Comparative studies of gametogenesis and sperm-egg interactions in the ticks, Dermacentor variabilis (Say) and Dermacentor andersoni (Stiles) (Ixodoidea: Acari)

Suleiman Ahmad Suleiman

Iowa State University
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Comparative studies of gametogenesis and sperm-egg interactions in the ticks, *Dermacentor variabilis* (Say) and *Dermacentor andersoni* (Stiles) (Ixodoidea: Acari)

by

Suleiman Ahmad Suleiman

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INTRODUCTION

Aristotle in the fourth century B.C. described ticks as "disgusting parasitic animals". These animals have held particular attention throughout recent history since they are associated with many disorders (dermatosis, envenomization, tick paralysis, otoacariasis, and exsanguination) of man and animals which can lead to secondary anaemia and even death. With the advent of modern science, ticks are known to be infected by various microorganisms and have a direct or indirect role in transmitting these organisms from wild or domestic animals to man. For example, they convey piroplasmas and parasitic worms, viruses and bacteria, anaplasmas, spirocheates and rickettsias. Particularly, their ability of transovarial and transstadial transmission of pathogens has assumed serious economic significance (Hoogstraal, 1966, 1967). In respect to their role in transmitted pathogens, ticks are uniquely suitable because of their dependence in all feeding stages on a blood meal for growth or reproduction. Their feeding habit affords many opportunities for pathogen acquisition and transmission.

Although ticks are generally classified in the class Arachnida and are included with mites in the order Acari, they are considered by some authors to form a separate suborder, Ixodides; and by others to form with a number of mite
families the suborder Metastigmata (Bequaert, 1946; Hoogstraal, 1956). The ticks per se constitute the superfamily Ixodoidea which is divided into two major families: Ixodidae, the hard ticks; and Argasidae, the soft ticks. The family Ixodidae consists of several medically important genera including *Dermacentor*, two species of which are examined in the present study. *Dermacentor* (D.) *variabilis*, the American dog tick, is the principal vector of tularemia and bovine anaplasmosis. *Dermacentor andersoni* (venostus), the Rocky Mountain wood tick, is one of the most infamous transmittors of diseases of man in the United States. This species is a vector of Rocky Mountain spotted fever, tularemia, Colorado tick fever and Q fever in addition to tick paralysis in both man and animals (Diamant and Strickland, 1965).

A fundamental knowledge of reproductive biology is a basic requirement to recognize and understand the differences in vector potential and epidemiological importance in ticks. In the present study, the male and female reproductive systems of *D. variabilis* and *D. andersoni* have been studied to determine the anatomy of the genital tract and the changes in this system during attachment and feeding on the host. In considering this approach, both species were examined for the following events: copulation and insemination, gametogenesis, sperm maturation, and sperm-egg inter-
actions. Since previous studies have demonstrated the presence of a unique motile system which differs greatly from the typical spermatozoon, special attention in the present study was given to this function.

The experimental procedures and results are preceded by a historical review of comparative male and female reproductive systems and gametogenesis. In addition and in order to relate ticks to other arachnid groups, a review is included concerning comparative sperm morphology, sperm transfer, and sperm storage of various arachnids.

Comparative Morphology of the Male Tick Reproductive System

Reports dealing with the anatomy and histology of reproductive systems in ticks date back over 100 years (Heller, 1858; Pagenstcher, 1861). The male reproductive system has been described for many species of argasids (Goroschenko, 1961; Roshdy, 1961, 1962, 1963, 1966; Khalil, 1969) and ixodids (Douglas, 1943; Khalil, 1970; Oliver and Brinton, 1972). From comparisons between male genital tracts of various species of both families, the male reproductive system, in general, consists of paired and elongated testes, vasa deferentia which join to form a seminal vesicle which is connected to the genital opening by an ejaculatory duct, and relatively large accessory
glands associated with the seminal vesicle.

