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Spermicide, cryptic female choice and the evolution of sperm form and function

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Abstract

Sperm competition and cryptic female choice profoundly affect sperm morphology, producing diversity within both species and individuals. One type of within-individual sperm variation is sperm heteromorphism, in which each male produces two or more distinct types of sperm simultaneously, only one of which is typically fertile (the 'eusperm'). The adaptive significance of nonfertile 'parasperm' types is poorly understood, although numerous sperm-heteromorphic species are known from many disparate taxa. This paper examines in detail two female-centred hypotheses for the evolution and maintenance of this unconventional sperm production strategy. First, we use game theoretical models to establish that parasperm may function to protect eusperm from female-generated spermicide, and to elucidate the predictions of this idea. Second, we expand on the relatively undeveloped idea that parasperm are used by females as a criterion for cryptic female choice, and discuss the predictions generated by this idea compared to other hypotheses proposed to explain sperm heteromorphism. We critically evaluate both hypotheses, suggest ways in which they could be tested, and propose taxa in which they could be important.

Sperm morphology is determined by a number of factors, including interactions with the ejaculates of other males and with the female reproductive tract (e.g. Eberhard, 1996; Birkhead & Møller, 1998). For instance, theoretical and empirical work suggests that sperm competition keeps sperm small in order to maximise the number of sperm that can be produced, as sperm number influences sperm competition success (e.g. Parker, 1982; Pitnick, 1996; Gage & Morrow, 2003). Sperm competition can select for larger sperm under some conditions and both comparative and empirical data suggest that this has happened in several species (see Snook, 2005). For example, a comparative study of frogs found a positive correlation between the probability of group spawning (and hence sperm competition) and sperm size (Byrne *et al.*, 2003). Sperm size may also be dramatically affected by female reproductive tract morphology (e.g. Briskie *et al.*, 1997; Miller & Pitnick, 2002; Minder *et al.*, 2005). As well as affecting sperm size, sperm competition and female reproductive tract morphology may also influence the shape of sperm. For example, the hook-shaped protrusions on the heads of some rodent sperm allow the sperm to link up to form 'sperm trains' that swim faster than lone sperm (Moore *et al.*, 2002).

Intriguingly, intra- and inter-sexual interactions may also be responsible for producing divergent sperm morphology within individuals as well as between species. Sperm heteromorphism is the simultaneous production of two or more distinguishable types of sperm by a single male in the same ejaculate. This striking phenomenon has been described in many species from a range of phyla, including chordates, annelids, molluscs and many different arthropods (see Silberglied *et al.*, 1984; Swallow & Wilkinson, 2002; Till-Bottraud *et al.*, 2005). Sperm types can differ in size, morphology and ploidy, and while there are typically two sperm morphs, occasionally there are more as in some prosobranch molluscs (Buckland-Nicks, 1998) and pentatomid bugs (references in Swallow & Wilkinson, 2002). In the majority of cases in which the fertilizing potential of the different sperm morphs has been assessed, only one of the sperm types is fertile while the remainder are

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Table 1 List of hypotheses for the evolution of infertile parasperm. The table also shows how parasperm investment is predicted to co-vary with sperm competition risk.

Hypotheses	Summary	Should investment in parasperm vary with sperm competition risk?
Nonadaptive	Parasperm have no adaptive function	No
Provisioning	Parasperm provide nutrition to the female, her eggs or the eusperm	Yes, males are predicted to provide smaller nuptial gifts when sperm competion risk is high
Facilitation	Parasperm aid eusperm transport or capacitation	Possibly, if the number of parasperm influences fertilisation success in competitive matings
Competition Offence Defence	Parasperm aid eusperm in sperm competition, either offensively (e.g. by displacing or killing rival eusperm) or defensively (e.g. by inhibiting female remating or blocking access by rival sperm)	Yes, as parasperm number/quality is predicted to be an important determinant of male reproductive success
Sacrificial sperm	Parasperm increase eusperm survival by diluting the effects of spermicide	Probably not. See main text
Cryptic female choice	Parasperm increase the chance that eusperm will be favoured in CFC	Yes, as parasperm number/quality is proportional to male post-copulatory reproductive success

infertile (Till-Bottraud *et al.*, 2005) and these types are called eusperm and parasperm, respectively (Healy & Jamieson, 1981).

