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Mechanisms of sperm competition in insects

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Sperm competition has been demonstrated in a variety of insects and is, in addition to ecological resource distribution and sex ratios, generally believed to play a major role in the evolution of insect reproductive strategies and mating systems. In this paper, I review the main theories and some of the empirical evidence regarding sperm competition in insects. Sperm utilization is shaped by selection on both males and females, sometimes in opposite directions. Here I focus mainly on adaptive mechanisms for sperm priority and paternity assurance, and consequences of such adaptations for females. I also evaluate the importance of the conflicts between the sexes for the evolution of mating behaviour from existing theory and available empirical evidence. Some urgent research areas for future workers are suggested. An explanation for the large intraspecific variation in last male sperm priority is still lacking. To this end, we need detailed studies of the mechanisms of sperm usage within the female, and to what extent females influence postcopulatory fertilization processes.

1. Introduction

According to Darwin (1871) sexual selection is the nonrandom differential reproductive success that results from competition for access to mates. However, the optimal strategy for males may not be to mate with as many females as possible, but rather to fertilize as many eggs as possible. Parker (1970) first pointed out that competition between males may continue inside the female, a mechanism he called sperm competition. Parker (1970) defined sperm competition as the competition within a single female between the sperm from two or more males for the fertilization of the ova. Sperm competition extends sexual selection beyond mating to the point of fertilization.

Postmating sexual selection includes both male-male competition (sperm competition) and female choice (cryptic female choice) (Thornhill 1983, Smith 1984, Eberhard 1996). Sperm competition results in selection for both offensive and defensive male traits. On one hand, evolution will favour a male that can affect the storage of other males' sperm or use his own sperm in such a way that his own fertilization success is maximized (male adaptations for sperm priority, *see* chapter 2). On the other hand, a selective advantage will be gained by males that are able to prevent or reduce subsequent competition from sperm of other males (male adaptations to prevent females from remating, *see* chapter 3). The outcome of a multiple mating will in part depend on how well these adaptations perform against each other (Parker 1970). Parker (1984) argues that these two lines of adaptations will be in some kind of evolutionary balance.

2. Male adaptations for sperm priority

Although sperm priority patterns have been studied in many insects, the mechanisms is still unknown in most cases. Five main sperm competition mechanisms have been suggested: sperm removal, sperm stratification, sperm dilution, manipulation of fertilization processes, and possession of adaptive sperm characteristics. These mechanisms are, however, not mutually exclusive.

2.1. Sperm removal

The first case of sperm displacement, i.e. the displacement of previously stored sperm followed by replacement with sperm from the last male to mate (Parker 1970), was suggested by Lefevre & Jonsson (1962) in their study of *Drosophila melanogaster*, although they were not sure about the mechanism. They saw that the number of sperm in the spermatheca was equal before and after a second mating event, but that the last male to mate gained most fertilizations.

2.1.1. Mechanical sperm removal

The mechanisms for sperm displacement have been known for a long time in dragonflies and damselflies (Odonata). Waage pioneered this field and he showed already in 1979 that the males of the damselfly *Calopteryx maculata* use the penis not only to transfer sperm to the female, but also to remove sperm deposited from earlier matings (Waage 1979). The penis is specially adapted and acts as a scrubbing-brush, catching and drawing out the stored sperm from previous males. Later studies provided evidence for a widespread occurrence of mechanical sperm removal in Odonata (Waage 1986, Michiels & Dhondt 1988, Siva-Jothy & Tsubaki 1989, Cordero & Miller 1992). All Orthectrum species studied so far are believed to remove sperm from female sperm stores during copulation by using the barbed flagellum of the penis, which fits into the narrow duct of the spermatheca (Siva-Jothy 1984, 1987, *see also* section 2.2. for additional position effects in Odonata). Another example of mechanical sperm removal has been seen in the mealworm beetle *Terebrio molitor* (Gage 1992). The penis of this beetle has a flexible sheath covered with reversed chitinous spines. As the central part of the penis extends within the female its covering of spines rolls back and scrubs out stored sperm, before the male transfers his own. In contrast to earlier examples only sperm in the female's copulatory bursa are affected. Once the sperm has reached the sperm storage organ it is not accessible for removal.

2.1.2. Flushing

Instead of using his penis, the male can flush earlier males' sperm out of the sperm storage organ by his own ejaculate. Flushing has been presented as a possible mechanism in the grasshopper *Locusta migratoria* and the tree cricket *Truljalia hibinonis* (Gregory 1965, Ono *et al.* 1989). The authors suggest that semen is ejaculated into the anterior part of the female's sperm storage organ. When the semen fills up the sperm storage, rival sperm is probably pushed towards the storage exit.

2.1.3. Chemical sperm removal

Chemical substances in the ejaculate of the last mating male, e.g., seminal proteins, can affect sperm displacement in two main ways. The substances can interact directly with sperm from earlier males, e.g., reduce motility or kill rival sperm. Such substances may also affect the female reproductive processes. In *Drosophila melanogaster* some chemical component in the seminal fluid causes the depletion of the earlier deposited sperm of rivals in the female storage organ (the seminal fluid effect), which probably is the reason for the high degree of sperm displacement in this species (Harshman & Prout 1994).