**Testes**

In general, the testes of argasid and ixodid ticks are separate and paired (cf. Arthur, 1962), however, noticeable exceptions exist in ticks of both families. For example the testes of some argasid ticks are either connected at posterior ends by a slender extension (Roshdy, 1961, 1962; Khalil, 1969) or are connected by a wide region forming a continuous single U-shaped structure (Roshdy, 1963). In a few ixodid ticks, the paired testes either have a narrow appendix-like projection in close proximity (Khalil, 1970) or have a posterior connection by means of a slender extension (Oliver and Brinton, 1972). With regard to anatomical position, the testes of argasid and ixodid ticks are laterally placed in the body and extend from the level of the brain to approximately the level of the posterior margin of coxa IV (cf. Arthur, 1962).

According to histological studies by Khalil (1970) and Oliver and Brinton (1972), the testes consist of several groups of germinal cells arranged in cysts. The testicular wall is a single layer of flattened epithelial cells overlying a thin layer of connective tissue. In cross section, the mentioned cysts are arranged radially around a small lumen which extends the length of the testes and is continuous with the lumen of the vas deferens.
During attachment of the tick to the host, the testes enlarge considerably, the sperm cell maturation is initiated, and new germinal cysts are formed (cf. Arthur, 1962). In regards to maturation, Oliver and Brinton (1972) reported that all developing sperm cells in each cyst are usually in the same stage of development. They also demonstrated that the posterior region of the testes contained more advanced stages than the anterior region. Accordingly from these observations, the testes were divided into six areas: the proximal region, connected to the vas deferens, was designated area 1 and each one-sixth of the testes proceeding posteriorly was numbered successively.

**Vasa deferentia**

In argasid and ixodid male ticks, each vas deferens arises from the anterior end of the testis and extends anteriolaterally towards the seminal vesicle (cf. Arthur, 1962). Although the length and diameter of the vas deferens can be quite variable, according to Roshdy (1961, 1962, 1963) the vas deferens of argasid ticks is much longer and wider than that of ixodids (Khalil, 1970; Oliver and Brinton, 1972). In both families, the vasa deferentia are usually extensively coiled before joining to form a seminal vesicle near the anterior end of the accessory glands (Roshdy, 1963, 1966).
**Vesicula seminalis and ejaculatory duct**

The vesicula seminalis or seminal vesicle in males of argasid or ixodid ticks is a small triangular sac formed by the union of the vasa deferentia. The seminal vesicle is located ventrally to the anterior end of the accessory glands and opens into an ejaculatory duct leading to the genital aperture. The ejaculatory duct is lined with a thin layer of cuticle which is a continuation of the external cuticle. One exception to the above reported by Khalil (1970) is the occurrence of a genital atrium in *Hyalomma anatolicum excavatum* into which the seminal vesicle opens instead of the ejaculatory duct.

**Accessory glands**

The accessory glands in males of all ticks are relatively large and comprise approximately one-third of the genital system. These glands consist of several lobes which can be classified as spongy or granular (Robinson and Davidson, 1914; Roshdy, 1961, 1962, 1963; Khalil, 1969, 1970). Although the number, size and arrangement of the lobes are variable, in general, the accessory glands consist of two pairs of spongy lobes (the short anterior lobes and the long posterior lobes) which are attached to the lateral sides of the ejaculatory duct and three granular lobes (the anteriodorsal, medial, and ventral lobe). The granular lobes differ from the spongy ones by having more
secretory products in their elongated cells (Douglas, 1943). In reference to the granular medial lobe, various numbers of lateral lobules are usually present. Although the chemical constituents of the secretions from the accessory glands were only examined in few species (Tatchell, 1962; Galun and Warburg, 1967), the general consensus is that these secretions are used to form the spermatophores in all ticks (Douglas, 1943; Feldman-Muhsam, 1967a; Feldman-Muhsam and Bourt, 1971).

Comparative Morphology of the Female
Tick Reproductive System

As with the male reproductive system, a thorough understanding of the female reproductive system is necessary in the study of the reproductive biology of ticks. In general, the female reproductive system consists of a single U-shaped tubular ovary, two variable length oviducts extending from each end of the ovary and which are usually coiled, a seminal receptacle or uterus, and finally a vagina which opens to the genital aperture (cf. Arthur, 1962).