Several hypotheses for the function of parasperm exist (Table 1; for reviews see Swallow & Wilkinson, 2002; Till-Bottraud *et al.*, 2005). The adaptive hypotheses are not mutually exclusive, as parasperm could perform more than one function. Despite this, parasperm are generally assumed to have only one function, likely to simplify testing their evolutionary significance. Sperm heteromorphism is thought to be adaptive in many taxa because: (1) parasperm production is tightly regulated (Friedlander, 1997; Till-Bottraud *et al.*, 2005) and (2) parasperm can represent a substantial proportion of the ejaculate and therefore would presumably be selected against in the absence of an adaptive function, freeing up the male's limited resources for other functions. However, the function of parasperm remains unclear in almost all known sperm-heteromorphic species (Till-Bottraud *et al.*, 2005). Parasperm function in the majority of sperm-heteromorphic species has never been investigated, but there are a few species from the Lepidoptera, the *Drosophila obscura* species group and the stalk-eyed flies (Diopsidae), which have been extensively studied. For example, in Lepidoptera, empirical support has been found for both the cheap filler hypothesis (Cook & Wedell, 1999) and the facilitation hypothesis (Sahara & Takemura, 2003). Research on the *obscura* group of Drosophilids, in particular *D. pseudoobscura*, has empirically rejected the nutrition (Snook & Markow, 1996) and blocking hypotheses (Snook, 1998) and cast doubt on the cheap filler hypothesis (Snook, 1998). In the Diopsidae, a comparative analysis found that parasperm and female spermathecal size are positively correlated, consistent with a number of hypotheses regarding the function of parasperm (Presgraves *et al.*, 1999).

Much recent work on the evolution of parasperm has focused on how sperm competition may have shaped their evolution (e.g. Snook, 1998; Wedell & Cook, 1999; Oppliger *et al.*, 2003). However, many puzzling male traits associated with reproduction are thought to be products of inter-sexual co-evolution (e.g. elongated sperm, Miller & Pitnick, 2002; traumatic insemination, Stutt & Siva-Jothy, 2001; copulatory courtship, Edvardsson & Arnqvist, 2000). Parasperm, too, may be involved in male–female interactions (Silberglied *et al.*, 1984; Till-Bottraud *et al.*, 2005), and in this paper we discuss two additional hypotheses for the evolution of parasperm, both of which suggest that parasperm have evolved in response to a female trait. We suggest that parasperm may represent a male defence against spermicide by females and investigate this idea using game theory. We also expand on the pre-existing but undeveloped idea that parasperm may be a criterion for cryptic female choice (CFC). In particular, we focus on the predictions generated by this hypothesis and compare these predictions to other adaptive scenarios. We evaluate both of these ideas using existing data, suggest ways in which they could be tested and recommend taxa in which they could apply.

The sacrificial sperm hypothesis for the evolution of parasperm

We define the sacrificial sperm hypothesis (SSH) as infertile parasperm functioning to increase the survivorship of brother fertile eusperm in spermicidal conditions. Males that invest some of their resources in parasperm may consequentially have more eusperm available for sperm competition and/or have an increased likelihood of fertilisation in matings with females that only mate once. The evolution of parasperm for 'sacrificial' functions has been modelled before, but this work models a situation where parasperm attack the sperm of rival males ('kamikaze sperm'; Kura & Nakashima, 2000). Our model also differs from the Kura & Nakashima (2000) model in that rather than simply demonstrating that

parasperm could evolve, we also estimate how much should be invested in parasperm. Another model on the evolution of sperm number in response to female spermicide (Greeff & Parker, 2000) only considers changes in sperm-monomorphic ejaculates.

The SSH is clearly dependent on the evolution of spermicide. Here, we adopt Greeff & Parker's (2000) definition of female spermicide; that is sperm dumping, sperm digestion/disabling, and phagocytosis. This definition is broad and would therefore include phenomena in which sperm are either actively or passively lost before or after storage. Despite this broad definition, our models below show that the male response may not depend on the mechanism of female spermicide. Females could evolve spermicidal reproductive tracts for a number of reasons. Spermicide could be important in safeguarding against either infection (Austin, 1975; Eisenbach, 2003), unfit sperm or polyspermy (see Birkhead *et al.*, 1993); facilitating female control of sperm storage and paternity (Birkhead *et al.*, 1993); fostering sperm competition (Keller & Reeve, 1995) and/or converting sperm into nutriment (Arnqvist & Nilsson, 2000). Spermicide could also be non-adaptive; for example, spermicide may occur as a by-product of a mating-induced immune response intended as a prophylaxis against sexually-transmitted infections (Barratt & Pockley, 1998), or because males transfer more sperm than females are selected to keep viable as sperm storage may be costly in many species (Miller & Pitnick, 2003; Roth & Reinhardt, 2003). The prevalence of spermicide across the animal kingdom cannot be estimated with much certainty but a number of species are thought to digest, disable or eject live sperm. Species that digest sperm include flatworms (Pongratz & Michiels, 2003), molluscs, including some that are sperm-heteromorphic (Haase & Baur, 1995; J. Buckland-Nicks, personal communication), and many mammals (Birkhead *et al.*, 1993). Sperm digestion may be mediated by enzymes or phagocytotic cells (Barratt & Pockley, 1998). Sperm are disabled by female accessory gland secretions in house flies (Degrugillier, 1985) and antibodies in many mammals (Birkhead *et al.*, 1993). Several species are known to eject sperm from storage (Eberhard, 1996), including some Lepidoptera (Etman & Hooper, 1979), Diptera (Otronen & Siva-Jothy, 1991; Snook & Hosken, 2004; Bonduriansky *et al.*, 2005) and several birds and mammals (Birkhead *et al.*, 1993; Pizzari & Birkhead, 2000; Wagner *et al.*, 2004). The 'insemination reaction' that occurs in some *Drosophila* after mating (Markow & Ankey, 1988), during which the uterus becomes filled with a large, gelatinous mass, may be symptomatic of spermicide. Sperm may be trapped or destroyed by the mass, and some species have been observed to expel the insemination mass 6–8 h after mating, along with a quantity of sperm (Patterson, 1947). Finally, the presence of protease-inhibiting seminal proteins in some mammals (e.g. Veselsky *et al.*, 1985; Schiessler *et al.*, 1976; Von Fellenberg *et al.*, 1985), birds (Lessley & Brown, 1978) and *D. melanogaster* (Lung *et al.*, 2002) may represent indirect evidence of spermicide; these proteins may be male adaptations against spermicidal protease enzymes in the female tract. A deficit of these seminal anti-proteases can cause infertility in male mice (Murer *et al.*, 2001) and humans (He *et al.*, 1999). Thus, spermicide may be intense and anti-spermicidal adaptations may be critical to male fitness.

