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# Why do sperm carry RNA? Relatedness, conflict, and control

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**Classically, sperm were seen as transcriptionally inactive vehicles for delivering the paternal haplotype to an egg. Yet, it has become apparent that sperm also carry thousands of different RNAs, and the functions of most of these are unknown. Here, we make four novel suggestions for sperm RNA function. First, they could act as relatedness markers facilitating sperm cooperation. Second, they could act as paternally imposed suppressors of haploid interests. Third, they could act as a nuptial gift, providing the female with resources that entice her to fertilise ova using the sperm of the gift-provider. Fourth, they could represent the contents of a Trojan horse, delivered by males to manipulate female reproduction. We discuss these ideas and suggest how they might be tested.**

## Sperm and their RNA

Sperm are remarkably differentiated cells whose primary function is to deliver the paternal haplotype to the eggs. Despite this seemingly simple mission, sperm are extremely variable in form, with all aspects of the sperm phenotype showing high levels of variation [1]. Studies documenting and trying to explain sperm variation have been undertaken for decades, but we still do not have a good understanding of many sperm attributes, including apparently simple characteristics such as sperm size. The fact that sperm have until now been presumed to only contribute DNA (plus structures such as centromeres) to eggs, makes the recent discovery of a complex sperm RNA payload [2–7] both surprising and hard to explain.

The RNA population carried by sperm is large and varied. It includes messenger RNA (mRNA; see Glossary), microRNA (miRNA), interference RNA (iRNA), and antisense RNA [5], and a study of human sperm detected more than 4000 different mRNAs alone [6]. These include transcripts for heat shock proteins, cytochrome P450 aromatase, and a range of receptors, including odour receptors [5]. Although initially identified in mammals, sperm RNAs have also recently been found in insects [7] and plants (pollen RNAs [4]), and are likely to be a universal attribute of the male gamete.

Sperm RNA is unlikely to be transcribed from sperm nuclear DNA because of the changes in chromatin structure that occur when protamines replace histones during sperm DNA compaction [7,8]. Hence, it was originally thought that sperm RNAs were simply relics of spermatogenesis [2,9,10]. However, two lines of evidence suggest that sperm RNAs are not merely discards from the sperm-building process. First, there are indications of translational activity in the sperm cells, using sperm RNA (here and subsequently this term refers to RNA packed into sperm rather than RNA produced by sperm) as the substrate [7]. Second, there is evidence that sperm RNA contributes to fertilisation and to embryo development [3–5,11]. All this implies that the presence of sperm RNA has fitness consequences for both males and females, and is there because of its adaptive value. However, unequivocal evidence of precise sperm–RNA function is rare (e.g., [11], but see [12]) and for most of the thousands of transcripts carried by sperm, functions are completely unknown [2,3,7].

## Glossary

**Apyrene sperm:** anucleate, nonfertilising sperm.
**Anisogamy:** a system of sexual reproduction in which the gametes that merge to form zygotes differ dramatically in size.
**Antisense RNA:** single-stranded RNA whose coding is such that it inhibits the translation of mRNA.
**Brother sperm:** the sperm present in the ejaculate of a single male.
**Eupyrene sperm:** nucleate, fertilising sperm.
**Greenbeard:** a locus, or group of tightly linked loci, that simultaneously yields a phenotypic trait, allows recognition of that trait in others, and causes the carrier to behave in a biased fashion (e.g., show favouritism) either toward other carriers of the greenbeard, or against noncarriers.
**Interference RNA (iRNA):** double-stranded RNAs that interfere with translation.
**Messenger RNA (mRNA):** RNA that specifies the sequence of amino acids in proteins.
**miRNA:** short RNAs that tend to silence gene expression by interfering with translation.
**Nuptial gift:** reproductive investment by males in the form of resource provisioning that entices females to mate with gift providers.
**Policing:** deliberate controlling of the behaviour of potentially selfish agents, to achieve the objectives of a more powerful agent.
**Polyandry:** a mating system in which females mate with multiple males per reproductive period.
**The raffle principle:** when sperm number is the primary determinant of ejaculate competitiveness.
**Relatedness:** a measure of kinship or sharing of genes.
**Sexual conflict:** evolutionary divergence of interest between males and females.
**Sperm competition:** when the sperm from multiple males compete to fertilise the ova of a female.
**Sperm competition risk:** the probability that a female will mate with more than one male.
**Spermatogenesis:** the process by which haploid spermatozoa are produced.
**Transcription:** the process by which RNA is produced using DNA as a template.
**Transfer RNA (tRNA):** RNAs that carry amino acids, allowing translation from mRNA into proteins.
**Translation:** the process by which proteins are produced from a mRNA template.
**Transmission asymmetry:** when genes are not transmitted with equal probability or via the same route.

