Size did matter -- evidence of giant sperm found in microfossils

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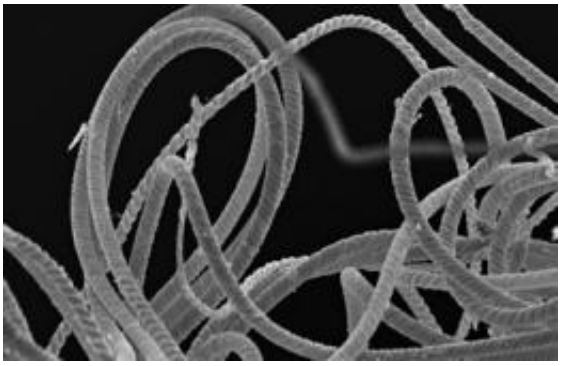

Scanning electron micrograph of a bundle of ostracod giant sperm cells. Some can be 10 times the length of the ostracod body. The synchrotron X-ray holotomography method can produce images of internal organs preserved in fossils. © Renate Matzke-Karasz

The mystery of giant sperm present in some living animal groups today has now taken on a new dimension -- in one group of micro-crustaceans new evidence shows that it is a feature at least 100 million years old.

In the competition for a partner, males typically have to vie with each other - be it with a colorful plumage, a large set of antlers or a seductive courtship dance. The females of some species, however, copulate with several males, so that rivals even after mating are still not defeated. So their sperm become rivals. Because greater size can increase the chance of fertilization, in some species truly giant sperm cells have evolved -

some grow to be even larger than the male that produced them.

Now, an international group of researchers led by Dr. Renate Matzke-Karasz, Ludwig-Maximilians-Universität (LMU) in Munich, has indirectly detected signs of giant sperm in fossilized ostracods. Using synchrotron X-ray holotomography, a highly complex imaging technique developed at the European Synchrotron Radiation Facility, the researchers were able to look non-invasively deep inside these tiny crustaceans, which measure only one millimeter in length. "In these microfossils, we detected organs that are required for transferring giant spermatozoa," reports Matzke-Karasz. "Since recent ostracods still produce giant sperm, and maneuver them with the same organs as 100 million years ago, it's safe to say that this distinctive feature evolved only once in this group. It seems to be an evolutionary successful reproduction strategy, even though it comes at an exceedingly high price for both genders." (*Science* Early Online Edition, 18 June 2009)

If a female allows more than one male to mate with her, then the males' rivalry has to continue after mating. This situation seems to result in enough pressure to uproot even the most valid theory of sexual selection: According to that, males who produce a large number of tiny spermatozoa quickly and cheaply better their chances for reproduction, while females invest in only a few, yet larger ova. But if the sperm have to compete inside the female's body, quality sometimes seems to trump quantity. In these cases chances of fertilizing an ovum can increase with the size of the sperm cell. This applies as much to the sperm of a single individual as it does to rival sperm of different males. So much so, that the animals invest a lot of energy in producing and carrying such enormous sperm.

This has led to some true giants evolving along the way. A human sperm would have to be 40 meters long in order to measure up against Drosophila bifurca, for example: the males of this fruit-fly are only a

few millimeters in size, but produce giant sperm around six centimeters long. Also other insects, as well as some primates, birds and worms are known for the production of giant sperm. Another example is one group of ostracods, whose sperm are up to ten times as big as the animals themselves. These aquatic crustaceans typically grow to only a few millimeters, and are - much like mussels - surrounded by a bivalve-like calcareous shell.

This protective armor fossilizes particularly well, so ostracods are some of the most common fossils found, some dating back to 450 million years ago. "They are an important group in that their remains store information about the environment they lived in," Matzke-Karasz says. "The fossilized shells of ostracods are therefore a kind of archive of earth's history, storing information on climate, ecology and geology thousands, even millions of years ago." Only in rare cases, however, remains of the soft parts of the body and its appendages are preserved along with the calcareous valves. Because these fossils are particularly interesting to evolutionary biologists, the group working with Matzke-Karasz investigated fossils of the Cretaceous Harbinia micropapillosa that still had intact remains of the soft body. Fortunately, these exceptionally rare fossils belong to the same group of ostracods that produces giant sperm today, providing an excellent opportunity to look for fossil evidence of giant sperm.

The highly complex, high-tech investigation was performed at the "European Synchrotron Radiation Facility (ESRF, Grenoble, France)", which also financed the project: "Holotomography is a non-destructive imaging technique like computer tomography, where powerful and coherent synchrotron X-rays are used," explains Dr. Paul Tafforeau of ESRF. "With this method, a three-dimensional image of the inner structures even of microscopically small objects can be reproduced without doing any damage, with contrast and precision levels not reachable with any other techniques." Holotomography has been only

very recently applied to imaging of fossils, but the recent results demonstrate that such technique will surely lead to many important discoveries on fossils. "We obtained an excellent image of the reproductive apparatus of the fossil ostracods and were in for a real surprise," reports Dr. Giles Miller of the Natural History Museum in London. "Our results show that these 100 million year old Cretaceous ostracods were already reproducing with giant sperm."

Recent relatives of these crustaceans have a complex reproductive apparatus that makes up about a third of their bodies in volume. In both sexes, the reproductive organs exist as two separately functioning units, one on each side of the body. In the males, these include two large sperm pumps called Zenker organs. Matching these, the female ostracods possess two long passages leading to two vaginal openings. These characteristic structures are a perfect adaptation to the transport of giant sperm. The X-ray investigation of the fossilized ostracods revealed pairs of hollow tubes in the males that correspond to Zenker organs.

"In the females, on the other hand, we found two elongated hollow cavities in the abdomen, which we also see in recent species," says Radka Symonova of Charles University in Prague. "These cavities are sperm storage receptacles. They only occur in ostracods whose females retain giant spermatozoa within their bodies until the moment of oviposition, when each ovum is fertilized by one sperm. From recent species we know that the seminal vesicles only obtain their typical shape when they are filled with giant sperm." Accordingly, the fossilized females must have mated shortly before their entombment in the sediment. "Our holotomographies actually revealed a fossil insemination," Symonova resumes.

"So, reproduction with giant sperm had already developed around 100 million years ago in this group of ostracods," Dr. Robin James Smith of Lake Biwa Museum in Shiga, Japan ponders. "Until now, it had been

unknown whether giant sperm ostracod sperm arose multiple times over the course of evolution, like those of Drosophila, or whether they have been a persistent feature in certain groups for millions of years," Matzke-Karasz continues. "This question can now be answered once and for all: giant sperm have been produced in at least some species over long periods of time, even though they come at an extremely high price for both, males and females. The next stage of our research is to try to understand why and how it has persisted for so long." The project led by the LMU Munich paleontologist was funded by ESRF (Grenoble), the European Union in the scope of the Marie Curie RT Network "SEXASEX" and the Lake Biwa Museum.

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