Models of the sacrificial sperm hypothesis

Given the likelihood of the importance of spermicide and its potential presence in a number of taxa, we developed three models to assess the plausibility of the SSH as an explanation for the evolution of sperm heteromorphism, and to investigate its predictions. For simplicity, we assume that parasperm function only to protect brother eusperm against spermicide, and do not have any other benefits to the male producing them.

Model 1: male responses to numerical spermicide by females

Model 1 investigates how the intensity of spermicide and the relative production costs of the sperm types affect the optimal investment in parasperm in species with two sperm morphs. The model assumes that one sperm morph is infertile and that females destroy, expel or otherwise incapacitate a certain number (as opposed to a proportion) of the sperm they receive. This type of spermicide could occur if sperm are moved into a fixed-volume receptacle for destruction, or if they are digested by a limited population of macrophages or enzyme molecules (Greeff & Parker, 2000).

Model 1 makes the following assumptions:

1. Males have a limited amount of sperm production resources per ejaculate, designated T, to invest in eusperm and parasperm. Each unit of T can produce either 1 eusperm or x parasperm; $1 < x < \infty$ if parasperm are cheaper to produce than eusperm, $0 < x < 1$ if they are more expensive. We only consider the simple case where all males have the same amount of resources for sperm production, and individuals expend equal amounts of resources on each of their ejaculates.
2. Investment in parasperm is traded off against investment in eusperm. Males invest a proportion, s, of the resources available for sperm production in parasperm, where $0 \leq s < 1$. Males therefore transfer $T(1 - s)$ eusperm and sxT parasperm in each ejaculate. It is reasonable to assume a trade-off between eusperm and parasperm production, because the two morphs must be produced in the same testis where space is limited and their production presumably consumes similar types of resources.
3. Females neutralize a certain number of the sperm they receive. This number is given by hT, where h is directly proportional to the intensity of spermicide. $0 < h < \infty$,

though high values of h (i.e. extreme spermicide) may be rare in nature because of associated energetic and fecundity costs. Note that if $h \geq 1$, the sperm of males producing only eusperm will all be neutralized. The number of surviving eusperm from male i is denoted E_i.

4. Eusperm and parasperm are equally likely to be affected by spermicide and both types are equally easy to neutralize (what happens when this assumption is invalid is investigated in model 2).
5. A proportion of a male's copulations, q, involve sperm competition with one other male while the remainder, $1-q$, are with females that only mate once. When two ejaculates meet, the proportion of offspring sired by each male is determined by 'fair raffle' sperm competition (Parker *et al.*, 1990), such that male A sires a proportion of the offspring equal to $E_A/(E_A + E_B)$. In nature, q and h may not be independent, as promiscuous species may have stronger spermicide. This potential nonindependence will be ignored for simplicity.

The evolutionarily stable value of s can be found by determining the fitness of a mutant male producing s' parasperm in a population of males producing s parasperm. The number of surviving eusperm belonging to the mutant, $E_{s'}$, is the total number of eusperm it produces minus the number that are lost to spermicide:

$$E_{s'} = T(1-s') - T(1-s')\left(\frac{hT}{(1-s')T + s'xT}\right).$$

Male fitness is thus:

$$W_{s'} = q\left(\frac{T(1-s') - T(1-s')\left(\frac{hT}{(1-s')T+s'xT}\right)}{T(1-s') - T(1-s')\left(\frac{hT}{(1-s')T+s'xT}\right) + T(1-s) - T(1-s)\left(\frac{hT}{(1-s)T+sxT}\right)}\right) + (1-q) \quad (1a)$$

We can use eqn 1a to calculate the evolutionarily stable investment in parasperm (s^*). Equation 1a is first differentiated with respect to s, then the mutant and wild type strategies s′ and s are set equal (i.e. $s' = s$). The derivative is set at 0 then solved for s, giving s^* (Maynard Smith, 1982). Inspection of the second derivative (not shown) confirms that s^* maximises rather than minimises W. s^* is given by either:

$$s* = \frac{1 - x - \sqrt{hx - 2hx^2 + hx^3}}{1 - 2x + x^2} \quad (1b)$$

or

$$s* = \frac{1 - x + \sqrt{hx - 2hx^2 + hx^3}}{1 - 2x + x^2}. \quad (1c)$$

Only solution 1b, which is plotted in Fig. 1, produces biologically meaningful values of s^*.