2.2. Stratification of sperm

The last male priority can be obtained by pushing previously introduced sperm to interior parts of the sperm store, so that the new ejaculate is placed closer to the site of fertilization. Because sperm normally leaves the spermatheca by the same duct as it entered, a male's sperm is more likely to fertilize the eggs if positioned near the entrance of the spermatheca, i.e. a last-in-first-out mechanism (Birkhead & Hunter 1990). One example is the libellulid dragonfly *Crocothemis erythraea.* Males lack the recurved spines used for sperm removal in other odonatas, instead an inflation of the penis medial lobe probably reposites or packs previously stored sperm away from the spermatheca's entrance. By placing his own sperm close to the entrance to the spermatheca, the male may gain high fertilization success by a last-in-first-out mechanism (Siva-Jothy 1988). Packing and repositioning has also been suggested in several other dragonflies and damselflies (McVey & Smittle 1984, Siva-Jothy 1984, Waage 1984, Michiels & Dhondt 1988, Siva-Jothy & Tsubaki 1989).

Walker (1980) proposed that a narrow, elongated spermatheca would promote a last-in-first-out mechanism, i.e. it might then be easier to move earlier males' sperm deeper into the spermatheca away from the advantageous position near the entrance (but *see* Ridley's comparative study, section 8.). There is a related idea suggesting that the length of the sperm in relation to the length of the spermatheca is important. When the storage organ and sperms are of equal length the sperms will orient themselves in a single layer, all having equal chance of fertilization. If the sperms are shorter than the spermatheca, they will probably become stratified within the spermatheca (Pitnick & Markow 1994). Siva-Jothy and Tsubaki suggest that sperm repositioning may have a selective advantage over sperm removal because it probably requires shorter copulation durations. Odonata reposition-species copulate for tens of seconds, compared with copulations that last for minutes or hours in Odonata species performing sperm removal (Siva-Jothy & Tsubaki 1994). There may be a trade-off between copulation duration and long-term fertilization success of the last male (Siva-Jothy 1988, Siva-Jothy & Tsubaki 1994; *see* section 2.3.). An important point is that estimating last male sperm precedence by measuring changes in sperm volume or sperm number only will underestimate P_2 in case of a last-in-first-out mechanism (Michiels & Dhondt 1988).

2.3. Sperm dilution

Effects of high sperm priority through position usually decrease with time after insemination (Siva-Jothy & Tsubaki 1989, Siva-Jothy & Tsubaki 1994). This pattern suggests sperm mixing within the spermatheca, i.e. sperm from multiple matings blend within the spermatheca. The rate of sperm mixing varies between species, from immediate mixing to several days, possibly depending on the mobility of the spermatozoa (McVey & Smittle 1984, Simmons 1987, Siva Jothy & Tsubaki 1994). If sperm mixing occurs immediately, male fertilization success should be directly proportional to the relative number of sperm in the fertilization set. Parker *et al.* (1990) named this as "the fair raffle game", i.e. all sperm has an equal chance of fertilization. In a fair raffle, fertilization success is related to the number of sperm transferred.

Parker *et al.* (Parker *et al.* 1990, Parker & Simmons 1991) have developed models for analysing the underlying mechanism determining sperm priority. The models require information about: the proportion of offspring sired by the last male to mate (usually denoted P_2), the number of sperm transferred by each male and the sperm storage capacity of the female, should this be limited. By testing how the sperm competition data fits the models one can distinguish between (1) raffle and (2) sperm displacement mechanisms.

2.3.1. Sperm competition in Scatophaga stercoraria

The dungfly *Scatophaga stercoraria*, presents one of the most thoroughly studied cases of sperm competition. In dungflies sperm removal is combined with instantaneous sperm mixing. The last male probably uses his own ejaculate to displace previously stored sperm (Simmons *et al.* 1996, Parker & Simmons 1991) and the proportion of eggs fertilized by the last male increases with copulation duration (Parker 1978, 1984). Instantaneous mixing of ejaculates within the female sperm stores yields exponentially diminishing returns with time spent copulating (Parker & Simmons 1991). Hence, males experience a trade-off between time spent copulating and time spent searching for new mates. Parker (1978) applied the marginal value theorem to predict optimal copulation duration. The fit between the predicted (41 min) and the observed (35 min) copulation duration was quite close, suggesting that copulation duration was optimized in response to this trade-off. Variation in copulation duration around the mean was partly explained by male size. Parker and Simmons (1994) showed that larger males had a higher rate of sperm displacement, and predicted that large males would interrupt copulation earlier than small males. The prediction was verified by a significant negative correlation between copulation duration and male size (Parker & Simmons 1994).

2.3.2. How much sperm should a male transfer at each mating?

Dewsbury (1982) challenged the general accepted idea that males produce unlimited numbers of gametes. Although sperm is vastly smaller and cheaper to produce than eggs, it is a fact that the quantities of sperm transferred by males do not equal the number of eggs laid by females. This focuses interest on the ejaculate size. There are several examples in literature showing that males transfer successively smaller ejaculates or that the number of transferred sperm increases with the number of days since last copulation (Dewsbury 1982, Svärd & Wiklund 1989, Eady 1995). In these cases sperm production probably limits the number of inseminated females.

In cases of sperm mixing male fertilization succeeds increasingly with the number of transferred sperm (Parker *et al.* 1990). But, because sperm is costly (Dewsbury 1982) there is a trade-off between the effort spent on producing sperm and the effort spent on obtaining matings (Parker 1990b). Parker used ESS models to examine optimal ejaculate size in different mating situations when sperm competition obeys the raffle principle (Parker 1990a, 1990b). The models predicted that males should conserve sperm reserves during matings when there is low risk of sperm competition, while larger ejaculates should be transferred when there is high risk of sperm competition (Parker 1990b).

