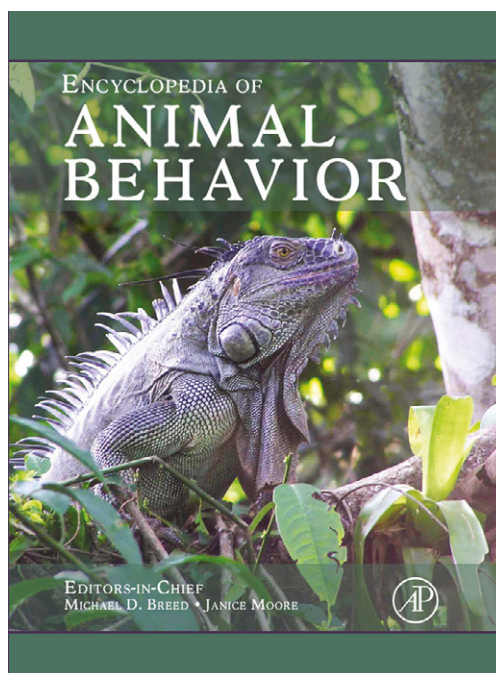


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Invertebrates: The Inside Story of Post-Insemination, Pre-Fertilization Reproductive Interactions

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Introduction

In organisms with internal fertilization, females serve as arenas for postinsemination, prefertilization interactions between the sexes. Although these interactions are not visible to the naked eye, from those species in which they have been studied, females are clearly the site of a rich intersexual dialog that considerably impacts the fitness of both sexes.

Among and within various invertebrate taxa are species with either external or internal fertilization (Table 1). Even where fertilization is internal, it is not necessarily preceded by copulation or by penetration of the female by an intromittent organ. For example, in some marine and freshwater invertebrates, sperm enter the females via the water column, while in others, females collect spermatophores and place them into their own reproductive tracts. Cases also exist in which insemination is referred to as 'traumatic' as it involves piercing of the female's body in order to deliver the ejaculate. This article will not concern itself with copulatory organs or copulation itself. Instead, the focus, regardless of how females come to be inseminated, will be the nature of intersexual interactions that take place inside the female. The intent is not to provide a complete review of all invertebrate taxa with internal fertilization, but instead to highlight some of the variability that exists in reproductive tract interactions in invertebrates with internal fertilization. This article is organized to first present what is known of the components interacting inside the mated female reproductive tract, including the sperm and any accompanying ejaculate as well as the structural and chemical features of the female. Following this, intersexual interactions, starting with the arrival of the ejaculate until the moment of fertilization, will be treated. The final section will address the evolutionary implications of the reproductive structures, substances, and processes that occur inside females.

Components

Male Ejaculate

What is the nature of the material inseminated females acquire from males? Typically, the ejaculate consists of the sperm and the chemical cocktail accompanying them.

In cases where there is a spermatophore, or packet of sperm, the chemical cocktail includes the covering of the packet. Both sperm and nonsperm constituents of ejaculates can be highly variable within and between species. Sperm can vary morphologically, chemically, and quantitatively. Nonsperm components also exhibit qualitative and quantitative variability. Furthermore, some of the ejaculate variability will be nongenetic: nutrition, age, mating status, and mating type (genotypes of each member of the pair) may influence the qualitative and quantitative features of what males pass to females.

Sperm

Sperm size and shape and surface chemistry are all features capable of interacting with the female. While we typically think of sperm as having a head, a midpiece, and tail, in some taxa, sperm have multiple or no flagella. In others, they are amoeboid. Among those species with tailed sperm, such as certain *Drosophila*, sperm can be many times the length of the male's body. The number of sperm transferred to females can vary within and among individuals or species by over a 100-fold.

The sperm surface contains substances that interact directly with the female or with the oocyte surface. Sperm plasma membrane glycosidases are implicated in gamete recognition in a range of invertebrates, including molluscs, ascidians, and insects, where they are considered as candidate proteins in binding with the egg surface at fertilization. In a detailed comparative study of 11 *Drosophila* species, the Perotti laboratory at the University of Milan has examined four sperm glycosidases, candidates for sperm-egg binding. While expression of all four glycosidases was observed in the 11 species, their distribution on sperm surfaces as well their activities were found to vary among species.