Results of model 1: Under the assumptions of model 1, $s^* > 0$ when $hx > 1$, which suggests that when parasperm are relatively inexpensive to produce and spermicide is intense, the evolution of parasperm under the SSH will be favoured (Fig. 1). As parasperm become very cheap, the evolutionarily stable investment in parasperm decreases because a small investment produces enough to provide near-total protection from spermicide (Fig. 1).

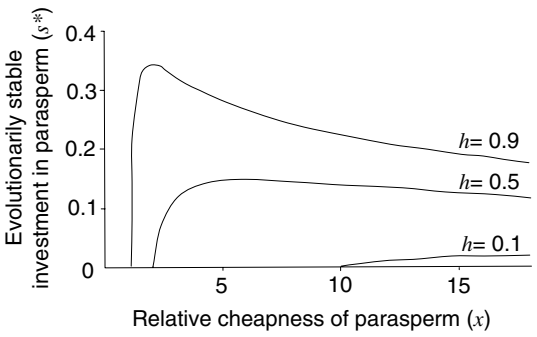

Fig. 1 Evolutionarily stable investment in parasperm (s^*) plotted against relative cheapness of parasperm (x) for three intensities of spermicide (h).

Because higher levels of spermicide in females may induce higher investment in parasperm by males (Fig. 1), an evolutionary arms race between the sexes is possible. Females might increase spermicide, perhaps to regain control of fertilisation, producing selection on males to increase parasperm production. Despite this, model 1 suggests that males should not invest the majority of resources in parasperm. Figure 1 shows that the optimal investment in parasperm under severe spermicide is typically less than a third of the available resources, but note that parasperm may still greatly outnumber eusperm under the SSH if they are cheaper to produce.

Figure 1 also shows that s^* drops off to negative infinity at $x = 1$, the point where parasperm cost as much to produce as eusperm. This result indicates that the SSH is only likely to be valid for this set of assumptions if parasperm are cheaper to produce than eusperm. Finally, model 1 suggests that the risk of sperm competition (Parker, 1998) should not influence how much males invest in parasperm if q is greater than zero, as indicated by the absence of the parameter q from eqn 1b. The reason for this is that it is always better to maximise the number of surviving eusperm, whether sperm competition occurs in 1 or 100% of matings. However, if $q = 0$ (i.e. there is complete monogamy) then the amount of resources a male invests in parasperm (his value of s) will not affect his fitness unless spermicide is so intense that there are not enough surviving eusperm to fertilise all the available eggs. Spermicide this intense seems unlikely to arise in nature

so the SSH is unlikely to be true in sperm-heteromorphic species with strict monogamy.

These conclusions are independent of the mechanism of spermicide, be it digestion, expulsion or incapacitation, so long as females neutralize a certain number of the sperm they receive. Although not investigated in the model, we also expect them to hold under both 'safe haven' spermicide, where sperm are only lost on their way to storage (so that a fixed number of sperm are lost) and 'continuous' spermicide, where females continuously destroy sperm in storage (so a fixed number are lost per unit time; Greeff & Parker, 2000).

Model 2: male responses to numerical spermicide when eusperm and parasperm are not equally vulnerable

Model 2 investigates what happens if eusperm and parasperm are not affected by spermicide in the same way, e.g. if they have a different chance either of being attacked by the spermicidal agent, or they take an unequal amount of time to neutralize. Factors such as these will determine the extent to which parasperm are able to protect eusperm from spermicide, and will affect the evolutionarily stable investment in parasperm. This model is similar to the first but contains a new parameter, k, which represents the value of parasperm as a 'spermicide absorber' relative to eusperm. $0 < k < 1$ means parasperm are poor absorbers; they could be quicker to neutralize, faster to handle and/or less prone to be affected by spermicide compared to brother eusperm. In contrast, $1 < k < \infty$ means parasperm are good absorbers; they are slower to neutralize, slower to handle, or are more prone to be affected by spermicide than eusperm. Model 1 is simply a special case of model 2; when $k = 1$, eusperm and parasperm are equally vulnerable to spermicide. Assumptions 1, 2 and 5 from model 1 are retained in model 2 but assumptions 3 and 4 have been revised:

3. Females neutralize a certain number of the sperm they receive, for any given value of k.

4. Eusperm and parasperm are not necessarily equally vulnerable to spermicide and the two types may not be equally easy to neutralize. In this case, parasperm have a spermicide absorption value k times that of eusperm, where $0 < k < \infty$. k and x may be nonindependent, but we have not modelled this since the qualitative predictions of the model would not change.

