

*Correspondence: jslefche@vims.edu (J.S. Lefcheck).

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Forum

Seminal Fluid and Mate Choice: New Predictions

Angela J. Crean,¹
Margo I. Adler,¹ and
Russell Bonduriansky^{1,*}

Recent evidence shows that seminal fluid can affect females and offspring independently of fertilisation in species lacking conventional 'nuptial gifts'. We argue that a hypothesis from paternal investment systems – that selection can favour changing female

preferences that maximise both sperm-borne and seminal fluid-borne benefits – could therefore apply much more broadly.

New Insights into the Multiple Functions of Seminal Fluid

In resource-based mating systems, female mate choice and polyandry have been assumed to evolve so as to allow females to take advantage of direct benefits or paternal investment provided by males. In some insects and other animals, limiting resources are transferred as 'nuptial gifts' of nutrients, defensive compounds, or water via the seminal fluid, with ejaculates sometimes comprising a substantial proportion of male body mass [1]. However, males of most species provide no obvious resources to females or offspring, with males' contribution to reproduction consisting of relatively tiny ejaculates that are usually assumed to be too small to contain substantial quantities of limiting resources (e.g., [2,3]). In such 'nonresource-based' mating systems, polyandry and female mate choice have typically been assumed to evolve via fertilisation benefits or genetic benefits to offspring, such as good or compatible genes, although the evidence remains equivocal.

Yet, even in species with small ejaculates, recent evidence shows that seminal fluid contains chemicals that can affect not only females themselves but also mediate nongenetic effects on offspring, and such effects can occur independently of fertilisation. In light of this evidence, we argue that theory on the evolution of female mate choice in resource-based mating systems could apply much more broadly, yielding new predictions for systems typically regarded as nonresource-based.

Effects of Seminal Fluid in Nonresource-Based Systems

Seminal fluid contains numerous proteins and peptides, RNA, salts, sugars,

enzymes, and hormones, and can contain pheromones, viruses, and bacteria [4]. The composition of seminal fluid is influenced by natural selection for sperm survival, as seminal fluid nourishes and protects sperm from oxidative damage and immune attacks in the female reproductive tract. In polyandrous systems, seminal fluid is also subject to sexual selection via its role in sperm competition, and may therefore be a sexually antagonistic trait [4]. Yet, despite the potential harmfulness of seminal fluid, females may benefit from some seminal fluid components. Seminal fluid can enhance female reproductive success through positive direct effects on fertilisation rate and female fecundity [5]. Moreover, rodent studies involving embryo transfer without exposure to seminal fluid, or mating to seminal-vesicle-deficient males, show that seminal fluid contains substances that are important for normal offspring survival, growth, and development [5]. Even in humans, acute exposure to semen at the beginning of a pregnancy, as well as cumulative exposure over time, has been shown to protect against recurrent miscarriage and pre-eclampsia, and significantly improve success rates of artificial reproductive technologies such as *in vitro* fertilisation (IVF) [5,6]. Given that seminal fluid appears to be costly to produce and variable among males (Box 1), this evidence suggests that seminal fluid composition can affect female fitness directly and via seminal fluid-mediated paternal effects on offspring.

In addition, recent evidence from insect studies shows that seminal fluid can influence traits of offspring sired by other males that mate subsequently with the same female ('non-sire effects'). In *Drosophila melanogaster*, exposure to non-sire ejaculates from different genetic backgrounds enhanced the fecundity of daughters [7]. In neriid flies, *Telostylinus angusticollis*, the environmentally induced condition of a female's first mating partner influenced the body size of offspring sired 2 weeks later by another male [8]. These

