trade-off could come at a cost of reduced mating rate, which is determined by accessory gland size
121 (37). Similar evolutionary compensation in sperm production may also be present in other taxa (e.g.
122 38; Figure 1), but it is currently unknown how widespread this is and likely to be shaped by the cost of
123 sperm production (e.g. sperm and ejaculate size). Nonetheless, it is clear there are male mitigation
124 strategies that reduce the cost imposed by SGEs.

125 *Mitigation by females*

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

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

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

150 **4. Evolutionary consequences**

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

155 *Suppressed SGEs and male fertility*

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

169 *SGEs and spermatogenesis*

170 The dissection of spermatogenesis at the cellular and molecular level has revealed some intricacies
171 that could be attributed to SGE-fuelled intra-genomic conflict. SGEs could contribute to complexity in
172 spermatogenesis in several ways. First, spermatogenesis-genes may be particularly vulnerable to
173 harbouring SGEs themselves, because suppression of SGEs may come at a cost to male fertility. For
174 example there are three different sperm-killing SGEs in *D. simulans*, some that are unsuppressed
175 (16,58). Second, if specific SGEs become suppressed or co-evolve with suppressors, over time, these
176 genes could become integral to achieving successful sperm production, with male fertility being
177 compromised if either the SGE or the suppression genes are lost (54,59,60). The co-evolution between
178 SGEs and their suppressors could thus lead to increasing number of genes being required for
179 successful spermatogenesis, as has been observed in the *Winters* meiotic drive system in *D. simulans*
180 (60). A third related explanation for increasing complexity is the evolution of a general defence against
181 SGEs, whereby genes critical to guarding spermatogenesis against SGEs accumulate (61). The
182 proliferation of certain testes-specific gene families, for example the argonautes in *Drosophila*, is
183 suggested to have evolved to suppress the activity of transposable elements during spermatogenesis
184 (60,61). The impact of SGE-fuelled genomic conflict could therefore contribute new testes specific
185 genes and promote diversification of gene-families associated with generally suppressing a variety of
186 SGEs during spermatogenesis (62,63). Haploid silencing of many genes during spermatogenesis has
187 been implicated as management by the diploid genome to avoid such intra-genomic conflict, (see
188 Sutter et al 2020 in this issue). As our understanding of spermatogenesis deepens, some of its
189 intricacies may turn out to be the result of SGE-fuelled intra-genomic conflict.

190 **5. Summary and future perspectives**

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

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

206 Another expanding area of research is the role of the microbiota in reproduction, and although we
207 know little about the male reproductive microbiome it can contain microorganisms from diverse taxa
208 (68–70) that can be transferred to the female at mating (71,72). There is evidence that the microbiota
209 within the male reproductive tract can adversely affect sperm performance. For example, in humans,
210 there is an association between the microbial community and sperm quality (73). However, it remains
211 unclear how these microbes influence sperm parameters or if this promotes their transmission (see
212 68 for review). Nonetheless, these impacts on male fertility have clear parallels with impacts of
213 vertically transmitted SGEs.

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

222

223

230   **Table and Figure**

231   **Table 1**

232   **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)

233

234 Sperm-competitive success of SGE males where a female is mated to 2 males unless otherwise stated. [1]P1

235 is the percentage of offspring fathered by the first of two males to mate with the same female. [2]P2 is the

236 percentage of offspring fathered by the second of two males to mate with the same female. [3]Overall

237 paternity is the mean of P1 and P2, and is the overall paternity expected under sperm competition when

238 SGE status does not affect mating order. [4] 2nd male only transferred seminal fluids and not sperm, an

239 estimate of 0.125 is therefore extrapolated from SR males producing 25% as many offspring as ST males

240 when exposed to the seminal fluid of a 2nd male. [5]Paternity estimated from competition of Sex-Ratio and

241 standard males against an inferior tester mutant strain, potentially causing an underestimated P1 and

242 overestimated P2. Table modified from (4). *The *Cyrtodiopsis* genus was synonymized with *Teleopsis* in

243 2001.

