Chapter 22
Postcopulatory Reproductive Strategies in Spermatozoa

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Abstract To reproduce sexually, males and females produce very different gametes (sperm and eggs) in many animals. This difference gives rise to very different strategies in the two sexes and in gamete cells from the two sexes. Sperm meet eggs in harmony; however, the male and female do not always have common interests in reproduction. The battle of the sexes continues even after copulation. Female promiscuity is key to the understanding of reproductive behaviors not only in male individuals but also in sperm cells, because sexual selection continues after mating through sperm competition. Here, we highlight multiple sperm traits—the sperm acrosome reaction in sea urchins, sperm storage in birds, and sperm dimorphism in squid—that are tightly associated with postcopulatory reproductive strategies.

Keywords Sperm acrosome reaction · Postcopulatory sexual selection · Sperm competition · Seminal receptacle · Sperm cooperation

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22.1 Introduction

In sexually reproducing organisms, fertilization—the integration of haploid paternal and maternal genomes—is primarily achieved by fusion between the spermatozoon and the egg. Because male gametes are generally produced and released more abundantly than female ones and yet sperm–egg fusion must occur at a ratio of 1:1, spermatozoa are always subject to strong selection or competition. This applies not only between spermatozoa from the same individual but also between those from different individuals. Thus, promiscuous mating by females can bring about the possibility of sperm competition: the process of spermatozoa from two or more males fertilizing a given set of ova (Parker 1970). Sperm competition theory has developed continuously over the last four decades, with empirical evidence from insects, birds, fish, and mammals (Birkhead et al. 2009). Currently, it is recognized as an evolutionary form of postcopulatory sexual selection. Sperm competition theory can explain why and how female promiscuity could drive the evolution of giant spermatozoa. However, recent studies have focused on more complicated situations where sperm size is well correlated with the structure of the female reproductive organs, which are known as sites for “cryptic” female choice. Thus, researchers have faced new problems in explaining the evolutionary forces that have driven the observed traits in sperm biology. In essence, the ultimate goal of a tiny swimming spermatozoon is to reach the surface of the egg at the right place, at the right time. It does not mean that faster-swimming spermatozoa are always superior in achieving fertilization; rather, adaptation to postcopulatory circumstances is mandatory for the ejaculates to maximize male fitness in terms of reproductive success. Male mating tactics also influence postcopulatory circumstances, and males often choose a favored tactic depending on the condition of rival males. In this chapter, we introduce representative processes in spermatozoa that could have evolved in complex postmating reproductive contexts.

22.2 The Sperm Acrosome Reaction As a Fertilization Strategy Differing Between Male and Female Organisms

In many species, the most relevant postcopulatory process is the sperm acrosome reaction (AR), by which spermatozoa discharge/expose the contents of the acrosomal vesicle located at the apical tip of their head upon certain stimuli derived from the egg’s extracellular investments (such as the jelly coat in echinoderms and newts, the vitelline envelope in birds and frogs, and the zona pellucida/cumulus oophorus in mammals) during fertilization. As a consequence of the AR, IZUMO1 in mice and bindin in sea urchins are exposed on the surface of the sperm; thereafter these proteins play a role in sperm–egg fusion. Thus, the AR is an essential exocytotic process leading to successful fertilization (Satouh et al. 2012). Other acrosomal contents might have fundamental roles in sperm–egg interactions or sperm penetration through the egg coat (lysin); however, the molecular identification of such participants has been extremely difficult. Not all species produce spermatozoa with an acrosome; for example, most fish do not, and those species produce eggs with a micropyle—a small opening in the egg coat, through which a spermatozoon can enter. Thus, the sperm AR is required for spermatozoa to penetrate the egg coat in general, with a few exceptions. Conversely, the presence of an egg micropyle is not always coincidental with the absence of an acrosome in the spermatozoa, as in the case of amphioxi (Morisawa et al. 2004), which show the acrosome or the AR in sperm.

In sea urchins, the AR is a species-specific process, which ensures conspecific fertilization (Vilela-Silva et al. 2008). In fact, the purple urchin, *Strongylocentrotus purpuratus*, produces sulfated fucans that differ in their pattern of sulfation from those of the sympatric sister species, *Strongylocentrotus franciscanus*, resulting in species-specific induction of the AR (Vilela-Silva et al. 1999). Hence, sperm–egg interactions play essential roles in prezygotic barriers, particularly for many broadcast spawners.