Box 1. Variation in Seminal Fluid Quality

Seminal fluid appears to be costly to produce, and the metabolic costs of regulating biochemical processes such as noncoding RNA expression or accessory gland protein synthesis may ensure that ejaculate composition is a highly variable, condition-dependent trait. Several lines of evidence suggest that seminal fluid quality can vary independently of sperm quality and genetic quality: frequent mating can rapidly reduce accessory gland size, and accessory gland products can become depleted before sperm stores [4]. Hence, the quantity and quality of seminal resources may be strongly dependent on male environment, mating history, and age (reviewed in [4]). However, the relationship between seminal fluid quality and other aspects of male mate quality could be complex. High-condition males (i.e., males that have been able to accumulate substantial stores of metabolic resources) may have more resources to invest in ejaculate production. Nonetheless, as seminal products can become rapidly depleted, high-condition males (who generally achieve higher rates of mating) may tend to deplete their stores of seminal fluid products more rapidly than low-condition males [4]. In addition, males could adjust the quantity or composition of their ejaculate based on their perception of female quality or mating status.

effects cannot be caused by epigenetic factors carried within sperm because offspring phenotype is influenced by a male who is not the sire. Such effects could potentially be mediated by females differentially allocating resources to developing offspring based on cues received from the non-sire mating partner [9]. However, in neriid flies, the effect was observed only when females actually mated, not when females were exposed to a male whose genitalia had been blocked to prevent semen transfer [8], implicating a seminal fluid-borne factor. These studies provide evidence that seminal fluid can affect offspring fitness independently of fertilisation.

Although the molecular mechanisms involved in paternal and non-sire effects remain largely unknown, two classes of semen-borne molecules – noncoding RNAs [10] and seminal proteins – are plausible candidates because of their potential to regulate embryonic gene expression. Proteins are released into the seminal fluid from accessory glands [4]. Moreover, proteins, RNAs, and other biomolecules are released by somatic cells into bodily fluids such as blood plasma and semen within extracellular vesicles (including exosomes, microvesicles, and apoptotic bodies), and such vesicles therefore have the potential to deliver proteins and RNA from any somatic tissue to the germ cells, providing a possible mechanism for the transfer of paternal somatic condition to offspring [11]. Vesicles in the seminal fluid could be absorbed by sperm from the same or

another male, or incorporated directly into oocytes or embryos.

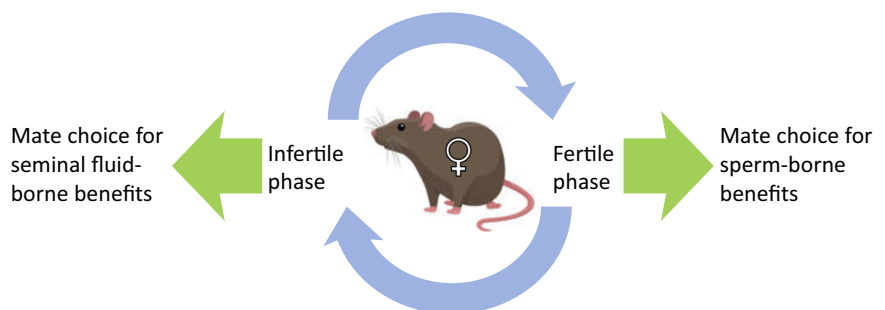
Mate Choice for Seminal Fluid in ‘Nonresource-Based’ Systems: A Matter of Timing?

Seminal fluid quality appears to vary independently of sperm quality and genetic quality (Box 1). Thus, if some males reliably signal seminal fluid-borne benefits via pre-copulatory or postcopulatory cues, then females may be expected to evolve preferences for such males [1], and female preference for the most beneficial or least harmful ejaculates may select on seminal fluid composition [1].

In species where males provision offspring, the ‘good parent’ hypothesis

proposes that, when at subfertile points of the cycle, females should seek males that provide paternal care benefits but, when likely to conceive, females should seek males that provide genetic benefits [12]. We suggest that this logic can be extended to mating systems where males provide no conventional nuptial gifts or other obvious resources to females or offspring.

