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Getting Under the Skin: Autonomous Implantation of Squid Spermatophores

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Squid spermatophores are complex structures that store sperm and, when transferred to the female, evert into spermatangia. Some deep-sea squid implant spermatangia into unmodified tissue of the female. Several hypotheses have been proposed to explain this peculiar implantation process. Here, through on board observation of freshly caught deep-sea squid (Moroteuthis ingens), *we show for the first time that spermatophores have an autonomous mechanism that enables them to migrate into body tissue.*

Cephalopod spermatophores are complex secreted structures that hold sperm masses and that consist, in part, of an ejaculatory apparatus for releasing the sperm mass and a cement body presumably for attaching the sperm mass to the female (1). Spermatophores are either transferred to the female by the male's hectocotylus, a specialized arm, or by a long penis, which is present in species that lack hectocotyli (2). After being discharged from the spermatophore in the spermatophoric reaction, the sperm mass is encased in a thin covering, with the cement body at one end. This discharged structure, the spermatangium, may attach to the skin of the female or be deeply embedded in her skin or muscle.

Many aspects of reproduction remain unknown for deep-sea squids owing to the inaccessibility of their habitat and the difficulty in obtaining large adult animals. In an extensive review on reproduction in oceanic cephalopods, Nesis (2) stated that "one of the most intriguing puzzles of spermatophore transfer is the rather common case of spermatophore embedding in the outer surface of the mantle—in special sites or simply in almost every place." Although common, the process of deep spermatophore implantation is highly controversial. It is not known what structure or process is responsible for spermatangia penetrating into body tissue. Because squid that implant spermatangia have a long penis and no hectocotylus, embedding has been suggested to involve the penis in a process by which the spermatophores are perhaps hydraulically forced into the tissue (3, 4). Since implanted spermatangia are sometimes associated with wounds or cuts, other hypotheses consider the action of the beaks or the hooks present on the arms and tentacles of many oceanic squid to be responsible for the implantation of spermatangia. Finally, an intrinsic chemical mechanism in the spermatophore's cement body has been proposed to be able to dissolve the female's tissue (5). All the above hypotheses are based solely on morphological observations of preserved male and female squid. This study presents the first observations on the process of spermatophore implantation in a deep-sea squid. In addition to showing that the spermatophores of the deep-sea squid *Moroteuthis ingens,* Smith 1881, are able to autonomously migrate through body tissue, we have determined the role of the penis in the transfer of spermatophores.

On board the research vessel *Dorada* of the Falkland Islands Government Fisheries Department, spermatophores were taken from the penis of a moribund specimen of the deep-water squid *M. ingens,* a species that embeds spermatangia (4). The spermatophores were positioned on the outer mantle of a dead male (Fig. 1a), and the spermatophoric reaction was induced by gently pushing on the oral end of the spermatophore, which contains the ejaculatory apparatus.

After pressure was exerted on the ejaculatory apparatus, the spermatophores started ejaculating (Fig. 1b); after a few minutes, spermatangia had autonomously embedded themselves into the tissue (Fig. 1c, d).

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Figure 1. (A) Placement of spermatophores on a dead male specimen of *Moroteuthis ingens* (ML ~300 mm) and initiation of the spermatophoric reaction by pressing on the ejaculatory apparatus with a forceps. (B) Same specimen, but submerged in seawater, showing the ejaculating spermatophores. (C) Exterior view of implanted spermatangia in tissue of a female, showing the site of penetration and part of the amber ejaculatory apparatus. (D) Interior view of same spermatangia, showing the sperm mass and the amber ejaculatory apparatus.

Each spermatangium entered the tissue through a small hole (diameter <1 mm) (Fig. 1c) and had an amber cylinder attached (a remnant of the ejaculatory apparatus), which in turn remained attached to the external casing. The everting ejaculatory apparatus has the first contact with the tissue and may facilitate adhesion or the first penetration into the tissue, perhaps by mechanical means. After eversion, the cement body is exposed and may dissolve (perhaps with the aid of proteolytic enzymes) the host tissue to allow further penetration of the spermatangium. The embedded spermatangia of our experiment resembled those normally found in female squids.

The penis, in addition to transferring spermatophores, initiates the spermatophoric reaction. This was seen in one moribund male in which the penis was expelling dozens of spermatophores, one by one. The aboral end of each spermatophore was expelled first. Then, with the oral end still inside the penis, the spermatophoric reaction began, triggered by mechanical stimulation by the penis. The mechanical stimulation probably involved the pulling of the thread, which is the oral extension of the membrane surrounding the spermatophore.

This study presents the first observations on the spermatophoric reaction of deep-sea squid spermatophores. We have shown that, after initiating the spermatophoric reaction, spermatophores of *M. ingens* are able to implant themselves wherever they are deposited.

Our observations also clarified the role of the penis. The penis initiates the spermatophoric reaction by pressing the oral part of the spermatophore or by pulling its thread. Another role of the penis is probably the precise positioning of the ejaculating spermatophore on the female before releasing it. After release, the everting spermatophore would implant itself at the place of deposition.

The fact that the spermatophore-embedding process occurs autonomously illustrates the sophisticated mechanisms deep-sea squid have evolved to ensure the successful reception of sperm by the female. Autonomous implantation of spermatophores could be an adaptation for species that have no seminal receptacles. Intradermal implantation of spermatangia would secure the sperm until the female reaches full maturity and is ready to fertilize her eggs.

Finally, this finding will permit researchers to investigate the implantation mechanism of spermatangia of deep-sea squid in detail because they no longer need to rely on the behavior of live squid but can examine spermatophores from fresh, dead squid.

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