Under the assumptions of model 2, W'_s is given by:

$$W_{s'} = q\left(\frac{T(1-s') - T(1-s')\left(\frac{hT}{(1-s')T+s'xTk}\right)}{T(1-s') - T(1-s')\left(\frac{hT}{(1-s')T+s'xTk}\right) + T(1-s) - T(1-s)\left(\frac{hT}{(1-s)T+sxTk}\right)}\right) + (1-q). \quad (2a)$$

Using the previous method to calculate the evolutionarily stable investment in parasperm gives two solutions. Again, only one gives biologically meaningful values of s^*:

$$s* = \frac{1 - kx + \sqrt{hkx - 2hk^2x^2 + hk^3x^3}}{1 - 2kx + k^2x^2} \quad (2b)$$

Equation 2b is plotted in Fig. 2 for the arbitrarily chosen $x = 5$.

Results of model 2: Similarly to model 1, model 2 predicts that $s^* > 0$ when $hkx > 1$, meaning that intense spermicide and cheap parasperm favour the evolution of parasperm. Again, an evolutionary arms race in which males produce more parasperm in response to increasingly strong spermicide by females is possible. High k, which means that parasperm are slower to neutralize or are more prone to spermicide than eusperm, also favours the evolution of parasperm. When k is high, lower levels of spermicide are required to make parasperm production advantageous. Interestingly, if spermicide is strong, low values of k can select for greater investment in parasperm than high values of k. This result suggests that if parasperm have low value as spermicide absorbers, then males might compensate by producing more of them, raising the possibility of a second type of arms race between the sexes. For example, males could benefit by increasing their investment in parasperm if the spermicidal agents of females began to preferentially target eusperm (perhaps because such discrimination between sperm morphs afforded females better control over paternity).

Model 2 also shows that, so long as spermicide is sufficiently intense and parasperm are sufficiently cheap to produce ($hk \leq 1$), parasperm can evolve even if they are neutralized more easily or attacked more frequently than eusperm (i.e. poor spermicide absorbers). As in model 1, the parameter q is not present in the ESS function, indicating that the risk of sperm competition should not influence the amount of resources males should invest in parasperm. Finally, model 2 also suggests that costly parasperm can evolve under the SSH as long as they are more difficult to neutralize or more affected by spermicide than eusperm ($k > 1$; i.e. good absorbers).

Model 3: male responses to proportional spermicide

Model 3 investigates whether parasperm can evolve if females neutralize a certain proportion of sperm rather than a certain number. This type of spermicide could occur if sperm are lost passively such that each sperm has a fixed, equal chance of being lost, or if they are killed by phagocytes or enzyme molecules in negligible 'handling time' such that the number of sperm killed increases with the number available to kill (Greeff & Parker, 2000). Proportional spermicide could also occur if the intensity of spermicide increases with the amount of sperm

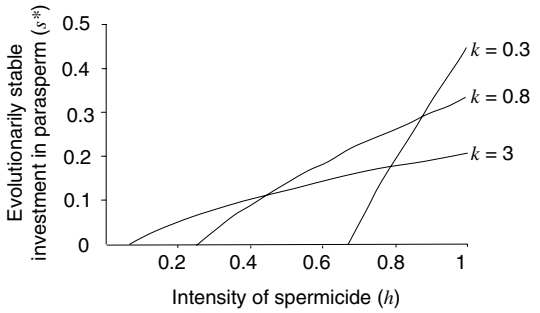

Fig. 2 Evolutionarily stable investment in parasperm (s^*) plotted against intensity of spermicide (h) for three different values of k when $x = 5$. Note that if k was fixed and the graph plotted with the same axes as those of Fig. 1, the plot would be qualitatively identical to the plot in Fig. 1.

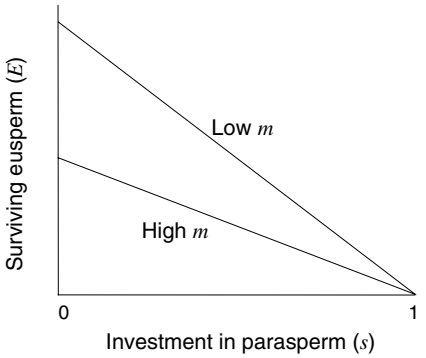

Fig. 3 Surviving eusperm plotted against investment in parasperm (s) under two different intensities of proportional spermicide (m) for an arbitrary value of T.

inseminated. Spermicide by sperm ejection might also fit the proportional model. Assumptions 1, 2 and 4 from model 1 have been retained, but assumption 3 has been modified and the sperm competition 'raffle' component is unnecessary (see eqn 3):

3. Females neutralize a fixed proportion of the sperm they receive. This proportion is given by m, where m is directly proportional to the intensity of spermicide. $0 < m < 1$, though high values of m (i.e. extreme spermicide) may be rare in nature because females employing such strategies would suffer reduced fecundity.

The number of eusperm lost to spermicide is given by the total number of sperm lost multiplied by the proportion that are eusperm:

$$m((1-s)T + sxT)\frac{(1-s)T}{(1-s)T + sxT} = (1-s)Tm.$$

This number is subtracted from the total number of eusperm males transfer, $T(1-s)$, to give the number of surviving eusperm:

$$E_s = T(1-s) - (1-s)Tm.$$

Simplified:

$$E_s = T(1-s)(1-m) \qquad (3)$$

Equation 3 is plotted in Fig. 3. Inspection of eqn 3 indicates that E_s decreases as both s and m increase.