Nonsperm

In many invertebrates, including worms, crabs, beetles, crickets, and butterflies, sperm are transferred in a spermatophore, or a discrete package. A spermatophore's contents may be compartmentalized into portions with and without sperm, depending upon the species. Furthermore, not all spermatophores are directly deposited into the female. In some species, females themselves insert spermatophores into their own reproductive tracts. Because nonsperm components of

Table 1 Internal fertilization in the invertebrates

Taxon	Fertilization	Copulation
Poriferans	Both	No
Colenterates	Both	No
Platyhelminthes	Both	Yes
Roundworms nematode	Yes	Yes
Echinoderms	Rarely internal But some stars and sea cucumbers	Rare if at all
Molluscs	Both	Yes
Crustaceans	Both	Yes
Arachnids	Internal	
Insects	Internal	Yes and no

spermatophores are in discrete packages, they lend themselves more easily to comprehensive chemical analyses than does seminal fluid passed directly, without any sort of membrane, to the female. Spermatophores vary tremendously in their size and composition. In some Orthoptera, spermatophores can be up to one-third of the male's body weight. Bush cricket spermatophores are mostly water and protein, with small amounts of glycogen and lipid, primarily hydrocarbons. Crab spermatophores are largely mucopolysaccharide, lacking glycogen. Specialized compounds such as alkaloids and carotenoids also are found in spermatophores of certain species, and may provide benefits to females or their eggs in the form of protection against parasitism or oxidative stress. In butterflies, spermatophore composition varies with adult diet.

In invertebrates lacking spermatophores, chemical analyses of seminal fluid are less advanced. Ejaculate quantities are small and the fluid itself is difficult to obtain in pure form. Some studies have utilized radioactive labeling of males to track and identify male-derived substances in females. Labeling studies have revealed the transfer of elements such as sodium and phosphorus. Studies of invertebrate seminal fluid, however, have tended to focus on seminal fluid proteins and the genes that code for them. Genomic and proteomic approaches have led to the characterization of male accessory gland and other seminal proteins in the honeybee *Apis mellifera*, in the malarial vector *Anopheles gambiae*, and in several species of *Drosophila*. These studies, despite using different approaches, have revealed over a 100 different male proteins in each of the foregoing taxa. Some proteins appear to be conserved across these diverse invertebrate taxa, and even share, in the case of the honeybee, considerable overlap with human seminal fluid. Other proteins, in *Drosophila*, for example, are so rapidly evolving that they cannot even be identified in other members of the same genus. Seminal fluid proteins fall into a number of functional categories, indicating their involvement in processes such as sperm energetics and immune defense.

Female Arena

Morphology

Some species such as poriferans and coelenterates have internal fertilization but typically lack specialized female tissue for receiving or storing sperm. In some sponges, however, oocytes develop in association with a cluster of nurse cells, one of which is specialized to capture sperm. Certain bivalve molluscs store sperm in gill chambers. At the other extreme, many molluscs and arthropods have highly developed female reproductive tracts, often with multiple types of structures. Reproductive tracts typically include a uterine structure or bursa where sperm enter and may or may not remain. Sperm storage organs can vary in shape, size, and number. Frequently, there are tubular receptacles and paired spermathecae, some of which have sphincter valves at the base and muscular coverings. Spermathecae and seminal receptacles also exhibit high levels of interspecific variability in shape and size. A wide variety of accessory glands occur in female tracts, although their specific functions typically remain unidentified. Lampyrid beetle female reproductive tracts, for example, have a certain structure that is assumed to be a spermatophore-digesting gland.

Chemistry

Reproductive tract morphology of female invertebrates has been studied far more extensively than has female chemistry. In those species in which female reproductive tract chemistry has been examined, however, it is proving to be complex and variable. Our information primarily derives from proteomic and genomic studies of economically or medically important insects and of *Drosophila*. In bloodsucking sandflies, spermathecae are filled with a mucopolysaccharide secretory mass. Honeybee spermathecal fluid has been found to have over a 100 proteins. In other cases, chemical interactions can be inferred from structural or ultrastructural studies. For example, in the lampyrid beetle, the spermatophore-digesting gland presumably contains digestive enzymes of some sort. In female houseflies, the posterior reproductive tract has at least six proteins with acid phosphatase activity, each of which exhibits specificity with respect to particular structures of the reproductive tract. Female reproductive proteins in several *Drosophila* species and in honeybees have been the most extensively studied of any invertebrates. Several hundred candidate female reproductive genes turned up in expression studies of whole *Drosophila* female lower reproductive tracts, while ~40 proteins were found in the spermathecae alone. Proteomic studies of the seminal fluid of honeybee queens revealed over a 100 proteins in this storage organ, most of which have energetic or antioxidant function.