Ovary

With the exception of Argas vespertiliones (Roshdy, 1961) which has two ovaries, females of all examined aragasid and ixodid ticks have a single U-shaped tubular ovary extending posteriolaterally on each side of the tick body with the middle of the ovary in front of the rectal
sac (cf. Arthur, 1962). The ovarian wall consists of two basic layers: an inner layer of epithelial cells which enclose the ovarian lumen and an outer layer which consists of interstitial cells and oogonia or oocytes (Khalil, 1969, 1970; Brinton and Oliver, 1971a). The outer layer is covered with a fine hyaline nonchitinous membrane, the tunica propria, which extends around the developing oocytes. As reported by Khalil (1970) and Brinton and Oliver (1971a), the ovaries of argasid ticks are relatively smaller than those of ixodids and differ in regards to location of egg production. Specifically in reference to the latter, eggs develop in the posterior portion of the argasid ovaries (Roshdy, 1961, 1962, 1963; Khalil, 1969), while in the ovaries of the ixodid females eggs develop along the entire length.

**Oviduct**

Oviducts arise from each end of the ovary (cf. Arthur, 1962) or in *Argas vespertilionis* (Roshdy, 1961) from the anterior end of each ovary as a narrow thick-walled tube which extends to the anterolateral area of the body cavity where connections are made with the vagina, seminal receptacle or uterus depending upon the species. According to the morphological studies by Roshdy (1961, 1962, 1963, 1966) coiled oviducts are characteristic of most ticks, although the length and number of coils are variable. In reference
to histological features, many investigators (cf. Arthur, 1962) have reported several basic layers comprising the wall of the oviduct: an inner epithelium, a muscular layer and an outer thin epithelium. The inner epithelium consists of columnar epithelium projecting into the lumen or of cuboidal epithelium as in *Hyalomma anatolicum* (Khalil, 1970). The muscular layer functions mainly to force the passing of eggs during oviposition. The distal attachment of the oviduct to the next organ shows numerous variations. For example, in the argasid *Argas persicus*, the oviduct leads directly into the vagina (Robinson and Davidson, 1914) while in all other examined argasids, the oviducts open laterally into a comparatively large uterus (Roshdy, 1961, 1962, 1963; Khalil, 1969). In *Ixodes ricinus* the oviducts join to form a short common oviduct which then opens into the vagina (Nordenskiold, 1909a) while in all other cases of examined ixodids, the oviducts open into a sac-like seminal receptacle (Khalil, 1970, 1972; Brinton and Oliver, 1971a).

**Seminal receptacle**

Although the two terms, seminal receptacle and uterus, are present in the literature they refer to a tick reproductive organ which has similar morphological and functional features. For example, in either case this organ is the site for sperm storage (Robinson, 1942; Feldman-Muhsam,
1967a, b; Gladney and Drumond, 1971) and in many tick spe-
cies sperm maturation also occurs in this organ (Sharma,
1944; Reger, 1962; Breucker and Horstmann, 1968). In gen-
eral, this structure is called the uterus in argasid ticks
(Williams, 1905; Rosshdy, 1961, 1962, 1963; Khalil, 1969)
and the seminal receptacle in ixodids (Khalil, 1970;
Brinton and Oliver, 1971a). This differentiation is due to
the relatively larger size of the argasid uterus and the
presumed presence of glandular epithelium (Khalil, 1969).
The uterus of argasids is triangular in shape and the base
ranges from slightly bilobed in *Argas boueti* (Rosshdy, 1962)
to deeply bilobed in *Argas vespertiliones* (Rosshdy, 1961).
The seminal receptacle of ixodids is sac-like and slightly
trilobed (Brinton and Oliver, 1971a). The uterus or seminal
receptacle narrows anteriorly to form a slender neck which
leads to the vagina.

**Vagina**

In most female ticks, the vagina is described as a
short tube which is connected to the genital opening an-
teriorly and is connected to the seminal receptacle (uterus)
posteriorly. Exceptions as previously mentioned are where
the posterior end of the vagina is either connected to the
oviducts as in *Argas persicus* (Robinson and Davidson, 1914)
or to the common oviduct as in *Ixodes ricinus* (Nordenskiold,
1920). The vagina is usually divided into two regions:
a posterior barrel-shaped or cervical region and a short vestibular region. The vagina is lined with a layer of folded columnar epithelium underlying a thick layer of cuticle (cf. Arthur, 1962) containing minute recurved spines. The vagina is ensheathed with a muscular layer and one or two pairs of accessory glands which are usually attached at the junction of the cervical and vestibular regions of the vagina.

