Primer

Sperm competition

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The existence and evolutionary significance of sexual selection through sperm competition was first realized by Geoff Parker in a prescient and influential review published in 1970. Parker recognized that competition between males for fertilizations will continue after mating, if the sperm of two or more males overlap near the site of fertilization in females. The process of sperm competition was originally defined as ‘the competition within a single female between the sperm of two or more males for the fertilization of the ova’. This definition was later broadened to ‘the competition between the sperm of two or more males for the fertilization of a given set of ova’. This was to reflect the fact that sperm competition is also possible in externally fertilising species that broadcast their gametes into water, such as sea urchins.

The prerequisites for sperm competition are simple: females must mate at least two males, whose sperm must exhibit spatial and temporal overlap. However, multiple mating by females, and even the presence of sperm from different ejaculates within females, do not necessarily guarantee that sperm competition will occur. For example, females may remate only when sperm stores are exhausted, thus minimising sperm overlap. There is also a variety of ways by which sperm from different ejaculates can be prevented from mixing inside females. For example, sperm from different males may be stored in different locations, or sperm from earlier matings may be displaced from storage before incoming sperm are stored. In its strictest sense, sperm competition occurs when sperm from different males are in direct contact with one another. However, sperm competition as a research field generally also encompasses adaptations that serve to minimize sperm competition.

Intra- and inter-ejaculate competition

Sperm competition can, in principle, occur both within individual ejaculates (intra-ejaculate) and between the ejaculates of different males (inter-ejaculate). Most work on sperm competition has investigated inter-ejaculate sperm competition. This process is a logical extension of sexual selection, as envisaged by Darwin. The selective pressure arising from sperm competition has led to numerous adaptations to assist males in gaining fertilizations (Boxes 1 and 2). Broadly speaking these can be grouped into adaptations that promote the success of sperm in direct competition, promote sperm success by preventing competition between different ejaculates (by preventing female remating or by blocking access to the female reproductive tract), or promote sperm success by removing, destroying or inactivating the sperm of rival males.

The fundamental problem for males engaged in sperm competition is that they face two opposing selective pressures. Successful sperm must be good at gaining dominance over those already in store and must be able to resist usurpation by the incoming sperm of later mating males. These phenomena are referred to as sperm ‘offense’ and ‘defense’ abilities. The relative investment made by males in offensive *versus* defensive adaptations will depend on the costs and benefits of each adaptation, and on the magnitude of the payoff as the investment in each adaptation increases.

Relatively little attention has been paid to intra-ejaculate competition. It is widely assumed that there is little expression of sperm haploid genotypes and, therefore, little realization of the differences between sperm within one ejaculate. It is worth noting that, on average, the relatedness of sperm within an ejaculate will be 0.5 and the relatedness of sperm in the ejaculates of unrelated males will be approximately zero. It may, therefore, be in a male’s genetic interest to repress competition between his own sperm, if this allows greater competitive ability against a rival male’s sperm. Hence, the repression of competition and minimal expression of the haploid genotype could reinforce one another to reduce the intra-ejaculate sperm competition.

Taxonomic distribution of sperm competition

Sperm competition is taxonomically widespread and has been reported in birds, mammals, fish, amphibians, reptiles and a huge number of invertebrates. In plants, rival pollen also compete, in a manner analogous to the process of sperm competition. Sperm competition is, however, particularly common in insects. This is for the simple reason that female insects often mate multiply with different males and have specialized organs in which sperm can be stored and maintained in a viable state until fertilization. Hence, sperm may be very long lived in the female reproductive tract and the temporal separation between mating and fertilization can be anything from minutes to years. In the sperm storage organs, sperm may also be protected against possible expulsion during egg-laying. However, this is not universally true, as, for example, female locusts lose sperm when eggs are laid.

Measuring sperm competition

The outcome of sperm competition is typically assessed by examining the paternity of offspring produced by a female after she has mated twice. This allows the calculation of the measure ‘*P2*‘, which is the proportion of offspring fertilized by the second of the two males to mate with the female. Thus, *P2* can vary from 0 to 1. A *P2* value of 0.5 is usually taken as evidence that the sperm of the two males are equally mixed in store. *P2* values of 0 or 1 may indicate that the sperm of the first or second males have gained complete precedence over the sperm of the other male, that sperm from the first or second male were not stored because of male infertility or female rejection of sperm, or that sperm from the first or second male have become depleted or lost.

Paternity patterns have been inferred in a number of ways: by irradiating the second male to mate and inferring his paternity via the number of unfertilized eggs laid (i.e., the number 'fertilized' by the sterile second male), by using morphological markers, by using allozyme markers and by using molecular markers such as microsatellites. Sperm labeled with green fluorescent protein have been used to distinguish the sperm of two males in storage, allowing the calculation of '*S2*' , the proportion of the second male's sperm that are resident in the sperm storage organs.