Interestingly, our (unpublished) observations suggest that spermatozoa of *S. purpuratus* are not able to penetrate—or can penetrate only with difficulty—the jelly layer of the egg from *S. franciscanus*. Spermatozoa of *S. purpuratus* can undergo the AR to some extent with sulfated fucans from *S. franciscanus* if high enough concentrations are applied (Hirohashi et al. 2002). Therefore, even if unfavorable collisions occur between heterospecific gametes, fertilization is normally strongly prohibited. Moreover, premature induction of the AR of foreign spermatozoa on the surface of the jelly layer might be beneficial for the eggs to reduce the risk of cross-fertilization, because of rapid loss in their fertilizing competence after the AR. Male organisms need to produce spermatozoa that recognize only conspecific eggs, possibly because of sperm competition, whereas females produce eggs that also can trigger the sperm AR of other species because of the presence of multiple reproductive barriers (Vilela-Silva et al. 1999; Hirohashi et al. 2002). Thus, a conflict of interest arises between the sperm and the egg. This hypothesis can be seen in the theory of sexual conflict, in which the two sexes have conflicting fitness strategies concerning reproduction. It is of particular interest to consider why only the patterns of sulfation on the largest carbohydrate polymer in the egg jelly confer this specificity of fertilization. It is possible that point mutations in genes encoding sulfotransferases could result in global and drastic changes in the sulfation pattern, thus giving a high potential for speciation.

22.3 Sperm Storage Is a Reproductive Strategy That Facilitates Fertilization Success

In marine animals that use broadcast spawning, such as sea urchins and ascidians, gametes from both male and female individuals are released in synchrony, and spermatozoa are then guided by a chemoattractant secreted from the eggs (Yoshida et al. 2008). Such chemotaxis, together with synchronized release of gametes, can yield better fertilization outcomes. However, in internal fertilizers, better fertilization success is primarily achieved by the coordinated arrival of both sets of gametes at the site of fertilization. It is unlikely that the sperm–egg encounter occurs accidentally in vivo, because only a small fraction of spermatozoa succeed in making the long journey through the female genital tract to reach the site of fertilization. Notably, the timing of ovulation does not always coincide with that of insemination except in some mammals such as rabbits and domestic cats, where copulation stimulates ovulation (Dal Bosco et al. 2011; Brown 2011). Occasionally, female animals store spermatozoa in the reproductive tract. Thereafter, sperm use is under the female's control and stored spermatozoa are released during the ovulation "window." This sperm-storing capacity in the female reproductive tract is well documented in a variety of animals—including insects, fish, amphibians, reptiles, birds, and mammals (Birkhead and Møller 1993; Holt and Lloyd 2010; Orr and Zuk 2012)—which possess specialized organs in their genital tracts, such as sperm reservoirs in mammals (Suarez 2008), sperm storage tubules in birds (Sasanami et al. 2013), spermathecae in amphibians (Watanabe and Onitake 2002), or spermathecae and seminal receptacles in insects (Heifetz and Rivlin 2010). These organs hold the spermatozoa until ovulation takes place or the oocytes become fertilizable.