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**Box 1. RNA as sperm transcripts**

The hypotheses presented here are based on sperm RNA being paternal transcripts. If sperm were found to be transcriptionally active, then the predictions become more complicated. Under these circumstances, RNAs contained in sperm cells could be a mixture of those transcribed there by the male, and those transcribed directly by the sperm cell. The sperm cell itself then becomes a battleground for sperm–male conflict. For example, paternal interests might not be served if sperm were able to transcribe RNA and express greenbeards because this could lead to interejaculate sperm cooperation that, from the paternal perspective, means helping sperm from rival males. The paternal response to this threat would in part depend on the frequency of greenbeards in the population. The ability of sperm cells to transcribe RNA would yield a whole new battleground for evolutionary conflict, and many new exciting areas for research.

Here, we present four novel hypotheses about potential functions of these sperm RNAs, all of which have their roots in relatedness and evolutionary conflicts of interest. Evolutionary conflicts are ultimately due to relatedness or transmission asymmetries, and any replicator exploiting the common good can increase in frequency [13]. Conflict can occur at any level, from genes to societies, and there are several ways to suppress or resolve evolutionary divergences of interests. For example, solutions can be kin based and/or involve policing or coercion [14].

Our hypotheses are all predicated on the assumption that RNA synthesis is costly, and on the fact that RNAs found in sperm are paternal transcripts and not the product of the sperm haplotype (although we briefly discuss the evolutionary complications that might arise if sperm were found to be transcriptionally active; Box 1). The first of our suggestions pertains to the relatedness of brother sperm: perhaps RNAs facilitate relatedness signalling, either generally or at specific loci. Such signals would facilitate the evolution and maintenance of cooperation among the sperm within the ejaculate of a male, or even between nonbrother sperm that share a common allele(s). The second idea is that RNAs are packed into sperm by the diploid male to suppress the selfish interests of the haploid cell. That is, the RNA acts as a standing police force to keep sperm under paternal control. Third, perhaps the RNA acts as a nuptial gift: the packaging of this gift within sperm prevents females from accessing the resource without having first used the sperm to fertilise her ova. Hence, the evolution of sperm RNA would be a male strategy resulting from sexual conflict over reproduction. Fourth, the RNA packaged within sperm might act like the contents of a Trojan horse: having accepted the sperm for fertilisation, the female might then suffer surprise manipulation of her fertilisation machinery, with benefits for the paternal haplotype.

## Signals of relatedness

As explained by Hamilton [15], it can pay replicators to sacrifice their own interests to help kin, in which case we have cooperation between replicators rather than conflict. Forfeiting one's personal fitness can even be beneficial, as long as the indirect genetic benefits of helping are greater than the cost of helping. That is, the benefits to the help-recipient weighted by their relatedness to the help-provider must exceed the costs paid by the provider. Given that this benefit–cost condition is easier to satisfy when interactors are more closely related, evolutionary conflicts can be reduced between close relatives or 'group' members, as long as within-group similarity is greater than between-group similarity. The ability to direct help appropriately requires that replicators follow rules that result in benefits to group members. This can be via direct recognition or some other behavioural algorithm (e.g., 'behave nicely toward individuals nearby because they are probably relatives').

Hamilton's inclusive fitness theory applies to any shared gene(s) regardless of genealogy, and can even apply to situations when individuals are dissimilar elsewhere in the genome [16]. Thus, relatedness is relative to some outgroup at some portion of the genome, and cooperation between related individuals should generally evolve more easily [16].

Sperm cooperation occurs in a range of taxa [1,17] and can be directed toward related sperm [18]. This includes the evolution of nonfertilising sperm-castes that support their fertilising brothers [19], loosely akin to the evolution of sterile worker castes in insects, and the forming of sperm pairs or sperm trains to facilitate migration to the site of fertilisation [1,17]. For example, the formation of sperm trains in wood mice increases the motility of the cooperating sperm over single sperm ([20], but see also [21,22]). However, for the trains to disaggregate, which is essential for fertilisation, some sperm need to compromise their own fertility by undergoing the acrosome reaction prematurely. The reaction releases proteolytic enzymes causing trains to disaggregate, but acrosome-reacted mouse sperm are no longer able to bind to ova and, therefore, can no longer fertilise [20]. Thus, the sperm causing disaggregation sacrifice themselves to help others.