Comparative studies using butterflies (Pieridae and Satyridae) showed that the ejaculate size increases with the typical degree of polyandry as predicted by Parker (Svärd & Wiklund 1989, Bissondath & Wiklund 1996). In polyandrous species males also produce sperm and accessory substances faster, and maintain the relative ejaculate mass longer (Svärd & Wiklund 1989, Bissoondath & Wiklund 1996). Gage (1994) compared testis size with female mating frequency in 74 butterflies, and found that the testis size increases with the risk of sperm competition. In insects, females are usually larger than males, but in some butterflies (Pieridae and Satyridae) male relative size increases with the degree of polyandry, with males being larger than females in some of the most polyandrous species (Wiklund & Forsberg 1991). This pattern may have evolved because polyandry intensifies sperm competition, and selects for larger males that produce larger ejaculates.

Parker's prediction has also been supported by intraspecific studies. In some species males adjust ejaculate size to the risk of being exposed to sperm competition. In the mealworm beetle *Tenebrio molitor* (Gage & Baker 1991), the fruit fly *Ceratitis capitata* (Gage 1991) and the crickets *Acheta domesticus* and *Gryllodes supplicans* (Gage & Barnard 1996), males transfer more sperm when there is a large number of potential competitors around (*see also* section 2.5.).

In the meal moth *Plodia interpunctella* there is a trade-off between the investment in testes and the investment in traits of importance for gaining mates (Gage 1995). These moths do not feed as adults and gather all the energy needed for reproduction during their larval stage. The larval derived resources are allocated among adult structures in different ways depending on the larval population density. Larval density is an indicator of future mating patterns and risk of sperm competition; at high densities females will mate more frequently, generating greater risk of sperm competition. Males allocate resources in accordance with Parker's theory; at high larval densities males develop relatively larger testes and transfer greater numbers of sperm. At low larval densities males instead invest more in survival and allocate more energy to head and thorax body components, traits that probably aid mate searching (Gage 1995). Also in the noctuid moth *Pseudaletia separata*, the population density at the larval stage influences male reproductive strategies. At high densities, males produce relatively large spermatophores (He & Tsubaki 1992).

In addition to being advantageous in case of sperm mixing, large ejaculates may improve paternity by inducing a longer female refractory period. The male beetle *Calosobruchus maculatus* benefits from producing larger ejaculates when mating with nonvirgin females by attaining a higher P_2-value (mechanism unknown) and a longer refractory period (Eady 1995).

In cases of extremely high last male sperm precedence, ejaculate size may instead decrease with sperm competition. Males experiencing strong sperm competition should conserve sperm reserves and transfer just enough sperm to ensure fertilization of all eggs laid before the females remate (Pitnick & Markow 1994).

2.3.3. Repeated matings

Although males are assumed to copulate with as many females as possible, repeated copulations with a female may be one way to ensure sperm precedence in case of intense sperm competition. Repeated matings would be expected, for example, when males are unable to guard the female continuously, or when the P_2-value declines with time due to sperm mixing (Alcock *et al.* 1977, Smith 1979, Thornhill & Alcock 1983). Such a behavioural strategy would also increase the number of transferred sperm in a fair raffle, when ejaculate size is constrained (Cordero *et al.* 1995).

2.4. Male manipulation of female controlled reproductive processes

A male can gain sperm priority by stimulating (manipulating) the female chemically or behaviourally, thereby initiating different female reproductive processes, e.g., sperm transport, ovulation, rejection of earlier males' sperm, or abortion (Walker 1980, Eberhard 1996). For example in the fly *Dryomyza anilis*, the female before oviposition always expels a droplet of sperm. By tapping the female's external genitalia, the second male can increase the proportion of earlier males' sperm in the expelled droplet (Otronen & Siva-Jothy 1991). This is an example of female-male coevolution rather than pure male-male competition. Males may however differ in their ability to manipulate the female processes.

2.5. Sperm size and sperm dimorphism

Parker *et al.* (1972) presented an explanation for anisogamy in animals with external fertilization, in terms of disruptive selection. But why is anisogamy maintained in species with internal fertilization? Parker (1982) suggested that anisogamy is maintained by sperm competition. He argued that large sperm does not significantly improve zygote survival (because of the high ratio of ovum size to sperm size), but large sperm size would be detrimental to males by reducing sperm number. In cases where there is no sperm competition, males that produce large sperm improving zygote survival will always be favoured. Parker's model of a fair raffle game predicted that anisogamy also will be maintained if the probability that a female remates is greater than 4 times the ratio of sperm size to ovum size. Presence of sperm displacement will call for higher probability of female multiple mating, but is unlikely to affect the general conclusion that sperms will be small and not contain nutrients for the zygote (Parker 1982).