Although the duration of sperm storage varies between species, it is known that spermatozoa can be stored in the genital tracts of reptiles for years, and in those of insects for decades (Birkhead and Møller 1993; Gobin et al. 2006). Nonetheless, the mechanisms by which spermatozoa can maintain their fertilization competence for such a long time at ambient or body temperatures remain elusive. Although it was believed that sperm storage is unique to animal phyla, recent findings suggest that plants can also store male gametes in the female until fertilization. In flowering plants, when the first pollen tube fails to fertilize, a second pollen tube that is quiescent in the pistil initiates migration toward the ovule and overcomes this fertilization failure (Kasahara et al. 2012). In addition, fertilization in the Fagaceae (*Fagus japonica*) is delayed by between 4 days and more than 1 year after pollination until the ovule becomes fully developed (Sogo and Tobe 2006). These phenomena appear to be similar to that of sperm storage in the female reproductive tracts of animals (Iwata et al. 2011). Sperm-storing phenomena typically occur in female individuals whose ovulatory cycles do not coincide with the timing of insemination. In some species, such as insects and birds, the mating opportunity is quite small relative to the female's reproductive period, and yet the oocytes quickly lose their fertilizability after ovulation. Thus, sperm must keep their ability to fertilize oocytes for a considerable length of time after ejaculation. Hence, the primary role of sperm storage is to adjust the timing of sperm arrival at the site of fertilization. In some mammalian species—such as sheep, pigs, and cows—spermatozoa are trapped in the lower isthmus of the oviduct (referred to as the sperm reservoir) until the time of ovulation (Suarez 2008). In pigs and cows, this trapping is mediated via cell adhesion molecules on the sperm head and carbohydrate moieties on the epithelial surface of the caudal isthmus (Ignotz et al. 2001). Spermatozoa are released from the sperm reservoir prior to ovulation, in response to ovarian steroids, which reactivate sperm motility sufficiently to break the bond between the spermatozoon and the sperm reservoir (Hunter 2008). In the Japanese quail (*Coturnix japonica*), the spermatozoa stored in the sperm storage organ are released in response to stimulation by circulating progesterone (Ito et al. 2011). Although the duration of sperm storage in mammalian species is relatively brief (up to several days), bats can store spermatozoa in the oviduct for 6 months (Racey 1979). These bats reside in temperate zones, where hibernation interrupts the reproductive cycle (Racey 1979). Birth occurs in early summer, which is suitable for raising offspring. In this way, bats are able to maximize the fitness of their reproductive cycle in terms of the timing of copulation and fertilization. A recent study in the greater Asiatic yellow bat (*Scotophilus heathii*) indicated that sperm storage is dependent on the maintenance of high levels of circulating androgens, produced by the ovaries interacting with androgen receptors in the uterotubal junction, where sperm storage occurs (Roy and Krishna 2010). Although the details are still unclear, these phenomena are linked to the expression of B-cell lymphoma factor 2 (Bcl-2), a key regulator of apoptosis in the uterotubal junction (Roy and Krishna 2011). From these results, the authors hypothesized that androgen-dependent expression of Bcl-2 might act as an antiapoptotic factor in the uterotubal junction and play a key role in long-term sperm survival at this storage site.

Storing spermatozoa for long periods over more than one breeding season (i.e., for more than 1 year) allows female individuals to produce offspring without the need for additional mating in the next breeding season. In general, mating is costly for females because of the risks of predation, infection, and injury (Watson et al. 1998; Archie et al. 2014; Hamilton and Zuk 1982). Furthermore, copulation itself can be harmful; in *Caenorhabditis elegans*, mating reduces a female's life-span (Gems and Riddle 1996). Thus, the sperm-storing system can ensure fecundity without payment of a precopulatory price during multiple breeding cycles. In fact, in reptiles such as turtles, snakes, and alligators, sperm storage can last for up to 7 years (Birkhead and Møller 1993; Mangusson 1979). In various elasmobranch species, the duration of sperm storage ranges from days to years (Fitzpatrick et al. 2012). Although sperm storage is an excellent strategy for producing offspring without unnecessary mating, it should be noted that parthenogenesis might be an alternative way to produce embryos if female animals have long been isolated from potential male partners. Therefore, we should carefully evaluate whether the offspring generated by these sperm-storing animals might actually result from parthenogenesis rather than from conventional sexual reproduction.

As noted, the period of sperm storage in mammals is usually short. In contrast, the queens of eusocial Hymenoptera (ants, bees, and wasps) often store spermatozoa for more than a decade (den Boer et al. 2009a; den Boer et al. 2009b). They store them in their storage organs (spermathecae) early in adult life after copulation and never mate again during the rest of their life. The spermathecae often have accompanying glands, and their secretions have been hypothesized to play a role in survival of stored spermatozoa. However, the chemical compositions of the secreted compounds—and their physiological actions—have not been elucidated. In these species, queens initially store several hundred million spermatozoa, which could reduce multiple risks such as predation and injury during copulation. Although a direct merit of sperm storage has not been established, it has been reported in *Drosophila melanogaster* that females with repeated mating survived for a much shorter time than virgin females. Furthermore, the exposure to seminal fluid products from the male accessory gland reduces the female's propensity to remate and increases the egg-laying rate (Chapman et al. 1995; Rice 1996). These findings indicate that females are discouraged from multiple mating by the first male's copulation.