Eggs

Outer membranes of freshwater and marine invertebrates, because desiccation is not a problem, are usually different from those of terrestrial species. Oocytes of terrestrial arthropods typically are covered not only with the vitelline membrane, but also a tough chorion, or shell, on the outside. In the vast majority of insect species, because of the impenetrable outer coverings, oocytes have a tubular structure or micropyle, through which sperm must enter. Oocytes of species in the Hemipteran family of Cemicoidae, where fertilization takes place in the ovariole even before the egg's vitelline membrane and chorion are complete, lack a micropyle. In most insects, oocytes have just one micropyle, but in some species there are two and even up to 70 micropyles, often arranged in a circle. In related taxa where the micropyle number differs, it is usually constant for a given species. Micropyles have unique surface carbohydrates that interact with sperm, purportedly the glycosidases mentioned earlier. Consistent with this idea, in *Drosophila*, the Perotti group has shown interspecific differences in the distribution of these carbohydrates in and around the micropyle. Spiders and other invertebrates, for the most part, lack any specialized sperm-guidance structure on the egg surface.

Processes

Once the ejaculate enters the female, one or more prezygotic processes occur, including sperm storage, various female physiological responses, and sperm retrieval and fertilization. These processes may happen quickly or last several years, depending upon the species, but all rely on interactions among the structural and molecular components discussed in the previous section.

Reaching Their Destination

Whether the immediate destination inside the female is the oocyte or a longer-term storage location, some mechanism (s) must guide sperm to their destination. In certain species, for example, the sponges previously mentioned, sperm are not stored. Rather, upon flowing through the body cavity with the seawater, a sperm is captured by a specialized female cell and quickly delivered to the oocyte where fertilization takes place. Nematode sperm are amoeboid and crawl from the uterus to the spermatheca where fertilization takes place. Oocytes of *Caenorhabditis elegans* use polyunsaturated fatty acids to control directional sperm motility within the uterus.

In species where sperm have tails, the storage process is assumed to be at least partially a function of their motility. Contraction of female reproductive tract musculature moves sperm in many insect species. As in *C. elegans*, however, chemical signaling is likely to play a role in

where sperm go. Even in taxa with flagellated, highly motile sperm, such as *Drosophila*, the Wolfner laboratory at Cornell University has shown using RNA interference and antibodies that certain seminal fluid proteins control the sperm storage process. In some cases, male proteins localize to specific regions of the female reproductive tract. Such specificity would be difficult without corresponding localized biochemical differences in the female reproductive tract. In another Dipteran, the sand fly, the mucopolysaccharide mass of the spermatheca appears to activate the sperm in the spermatophore, promoting their uptake into storage. In certain insects, females appear to dump or kill sperm, although the underlying mechanisms are unknown.

Traumatic and hypodermic insemination represent special cases of sperm delivery. A number of invertebrate taxa, including molluscs, insects, and spiders, have traumatic insemination. In most cases of traumatic insemination, sperm are delivered directly to the female reproductive tract. Hypodermic insemination, in which the females' body is pierced and sperm are delivered to the female hemolymph, requires some mechanism by which sperm navigate through the female's body to her reproductive tissue. In the Dysderid spider, *Harpactea sadistica*, for example, where sperm storage organs are atrophied, sperm travel directly to the ovary where fertilization occurs. In this and other cases, the mechanisms guiding sperm to their destination remain unknown.

Maintenance in Storage

In those invertebrates where sperm are stored, what does maintenance of sperm in storage require and who provides it? Duration of sperm storage can last less than an hour or several years as in some species of hymenoptera. In honeybees and leaf cutter ants, both the seminal fluid and spermathecal secretions are important in the long-term viability of sperm in the sperm storage organs. In many invertebrates in which females store sperm, they also mate multiply. The presence of multiple ejaculates in the storage organs sets the stage for interejaculate interactions as well as more complex, multiindividual interactions that include the multiply inseminated female and influence the fitness of all parties.

Physiological Changes in the Female

Male materials produce a variety of changes not only in the female reproductive tract but in somatic tissues as well, either by mechanical or chemical means or both. Innervation of the female reproductive tract can detect the presence of sperm and signal responses leading to ovulation and fertilization. In isopods, mating itself triggers remodeling of the sperm storage organs. In some *Drosophila* species, especially those of the mulleri complex of the repleta group, a large mass forms in the uterus

following mating that can last up to 10 h in intraspecific and indefinitely in interspecific matings. *Drosophila* species have proved highly informative regarding the action of specific seminal proteins in triggering oogenesis, ovulation, and delaying remating. While the well-known sex peptide is found in many *Drosophila* species, other proteins produce similar responses in mated females. Microarray and other experiments have revealed postmating changes in the expression of many functional categories of genes in females. Immune responses to mating may include upregulation of immune-response genes as in *Drosophila* as well as their downregulation as in *Tribolium*.