Why then do males of some species produce few and long sperm despite the presence of sperm competition? One reason may be that size (length) represents a nonnumerical adaptation to sperm competition (Pitnick & Markow 1994, also alternative hypotheses). In a comparative study including 74 butterfly species, Gage (1994) found that the length of fertile sperm (eupyrene sperm, *see* below) increased with sperm competition intensity (after control for body size), and he suggested that longer and faster swimming sperm may be of selective advantage in sperm competition. In some extreme cases, insect spermatozoa are much longer than the distance they travel within the female. In these cases, a more plausible advantage than faster swimming speed is that the longer sperm can prevent rival sperm from entering the spermatheca or resist displacement attempts of other males (Dybas & Dybas 1981, Pitnick & Markow 1994). Large sperm may also contain greater energy reserves or have better defensive ability and thereby increased survival potential. The latter may be especially advantageous when a female mates with several males but fertilization takes place after some time (Parker 1993). Statistically significant intraspecific variation among males in sperm size has been observed in several species, in one case with effects on male fitness (Ward 1998). In the bulb mite *Rhizoglyphus robini* there was a positive relationship between sperm size and fertilization success (Radwan 1996, *see also* Otronen *et al.* 1997).

In some species males produce more than one type of sperm and this is sometimes interpreted as an adaptation to sperm competition. Lepidoptera (moth and butterfly) males normally produce two distinct types of spermatozoa: eupyrene, typical insect sperms that fertilizes the eggs, and apyrene, smaller sperms without nuclear material and therefore incapable of fertilization. Apyrene sperms appear maladaptive since they cannot fertilize eggs. Nevertheless, apyrene sperms usually predominate in the ejaculate. Ever since their discovery in 1902, their function is still rather enigmatic. Silberglied *et al.* (1984) proposed two functional explanations related to sperm competition (*see* Silberglied *et al.* 1984 for a review of alternative hypotheses): (1) apyrene sperms displace or inactivate nuclear sperm from previous matings, so-called killer sperms, and (2) apyrene sperms delay further mating by the female. The highly motile apyrene sperms are simpler and develop more rapidly than eupyrene sperms. Female sexual receptivity in Lepidoptera is negatively correlated to the presence of sperm movements in the spermatheca (Drummond III 1984, He *et al.* 1995) and apyrene sperms would therefore offer a cheap way to simulate a full spermatheca (Silberglied *et al.* 1984, Cook & Gage 1995). Eupyrene sperms conserve energy by remaining encysted and immobile (Cook & Gage 1995). In the moth *Plodia interpunctella* and the butterfly *Pieris rapae* males increased the proportion of eupyrene sperm within the ejaculate when mating with nonvirgin females (Cook & Gage 1995, Cook & Wedell 1996). This pattern contradicts the suggestion that apyrene sperms serve as "killer sperms". Sperm dimorphism also occurs in *Drosophila pseudoobscura.* Males produce both long and short sperm of which only the longer sperm morph participates in fertilization, although both sperm types possess the correct amount of DNA (Snook *et al.* 1994). Suggested functions of the short infertile sperms are the same as for the apyrene sperms (Snook *et al.* 1994).

3. Male adaptations to prevent females from remating

There are many adaptations, morphological, behavioural and chemical, which prevent later males' sperm from outcompeting self's sperm. Last male priority and females that remain receptive after mating will add to the selective advantage of mechanisms that reduce subsequent sperm competition. However, as Thornhill (1984) emphasized, one should always consider alternative hypotheses and remember that one specific male behaviour may serve multiple adaptive functions. For example, a behaviour that reduces the risk of female remating may also serve as stimulation for cryptic female choice.

3.1. Mating plugs

Mating plugs are structures placed after insemination by the male, to prevent a second insemination (Parker 1970). Production of mating plugs has been observed in several insect families (Parker 1970, Aiken 1992). Usually the male smears accessory gland material over the female's genital opening and after some time the product hardens and forms a mating plug (*see* for example Labine 1966, Dickinson & Rutowski 1989, Aiken 1992). Some authors have suggested that mating plugs may have other functions, for instance "the leaking hypothesis" suggest that plugs assist insemination by preventing passive loss of semen from the female genital tract (Parker 1970).

3.2. Prolonged copulation and postcopulatory mate guarding

Prolonged copulation (i.e. copulation that continues after completed insemination) functions more or less in the same way as mating plugs, i.e. to block the female tract. Also, mate guarding prevents the female from remating, but without maintaining genital contact. The male pays a high price for the extra time spent on prolonged copulation or mate guarding, in terms of reduced time for mate searching. Mating plugs, on the other hand, are expensive in terms of energy, but after the copulation the male is free to search for new partners. Post-insemination associations are expected to evolve when the fitness benefits for males remaining with their mate exceed the cost in terms of lost mating opportunities (Parker 1970, 1984, Thornhill & Alcock 1983).

Since males that are successful in "take-over" attempts almost invariably mate with the female, selection favours males that are able to reduce the risk of take-over events. Male adaptations such as special organs that serve to keep the sexes together during copula, threatening and aggressive behaviour and emigration from areas with high density of rivals, are used to prevent take-overs during the copulation and the guarding phase (Parker 1970, 1984, Thornhill & Alcock 1983).

Mate guarding behaviour may have additional functions related to sperm competition (Thornhill & Alcock 1983). When mating the male cricket *Teleogryllus commodus* transfers a spermatophore to the female, and the male guards the female after mating. This behaviour does not only prevent females from remating, but also keeps the female from eating the attached spermatophore before the sperm have migrated into her spermatheca (Loher 1981).