22.4 Promiscuity Drives Multiple Reproductive Tactics

Paradoxically, females do not always mate with one partner (monogamy); rather, they mate with multiple male partners (polyandry) during the reproductive season or even during a single spawning event. Female promiscuity is common in various animal taxa, from insects to humans. Females can receive a sufficient number of spermatozoa from a single copulation to fertilize all of their eggs, but they still mate with multiple males. Thus, promiscuous mating does not increase overall fertilization success; rather, it increases genetic variation in the offspring and therefore offspring fitness (Mays and Hill 2004). When a female mates with more than one male partner, spermatozoa from different males may compete for fertilization. This is called sperm competition and is one of the strongest selective forces driving the evolution of reproductive strategies (Parker 1970). Where sperm competition occurs varies among different mating systems. In external fertilizers—such as marine invertebrates, frogs, and fish—sperm competition may occur in the water environment (Byrne 2004; Gage et al. 2004). Here, the rate of sperm release, the rate of sperm dilution, and the timing of gamete release from both sexes impact on fertilization success (Levitan and Petersen 1995). In internal fertilizers—such as insects, birds, and mammals—sperm competition occurs in the female's reproductive tract. The mating order, the number of transferred spermatozoa, successful arrival in the sperm storage organs, and the survival duration in these organs are the factors that may influence fertilization success. Furthermore, sperm–female interactions lead to more important and complex situations. When females store spermatozoa from more than one male, they frequently select only "favorable" ones by eliminating others either actively (via direct sperm displacement (Sato et al. 2014)) or passively (via interactions with the female seminal receptacle (Manier et al. 2010)). Such female preference after copulation is called cryptic female choice and is regarded as the other strongest postcopulatory force in sexual selection besides direct sperm competition (Eberhard 1996). Indeed, mice lacking the seminal vesicle secretion 2 (SVS2) protein are infertile, and their spermatozoa are killed by uterus-derived cytotoxic factors. Thus, the SVS2 protein coats the surface of spermatozoa and protects them from attack by the uterus (Kawano et al. 2014). Those spermatozoa that can protect themselves from such female attack increase their chance of winning the race to the ova. This male/sperm selection by the female might maximize offspring fitness and the number of offspring by selecting among spermatozoa in the oviduct, deposited by different males at different times (Eberhard 2009; Holt and Fazeli 2010). Hence, the female reproductive tract has been recognized to serve as a site not only for sperm storage but also for sperm selection in favor of the female's cryptic preference.

22.5 Characteristics of Alternative Reproductive Tactics and Postcopulatory Sexual Selection in the Squid

It is not unusual to have more than one reproductive phenotype in a population. Such phenomena involve alternative reproductive tactics (ARTs)—one of the major current issues in evolutionary and behavioral ecology. The most commonly observed ART is a case in which large "consort" male individuals compete with each other to gain access to females, whereas small "sneaker" male individuals attempt to "steal" mating and avoid direct male–male competition. ARTs are manifested in a wide variety of behavior, morphology, physiology, and life history among individuals, and lead to male polymorphism; thus, secondary sexual characteristics that engage precopulatory sexual selection are highly developed in consorts but poorly in sneakers (Oliveira et al. 2008). Substantially more prevalent and prominent in consorts are not only a male individual's body display, such as bright colors or enlarged ornaments, but also postmating behaviors, such as mate guarding and male parental care. In contrast, sneakers usually have at least one dominant competitor at the moment of copulation and thus exhibit elaborate behaviors in order to access the female, such as sneak or parasitic mating, and female mimicking (Taborsky 2001; Parker 1990b). In addition, sneaker males would have a higher sperm competition risk than consort males, which could lead to adaptive trait evolution in sperm.

