Spermatophore Formation and Sperm Transfer in Ornithodoros Ticks

Abstract. *In seven species of* Ornithodoros *and two species of* Argas *ticks the spermatophore is formed outside the male body in three consecutive stages: (i) the outer bulb, the ecto-spermatophore, is formed; (ii) it is filled with sperm; and (iii) the inner capsules, the endo-spermatophore, are extruded into the ecto-spermatophore. The morphologies of the ecto-spermatophore and the endo-spermatophore are related to their biological functions. Eversion and evagination of the endo-spermatophore after deposition of the spermatophore on the female genital opening, as well as transfer of sperm from ecto-spermatophore to endo-spermatophore, are described.*

Copulation in ticks has been studied by a few workers. Nuttall and Merriman (*1*) described in general terms copulation in *Ornithodoros moubata*. Robinson (*2*) studied the morphology of the spermatophore in this species and described its components, namely the bulb, which remains outside the female body, and the capsules containing the sperm, which are found in the uterus after copulation. Wagner-Jevseenko (*3*) speculated on the formation of the spermatophore in *O. moubata*; and the histochemistry of the spermatophore in *Argas persicus* has been studied, and its transfer from the male to the female has been briefly described (*4*). But the mechanisms of the formation of the various components of the spermatophore and of the transfer of sperm from the spermatophore to the female uterus have hitherto not been closely observed and described in detail.

It has been claimed that the spermatophore is built inside the male body and that it is fully formed when extruded by the male (*1, 3, 4*). However, Robinson noted that the spermatophore is formed outside the male body. He observed that at first the bulb only is pushed out from the male sexual orifice, but he thought that the capsules were squeezed into the bulb after they had been filled with spermiophores in the ductus ejaculatorius. Thus, he assumed that the sperm remains enclosed in the capsules throughout their passage from the male genital tract to the uterus of the female. This opinion was shared by Wagner-Jevseenko. Although our main observations were made on *O. savignyi* and *O. tholozani*, while those of Robinson and Wagner-Jevseenko were of *O. moubata*, some observations which we made on *O. moubata, O. erraticus, O. delanoei, O. coriaceus, O. canestrinii, Argas persicus*, and *A. reflexus* leave no doubt that, in the main, the processes involved in all species of the family *Argasidae* are identical, although some minor differences do exist.

We were unable to confirm Tatchell's (*4*) claim that the male cuts the tip of the protuberance of the spermatophore with its cheliceral digits to enable the contents of the spermatophore to flow out into the female genital tract.

Definition of the appropriate terms for the various parts of the spermatophore appears desirable because the term "spermatophore" has been applied by acarologists as a very natural extension of its traditional use in relation to insects. The term spermatophore does designate the container used for transport of sperm. However, to our knowledge, in insects the function is performed by one container, while in ticks two containers are used. One, which we propose to call the ecto-spermatophore (Robinson's bulb), serves as a container for the transfer of sperm from the male genital opening to the female genital opening, while the other one, the endo-spermatophore (Robinson's capsules), is used to transport sperm from the ecto-spermatophore attached to the female genital opening to the uterus and to store it there.

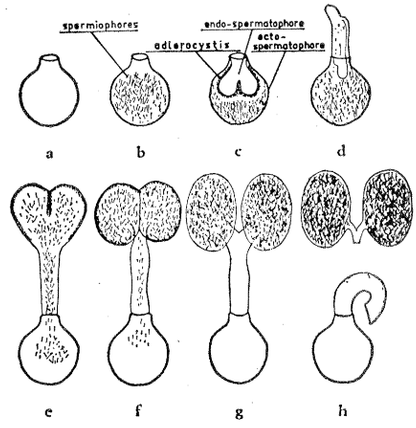

Fig. 1. Consecutive stages in the formation of a spermatophore and evagination of the endo-spermatophore in an undisturbed copulation of *O. savignyi* (schematic presentation). (a) Stage 1, still empty ecto-spermatophore; (b) stage 2, ecto-spermatophore filled with sperm; (c) stage 3, a fully formed spermatophore containing sperm, the endo-spermatophore, and *Adlerocystis*; (d) beginning of evagination of endo-spermatophore; (e) a later stage in evagination; the bilobated endo-spermatophore has completely everted; (f) the capsules almost entirely full of spermiophores; (g) the two capsules of the endo-spermatophore now separated; (h) an empty ecto-spermatophore as it is found on the female genital aperture after copulation; and (i) the two connected capsules of the endo-spermatophore, as they are found in the uterus immediately after copulation.