3.3. Male-induced female non-receptivity

A female refractory period, i.e. a non-receptive time after copulation, is often induced by sperm or seminal products inseminated by the male (Eberhard 1996: tab. 6.1). Males may in this way manipulate the female to reduce the risk of sperm competition. Males can also make the female less attractive by reducing her output of pheromones or by applying antiaphrodisiac substances that reduce her attractiveness (Riddiford & Ashenhurst 1973, Thornhill & Alcock 1983, Kukuk 1985, Eberhard 1996).

4. Sexual conflict over fertilization control

Already in 1970, Parker (1970) pointed out that the female should not be regarded as an inert environment around which male adaptations for sperm competition evolve. Recently Eberhard (1996) expounded his views on the subject. He argues that females are not passive but rather dictate "the rules of the game". As soon as male adaptations are costly to females, we must consider the female as a player of the game (Walker 1980, Parker 1984). Conflicts between male adaptations to sperm competition and female interests can be classified into two main groups (Parker 1984). Firstly, there are cases when multiple matings are costly to the female; i.e. there is a conflict between male adaptations for courtship persistence or sperm displacement, and female interests. Secondly, there are cases when the female benefits by multiple matings; i.e. there is a conflict between male adaptations to prevent remating of the female and female interests. As Yamamura & Jormalainen (1996) noticed, one should remember that even if a behavioural conflict between the sexes is not evident, conflict of interests may still exist.

5. Mating costs experienced by females

There are several examples of female mating costs. Matings will involve time and energy losses (Alcock *et al.* 1977, Daly 1978, Thornhill & Alcock 1983) and the female may run the risk of being injured or killed by predators (Arnqvist 1989, Fairbairn 1993, Rowe 1994), competing males (Daly 1978, Parker 1979), or male-transmitted pathogens (Daly 1978, Thornhill & Alcock 1983). There may also be a conflict between female optimal reproductive rate and male interests. Selection will favour males that induce females to make an especially large reproductive investment soon after copulation. But too high rates of reproduction may shorten the female's life and her lifetime reproduction (Fowler & Partridge 1989, Chapman *et al.* 1995, Eberhard 1996, Rice 1996). Mating plugs obstructing oviposition (Knowlton & Greenwell 1984) and overly aggressive sperm causing polspermy (Eberhard 1996, Rice & Holland 1997) are examples of male sperm competition adaptations that lower female fitness.

6. Possible females benefits of multiple matings

Few studies have shown clear evidence of female benefits through multiple matings. Proposals for the adaptive value of multiple matings to females include: direct benefits, genetic benefits and life history benefits.

6.1. Nutrition

Males of many species provide females with nutrition in some way (prey as a nuptial gift, male secretions during courtship or nutrition through ejaculates) which may represent a form of paternal investment that increases female reproductive success (Thornhill & Alcock 1983, Fincke 1984, Gwynne 1984, Knowlton & Greenwell 1984, Sivinski 1984, Kaitala & Wiklund 1995). Females may experience nutrition losses if males efficiently prevent remating. Also, copulation may function as a payment for gaining access to some male controlled resource, e.g., permission to forage or oviposite in the male's territory (Alcock *et al.* 1977). Females may also receive defensive substances from males during insemination (Sierra *et al.* 1976). One would then perhaps expect females to gain by multiple matings in those species where females receive some kind of resource. But, in a comparative study of insect species, Ridley (1988) did not find any clear connection between paternal investment and fecundity from repeated matings. However, multiple matings in polyandrous species often lead to increased fertility and/or fecundity.

6.2. Sperm replenishment

Multiple matings are necessary when a single insemination does not provide the female with sufficient sperm to insure high fertility during the female's reproductive period (Walker 1980, Knowlton & Greenwell 1984, Halliday & Arnold 1987). The reason may be limited sperm storage capacity. This may be an economic optimization by which the female reduces the energy cost of keeping sperm alive for a long time (Ridley 1988).

6.3. Guarding services

A female may benefit from services provided by a guarding male. In the damselfly *Enallagma hageni*, females are protected by guarding males. After submerged oviposition the female sometimes gets trapped on the water surface. If she is not grabbed and carried away by a guarding male she risks drowning or predation. Thus by trading matings for guarding protection females are able to increase their life time reproductive success (Fincke 1984). In some cases the guarding male aids female foraging or facilitates oviposition by repelling copulatory attempts by other males. However, in these cases females may just do the best of a bad job. Although mating is costly for the female, it can be even more costly to repel unwanted males. By decreasing her reluctance to mate and so accepting superfluous matings, the female acts to balance these costs. In several species, this convenience polyandry hypothesis (Thornhill & Alcock 1983) seems to fit, e.g., in megachilid bees (Alcock *et al.* 1977), water striders (Rowe *et al.* 1994) and odonates (Fincke *et al.* 1997).

6.4. Genetic diversity

Genetic diversity in progeny may be advantageous in unpredictable environments with strong sib competition and multiple mating females may then be favoured over single mating females (Knowlton & Greenwell 1984, Parker 1984, Maynard Smith 1989). However, by increasing genetic diversity in offspring, any restraints on sib competition due to kin selection will weaken as well, and thus increase sib competition (Reynolds 1996). Williams (1975) claimed that one mating would supply the female with a potential progeny diversity equivalent to one-half that of the whole population. A female could then, by mating with only a few males, obtain almost the total range of genetic diversity present in the population, and should gain very little by further matings. Besides, females probably seldom produce enough eggs to use up even the diversity from one mating (Yasui 1997).