Many species have ARTs with two different male types—consorts and sneakers—and studies have focused on the differences in the modes of sperm competition between these different tactics (Oliveira et al. 2008). In contrast, some squid species in the Loliginidae exhibit unique reproductive biology: female squid have their seminal receptacle near the mouth in their array of arms and receive spermatophores from sneakers with "head-to-head" copulation. Thus, mature female squid that arrive in the spawning areas have already stored some spermatozoa in their seminal receptacle (Drew 1911). After migration to the coastal spawning areas, consort male squid fight with rivals and court females using body color display (DiMarco and

Hanlon 1997). Thereafter, they copulate with the females in a “male-parallel” position—that is, males hold females from the dorsal side and transfer their spermatophores directly into the opening of the oviduct in the mantle cavity. Sneakers rush to the paired squid and attempt to transfer their spermatophores around the seminal receptacle in a head-to-head manner (Hanlon 1996). This difference in the site of sperm deposition determines the fate of the sperm’s journey. Because the site of sperm transfer by consort males is near the ovipositor, their spermatozoa acquire a positional advantage in gaining access to eggs. In contrast, although the exact site of fertilization by sneaker spermatozoa is unknown, it is thought that it occurs when the female holds the egg mass in her arm crown (Iwata et al. 2011). This positional advantage results in greater fertilization success for consorts, as revealed by DNA paternity analysis (Iwata et al. 2005). Spermatozoa from sneaker males are stored in the seminal receptacle and participate to some extent in fertilization, suggesting that both consort and sneaker male squid have a constitutive risk of sperm competition, but sneakers have a higher risk than consorts. In squid, ARTs result in two sperm placement and storage locations, with possible differences in fertilization success.

22.6 Evolution of Sperm Number Strategies Associated with Alternative Reproductive Tactics

Theoretical modeling predicts that males exposed to greater competition from other males allocate greater resources to sperm production in order to compensate for their reduced chances of fertilization success (Parker 1990a, b; Parker et al. 1997); the results of several studies of external and internal fertilizers support this prediction (Gage and Barnard 1996; Evans et al. 2003). In the bluegill fish, *Lepomis macrochirus*, Leach and Montgomerie (Leach and Montgomerie 2000) showed that the milt of sneaker males contained 50% more concentrated sperm than that of consort males. In the squid *Heterololigo bleekeri*, Iwata and Sakurai (Iwata and Sakurai 2007) analyzed morphological characteristics both inside and outside the adult body to look for adaptive traits in each ART. Although no dimorphism was observed in external body characteristics, clear dimorphism was observed in spermatophores. The length of the spermatophore is generally associated with body size, but this relationship was lost between sneakers and consorts. The long-type (consort) spermatophores contained around five times more spermatozoa than did the short-type (sneaker) spermatophores (Iwata et al. 2011). This finding was inconsistent with those of previous studies showing that males in the species with a high sperm competition risk have a higher gonadosomatic index (ratio of gonad to body size) to produce more sperm (Harcourt et al. 1981). The other model in conjunction with empirical evidence (Pilastro et al. 2002) predicted that sperm expenditure should decrease when there are more than two competing male individuals (Parker et al. 1996). In *H. bleekeri*, spermatozoa from a consort and from multiple sneakers can be involved in fertilization during a single spawning episode. Indeed, a paternity analysis identified that more than two male squid were involved in the fertilization of eggs spawned as a single clutch (Iwata et al. 2005). The small sneaker-derived spermatophores contained fewer spermatozoa than the larger consort-derived ones, which is consistent with the prediction that sneakers would have greater exposure to sperm competition and lower sperm expenditure. Such dimorphism in spermatophore morphology could have resulted from adaptation to the structure of the seminal receptacle rather than via sperm allocation. During mating, the spermatophores of cephalopods stimulate a spermatophoric reaction, which involves extrusion of inner sperm sacs called spermatangia (Mann et al. 1966; Marian 2012). The extruded spermatangium also shows a clear dimorphism between sneakers and consorts in *H. bleekeri* (Iwata et al. 2015). The morphology of the spermatangium is "drop-like" in the short-type (sneaker) spermatophore and "rope-like" in the long-type (consort) spermatophore. The design of the drop-like spermatangia reduces mechanical tension, so the risk of detachment from the female tract is reduced. Moreover, the drop-like spermatangium has an anchor on its base, enabling it to hook onto the female body surface near the seminal receptacle (Iwata et al. 2015). Such spermatophore dimorphism is also known in *Euchaeta norvegica* (a copepod) (Hopkins and Machin 1977) and *Chionoecetes opilio* (the snow crab) (Beninger et al. 1993). Males of these species produce two different types of spermatophore at the same time: one for long-term fertilization opportunities and the other for a short-term strategy (Eberhard 1996). Spermatophore dimorphism in squid might also be an adaptation to male mating behavior associated with sperm transfer and storage, but it differs from the cases above in that individual male squid produce only one of the two types of spermatophore associated with each mating tactic.