**Accessory glands**

One or two types of accessory glands are associated with the female genital tract in ticks. One type is universal among ticks and opens into the vagina (Roshdy, 1961, 1962, 1963; Khalil, 1969, 1970, 1972). These accessory glands are tubular and consist of columnar or wedge-shaped cells which were demonstrated by Douglas (1943) and Lees and Beament (1948) to produce a lubricant fluid facilitating the extrusion of eggs through the vagina. The second type of the accessory glands is present only in few tick species, is lobular, and forms a large median unpaired sac-like structure opening into the vagina. This type of glands consists of large columnar or polygonal cells which also produce a lubricant fluid (Lees and Beament, 1948).

Another secretory structure commonly found in female ticks is the so-called Gene's organ (cf. Arthur, 1962). This structure is spherical and is located under the cuticle
anterior to the vagina and opening above the genital aperture. This structure is involved with the production of waxy material during oviposition (Lees and Beament, 1948). During oviposition, this organ becomes considerably distended and receives eggs from the vagina. This organ then inflates and deflates several times forming a wax layer around the egg (Lees and Beament, 1948).

Gametogenesis in Ticks

Gametogenesis can be considered the first step in the reproduction of ticks, the others being fertilization and oviposition. During the first step, the primordial germ cells or gametogonia are transformed into specialized cells: the egg in the female and the spermatozoon in the male. As with many other species, the early steps of gametogenesis occur during immature stages, i.e., the larval and nymphal stages and in the case of oogenesis is not completed until after full engorgement of the adult female tick. In considering spermatogenesis, with the exception of Ixodes, this process is completed only in the female genital tract after insemination. In general, ticks attach to the host for four to eight days as a larva, four to seven days as a nymph, and eight to 12 days as an adult, with varying periods in between attachment ranging from a few days to several weeks depending on the species and the availability
of the host.

Oogenesis

Ovarian development and oocyte maturation are being studied in great depth in metazoan phyla by an increasing number of investigators. Such interest has resulted in elucidating changes in intracellular fine structure, vitellogenesis, uptake of extracellular vitellogenesis precursors, and in emission of nucleoproteins by the nucleus.

In newly hatched larvae, Khalil (1969, 1970) has studied the genital primordium and has observed that this structure consists of two types of cells distinguished by the type of nuclei; either a large rounded vesicular nucleus with two dark-staining nucleoli and a fine chromatin network or a small oval nucleus containing chromatin clumps, but no nucleoli. She speculated that the cells with large nuclei are derived from primordial germ cells and the cells with smaller nuclei are of primordial supporting elements, an observation agreeing with other taxa, for example, vertebrates. As the newly hatched larva feeds and develops, the genital primordium becomes crescent-shaped with a flattened central lumen surrounded by undifferentiated cells which are derived from the previous mentioned cells with large nuclei. As this feeding period progresses, these cells perform extensive mitotic division eventually culminating in the formation of the oogonia (Khalil, 1969).
In the nymphal stage of tick development, Khalil (1969, 1970) and Brinton and Oliver (1971a) observed elongation of the ovary and actively dividing oogonia. After five days of attachment of the nymph to the host, many of these cells begin to differentiate into primary oocytes, an endpoint for oogenesis during the nymphal stages. Several investigators (Khalil, 1969, 1970; Brinton and Oliver, 1971a) have observed in the newly molted adult females arrested primary oocytes. No further development occurs unless these adult females attach to the host for engorgement.