6.5. Avoidance of defective sperm and genetic incompatibility

Females may mate with more than one male to guard against genetic incompatibility. Genomes of species are dynamic entities constantly evolving partly as a result of intragenomic conflicts (Rice & Holland 1997, Parker & Partridge 1998). Selfish genetic elements such as cellular endosymbionts, transposable elements and segregation distorters, can modify maternal and paternal haplotypes enough to make them unable to combine and produce a viable zygote (Zeh & Zeh 1996). Multiple mating females could by postcopulatory mechanisms, e.g., sperm competition, sperm choice or reallocation of resources from defective to viable offspring, minimize the risk or cost of being fertilized by genetically incompatible sperm (Zeh & Zeh 1997). Females may also mate with several males so as to guard against abnormal chromosome numbers, defects due to long-term sperm storage, or sterile or subfertile ejaculates (Dewsbury 1982, Halliday & Arnold 1987).

6.6. Good genes

Assuming that males differ in genetic quality, females will try to combine their own genes with male genes of as high quality as possibly. Multiple mating may then enable females to exercise sequential mate choice. When a female encounters a male that is better than previous ones, she will remate and use the last male's sperm for fertilization (Dickinson & Rutowski 1989, Eberhard 1996, Yasui 1997). In species with sperm mixing (fair raffle game) females may devalue the sperm obtained by earlier mates by performing repeated matings with a preferred later male (Hunter *et al.* 1993). Yasui (1997) presented a good-sperm model providing a mechanism for maintaining costly multiple mating by females. Multiple mating will induce sperm competition among the female's mates and male fertilization success was assumed to be determined by the level of investment in sperm competition traits. It was also assumed that males with greater general viability can invest more resources in sperm competition. Thereby multiple mating females receive good genes, because males with greater sperm competitive ability also possess genes for greater general viability.

6.7. Sexy sperms

The sexy-sperm hypothesis proposes another selective advantage to multiple mating females over single mating females. By multiple mating and so mixing the sperm of several males, females ensure that they are fertilized by the most competitive sperm, thereby increasing the probability that their sons also will have competitive sperm (Knowlton & Greenwell 1984, Harvey & May 1989, Curtsinger 1991). This proposed payoff in the next generation is analogous to that in the sexy-son hypothesis (Weatherhead & Robertson 1979). A similar argument can be used for paternity assurance mechanisms. Male paternity assurance mechanisms will prevent females from remating, but females will also benefit by having sons with effective paternity assurance mechanisms. Curtsinger (1991) studied a genetic two-locus model of the sexy sperm processes and found that the conditions necessary for sperm competition to solely promote the evolution of multiple matings were rather restricted. The sexy-sperm mechanism failed if there were any fitness costs associated with multiple matings.

6.8. Variance in offspring numbers

Gillespie (1977) modeled the population consequences of variance in number of surviving offspring. One conclusion from these models was that if one compares two strategies producing the same mean number of offspring, the strategy with the lower variance in offspring number will be favoured. Multiple mating females should have lower within-generation variance in expected number of offspring than single mating females (Knowlton & Greenwell 1984). But the selective advantage of low within-generation variance in offspring number is difficult to evaluate as it depends on population stability and population size. Multiple matings may however be favoured in small populations or in populations that experience founder effects (Parker 1984).

7. How is the sexual conflict resolved?

Apparently, there is an evolutionary arms race not only between sperm displacement and paternity assurance strategies in males, but also between male traits to achieve matings or to prevent female remating, and female counteradaptations (Parker 1984, Choe & Crespi 1997, Rice & Holland 1997). The sperm competition system will hence have complex dynamics. Two lines of argument may be used to predict the outcome of the sexual conflict (Knowlton & Greenwell 1984). (1) The party experiencing the strongest selection pressure will win the conflict. (2) The party that is in a superior position to manipulate the other (based on existing adaptations) will win the evolutionary race. Any costs to females from mating with males with traits to improve the sperm competition ability, will also be felt by the male through reduced reproductive success. But generally the cost will not be symmetric; the male trait may increase the male's total progeny although it reduces the number of offspring produced by each inseminated female (Parker 1984). But females can only gain indirectly by producing sons that inherit the advantageous trait (sons effect, Weatherhead & Robertson 1979). This benefit to the female is much diluted compared to the direct benefit experienced by the male (Parker 1984). Selection for male paternity assurance is thus expected to be stronger than selection for female resistance to mating or male adaptations (Knowlton & Greenwell 1984, Parker 1984). On the other hand, since fertilization takes place within the female, she has the opportunity to control reproductive processes and thereby influence sperm use (Knowlton & Greenwell 1984, Parker 1984). This argument has been emphasized by several authors, e.g., Eberhard (1996) maintains that selection on male adaptation is constrained mainly by female interests.

Knowlton & Greenwell (1984) showed that, although the costs to females generally do not exceed the selective advantage to males, these costs may be so high that they favour female counteradaptations. One example of such an adaptation would be a female ability to influence paternity by terminating the copulation prematurely. The selection acting on males should then be reversed, i.e. the fitness of active males should decrease relative to that of passive males. If such a strategy required less energy than just accepting active males, it would spread among females and prevent or slow down the selection for paternity assurance mechanisms (Knowlton & Greenwell 1984). Several female counteradaptations for reducing male imposed limitations to their reproductive success, have been proposed. For instance females may lower their conspicousness to males by adapting a male-like morph, physically resist mating attempts, possess specialized organs for rejecting males, remove mating plugs, disguise their reproductive status and have barriers in the reproductive tract to resist overly aggressive sperm (Krupa *et al.* 1990, Arnqvist & Rowe 1995, Clutton-Brock & Parker 1995, Eberhard 1996, Stockley 1997).