22.7 Evolution of Sperm Size Associated with Alternative Reproductive Tactics

Sperm competition theory predicts that intense sperm competition will lead sperm morphology toward an optimum form (Varea-Sanchez et al. 2014). In addition, sperm morphology must match a particular mode and strategy of fertilization (Higginson et al. 2012; Scharer et al. 2011). Therefore, morphological diversification within a species is severely limited by both sperm competition and the mode of fertilization. Interestingly, comparisons among closely related species in some groups have demonstrated that male individuals exposed to more intense sperm competition make larger spermatozoa (Gomendio and Roldan 1991; LaMunyon and Ward 1999). Interspecific comparisons among cichlid fish have demonstrated that the spermatozoa of highly promiscuous species are larger, and a positive relationship is seen between sperm size and sperm swimming velocity (Fitzpatrick et al. 2009). In addition, being larger is better for spermatozoa to occupy a female's storage organ, possibly outcompeting those of others. Hence, males facing bad situations in mate access tend to make larger and/or faster sperm to improve their chances of fertilizing eggs. In this context, comparisons between consorts and sneakers have attracted particular attention because both types of spermatozoa share the same fertilization environment but are exposed to different intensities of sperm competition. However, no clear dimorphism has been found in sperm size between consorts and sneakers (Pitnick et al. 2009) except in the case of the bluegill, where sneaker spermatozoa are slightly longer and swim faster than consort spermatozoa (Burness et al. 2004).

What about the case of the male squid that have two different fertilization strategies and two different sperm competition risks? In *H. bleekeri*, clear sperm dimorphism was found, in that the sneaker spermatozoa had a ~50% longer flagellum than consort spermatozoa (Iwata et al. 2011). However, there was no significant difference between sneaker and consort spermatozoa in their initial swimming speed (Iwata et al. 2011). Fertilization competency was examined in both types of spermatozoa and was much greater in sneaker spermatozoa than in consort spermatozoa when assayed in vitro. Although this difference could be attributed to the duration of sustainable motility after initial dilution, it is unlikely that a longer flagellum is associated with sperm longevity. The other possible explanation for the divergence in sperm types is that it arose in coevolution with the female seminal receptacle because only sneaker sperm can be stored in the seminal receptacle. Recent studies have demonstrated that morphological variations in male genitalia and sperm characteristics have coevolved with the morphology of the seminal receptacle (Pitnick et al. 2003; Hosken and Stockley 2004). In the squid, comparative studies with closely related species are needed to clarify this issue. Although the adaptive significance of sperm dimorphism is still elusive, manifold insemination strategies undoubtedly play essential roles in this phenomenon. In turn, basic information on postmating events is needed to estimate the intensity of competition in sperm transfer, sperm precedence in the seminal receptacle, and fertilization success in squid species.

22.8 Evolution of Sperm Cooperation Associated with Alternative Reproductive Tactics

Sperm competition can sometimes drive cooperation among sperm. Cooperation is seen in changes in sperm behavior that enable other spermatozoa from the same individual to increase their chances of successful fertilization. A representative case of sperm cooperation is in sperm aggregates, which are found in opossums (Moore and Taggart 1995), rodents (Moore et al. 2002), and insects (Hayashi 1998). Spermatozoa in a bundle can swim faster than single spermatozoa under experimental conditions (Immler et al. 2007). However, whether spermatozoa ascend the oviduct to reach the oocytes in such cooperating assemblages is purely hypothetical. In deer mice, spermatozoa tend to assemble with “brothers,” suggesting that kin selection leads to cooperation (Fisher and Hoekstra 2010). To our knowledge, this phenomenon of sperm cooperation has been reported only for species with internal fertilization. In the squid *H. bleekeri*, only sneaker spermatozoa show chemotaxis toward CO_2, and respiratory CO_2 emitted from spermatozoa induces self-clustering

(Hirohashi et al. 2013). Unlike other cases of sperm assembly, the clustering of squid spermatozoa does not involve their physical contact with each other; rather, each one swims independently. In addition, the clustering of sneaker spermatozoa does not facilitate enhanced movement in any particular direction. The physiological significance of sperm clustering in squid remains to be determined.