Results of model 3: Figure 3 indicates that as males invest more in parasperm (and as females become more spermicidal), the number of surviving eusperm will decrease. Thus, parasperm are not protecting eusperm from proportional spermicide. The reason for this is that the probability of death is the same for each sperm regardless of how many sperm are present. Consequentially, it is not possible for males to overwhelm the spermicide systems of females by strength of numbers; if $m = 0.5$ then half the eusperm will be lost irrespective of how many parasperm are present. This model highlights that although the presence of spermicide is essential for the evolution of sperm heteromorphism under the SSH, parasperm are not an effective defence against proportional spermicide. As spermicide via sperm ejection may best fit the proportional model, this result suggests that the SSH may not apply to species that only kill sperm by dumping them.

Implications of the models for the sacrificial sperm hypothesis

Models 1 and 2 suggest that the SSH is most likely to apply to species with parasperm that are cheaper to produce than eusperm and are relatively good at absorbing spermicide (although under some conditions, parasperm can evolve even if more than one parasperm is needed to save one eusperm). A model examining whether parasperm could evolve as 'soldiers', killing eusperm of rival males, also concluded that parasperm have to be cheap to produce and that they do not necessarily need to kill one or more eusperm each (Kura & Nakashima, 2000). Several sperm-heteromorphic groups possess parasperm that are smaller, and presumably cheaper, than the eusperm, including stalk-eyed flies (Presgraves *et al.*, 1999), the *D. obscura* species group (Snook, 1997) and the Lepidoptera (whose parasperm are also free of expensive DNA; Silberglied *et al.*, 1984). However, the parasperm of some molluscs (Buckland-Nicks, 1998) and carabid beetles (Fain-Maurel, 1966) are much larger, and thus presumably more costly, than the eusperm. Costly parasperm may still evolve in response to spermicide, as shown in model 2, because expensive parasperm may be more difficult to destroy or expel and thus might be very effective at occupying macrophages and enzyme molecules or preventing smaller eusperm from being expelled. The SSH could also conceivably apply to taxa with expensive parasperm if their parasperm act as 'spermicide recruiters' that attract spermicidal agents

more than the eusperm. The extent to which parasperm are vulnerable to spermicide relative to eusperm may be difficult to assess, but some indirect data are consistent with the idea that parasperm are more prone to spermicidal effects. In *Littorina* and *Fusitriton* snails (J. Buckland-Nicks, personal communication), *D. pseudoobscura* flies (Snook, 1998) and *Graptopsaltria nigrofuscata* cicadas (Kubo-Irie *et al.*, 2003), parasperm have been observed to disappear from the female tract long before eusperm. Also, in the butterfly *Papilio xuthus*, <1% of inseminated parasperm reach storage compared with 11% of eusperm (Watanabe & Hachisuka, 2005), and in *Pieris napi* eusperm are more than twice as likely to reach storage and remain there as parasperm (Cook & Wedell, 1999). These results suggest that parasperm may be neutralized more easily or preferentially in these species. Alternatively, eusperm might simply stay alive or retain motility longer, or take less time to reach the relative safety of storage. The latter is not a viable explanation in *D. pseudoobscura* as the two morphs arrive in storage simultaneously (Snook, 1998).

Our models show that parasperm numbers should not vary with the risk of sperm competition, assuming that parasperm function only to protect brother eusperm against spermicide. The kamikaze sperm model, which assumed that parasperm destroy rival eusperm, obviously has a specific sperm competition requirement for the evolution of parasperm (Kura & Nakashima, 2000). This finding also differs from the predictions of the competition and provisioning hypotheses for the evolution of parasperm, which both suggest that males should adjust the number of parasperm they transfer in response to the risk of sperm competition (Table 1). These latter hypotheses suggest that males should adjust the number of parasperm they transfer in response to the risk of sperm competition. Studies of *D. pseudoobscura* (Snook, 1998), *Plodia interpunctella* (Cook & Gage, 1995) and *Viviparous ater* snails (Oppliger *et al.*, 2003) have shown that males do not appear to adjust the amount of parasperm transferred in response to elevated sperm competition risk. Counter-examples are provided by studies of *Pieris rapae* (Wedell & Cook, 1999) and other studies of both *P. interpunctella* (Gage, 1995) and *V. ater* (Oppliger *et al.*, 1998).

Predictions of the SSH

Our models predict a positive relationship between female spermicide intensity and male investment in parasperm. Spermicide can be quantified by assessing the survival of sperm after *in vivo* and *in vitro* exposure to the female reproductive tract relative to controls, using live/dead staining (e.g. Bernasconi *et al.*, 2002). If spermicide is found, it should be possible to test whether eusperm survival is influenced by parasperm number when the ejaculate is exposed to spermicide. While there is some evidence that spermicide occurs in sperm-heteromorphic species (e.g. *D. obscura* flies, Markow & Ankey, 1988; snails, J. Buckland-Nicks, personal communication), there has been no formal test of this idea.

