

Quick guide

Sperm storage

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What is sperm storage? Sperm storage is most commonly defined as the maintenance of sperm inside a female's reproductive tract for an extended period of time. Because the fate of sperm post-copulation is important for understanding sexual selection, sperm storage is a particularly interesting characteristic that may have drastic impacts on the operation of post-copulatory sexual selection. Sperm storage aside, there is enormous variation among animals in the fate of sperm, including sperm that are viable years after mating and others that have very short periods over which sperm can fertilize eggs. For example, spawning organisms like sea urchins or salmon don't store sperm at all, but honeybee and termite queens can keep sperm for years. Thus, sperm storage has clear implications for the evolution of mating systems and sexual conflict.

A wide array of animals store sperm, including: earthworms; arthropods such as insects and spiders; birds, such as falcons, quail, finches and geese; reptiles, including turtles and tortoises, lizards, crocodiles and snakes; and a few mammals, for example bats, hares, horses and dogs (Figure 1A–D). In these taxa, sperm may be stored for a few hours to several years. In cases where sperm are only 'stored' for a few hours it may be more likely the trait observed is actually sperm longevity rather than female sperm storage. Sperm longevity is the period of time spermatozoa are able to survive independent of female maintenance in an environment with the correct conditions for sperm survival but differing from the natural site of sperm storage.

Sperm are frequently observed within a female's reproductive tract long after mating. This characteristic is often dubbed 'sperm storage', but for true female sperm storage the female must provide a reproductive tract environment conducive to sperm survival (with the requisite pH, immunological activity and so on).

Thus, sperm storage results from selection operating on both male (the ability of sperm to live a long time) and female (the ability to maintain sperm) traits. It is interesting that neither the simple definition of sperm storage nor the dichotomy of male versus female roles in sperm storage have received much attention. While both sperm storage and longevity are necessary for sperm maintenance in a female's reproductive tract, careful use of terminology is necessary for researchers interested in understanding the biology (particularly co-evolution) of either trait. This problem extends to how researchers determine if a species exhibits sperm storage.

The usual criteria for determining that a species stores sperm range from simply finding sperm within a female's reproductive tract long after she has mated to more thorough evaluations of both the female's physiology and the sperm's viability. Indeed, implied in the definition of sperm storage above, though not always tested, is that the sperm must be viable (able to successfully fertilize an egg) at the termination of sperm storage. Thus, there are varying degrees of certainty that different taxa actually store sperm. Studies aimed at understanding the evolutionary origins or consequences of sperm storage need to be certain that species are only said to store sperm after rigorous inquiry. We suggest sperm storage requires that the female plays a role in maintaining sperm, otherwise it would be a male process (sperm longevity) occurring independent of selection on the female.

For sperm storage to evolve, sperm must gain an ability to survive for long periods of time and remain viable, but the female must provide an environment conducive to sperm survival. Thus, sperm storage results from selection operating on both male and female traits. What is the use of sperm evolving long lives when survival in the environment of the female's reproductive tract is low? Likewise, females are unlikely to evolve reproductive tracts able to maintain sperm if the sperm are unable to live longer and thus correlated evolution is a must for sperm storage to evolve. As a result, both sperm storage and longevity exist

on a continuum ranging from a few hours to years.

Why would a female store sperm?

In many cases, sperm storage is an essential part of reproduction. Because sperm storage enables mating and fertilization to be asynchronous, it could be extremely advantageous to females, who could thus mate before it is time for ovulation and seize the opportunity for post-copulatory sexual selection. Indeed, sperm storage may have evolved because it provides an advantage to females of taxa with uncertain mating opportunities, as, for example, where males and females have large home ranges or migrate separately. Sperm storage may also be an integral part of the mating system of species with different male and female life histories (longer-lived females, for example). Some bees and spiders mate immediately after a female's imaginal molt, but the female can use the sperm of her often long-dead mate to fertilize her eggs years later. These females are likely to have an extremely high reproductive output because they can continually produce fertilized eggs without re-mating.

How do animals store sperm?