22.9 Conclusions

In this chapter, we have reviewed the postcopulatory fertilization strategies observed in various externally and internally fertilizing species (Fig. 22.1). In general, because the sperm AR is crucial for fertilization, it has been thought that timely commitment of this reaction would increase reproductive fitness in both male and

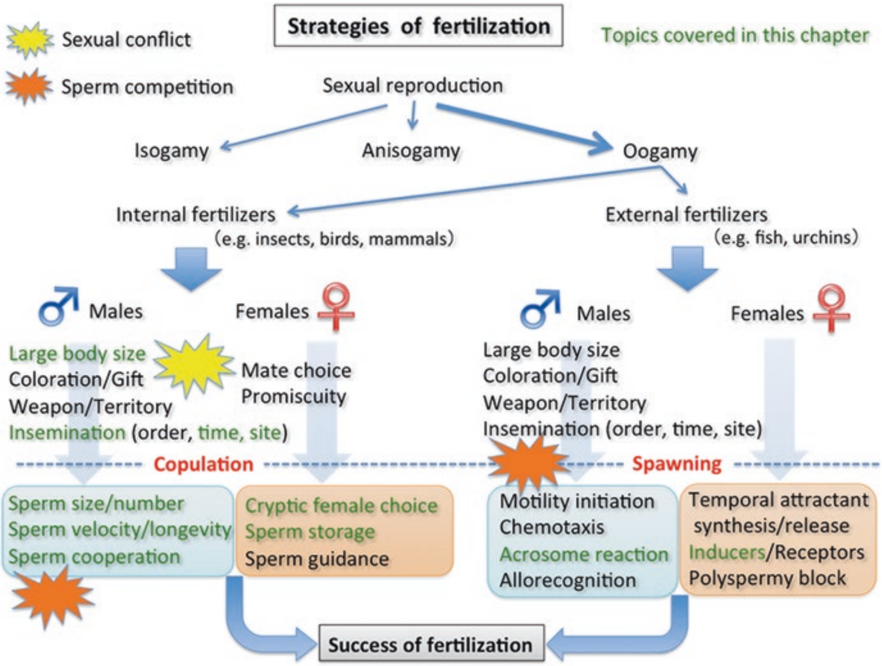

Fig. 22.1 Summary of sex-biased fertilization strategies. The most successfully widespread reproductive system is oogamy, where each sex makes either spermatozoa or eggs to pass on genes between generations. In essence, eggs are always more costly to produce than spermatozoa because of their huge difference in size. Such a difference in each gamete's cost performance drives a conflict of interest between the sexes (sexual conflict), resulting in phenomena such as sexual dimorphism, male–male competition, and female choice. These differences in morphological and behavioral characteristics are consistently observed in internal and external fertilizers. Notably, sexual conflict continues even after copulation/spawning, which is now recognized as postcopulatory sexual selection (sperm competition and cryptic female choice)

female individuals. However, as discussed in the case of sea urchins, specific induction of the AR is much more important for male individuals (spermatozoa) than for female individuals (eggs). In contrast, the threat of cross-fertilization within closely related species is more serious for females than for males because of the higher cost of egg production. Therefore, we should consider the possibility of a conflict of interest between sexes at a molecular level, which has been largely overlooked. In this regard, the limited structural variation of the AR inducer might represent the strategic regimen of oogamy. One adaptive explanation for successful sperm storage is that there are benefits gained by temporal synchronization between copulation and ovulation. These events are thought to be regulated during the ovulatory cycle, which allows sperm release from the storage site to synchronize perfectly with ovulation. Because the ability to promote sperm survival at the storage sites is astonishingly long in some taxonomic groups, elucidation of the mechanisms of sperm storage in different species must include examination of possible solutions to the problem of keeping sperm alive for long periods, which might lead to the development of new strategies for sperm preservation at ambient temperatures. We have also discussed unique sperm traits—sperm flagellar dimorphism and CO_2-mediated self-clustering—that might be associated with ARTs in the squid *H. bleekeri*. In the other species that exhibit ARTs, it is common that consort and sneaker male individuals attempt different mating tactics but release spermatozoa in the same place. In this situation, the sperm traits are still the same with the two tactics. For these squid, differences in the route to reach the ova, the storage sites, the storage period, and fertilization environments between different ARTs would lead to phenotypic variation in sperm biology. It will be important to dissect the evolutionary forces that give rise to new traits in spermatozoa.

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