As with other forms of cooperation, these altruistic sperm behaviours should be directed toward related sperm. However, polyandry is the most common mating system in nature [23–25], meaning that ejaculates from more than one male are often present in the female reproductive tract at any one time [26–29]. Selection for cooperation between related sperm could be strong in such circumstances [30], but the mixture of related and unrelated sperm makes kin-directed altruism more difficult. One way to facilitate cooperation between brother sperm may be to load them with RNAs that produce recognisable relatedness markers. This could be especially important from a paternal perspective because sperm within a single ejaculate will vary in the precise genes they share with brother sperm, so by loading them all with RNA to act as relatedness markers, males could facilitate relatedness signalling and recognition. Markers could be general indicators of relatedness, or could be at specific transcripts that do not necessarily reflect kinship, a phenomenon known as 'greenbeards' [16]. From a paternal perspective, greenbeard signalling is unlikely (as sperm are either 0% or 100% related at any particular locus), but selfish genes could use this type of signal.

Sperm express a range of cell surface proteins and receptors, possibly including major histocompatibility complexes (MHC), and at least some of the sperm RNAs seem to be linked to cell surface products [30–33]. Additionally, the cell surface odour receptors could in principle help sperm 'smell' related sperm, perhaps using the MHC genotype, as reported for whole organisms [34,35].

Indeed, fertilisation biases have been linked to the MHC genotype [36]. Hence, there is potential for directly displaying attributes that signal relatedness or shared genes, and differences in miRNA expression are associated with different sperm phenotypes [9]; thus, it is plausible that sperm phenotypes could reveal information about sperm relatedness. Clearly, this need not be only MHCs (which are vertebrate specific), but merely needs to involve factors that are variable across males, and we note that approximately half of the sperm RNAs appear to be variable across sperm samples [37]. Even if RNAs are not directly detectable externally, these arguments apply as long as RNAs transcribe activities that cause sperm to direct a greater proportion of their cooperation toward related sperm and, therefore, we tentatively suggest that this idea does not apply to RNAs that block translation (e.g., iRNA).

## Suppression of selfish interests: sperm–male conflict and control

It is usually assumed that sperm are transcriptionally inactive and, therefore, that their phenotypes are determined by the diploid male [7]. However, there is some evidence of transcriptional activity in sperm [38,39], although this is controversial and probably reflects translation of male transcripts packed into the gametes [7]. Nonetheless, sperm potentially have some control over their own behaviour. Indeed, segregation distortion is an example of the phenotype of a sperm being determined by its haploid genotype [30] (and see below). That is, the gene content of a sperm determines its behaviour and fate. Furthermore, the fitness interests of haploid sperm and diploid male do not totally overlap: males do not care which sperm fertilises an egg, whereas each individual sperm has a selfish interest in fertilisation [40]. This sets the scene for evolutionary conflict between males and the sperm that they produce because individual sperm gain more by being selfish, whereas the males can gain more from unselfish sperm.

A drive system that exemplifies all the above is segregation distorter (SD) in *Drosophila melanogaster* [13,41]. During spermatogenesis in heterozygote males, sperm carrying a driving gene complex target noncarrier sperm and damage them, resulting in more than 90% of sperm having the driver. This clearly favours sperm with the driver, but because the number of sperm produced by the male is reduced, paternal fertility and sperm competitiveness is lowered.

What could the diploid male do to ensure that sperm more generally behave in a manner that is commensurate with paternal fitness interests? Driver systems point to the answer, because suppression of drive typically evolves rapidly [41]. That is, other genes evolve to suppress selfish behaviour. So, perhaps males prevent the selfish behaviour of sperm by loading them with RNA whose task is to facilitate translation in paternal interest, or perhaps to stop sperm-interest gene transcripts from having an impact on the sperm phenotype. We know that at least one class of iRNA (Piwis) functions to control rogue genetic elements [42]. This 'policing' scenario could explain the diversity of RNA found in sperm. For example, antisense RNAs and miRNA can inhibit translation by binding to mRNA, blocking the translational machinery. Thus, the diploid male could police the behaviour of the haploid sperm cell remotely, by loading it with RNA molecules that keep sperm under paternal control long after they have left the male.