It should be noted that methods for analyzing paternity may inadvertently influence the outcome of sperm competition. Marker strains with visible mutations generally have lower fitness than the wild-type. They may transfer fewer sperm at a slow rate and fewer sperm may be stored. Hence, the use of marker strains can artificially inflate estimates of sperm competitive ability in tester strains. Sperm competition is also likely to be altered when sperm numbers are low. It is also important to be aware of potential fitness differences between larvae fathered by marker *versus* tester males. Low fitness of larvae fathered by marker males, for example, could inflate the apparent sperm competitive ability of tester strains. Irradiated sperm may also be less successful at 'fertilization', which could again tip the balance in favor of the males with which they are in competition.

Box 1

Male strategies to increase success under direct sperm competition.

Sperm number

Theory predicts that males should produce greater numbers of sperm when the risk of competition is high. This can be achieved by delivering single ejaculations containing many sperm or, as in socially monogamous birds and other bond-forming animals, by copulating frequently. If sperm from different males are mixed inside females, then the ejaculation of a large number of sperm may give males a proportional gain in fertilizations. Consistent with these predictions, increases in sperm competition across species (as indicated by frequent female remating) are often associated with increases in the relative size of male testes. Male responses to sperm competition risk may also be strategic and there is evidence from orthoptera, beetles, flies, hymenoptera, lepidoptera and chickens that males ejaculate more sperm when the risk of sperm competition is perceived to be high and *vice versa*.

Sperm size/length

Sperm competition may explain dramatic variation seen in sperm length. For example, sperm can range from a few tenths of a mm in dung flies, to nearly 6 cm in *Drosophila bifurca* (over 30 times this fly's body length). The role of sperm competition in the evolution of giant sperm is unclear, as increased sperm length is normally associated with decreased sperm number. Theory predicts lower sperm numbers only as the probability of paternity increases. Hence, one would not predict selection for longer sperm under intense competition, unless longer sperm had an increased probability of fertilization. In fact, in species exhibiting sperm gigantism, sperm mixing may be higher than in small-spermed species in which displacement is common. Hence, sperm gigantism may be associated with increased direct sperm competition. Increased sperm length could be favored by sperm competition if it leads to the production of more competitive sperm. For example, long sperm might gain a competitive advantage through swimming faster. Alternatively, long sperm may be better at blocking up the female sperm storage organs and promoting sperm retention. Evidence from comparative studies of butterflies and moths shows that the length of eupyrene (fertilising sperm) is positively associated across and within species with the risk of sperm competition, lending support to the view that long sperm are selected by increased sperm competition.

Sperm polymorphism

Sperm competition could favor alternative forms of sperm, if they increase the ability of sperm to compete. Sperm polymorphism appears universal in the Lepidoptera, whose ejaculates contain nucleated, fertilising 'eupyrene' sperm and non-nucleated 'apyrene' sperm. The apyrene sperm can form as much as 90% of the ejaculate. Long and short forms of nucleated sperm are common in the ejaculates of stalk-eyed flies and the *'obscura'* group of fruit flies. Although evidence that sperm dimorphism is selected through direct sperm competition is lacking, the non-fertilising sperm of Lepidoptera do seem to play a role in delaying female remating (Box 2).

Sperm storage

The rate or efficiency of sperm storage could be selected by sperm competition, if it results in more competitive ejaculates. Sperm storage may be affected by the rate at which sperm are transferred to females, the size and the morphology of the female sperm storage organs and the hormonal/chemical environment in the ejaculate or in the female reproductive tract. For example, in *Drosophila melanogaster*, a single ejaculate protein is necessary for effective sperm storage.

Great care must be taken in analyzing and interpreting *P2* values. It is often overlooked that *P2* does not necessarily give any indication about the occurrence or intensity of sperm competition. *P2* is a derived measure that reflects the performance of both the first and second mating males. For example, high values of *P2* would occur, if the sperm of different males mixed, but the sperm of the second male were superior competitors; if the sperm of the first male were displaced from storage; if the sperm of the first male were lost from storage; or if the sperm of two males were stored, but only the sperm of one of them were used. In the first case, sperm competition would be intense, but in the other cases it would be low or even absent. The distribution of *P2* values for individual females must also be considered. For example, in a group of females of which one half used only the sperm of the first male and the other half only the sperm of the second male, the average *P2* value would be 0.5. If this bimodal distribution of *P2* values was not identified, this could lead to the erroneous conclusion that sperm were perfectly mixed in doubly mated females, whereas in fact there was almost a complete precedence of sperm of the first or the second male. Thus, we must know the mechanism of sperm competition to assess its intensity, specifically the extent to which the sperm from different males show spatial and temporal overlap. Factors that affect sperm competition intensity include the number of sperm transferred and stored, the efficiency of sperm storage, the inter-mating interval, sperm retention time, survival rate of stored sperm, the extent to which sperm mixing is random and the mode in which multiple sperm sets are stored.