Another related prediction is that if spermicide continues throughout storage, males should transfer more parasperm to young females than to older females, because males mating with a young female should lose more sperm (i.e. experience stronger spermicide) than males mating with a female near the end of her life, at least in short-lived invertebrates. Males could thereby maximize the number of viable eusperm stored by the female at any given moment after mating. Problematically, this prediction is the same as when parasperm function as 'cheap filler'. These predictions assume that males are able to assess female age and adjust the composition of their ejaculates (Wedell *et al.*, 2002) and that spermicide is continuous; if males are unable to adjust ejaculates or there is a 'safe haven' for sperm, then males will be expected to transfer a similar amount of parasperm to each of their mates, all else being equal. Males of the sperm-heteromorphic moth *P. interpunctella* transfer fewer sperm of both types to older females than to younger females (Cook & Gage, 1995). While this result is consistent with the SSH, there are alternative explanations; e.g. *P. interpunctella* is a short-lived species in which males are sperm limited and should be expected to allocate fewer sperm to less fecund females (Cook & Gage, 1995; Wedell *et al.*, 2002).

A potential criticism of the SSH is that males could increase the number of fertile sperm that survive spermicide simply by producing more fertile sperm instead of producing an additional, infertile morph. To increase the number of fertile sperm, males would have to either increase the amount of resources allocated to sperm production at the cost of some other traits or reduce the resources invested in each sperm, probably by making them smaller. Changing sperm size might not be beneficial as sperm size is likely to be constrained by many other factors; for example, smaller sperm may be less effective in sperm competition (e.g. Snook, 2005). Increasing the overall investment in sperm production may be possible up to a point, but resources must be taken away from other functions. By allocating some resources to parasperm production, males can increase the number of eusperm that survive spermicide for any given sperm production resource budget, without changing eusperm size.

Finally, it is possible that parasperm serve more than one function in some taxa. Indeed, in Lepidoptera, empirical data have supported both the cheap filler and facilitation hypothesis (Cook & Wedell, 1999; Wedell, 2001; Sahara & Takemura, 2003). If parasperm do serve more than one purpose, then it will be difficult to discern their function by looking at ejaculatory responses to mate age and mating status or risk and intensity of sperm competition. Most studies of ejaculatory adjustment in sperm-heteromorphic species (e.g. Cook & Gage, 1995; Oppliger *et al.*, 1998; Snook, 1998; Wedell & Cook, 1999)

have not tested or discussed the possibility that sperm heteromorphism is maintained by more than one mechanism, and have thus neglected some of the potential interpretations of their data. For instance, Snook (1998) found no difference in the number of parasperm transferred to virgin and nonvirgin female *D. pseudoobscura* and concluded that parasperm are probably not involved in sperm competition in this species. However, the same result could be produced if parasperm function in both sperm defence (e.g. cheap filler) and sperm offence (e.g. displacement of rival eusperm) because these mechanisms are expected to have opposite effects on the amount of parasperm males should transfer to virgin and mated females. Similarly, parasperm functioning in the SSH is not mutually exclusive to other functions. For example, sacrificial parasperm might also supply nutrition following digestion or act as cheap filler, displacers or facilitators, before they have been affected by spermicide.

The cryptic female choice hypothesis for the evolution of parasperm

Cryptic female choice (CFC) is the female component of post-copulatory sexual selection. Females may bias paternity after copulation in a number of ways (reviewed in Eberhard, 1996). Other authors have suggested that sperm and nonsperm components of the ejaculate may serve in CFC (e.g. Eberhard & Cordero, 1995; Cordero, 1998) but these works do not specifically discuss parasperm. Only recently have parasperm been suggested to evolve in response to CFC (Swallow & Wilkinson, 2002; Oppliger *et al.*, 2003; Till-Bottraud *et al.*, 2005). Parasperm may function in CFC by, for example, increasing the size or motility of the ejaculate, thereby inducing females to select their sperm for fertilisation. Alternatively, females might judge males by the number, quality or proportion of parasperm they produce. Parasperm would thus represent an unusual epigamic trait.

In order to conclude that parasperm are used as a criterion for CFC: (1) females must not be exclusively monandrous; (2) parasperm number/quality must explain a portion of the variance in paternity, such that males with more/better parasperm fertilise more ova when all else is equal; and (3) this paternity increase must be mediated by the morphology, behaviour or physiology of the female (e.g. Eberhard, 1996; Pitnick & Brown, 2000) rather than male–male competition. The first requirement is satisfied in many species from all the well-studied sperm-heteromorphic taxa (Swallow & Wilkinson, 2002; Oppliger *et al.*, 2003). The second requirement necessitates a positive correlation between paternity and some parasperm trait, all else being equal. Many female insects appear to be sensitive to the quantity or quality of ejaculate in their reproductive tracts (Raulston *et al.*, 1975; Sakurai, 1998; Cook & Wedell, 1999; Mossinson & Yuval, 2003), potentially allowing females to identify males with high quality or abundant parasperm. Sperm may stimulate the female either by moving (Miller, 1950; Sugawara, 1979) or through chemicals bound to their surface membranes (Chapman *et al.*, 2003; Liu & Kubli, 2003; Wigby & Chapman, 2005). Such sensitivity to sperm in storage could allow females to identify males with high quality or abundant parasperm. Additionally, a study of the sperm-heteromorphic *V. ater* found a positive association between paternity and parasperm length (Oppliger *et al.*, 2003). Demonstrating the last requirement, female post-copulatory control over paternity separate from male to male effects, is often difficult (Birkhead, 1998; Eberhard, 2000; Pitnick & Brown, 2000) and has not been specifically examined in any sperm-heteromorphic taxa.