Male–sperm conflict over sperm phenotypes has not been the subject of much empirical investigation, although theory does suggest that variation in the sperm phenotype could be explained by male–sperm conflict [30]. So do sperm RNAs help males to control their own sperm? At present, we do not know, but it seems the conditions required to select for such control exist, and the RNA are there. It is even possible that loading sperm with policing RNA has ultimately been superseded in some instances by the production of sperm classes that do not contain the normal haploid DNA content and, hence, have limited ability to behave selfishly, instead serving their producer by helping brother sperm to fertilise eggs [19]. Clearly, by producing DNA-deficient sperm types, males are already ensuring that haploid gene expression is severely limited.

## Nuptial gifts

As with evolutionary conflicts generally, sexual conflict occurs because the evolutionary interests of males and females rarely, if ever, perfectly align [43]. One major form of sexual conflict occurs over reproductive investment. Either sex would do better if it could coerce the other sex into increased investment in their mutual offspring. Therefore, selection can favour one sex manipulating the reproductive investment of the other. This conflict over reproductive investment has resulted in the evolution of strategic reproductive allocation, including adjustment of the size of nuptial gifts.

Nuptial gifts can be thought of as a form of male reproductive investment (or mating effort) provided to females in return for matings [44,45]. They are found across a large number of taxa, and males have been found to tailor these gifts in relation to the likelihood of siring success [45]. For example, male bush crickets (*Requena verticalis*) allocate more resource to younger females because their siring success will be greater with them [46]. The problem for males is that females can accept gifts and then use them in ways that do not benefit the gift provider [45].

One way to circumvent the problem of gift abuse is to tie the gift directly to sperm use. That is, selection favours males able to ensure that gifts are spent on their offspring and, by providing gifts in sperm, they ensure that females not using these sperm to fertilise ova cannot access the resource (although there is evidence that females can digest sperm to use the materials they contain according to female interests [47]). Although theory suggests that male provisioning via sperm is unlikely [48], there is some evidence for it in *D. melanogaster*, where whole sperm enter the egg and are then stripped down during embryo development [49].

Sperm RNA are putatively involved in a range of post-fusion and/or early developmental events that clearly benefit females [5,11] and, following the gift logic, sperm RNA could be a male response to sexual conflict over reproduction, ensuring females use the sperm directly.

Although the trait itself (sperm RNA) has now fixed in many taxa (all sperm contain RNA), there is variation in sperm RNAs among males [37], so perhaps the quality or quantity of the nuptial gift(s) impacts male fitness. This idea may only apply when most sperm in an ejaculate ultimately fertilise an egg, otherwise the resources wasted by males could make this approach too costly (and see [48]). Alternatively, it may be that females have simply shifted some of the costs of reproduction back onto males, and the RNA represents this shift. In any case, it is clear that at least one sperm RNA is essential for early developmental events [4,11], which is consistent with either scenario and we cautiously suggest that iRNA and antisense RNAs seem least suited for a nuptial gift functions: females are likely to benefit more from promoters of functions, rather than inhibitors.

## Trojan horses

It is also possible that sperm RNAs are used to manipulate female reproductive investment in some way. That is, sperm act as Trojan horses delivering manipulative RNAs to the egg. There are many examples of male-derived ejaculatory substances that alter female reproduction [27–29]. The benefit of hiding such agents of manipulation within the sperm, in the form of functional RNA molecules, is that the manipulation would be invisible to the female until it is too late to prevent fertilisation. The conditions favouring the Trojan horse hypothesis are also probably restrictive, because it may be costly for the male to invest manipulative RNA into every sperm cell, many of which will never even reach an ovum. Nonetheless, all types of RNA delivered by sperm could act as male agents of manipulation.

## Tests

Tests of the validity of these ideas are possible and we suggest a few approaches here. The location of sperm RNAs, their transcripts, and the transcriptional activity will narrow the possibilities that RNAs serve as either relatedness markers or as policing agents, for instance. Additionally, if a RNA is only ever active in the ovum, it is unlikely to be involved in policing sperm behaviour. However, if RNAs serve as relatedness markers, then clearly there must be more variation in transcripts among than within males, which may be true in humans [37]. This is testable. Additionally, in species producing two sperm types, if some transcripts are lacking from the apyrene sperm population, but found in eupyrene sperm, then these are likely to be related to fertilisation and/or policing.