244

Segregation distorters, that kill sperm, act at different stages of spermatogenesis.

The *Paris* system in *Drosophila simulans* acts early in meiosis causing failure of chromosome segregation (16). The *SD* system in *D. melanogaster* acts later during sperm differentiation (85).

When and how SGEs disrupt sperm could differentially affects male fertility.

Post- segregation distorters can act by depositing a toxin in sperm that has downstream effects by killing zygotes that do not carry endosymbionts providing the rescue antidote. This is known as Cytoplasmic Incompatibility (CI).

Wolbachia in *Drosophila melanogaster* is a well characterised example of CI. The impact of *Wolbachia* on sperm appears to spread from infected to uninfected mature sperm (86) and longer exposures to the *Wolbachia* 'toxin' seem to result in greater embryo lethality (31).

SGEs that kill and damage sperm can reduce sperm numbers in ejaculates by 50%.

Some SGEs may also harm SGE carrying sperm, for example *t-haplotype* in *Mus musculus* (83).

However, some SGEs show no apparent damage to sperm, e.g. some viruses in sperm (9).

Sperm limitation caused by SGEs could cause increased polyandry in populations, particularly where sex-ratios are also biased (87).

Sperm damage by SGEs can also affect ejaculate performance under abiotic stresses (11).

1 – Testes
2 – Seminal Vesicle
3 – Male Accessory Gland
4 – Ejaculatory Bulb
5 – Sperm Storage Organs
6 – Ovaries

SGEs EVOLVING TO BE LESS COSTLY?

The effects of SGEs on male fertility varies and many estimates come from assays without abiotic or biotic challenges.

In some taxa, sperm death is compensated by increased production (e.g. stalk-eyed fly, *Teleopsis dalmanii*) (35, 36).

Do SGEs generally co-evolve with their hosts to be less damaging to enhance their persistence?

MITIGATION STRATEGIES

Costly SGEs exert selection on males and females to adopt mitigation strategies.

Whether males that carry SGEs may be adopting different behaviours and roles during mating remains to be tested (34).

Could the diversity of mating behaviour strategies found in nature be influenced by SGEs?
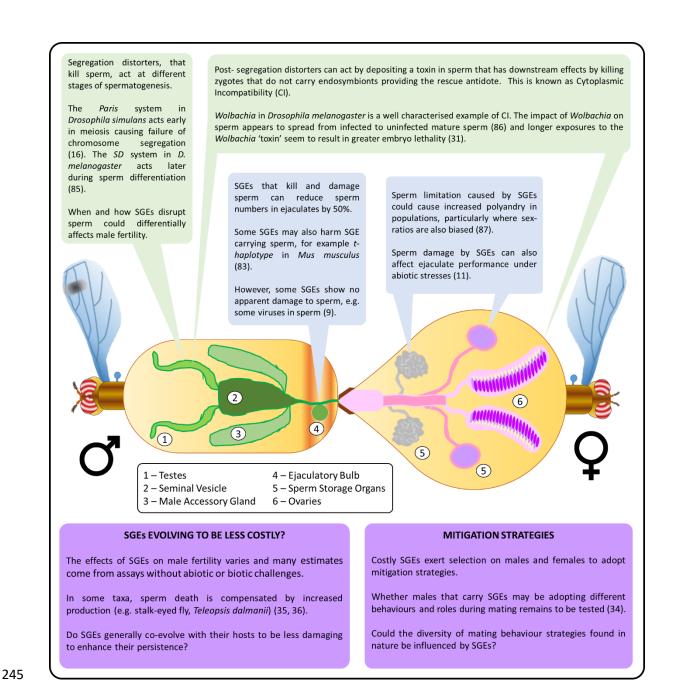
245

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.

250

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