Female choice

Sperm competition between males has traditionally been viewed as a logical extension of male–male competition. However, Thornhill and Eberhard have argued that this view neglects an important possibility, that the arena in which sperm competition occurs (i.e. the female reproductive tract or fertilization site) permits female choice. ‘Cryptic female choice’ refers to female-influenced processes before and/or after mating, which skew offspring production toward one male at the expense of another. This includes the idea that the mixing of sperm from two or more males somehow provides females with the opportunity to select the ‘best’ from those on offer.

It is not possible to conclude that cryptic female choice is operating simply by examining the patterns of paternity after multiple matings. For example, a particular male could gain a disproportionately large number of fertilizations because a characteristic of his ejaculate promotes effective sperm transport. His sperm would, therefore, reach the fertilization site more quickly than those of other males and hence fertilize a greater proportion of eggs. In this scenario, females play no part in generating non-random paternity. Therefore, without knowledge of the mechanism, it is difficult to determine with certainty to what degree sperm competition or cryptic female choice influence paternity patterns. One approach is to determine whether there is genetic variation for female or male effects on the outcome of sperm competition. In *Drosophila melanogaster*, research by Clark and colleagues has shown that the outcome of sperm competition is significantly affected by male genotype, female genotype and the interaction between male and female genotype. Part of the problem in attributing variation in sperm competition success to males, females or both is that it is difficult to design experiments that will unequivocally distinguish between these possibilities.

Female remating is a requirement for cryptic female choice. But remating in itself cannot be taken as evidence that female choice is occurring, as females may remate for a number of different reasons. Females can gain direct benefits by acquiring resources from males, by topping up sperm supplies and by reducing harassment costs. Indirect genetic benefits from multiple mating may include increased genetic diversity of the offspring, decreased genetic incompatibilities, acquisition of sperm of high quality males and the promotion of sperm competition or cryptic female choice. It is a difficult task to detect the difference, if there is one, between remating to promote sperm competition and remating to allow cryptic female choice.

Box 2

Male strategies to avoid direct sperm competition.

Prevention of female remating
Males can avoid sperm competition by preventing females from mating with rivals. Mate guarding can occur before or after copulation. Males of several insect species, particularly those in which first male sperm precedence occurs, attempt to hide their mates from other males before copulation. In other species males extend copulation as a way of excluding rival males until their mate is unreceptive or ready to lay eggs. In species with last male sperm precedence (e.g., the Odonata) post-copulatory mate guarding is favored. Methods of preventing females from remating other than physical guarding are also common. Mate guarding may also be remote and induced by substances transferred in the male ejaculate. In *Drosophila melanogaster*, for example, a seminal fluid protein suppresses female remating for several days, ensuring paternity for at least that length of time. In the housefly the refractory period can last the entire lifetime of the female.

Mating plugs
Mating plugs are formed by coagulation of the ejaculate in the female reproductive tract. They may be effective in physically preventing the sperm of rival males entering sperm storage. In addition, it is also suggested that they may act to localize newly ejaculated sperm in the correct location to ensure efficient storage in the female sperm storage organs.

Sperm displacement
Particularly common are methods of displacing the sperm of previous males from the storage organs of mated females. The ejaculation of large numbers of sperm, as well as increasing fertilization success when direct competition occurs, can also act to displace the sperm of previous males, thus reducing the intensity of competition. Similarly, by copulating frequently males transfer ever greater numbers of sperm, which may act to flush out the sperm of rival males. Thus, by overwhelming with numbers the sperm of any other males, sperm competition can be greatly reduced. Seminal fluid proteins of male *D. melanogaster* can somehow displace or inactivate the sperm of previous males. Male Odonata (dragonflies and damselflies) are physically able to remove the sperm of rival males with part of their intromittent organ.

Mechanisms of sperm competition

Despite over 30 years of experimental research on the subject, relatively little is known about exactly what happens when sperm meet under competitive circumstances. Many of the methods to measure sperm competition are indirect and thus do not illuminate precise details of the process. However, the advent of new molecular techniques may go some way to revealing some of these hidden mechanisms. Tools are needed to determine the identity and location of sperm from different males within females. Such tools may come from markers such as microsatellites or GFP-markers to distinguish sperm from different males in female sperm storage organs. It will be exciting to see exactly how different sperm interact and discover the means by which some sperm predominate over others.