The ecto-spermatophore is the exterior part of the fully formed spermatophore; it is a bulb-shaped capsule with a short neck, and it is closely packed with spermiophores. The endo-spermatophore is a bilobate bottle-like, membraneous structure inserted in the neck of the ecto-spermatophore. The endo-spermatophore also has a neck, and the rims of both necks are tightly sealed together. The sperm symbiotes *Adlerocystis* sp. Feldman-Muhsam and Havivi (*5*) densely cover the endo-spermatophore.

The spermatophore is formed outside the male body in at least three consecutive steps, its various components being secreted by the sex glands and the accessory lobes of the genital system.

1) Two to three minutes after the male introduces his capitulum into the female genital opening, the ecto-spermatophore is extruded from the male genital opening. The ecto-spermatophore is retained on the male genital aperture by its neck. At this stage it is white, transparent, and contains no sperm (Fig. 1a).

2) Immediately after the extrusion of the ecto-spermatophore, the male ejaculates into it sperm, spermal fluid, and large proteinic granules. Consequently the ecto-spermatophore becomes opaque. This stage lasts for 3 to 13 seconds (Fig. 1b).

3) The male ejects the *Adlerocystis* into the proximal part of the ecto-spermatophore, which is already filled with semen, and at the same time, or a fraction of a second later, extrudes the endo-spermatophore into it. At this stage, the endo-spermatophore closes the ecto-spermatophore like a stopper, and its lobes are very small. The adlerocysts surround the endo-spermatophore and do not intermingle with the sperm (Fig. 1c).

With his chelicerae, the male then catches the fully formed spermatophore at its neck and deposits it on the female genital aperture. One to two minutes later the contents of the ecto-spermatophore are pushed out into the female uterus. The ecto-sper-

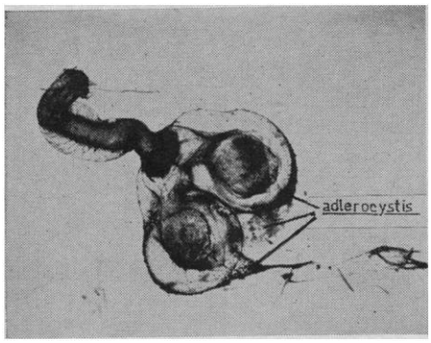

Fig. 2. The endo-spermatophore of *O. savignyi* of an interrupted copulation (× 57). The endo-spermatophore was extruded by the male after removal of the ecto-spermatophore. Preparation vitally stained with Janus green. The *Adlerocystis* were spread in the preparation.

matophore collapses, hardens, and becomes yellowish brown. It may remain attached to the female genital opening for a few hours or days.

The various stages of the formation of the spermatophore were closely followed under a stereoscopic microscope. I ascertained their temporal sequence by interrupting copulation at various stages. Although the whole process of extruding the ecto-spermatophore, filling it with sperm, and extruding the endo-spermatophore takes only 20 to 30 seconds, by close observation and quick manipulation it was possible to interrupt spermatophore formation when the ecto-spermatophore was either empty or filled only with sperm. In either case, once the male had started to copulate, it continued the chain of instinctive actions, even if they were in vain.

If, at the right moment, the still-empty ecto-spermatophore is removed from the male genital aperture, the male will eject sperm, large proteinic granules, and adlerocysts, and will extrude the endo-spermatophore on the surface of its own body (Fig. 2).