We predict that, in the absence of long-term sperm storage and first-male sperm precedence, females will be selected to prefer males that provide beneficial seminal fluid when fertilisation is unlikely (e.g., in insects, when eggs are immature; in mammals, when females are at nonfertile points of the reproductive cycle). Although such males will probably not sire offspring, obtaining their semen may result in positive direct effects on females themselves, or beneficial nongenetic effects on future offspring, which could be sired by subsequent males that mate at a fertile point in the female's reproductive cycle. Once eggs are ready to be fertilised, females are expected to shift their preferences towards males that also provide sperm-borne benefits, such as a high fertilisation rate, or good or compatible genes or epigenetic factors



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Figure 1. Female Preferences May Change over the Reproductive Cycle to Take Advantage of Both Seminal Fluid-Borne and Sperm-Borne Benefits. When fertilisation is unlikely, we predict that females will prefer males that provide seminal fluid that confers direct benefits to females themselves, or nongenetic benefits to future offspring, which might be sired by a different male. By contrast, when fertilisation is likely, females should prefer males that also confer sperm-borne benefits, such as high fertilisation success, good genes, compatible genes, or good epialleles.

(Figure 1). By contrast, in species with long-term sperm storage and first-male sperm precedence (e.g., some insects), we predict that females will seek sperm-borne benefits from their initial mate, who is likely to fertilise eggs, but seek seminal fluid-borne benefits from subsequent mates who are unlikely to achieve fertilisations.

Although sperm-borne factors are likely to be important only when fertilisation is possible, seminal fluid quality could be important throughout life. In mammals, eggs and even early embryos may be permeable to seminal fluid-borne molecules. In insects, although mature (chorionated) eggs may be impermeable except via the specialised opening (micropyle) used for sperm entry [8], the female's immature eggs may nonetheless be susceptible to seminal fluid-borne factors. At the fertile stage, females may therefore seek males that provide an optimal combination of both sperm-borne and seminal fluid-borne factors.

Selection on females to acquire and utilise seminal fluid without fertilisation could explain why females in some species mate and exhibit preferences even when they are immature or outside of their fertile window [8]. However, such female strategies will be sexually antagonistic because seminal fluid donation without fertilisation represents wasted investment for males, perhaps driving the evolution of male counterstrategies. This situation is similar to the cuckoldry that occurs in many birds with biparental care, in which females may seek extra-pair sires for their offspring while benefiting from the parental investment of their social mate. In such situations, males may be selected to assess the risk of cuckoldry and reduce investment if their perceived risk of investing in another male's offspring is high, while females may evolve to deceive males to take advantage of valuable seminal fluid products. Such cuckoldry could also select for manipulative seminal fluid

components that delay or reduce the probability of female remating, or even male mate preferences that exploit seminal investment by previous males. A number of questions need to be answered to understand the role of seminal fluid in the evolution of mate choice (see Outstanding Questions).

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¹UNSW Australia, Evolution and Ecology Research Centre and School of Biological, Earth, and Environmental Science, Sydney, NSW 2052, Australia

*Correspondence: r.bonduriansky@unsw.edu.au (R. Bonduriansky).

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Outstanding Questions

What are the environmental factors that induce seminal fluid-mediated paternal and non-sire effects, and what are the offspring traits affected in various taxa?

Which seminal fluid components mediate paternal and non-sire effects in nonresource-based systems, and what are the molecular/developmental mechanisms involved?

How costly and condition-dependent is the production of various seminal fluid components, and how does seminal fluid quality relate to other aspects of male mate quality, such as genetic quality and sperm quality?

Are females able to assess semen quality in nonresource-based systems and, if so, what are the key phenotypic signals? Are males able to assess the semen quality of a female's previous mates?

How important is parental investment (i.e., investment in the quality of a male's own offspring) versus mating investment (i.e., investment in securing fertilisations) in the evolution of seminal fluid components?

Spotlight

Causality and the Levels of Selection

D.B. Krupp^{1,2,*},@

When is it sensible to say that group selection has shaped organismal design? This question has prompted many replies but few credible solutions. New work that exposes the causal relationships between phenotypes and fitness may finally settle the matter – and a few other things besides.

Despite its considerable age, group selection remains a woolly concept. Historically, it has been defined as heritable variance in a phenotypic character that causes variance in group fitness [1,2], but it has also been bound up with notions of frequency dependence, emergence, and the appearance of group functionality (e.g., [3–5]). Consequently, there is an idiosyncratic quality to the literature.