Thus, after the attachment of the adult female to the host, the final stages of oogenesis are initiated. The oocytes become involved in the "first growth phase" (Khalil, 1969, 1970) or "intra-oocytic phase" (Brinton and Oliver, 1971b) which lasts from five to eight days. A unique phenomenon occurring at this time is nuclear emissions in the form of dense granules which have been reported by various investigators (Sareen, 1965b; Aeschlimann and Hecker, 1967; Brinton and Oliver, 1971b). Sareen (1965b) showed that these nuclear emissions are nucleoproteins and participate in yolk formation. Also during the first growth phase, multivesicular bodies which are believed to be (Brinton and Oliver, 1971b) aggregations of ribosomes and groups of Golgi vesicles appear in the ooplasm. Finally, appearing along the oocyte periphery are microvilli which project from the
oolenum (Aeschlimann and Hecker, 1967, 1969; Brinton and Oliver, 1971b) and will gradually increase in number until they form a brush-like border.

After the oocyte becomes considerably large, the second phase of growth occurs. Brinton and Oliver (1971b) called this phase "extra-oocytic phase" since this involves, among other functions, micropinocytosis or obtaining nutritive materials from the haemolymph through the surface of the oocyte. According to these authors, this phase occurs during the last three days of adult feeding and after full engorgement and is assumed equivalent to the "second growth phase" reported by Khalil (1969, 1970). Another important aspect of the second growth phase is vitellogenesis. As reported by Brinton and Oliver (1971b) this process begins with the formation of small vitelline bodies which gradually enlarge and readily fuse resulting in larger yolk bodies. Further accumulation of the yolk bodies causes crowding and compressing. As observed by Sareen (1965b) and Brinton and Oliver (1971b), formation of yolk bodies may involve the absorption of nuclear emissions and the utilization of mitochondria which are trapped between the enlarged vitelline bodies. In general, as is also true with many other taxa, intra-oocytic and extra-oocytic sources of materials participate in the vitellogenesis process in ticks.

While vitellogenesis is proceeding, the formation of
the cuticle around the oocyte is also taking place. However, only a few reports about the actual time of cuticle deposition are available. Brinton and Oliver (1971b), for example, reported in *D. andersoni* the occurrence of this process approximately six days after feeding. In addition, these investigators reported that the deposition of the cuticular envelope is initiated between the bases of microvilli and speculated an intra-oocytic contribution. They also observed that as the cuticular deposition progresses, the number of microvilli are reduced, presumably being retracted from the cuticle. Although the time and the site of fertilization were not reported, Khalil (1969) and Brinton and Oliver (1971b) suggested that fertilization occurs in the ovary before the deposition of cuticle around the oocytes.

Fertilized eggs are released in the lumen and then pass through the oviduct to the seminal receptacle. After the eggs leave the vagina, they obtain a waxy coat from the so-called Gene's organ (Lees and Beament, 1948). The size of oviposited eggs ranges from 300 µ in *Ixodes hexagonus* to 600 µ in *Ornithodoros moubata* (cf. Arthur, 1962).

Aeschlimann and Hecker (1967) and Brinton and Oliver (1971b) have observed material from the haemolymph passing into the cortical region of oocytes by micropinocytosis. In addition to these sources of contribution to the egg growth, Chinery (1965) reported yolk bodies in oocytes of
**Hemophysalis spinigera** containing basic proteins possibly derived from the epithelial cells of the gut. Aeschlimann and Hecker (1967) concluded that the funicle cells, by which oocytes are attached to the ovary, are one of the primary sources of extraoocytic protein for developing oocytes.

A relationship between insemination, engorgement and egg maturation has been suggested by various investigators. Gregson (1944, 1947) showed a positive correlation between engorgement and mating in *D. andersoni*. In general, females of ixodid ticks, excluding *Ixodes* would not fully engorge until mating occurred. In addition, complete oocyte maturation in some species of ticks is also dependent on insemination. Specifically, Tatchell (1962) and Galun and Warburg (1967) have shown that the accessory glands of male *Argas persicus* and *Ornithodoros tholozani* contain a pharmacological agent which is carried in the seminal fluid to the female and presumably stimulate the ovary to cause oocyte maturation.

**Spermatogenesis**

In order to recognize the sperm components and to know their function, the process of spermatogenesis in ticks must be understood. Probably because of ease of obtaining material, most reports on spermatogenesis are restricted to
the adult male stage (Christophers, 1906; Oppermann, 1935; Sharma, 1944; Reger, 1961, 1962). In reference to preadult stages, Khalil (1969) and Oliver and Brinton (1972) have described the development of primordial germ cells of *Argas arboreus* and *D. occidentalis* respectively. These authors reported that during attachment of the larvae to the host, the primordial germ cells undergo mitotic division and eventually form spermatogonia.