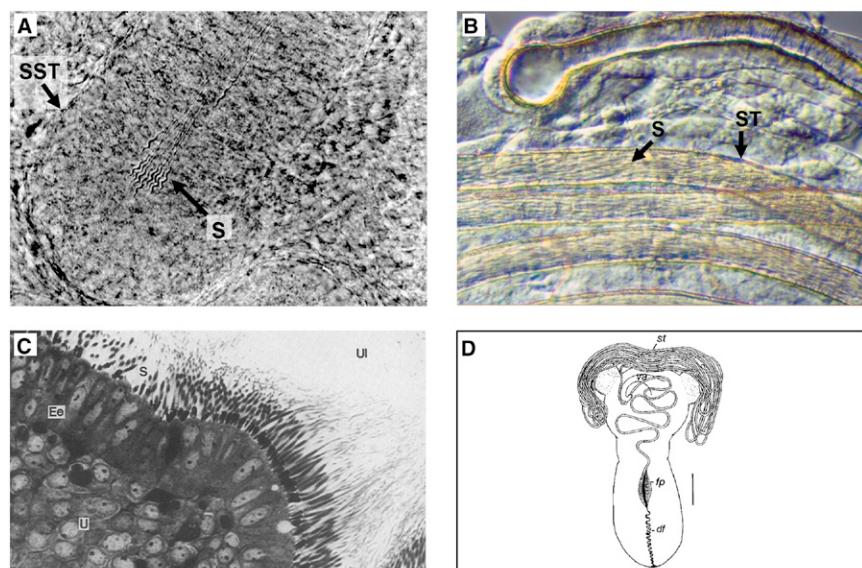
Females often have specialized structures for sperm storage (Figure 1D). Insects have organs, called spermathecae, that extend from the female reproductive tract and function in sperm storage. Similarly, amphibians store sperm in glands (spermathecae) located above the cloaca. Birds and reptiles store sperm in blind-ended grooves referred to as sperm storage tubules (Figure 1A). In addition to providing a location for sperm, the biochemical attributes, such as the pH, of the storage site must not damage sperm. Female honey bees actually produce proteins aimed at maintaining sperm during their long period of storage. Sperm storage may place other unexpected demands on females. For example, an interesting quandary is that a female's immune system must not destroy sperm while remaining sufficiently active to prevent reproductive tract infections and, in some cases, protecting stored sperm from bacterial or fungal attack.

Spermathecae, however, are not required to store sperm. Some female vertebrates store sperm within the confines of their reproductive tracts by co-opting pre-existing structures such as the lining of the uterus, cervix, oviducts and vagina. This ‘make-do’ approach to sperm storage has been observed primarily in mammals but also occurs in sharks and some bony fish. Interestingly, even though the structures for sperm storage in these taxa may not be complex, the spermatozoa themselves often exhibit very specific and organized arrangements. In many bats, including the little brown bat (*Myotis lucifugus*), sperm are aligned with their heads in close association with the epithelial lining of the female’s uterus and their tails in the lumen (Figure 1C).

Perhaps because vertebrates often lack special sperm storage structures, this phenomenon is referred to as both sperm storage and sperm longevity in the literature. One way to distinguish between these two concepts in species without sperm storage organs may be to evaluate the proximity of sperm relative to the site of fertilization and consistent location of ‘storage.’ The mere presence of sperm in the female reproductive tract after long periods of time is not enough to declare that females store sperm. Likewise, the production of young after long periods of seclusion overlooks alternative explanations, such as parthenogenesis or embryos that were in diapause (delayed implantation). Indeed, parthenogenesis has recently been noted in species previously thought to store sperm (Komodo dragons and some sharks).

Animals that experience periods of lower body temperatures may be better able to store sperm because of the resulting reduced metabolic rates of sperm. Indeed, many species that store sperm are poikilothermic, although some mammals that store sperm have high body temperatures. The most extreme exception to this correlation between body temperature and ability to store sperm is seen in many birds (Figure 1A) that store sperm, such as albatrosses, eagles and turkeys, which have famously high body temperatures (40°C).

I didn’t want to ask, but can humans store sperm? Humans can maintain viable sperm in the female’s



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Figure 1. Examples of sperm storage and sperm storage organs in several taxa. (A) Sperm (S) stored inside sperm storage tubules (SST) of a female Chaffinch (*Fringilla coelebs*). Reproduced with permission of T. Birkhead. (B) Sperm bundles (S) stored in water-strider (*Aquarius remigis*) spermathecal tubes (ST). Reproduced with permission from Campbell and Fairbairn (2001). (C) Sperm (S) storage in a little brown bat (*Myotis lucifugus*), showing the uterine wall (U), endometrial epithelia cells (Ee) and uterine lumen (Ul). Reproduced with permission from Racey et al. (1987) © Society for Reproduction and Fertility (1987). (D) The reproductive anatomy of sperm storage organs in water striders (*Aquarius remigis*) reproduced with permission from Campbell and Fairbairn (2001), © 2008 Canadian Science Publishing or its licensors. Illustrated are the spermathecal tube (st), veriform appendix (va), fecundation pump (fp) and the distal portion of the fecundation canal (df).

reproductive tract for about seven days. This is a fairly short period of sperm storage, and may be due more to the natural longevity of human sperm rather than the active storage by the female. For this to be considered short-term sperm storage, sperm must be unable to live equally long in a similar environment outside the female’s reproductive tract.

Are certain taxa more likely to store sperm? Female sperm storage has been observed in many taxa, as evidenced by the examples above, but is more common in invertebrates than in vertebrates. At least in vertebrates, sperm storage appears to have had several independent origins. Potential costs associated with sperm storage include metabolic and immunological costs, or those associated with sperm degradation. However, females who store sperm may forego the costs of repeated mating or fecundity costs associated with missed-mating opportunities. Thus, there is a likely adaptive value for females to store sperm as well as potential costs or physiological restraints that may result in the loss of this trait. The repeated

appearance of sperm storage suggests that the mechanisms of sperm storage (for example, the location of stores) may differ substantially between groups but also that the trait must be relatively easy to evolve.