Parker and colleagues suggested a very useful modelling approach, which has been used with some success to gain insight into the mechanisms underlying sperm competition. The potential outcomes of different sperm competition scenarios are modeled and the patterns compared to observed data. For example, the outcome of sperm competition could be a raffle, in which paternity is exactly related to the number of sperm a male gets into store. Alternatively, the outcome could be more like a loaded raffle, in which the sperm of one male have an advantage over the sperm of another, hence paternity is not simply related to the number of sperm in store. Sperm could also mix instantaneously following ejaculation, or there could be some displacement of stored sperm followed by mixing with whatever sperm is left behind.

The evolutionary potential of sperm competition

Sperm competition clearly has the potential to influence a huge variety of different sexual traits in males and females (Box 1 and 2, Figure 1). It seems likely that sperm competition also plays an important role in population divergence and, ultimately, in speciation itself. Consistent with this idea is the strikingly higher divergence of male genitalia, compared to other, non-sexual traits. In addition, many genes of male reproduction exhibit rapid evolutionary change. Whether these changes are driven by sperm competition and are causal or merely associated with divergence is a hot topic of debate.

Conclusions

The simplicity of the concept of sperm competition contrasts with the complexity inherent in understanding the significant and widespread impact that it can have (Figure 1). As with many topics in evolutionary biology, the mechanism really matters, and future insight in this field is likely to rely on intimate knowledge of patterns of sperm storage and of sperm utilization.

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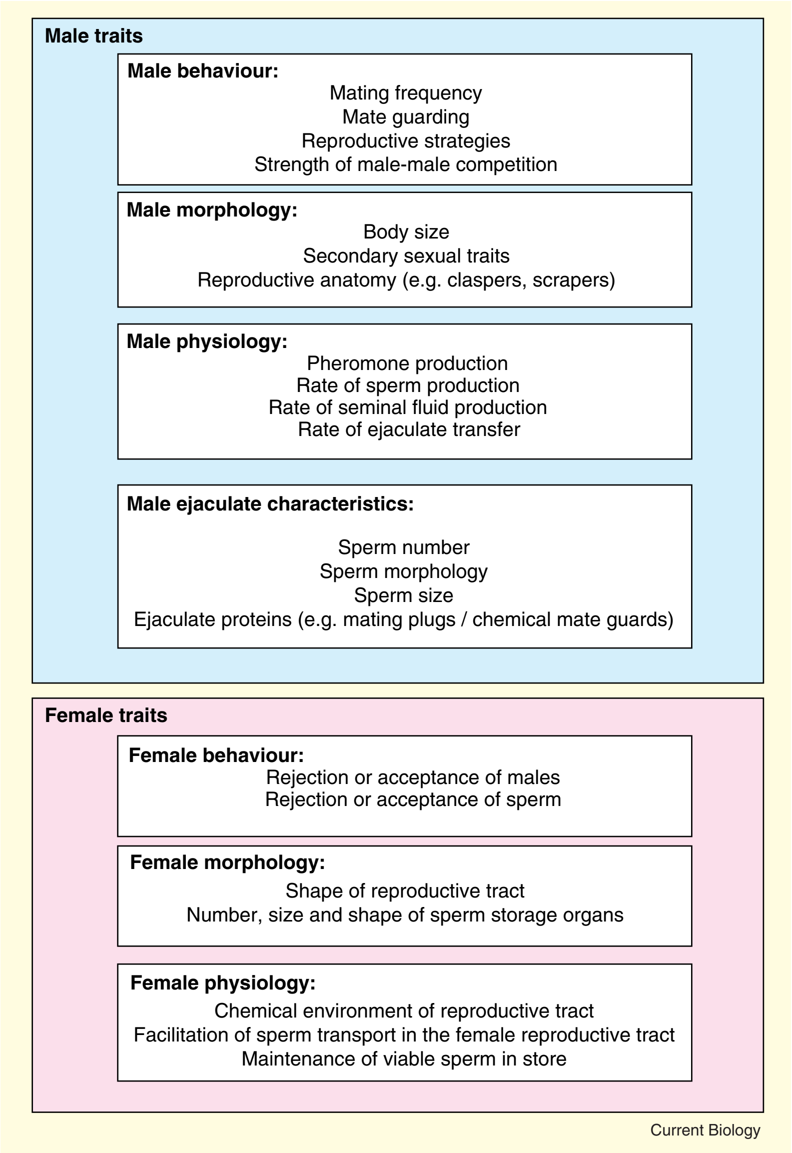

Figure 1. The complexity of sperm competition: traits influenced by sperm competition.

Further reading

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