Another way to differentiate between the putative functions suggested here is to compare related species that vary in sperm competition risk. With high risk of sperm competition, male and sperm interests become more closely aligned [30], in that neither wants the sperm of rival males sperm to fertilise ova, and less policing is needed. Conversely, if RNAs are markers of relatedness then, from a paternal perspective, the need for such markers should increase with increasing risk of sperm competition. The intercept of this relation should pass through the origin because, when there is no risk of sperm competition, all sperm in a female are equally related from a paternal perspective. Furthermore, if a species produces nonfertilising sperm, we might still expect the relatedness marker and policing hypotheses to favour the presence of RNA in these cells. However, if the RNA carried by sperm are greenbeards (or sperm transcripts: Box 1), then, with no sperm competition risk, they will still be present, but how quantities vary with risk may depend on allele number and frequencies at the greenbeard locus. If sperm RNA is a nuptial gift, then with increasing competition among males for reproductive opportunity, female choice could also drive selection for increasing magnitude of the nuptial gift. However, because gifts can also be thought of as mating effort (here fertilisation effort), we predict that the intercept of the gift–risk relation should be higher than the relatedness–risk relation: even with no risk of sperm competition, females could still select for some male investment via an RNA gift. Finally, if sperm RNA are agents of manipulation (the Trojan horse idea), they should not be present in monogamous taxa or in nonfertilising sperm. Combinations of these experimental and comparative approaches could be used and will be revealing because predictions for the possibilities outlined are reasonably distinct and readily testable (Figure 1).

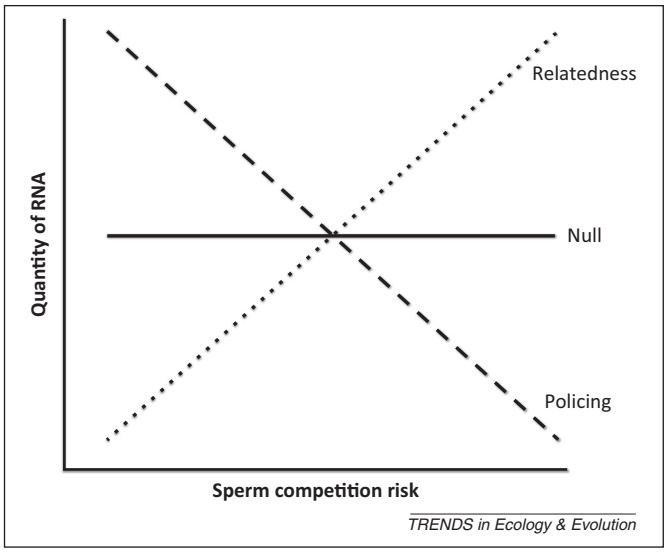


**Figure 1**. The predicted associations between RNA quantity and sperm competition risk under three of the hypotheses presented here. Note that we have shown relations as linear functions, but this need not be true: it is the sign of the associations that is key. For the inclusive fitness hypothesis (dotted line), we expect more RNA as risk increases. This is because the difficulty in, and need for, identifying brother sperm becomes more acute with increased risk of sperm competition. The association between risk and the size of nuptial gifts is also likely to be positive, but unlike the inclusive fitness association, may not pass through the origin (i.e., the intercept should be higher: see main text for an explanation). For the policing hypothesis (dashed line), we expect a negative association because sperm–male interests become more aligned as sperm competition risk increases. As a null model, we expect no association between risk and RNA content, although we have no *a priori* expectation about the intercept, which may be as shown here, or take some higher or lower value.

## Concluding remarks

Sperm are loaded with RNAs. To date, functionality has been ascribed to a few of them, but in most cases RNA roles are unknown. Based on evidence that these transcripts are paternally derived, we provide four novel suggestions for their evolutionary functions: badges of relatedness, policing, nuptial gifts, and Trojan horses. These ideas can be tested. The first will be most likely when sperm populations are

mixtures sourced from several males. Paternal control over sperm could be difficult to maintain once they have been ejaculated. This would seem to provide ample opportunity for any mutation that could remain transcriptionally active to cause sperm to act selfishly. However, the likelihood of selfishness would be reduced by a RNA police force ready to club any miscreants that defied the common good. Our suggestions that sperm RNAs might contribute nuptial gifts or Trojan horses to females are perhaps the least likely, because the cost of packaging gifts into each spermatozoon will be wasted if only a few sperm fertilise ova.

If it is found that sperm transcribe their own DNA, then some hypotheses become more complicated than described here (Box 1) and, because different RNAs may have different roles, our hypotheses need not be mutually exclusive. Time will tell if the ideas proposed here have any merit, but we think that they are worth investigating and look forward seeing tests of them.

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