When selection acts in separate directions on a homologous character in males and females, a genetic correlation between the sexes for this trait will slow down the rate of divergence and an evolutionary optimum may never be reached (Halliday & Arnold 1987, Partridge 1994). For example if there is a genetic correlation between the sexes in mating tendency, females may mate repeatedly as a correlated response to stronger selection on males to do so.

Rice (1996) made a clever experiment using the genetic features of *Drosophila melanogaster*, and so presented empirical evidence for an evolutionary arms race between the sexes. By artificial selection, females were experimentally prevented from coevolving with males. Males then rapidly (within 30 generations) adapted to the static female phenotype and increased their abilities to remate with nonvirgins, to prevent the female to remate, and to displace rival sperm. This increase in fitness of the males was accompanied by a decrease in survival of their mates, suggesting counteracting adaptations in the two sexes.

To summarize, the outcome of an arms race between female and male can only be decided by the strength of the selection pressure acting on each sex and by the ability of the sexes to manipulate each other. These two aspects of the sexual conflict over sperm competition are likely to be biased in favour of one sex; selection is more intense on males, but females have more physical control over their bodies (Knowlton & Greenwell 1984, Parker 1984). Recent empirical evidence suggests that the two sexes experience a perpetual coevolution, just as is the case with predator vs. prey or parasite vs. host (Rice 1996).

8. Mating order: last male advantage is the rule in insects

Since mating order seems to be of fundamental importance, sperm priority patterns are generally classified either as first male advantage or as last male advantage. In insects, last male advantage seems to be the main rule (Parker 1970, 1984, Gwynne 1984, Ridley 1989, Birkhead & Hunter 1990). Usually sperm priority is quantified from double matings with virgin females, and expressed as a P_2-value, which is the proportion of offspring fathered by the second male to mate in double matings (Boorman & Parker 1976). Gwynne (1984) showed that last male precedence occurs in at least seven insect orders, and Parker (1984) showed that in many insect species the last male often fertilizes 75%–100% of the eggs.

However, the degree of last male priority varies considerably among species, with average P_2-values ranging from 0.02 to 1.00 (Gwynne 1984, Ridley 1989). Several explanations have been suggested for this huge interspecific variation in sperm displacement capacity of the last male. Referring to a literature survey including 21 species, Boorman and Parker (1976) suggested that the degree of sperm displacement should be lower in species with mating plugs. Walker's analysis (1980) based on 30 species indicated low degrees of displacement in species with spheroid spermatheca, and high degrees of displacement in species with elongate spermatheca. He suggested that sperm precedence is mainly achieved through sperm stratification (*see* section 2.2.), and that rival sperm stored in elongated spermathecas may more easily be pushed away into its far end. Gwynne (1984) argued that males that invest in offspring or provide other kinds of direct benefits would compensate loss of mating opportunities with high confidence of paternity. Sperm competition data from 37 species support his hypothesis; the highest degree of sperm displacement was found in species providing, or likely to provide, direct benefits to their mates or progeny. Knowlton & Greenwell (1984) presented a model in accordance with Gwynnes hypothesis, predicting a correlation between male parental investment and high certainty of paternity. These authors stress the fact that in this model high paternal investment promotes the evolution of paternity assurance traits and not the other way around. Ridley (1989) suggested that the natural mating frequency of females could explain most of the interspecific variance in sperm displacement rate. He reasoned that if the first male was successful, the species should evolve back to monandry. In cases with last male precedence, selection for increased mating frequency would occur. High degree of sperm displacement would then be associated with polyandry, and low degree of displacement with monandry. In a comparative study of 57 species, Ridley (1989) tested the four alternative hypotheses mentioned above, and found support only for the mating frequency hypothesis.

9. Intraspecific variation in sperm priority

The within species variation has often been ignored, as pointed out by several authors (e.g., Gwynne 1984, Eberhard 1996), when classifying species; sperm priority patterns are not always as clear as suggested above. Lewis & Austad (1990) presented a review of insect studies dealing with the intraspecific variation in P_2-values. In nearly all the fourteen species P_2-values varied extremely, in some species between 0 and 1.0.

Intraspecific variation in male fertilization success has been found to correlate with a number of male traits and mating properties, e.g., copulation duration (Rubenstein 1989, Siva-Jothy & Tsubaki 1989, Simmons & Parker 1992), male size (Lewis & Austad 1990, Simmons & Parker 1992, LaMunyon & Eisner 1993, 1994, Ward 1993, Otronen 1994, Bissoondath & Wiklund 1997), spermatophore size or spermatophylax size (Wedell 1991, LaMunyon & Eisner 1994), sperm size (Radwan 1996), male genital morphology (Arnqvist & Danielsson 1999), courtship behaviour (Otronen 1990, 1994, Otronen & Siva-Jothy 1991), male olfactory attractiveness (Lewis & Austad 1994) and seminal protein contents (Harshman & Prout

1994, Clark *et al.* 1995). Considerable variation has been shown between *Drosophila* male genotypes in sperm competition ability (Clark *et al.* 1995, Hughes 1997). It is impossible to decide whether these intraspecific patterns are due to male differences in competitive or manipulative ability, or to female choice. However, traits such as copulation duration and male size are likely to correlate with ejaculate size (rate of sperm transfer being usually constant and increasing with male size), and differences in the number of transferred sperm may be a likely explanation for the correlation between P_2 and copulation duration or male size (Wedell 1991, Simmons *et al.* 1996, *see* fair raffle section 2.3.). Although empirical evidence for postcopulatory female choice is scarce, Eberhard (1996) has pointed out many possible mechanisms for female discrimination between sperms, e.g., rejection of semen, slow or inhibited transport of sperm to storage organs, lack of sperm nourishment, digestion of current male's sperm, control of ovulation and selective use of stored sperm.