If the ecto-spermatophore is removed when it is already full of sperm, the male will eject adlerocysts and extrude the endo-spermatophore, the adlerocysts adhering closely to the exterior surface of the latter. When the endo-spermatophore is extruded from the male genital aperture to the outside (after the ecto-spermatophore has been removed), its shape is also that of a bilobate bottle, but the two lobes are considerably larger than they are inside the ecto-spermatophore (Fig. 2), although they are not as large as when they are filled with sperm in the uterus. If the endo-spermatophore is dropped into saline on a slide, one can see the huge number of adlerocysts which cover the lobes of the endo-spermatophore.

The processes that normally occur in the female genital tract after the deposition of the spermatophore on the female genital aperture can be studied in vitro. If a completely formed spermatophore is removed from the male before it is deposited on the female and if it is put into saline or left on a dry slide, within 1 to 2 minutes the endo-spermatophore evaginates from the ecto-spermatophore. The evagination occurs whether the spermatophore was taken off the male genital opening or off the male chelicerae. The evagination starts without any intervention. At first, a stem pops out (Fig. 1d), and immediately afterwards the two lobes of the endo-spermatophore are everted and evaginated (Fig. 1e). At this stage arise most of the minor differences between *O. savignyi* and *O. tholozani*, to which this description refers, and some other species of Argasid ticks. The everted neck of the endo-spermatophore extends and elongates. During evagination, the lobes turn inside out, and adlerocysts and sperm which were in the ecto-spermatophore, but outside the endo-spermatophore, stream quickly into the two everted lobes of the endo-spermatophore (Fig. 1f) until no sperm remains in the ecto-spermatophore. Only after the lobes have entirely been filled up with sperm do they separate slowly to form two distinct capsules, but both are still connected to the ecto-spermatophore by the neck (Fig. 1g). The sperm and adlerocysts, which were entirely separated in the ecto-spermatophore, are mixed in these capsules. Thus, during the whole process of transfer of sperm from the male to the female, the semen remains in a closed system.

In the normal course of copulation (but not in vitro), the two capsules are soon disconnected from the ecto-spermatophore. The separation occurs at the neck, near the capsules. The longer part of the neck, which originally belonged to the endo-spermatophore, remains attached to the ecto-spermatophore and can be seen when it falls off the female (Fig. 1h). The shorter part of the neck remains attached to the two capsules and forms a thin tube connecting them (Fig. 1i). This is the form in which the endo-spermatophore reaches the uterus. There the two capsules remain attached to each other for various lengths of time depending on external conditions, such as subsequent copulation and feeding by the female. The capsules then separate, and every two capsules found in the uterus represent one copulation. The sperm remains viable in the capsules for many months (*6*).

B. FELDMAN-MUHSAM
Department of Parasitology, Hebrew University, Jerusalem, Israel

References and Notes

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7. Supported by NIH grant R.G. 4531. I thank Miss M. Hirsh for technical help.

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Submaxillary Gland of Mouse: Properties of a Purified Protein Affecting Muscle Tissue in vitro

Abstract. *A protein from the salivary gland of mice has been highly purified. It affects embryonic muscle tissue in vitro and has both esterase and peptidase activities. Addition of the pure protein to tissue culture in synthetic medium causes dissociation of muscle fibers in individual myoblasts with loss of myosin. This biological activity, as well as the esterase activity, is inhibited by low concentrations of phenylmethanesulfonyl fluoride; this suggests that the effect on the tissue is a consequence of the protein's enzymatic activities.*

The submaxillary gland of the male mouse contains a number of proteins that have specific growth-promoting properties. One of these, the nerve-growth factor, stimulates the growth and differentiation of embryonic sensory nerve cells and embryonic and mature sympathetic nerve cells (*1*). Subsequently, another protein, the epidermal-growth factor which promotes the growth of epidermal cells, was purified from the submaxillary gland (*2*). More recently, a fraction from extracts of the salivary gland was reported to stimulate in vitro the growth of mesenchymal cells and cause loss of differentiative marks in muscle and cartilage (*3*). The material responsible for the latter effects was also considered to be a pro-