Females of several taxa take up seminal fluid substances, including proteins, sodium, and phosphorus, to incorporate them into their somatic tissues and ovaries. In *Drosophila melanogaster*, some seminal proteins appear to be associated with reduced female lifespan, while in the majority of *Drosophila* examined, no lifespan reduction seems to result from mating. In certain ant species, however, queens mating with either fertile or sterile males experience significantly longer lifespans than virgin queens.

Retrieval/Fertilization

Fertilization in invertebrates with stored sperm requires (1) the retrieval of sperm from storage, (2) ovulation, and (3) fusion of the sperm with the oocyte. In the majority of species studied, females release from their ovaries mature oocytes, which then are fertilized by stored sperm. What triggers the processes leading to fertilization? Signals may consist of environmental cues, internal cues, and/or their interaction. Environmental cues may be abiotic, such as light or temperature, or biotic, such as the availability of suitable oviposition sites. Internal factors can include the nutritional state of females, such as a recent blood meal in hematophagous species, and its influence on the production of mature oocytes. Female reproductive tracts frequently are innervated, by stretch or proprioceptors, signaling to a female that she is inseminated. Chemical features of seminal fluid, particularly male accessory gland proteins, also can initiate the steps toward fertilization via the female nervous system. Oviposition decisions, therefore, while they may appear to be female controlled, can be under the morphological or biochemical influences of both sexes long after copulation. In *Rhodnius*, male secretions cause contractions of the female oviduct.

An unidentified product of the paired sex accessory glands of the posterior reproductive tract of female houseflies allows penetration of the eggs by sperm, either by 'activation' of the sperm or alteration of the egg membrane. *Tribolium* females exert muscular control over sperm storage, although there is no evidence to date that females use this to differentiate among mates. The development of sperm storage organs allows females control over sperm storage and subsequent utilization.

Molecular mechanisms are implied to be the male's way of controlling the same processes, but in ways that benefit the male.

Evolutionary Implications of Reproductive Tract Interactions

A major challenge remains to understand the evolutionary significance of the observed intra- and interspecific variability in the processes that take place within inseminated females. Postmating control over reproduction frequently is discussed in different contexts such as cryptic female choice or sexually antagonistic co-evolution. These processes presumably occur inside the mated female and ultimately influence the genotypes of the fertilized eggs, embryos, or even older stages of the progeny that subsequently issue from the female. If we are to understand the evolution of these processes at their most fundamental levels, their underlying genetic architecture first must be identified. For example, if multiply mated females are able to selectively store and or use the sperm from one male over another, some heritable characteristic of the ejaculate must be not only variable among males, but it must also be detectable to females. Females also must have heritable mechanisms by which to detect male ejaculate variants and in order to differentially respond to them. Sperm uptake, storage, and retrieval each represent critical control points if female sperm choice is a reality. A firm grasp of the genetic underpinnings of each step will allow stringent and manipulative tests of the evolutionary processes hypothesized to explain the particular intersexual interaction.

Currently, we know only parts of the story. For example, while there is strong evidence for selection on genes encoding both male and female reproductive proteins, especially in *Drosophila* and there is evidence of assortative fertilization in several taxa, including *Drosophila*, no connection currently exists between these different bodies of evidence. What is the role of precopulatory factors, such as whether a mating was preferred by one or both partners, on postcopulatory processes? Are certain steps in the postcopulatory sequence of interactions more probable than others to influence fertilization success? In other words, is selection stronger on particular types of internal interactions than on others? Are certain components more variable than others either at a genetic or nongenetic, including the environmental level?

What is the relationship between fertilization success within a species and the evolution of postcopulatory-prezygotic reproductive isolation?

Much of our information on proximate mechanisms comes from laboratory studies of model organisms such as *Caenorhabditis*, *Drosophila*, *Tribolium*, or *Apis*. These model organisms exist in nature as well, and are not especially

difficult to study in the wild. Furthermore, with next-generation sequencing technologies and other molecular approaches becoming affordable, a larger range of ecologically interesting taxa can be added to the pioneering studies on model organisms. Perusal of **Table 1** shows that in the porifera, coelenterata, platyhelminthes, mollusca, and crustea both internal and external fertilization occurs. How many times within one of these groups has internal fertilization evolved and what are the ecological, behavioral, and internal correlates? Carefully selecting new taxa to study should generate hypothesis-driven investigations of the relationship between mating system, reproductive tract interactions, and fitness.

See also: Cryptic Female Choice; Forced or Aggressively Coerced Copulation; Social Selection, Sexual Selection, and Sexual Conflict; Sperm Competition.

Further Reading

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