Which species stores sperm the longest? In terms of absolute duration of sperm storage, the domesticated dog can store sperm for 11 days, and some turtles and tortoises can maintain sperm for an impressive four years. Perhaps the most remarkable duration of sperm storage is seen in the Javan wart snake, *Acrochordus javanicus*, which can store sperm for as long as seven years. However, considering that some bees can store sperm for nearly their entire life (several years), and that some ants can store sperm from a single mating for their entire lives, which can last over a decade, insects provide much more impressive examples of sperm storage.

What about the guys – do they have a say in sperm storage? Actually, males have a lot to do with the female’s ability to store

their sperm. As mentioned above, if sperm were not adapted to survive (sperm longevity) inside the female's reproductive tract, the female would be limited in her ability to maintain sperm. Males may face a trade-off between sperm competitive ability (fast-swimming sperm) and ability to be stored (long-lived and slow-swimming sperm). Sperm pre-adaptations for storage include a decreased sensitivity to sperm activation specifically, acrosomal reaction desensitization, and the presence of antioxidative enzymes. Also commonly observed and apparently important for storage is a decrease in sperm motility. In addition to changes to the spermatozoa, males may produce chemicals that increase sperm viability during their stay in the female's reproductive tract. The notion that mate choice only occurs prior to copulation has been rejected for well over 40 years. However, the complexity of post-copulatory sexual selection and the potential for sexual conflict may be even greater than currently appreciated if the time-frames over which events occur are lengthened by weeks, months or even years through the storage of sperm. Sperm storage remains an exciting area for future research, including the refinement of associated terminology as well as studies relating to co-evolution.

Where can I learn more?

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Genetic rediscovery of an 'extinct' Galápagos giant tortoise species

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Genes from recently extinct species can live on in the genomes of extant individuals of mixed ancestry. Recently, genetic signatures of the giant Galápagos tortoise once endemic to Floreana Island (*Chelonoidis elephantopus*) were detected within eleven hybrid individuals of otherwise pure *Chelonoidis becki* on Volcano Wolf, Isabela Island [1]. Movement of tortoises between islands by pirate and whaling ships was not uncommon during the 1800s [2], representing a likely mechanism by which individuals from Floreana were translocated to northern Isabela, despite being presumed extinct soon after Charles Darwin's historic voyage to the Galápagos Islands in 1835. These eleven hybrid individuals with *C. elephantopus* ancestry were thought to be the last genetic vestiges of a unique evolutionary lineage in the wild. Here, we report that reproductively mature purebred tortoises of the recently 'extinct' *C. elephantopus* from Floreana Island are very likely still alive today, as identified and tracked through the genetic footprints left in the genomes of very recent hybrid offspring on Volcano Wolf. If found, these purebred *C. elephantopus* individuals could constitute core founders of a captive breeding program directed towards resurrecting this species.

Alerted by our previous discovery of hybrid individuals [1,3], we returned to Volcano Wolf, sampled its tortoise population intensively (1669 individuals, approximately 20% of

the estimated current population), and screened them for genetic variation using fast-evolving nuclear DNA markers (12 microsatellite loci). Each tortoise was then assigned to one or more parental gene pools by comparison to a genetic database of all extant and extinct Galápagos tortoise species. Genetic marker-based assignments can be challenging, however, in systems with a history of hybridization. In such cases, hybridization generates offspring with genomes that include parts of both parental gene pools, and subsequent crosses between hybrids and purebreds, or between two hybrids, lead to mosaic-like genomes. Indeed, there is an ever-increasing role of chance in shaping the genetic make-up of second-generation hybrids. To account for this complexity, we used computer simulations to determine the range of possibilities for the genetic make-up of hybrid tortoises resulting from an array of different hybridization scenarios that may be occurring on Volcano Wolf. Having quantified this inherent variability, we were able to identify hybrid tortoises as well as determine the most likely parental cross that generated them (Supplemental information).

We determined that the genotypes of 84 Volcano Wolf tortoises result from hybridization events that involved a purebred *C. elephantopus* as one of the immediate parents (Figure 1). Moreover, these events were very recent – 30 of the 84 tortoises are less than 15 years old (Supplemental information). Given the documented lifespan of Galápagos tortoises of more than 100 years, there is a good chance that purebred *C. elephantopus* tortoises are still alive. The minimum number of equally contributing *C. elephantopus* founders needed to produce the same genetic diversity observed in the 84 hybrids was 38, as estimated via founder genome equivalents (Supplemental information). Theoretically, 20 or more founder genome equivalents are the approximate genetic base necessary for a viable *ex situ* population [4]. Consequently, our findings offer hope for an attempt at species recovery via captive breeding.

Of the 84 hybrids identified from patterns of nuclear genetic