Any trait that influences male fertilization success, either through sperm competition or postcopulatory female choice, should have a great impact on male fitness. In this perspective, it is interesting to note the great intraspecific variation in sperm precedence. All else being equal, the most competitive sperm alleles should go to fixation. How then is the large variation in sperm precedence maintained? Prout and Bundgaard (1977) presented a population genetic model showing that stable polymorphism in a diallelic sperm-displacement locus can be maintained either by overdominance or nontransitivity. Prout and Clark (1996) showed later that stable polymorphism could also be maintained if the alleles affecting sperm displacement have pleiotropic effects on fecundity and mating ability. Variation in male performance may also reflect interactions between sperm precedence ability in males and female genotypes. No clear correlation between male traits and paternity would then be expected, because a male would successfully fertilize only certain female genotypes. A few insect studies have shown that female genotypes affect sperm displacement (e.g., Wilson *et al.* 1997, Clark & Begun 1998). It has also been suggested that an intergenomic conflict can lead to an antagonistic coevolution between loci within the genome of a single species, for instance between offensive and defensive sperm competition traits, analogous to the Red Queen process among species (Rice & Holland 1997).

The choice of experimental design may also affect the outcome and produce considerable variation in experimental results. In almost all studies of sperm priority so far virgin females have been mated with two males only (Zeh & Zeh 1994). However, the assumption that sperm priority patterns are independent of the number of subsequent matings probably does not always hold (Zeh & Zeh 1994). When performing double matings with the pseudoscorpion *Cordylochernes scorpioides,* a clear pattern of last mating male advantage occurred, but when the number of successive mates was increased from two to three, the previously observed pattern of last male sperm precedence broke down. Instead the result indicated complete sperm mixing; each male fertilizes about one-third of the eggs. Time interval between matings may also influence sperm priority (e.g., Bartlett *et al.* 1968, Boorman & Parker 1976, Walker 1980, Ridley 1989). There are examples of both increase and decrease of last male priority with a longer mating interval. When P_2 increases with a longer time interval one possible explanation could be that females run out of sperm over time, resulting in higher fertilization success by the latest male when remating occurs (Ridley 1989, Yamagishi *et al.* 1992). If P_2 decreases with an increase in the mating interval this may be due to sperm migration. When sperm has reached the spermatheca the later male may not be able to remove sperm (i.e. only sperm in the bursa copulatrix can be replaced by later males) (Retnakaran 1974, Gage 1992). Usually females in optimal condition are used in double mating experiments, i.e. virgin females fed *ad libitum* and highly receptive. In nature, males probably often meet females outside the peak of receptivity, i.e. nonvirgins, young or old females, malnourished females, etc (Eberhard 1996). Sperm priority patterns are thus affected by several factors concerning the mating situation. The experimental mating should preferably correspond to the natural situation in order to eliminate misinterpretation of how females utilize sperm in nature.

10. Future research: some suggestions

Early studies of sperm priority studies focused on competition between males and led to the discovery of many adaptive responses to handle sperm competition. The introduction of new ideas about how females can exert influence on postcopulatory processes has put the problem into another perspective. The evidence for cryptic female choice is so far scanty, and more empirical research is clearly needed in this area. Future research may possibly lead to a revaluation of some processes presently interpreted as being controlled purely by the male. To distinguish experimentally between male manipulation and cryptic female choice is a difficult task and will constitute a major challenge for future workers.

Detailed knowledge of how sperm is transported and used by the female is also urgently needed. Studies reviewed in this paper suggest that there is a great taxonomic variation in genital morphology and sperm storage processes, which makes generalizations impossible. The morphology of the reproductive organs is well described in most insect groups, but very little is known about their function. This is partly due to the difficulties associated with dynamic processes, and to the fact that morphologists have focused mainly on phylogeny reconstruction. Surely, ecologists have studied sperm utilization for a long time, but progress has been restrained by the lack of knowledge about how the female reproductive organs are functioning. Co-operation between ecologists and morphologists to fill these gaps would be rewarding.

Previous studies of sperm priority patterns were mainly performed at the species level, and contributed to the explanation of the interspecific variation. Recent studies indicate a great variation also within insect species, and these investigations should be pursued further. Also studies of paternity patterns with molecular methods may come up with important clues to the selective forces influencing sperm usage. Possible interactions between male adaptations and female genotypes add to the complexity of this problem and will call for well planned experiments. Another important reason to look closer at individual variation is the recent discovery that the experimental protocol affects the results. This should lead us to question how well the laboratory conditions mimic the natural situation in which the reproductive strategies have evolved, and may ultimately call for modification of the experimental design.

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