Here, we will focus on predictions arising from the CFC hypothesis of the evolution of parasperm and compare these predictions to those of other hypotheses suggested for parasperm evolution. This discussion shows there is considerable overlap between predictions that make supporting the evolution of parasperm in response to CFC problematic. We draw attention to these problems so that future work can either take these into account or attempt to avoid them.

Comparison of the CFC and other parasperm evolution predictions

The key prediction of the cheap filler hypothesis (Silberglied *et al.*, 1984) is that females receiving or storing more parasperm should have a greater remating latency; parasperm production would thereby allow a male to sire more offspring by reducing the future risk and/or intensity of sperm competition. However, adjusting remating latency in response to the attractiveness of the male or his ejaculate is one way in which females could exercise CFC (Eberhard, 1996). Parasperm could therefore function as cheap filler in male–male competition and be considered as one version of the CFC hypothesis, where the mechanism of CFC is stated explicitly. It may therefore be very challenging to distinguish between these two hypotheses in species where parasperm number influences female remating propensity (Cook & Wedell, 1999; Wedell, 2001).

The displacement, blocking and elimination (Silberglied *et al.*, 1984; Swallow & Wilkinson, 2002) hypotheses (Table 1) are distinct from the CFC hypothesis, but all of them have a common prediction: that males producing the most (or highest quality) parasperm should sire the most offspring in matings with polyandrous females. A potential problem with distinguishing between these hypotheses is that processes like sperm displacement may be influenced by the behaviour and morphology of females as well as males (e.g. Eberhard, 1996, Snook & Hosken, 2004), but sperm competition experiments may be informative. For example, parasperm number/quality should only explain a portion of the variance in paternity

for the second male of two to mate with a female if parasperm function in either displacement and/or elimination of rival male sperm (after controlling for eusperm numbers, time between inseminations, etc.). In contrast, parasperm number/quality should only influence paternity for the first of two males to mate with a female if parapserm function to block rival sperm. Unique to the CFC hypothesis however, paternity should be affected by parasperm number/quality in both the first and second male roles. Sperm-heteromorphic *V. ater* snails with larger parasperm had increased success in sperm competition irrespective of whether they were the first or second male to mate (Oppliger *et al.*, 2003). This result supports the CFC hypothesis, the facilitation hypothesis (parasperm might transport/capacitate eusperm with size-dependent efficiency) or a pluralistic interpretation in which parasperm function in both sperm offence and defence (Oppliger *et al.*, 2003).

The CFC hypothesis may also be difficult to distinguish from the facilitation hypothesis. A study of silkworms, *Bombyx mori*, found that when either only eusperm or parasperm were artificially inseminated, fertilisation did not occur, but when the two morphs were inseminated together around 95% of the eggs were fertilised (Sahara & Takemura, 2003). The authors concluded that the presence of parasperm is essential for eusperm fertility. An alternative explanation is that the females rejected the eusperm-only ejaculates because of some missing quality. For example, the eusperm-only ejaculates could have been less motile than the ejaculates containing parasperm because most of the eusperm were in nonmotile bundles, so the females may have not used these sperm for fertilization sperm because the ejaculate did not provide sufficient stimulation.

If parasperm function in CFC, then we expect that males might produce and/or transfer more parasperm when the risk or intensity of sperm competition is elevated, because the hypothesis states that males with many or better parasperm should father more offspring in matings with multiply mated females. Consistent with this prediction, male *P. rapae* (Wedell & Cook, 1999), *P. interpunctella* (Gage, 1995) and *V. ater* (Oppliger *et al.*, 1998, but see Oppliger *et al.*, 2003) can transfer more eusperm and parasperm when facing elevated sperm competition. However, these results are also consistent with both the sperm offence and defence hypotheses (Table 1). Like the SSH, the CFC hypothesis could act concurrently with other mechanisms. For example, parasperm might induce females to favour their ejaculate in CFC as well as assisting eusperm transport, providing nutrition or displacing rival sperm.

Conclusion

Sperm heteromorphism, though widespread, remains a conundrum despite a number of studies testing existing hypotheses for the evolution of parasperm that focus on the benefit to males. We suggest that parasperm production may be an adaptation to the female reproductive tract. Specifically we examine whether parasperm could evolve either to increase eusperm survival in spermicidal female tracts or induce females to bias paternity in favour of their brother eusperm. The SSH is most likely to explain the maintenance of sperm heteromorphism in species with cheap parasperm and strongly spermicidal females. The CFC hypothesis remains a possibility in all sperm-heteromorphic species studied to date, and several recent studies have produced results consistent with it.

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