Although slight differences occur between argasid and ixodid ticks, in general, the spermatocytes are formed during the nymphal stage. One of the minor differences reported by Khalil (1969) is the presence of primary and secondary spermatocytes in the nymphal stage of argasid ticks while only primary oocytes are found in the nymphal stage of ixodids. Secondary spermatocytes are found only in the newly molted adult male of ixodids. A surprising exception is reported in the genus *Ixodes* where Nordenskiiold (1920) and Tuzet and Millot (1937) have observed the formation of mature spermatozoa shortly before the engorged nymph drops off. Consequently, the testes of newly molted males contain mature spermatozoa.

Spermatocytes have been described by many investigators (cf. Arthur, 1962). In general, primary spermatocytes are large round cells, but may appear irregular in shape or distorted due to the overcrowding or presence of
other organs. Secondary spermatocytes can be described in various ways. For example, while Oppermann (1935) reported that secondary spermatocytes of *Argas reflexus* differ from primary spermatocytes only in their smaller size, other investigators characterized the secondary spermatocytes by the presence of the unique peripheral striations. These striations have received a great deal of attention in regards to their significance and development. In earlier studies they were recognized but considered abnormal degeneration (Christophers, 1906) or merely thick ectoplasm (Nordenskiold, 1920). Tuzet and Millot (1937) and Till (1961) believed these striations to be lipid granules and applied the name "lipoplasm". The first accurate interpretation was by Reger (1961, 1962) who described these striations as small oblong subsurface cisternae, an observation confirmed by Oliver and Brinton (1972) and Breucker and Horstmann (1968, 1972). Besides these interesting striations, the secondary spermatocytes are also characterized by "intercellular bridges" which are believed to facilitate exchange of materials between spermatocytes of *A. reflexus* (Oppermann, 1935) or to keep the development of spermatocytes of *D. occidentalis* in synchrony (Oliver and Brinton, 1972).

As the spermatocytes begin to differentiate they are referred to as spermatids. The development of spermatid to
a mature sperm is spermiogenesis or sperm maturation. Each spermatid is typically spherical and is characterized by a relatively large accumulation of subsurface cisternae. In addition, each spermatid is usually enveloped by nutritive cells (Reger, 1962; Khalil, 1969). During spermiogenesis, the spermatid passes through two important morphological transformations: polarization and invagination. Polarization involves the movement of the nucleus to one end of the cell and the subsurface cisternae to the other end (Sharma, 1944; Reger, 1961, 1962). Reger (1962) and Breucker and Horstmann (1972) have shown after polarization that the subsurface cisternae enclose a cavity, a structure which plays a significant role in the second transformation, invagination. After the cavity formation, the spermatid nuclear end invaginates into the cisternal cavity and, although this process has been poorly described, several authors have correctly reported that the process of invagination causes the formation of an inner and outer tube in the developing spermatid. In addition, Sharma (1944) and Reger (1962) observed that most of the cytoplasmic structures flow into the inner tube while some flow into the outer tube. The nucleus was observed to migrate to the outer surface of the outer tube. Another observation of nucleus migration was by Khalil (1969) who claimed the nucleus became attached to the cytoplasmic membrane. Also during the process of invagination, Sharma (1944) claimed
that the centrosome migrates into the inner tube and becomes located at the anterior tip. However, Breucker and Horstmann (1972) proved that no centrosome migration occurred during invagination.

A little-known tick sperm organelle is the acrosome. Before and during invagination of the spermatid, an acrosome is formed by fusion of vesicles from the Golgi complexes (Sharma, 1944; Reger, 1961, 1962; Breucker and Horstmann, 1972). This organelle becomes located in the posterior region of the mature spermatozoon. As reported by many investigators, the spermatids become elongated during invagination and upon completion of this process pass to the seminal vesicle and are stored until transferred to the female during insemination.

After the transfer of these elongated spermatids (immature spermatozoa) to the female seminal receptacle, the final stages of spermiogenesis occur. These stages of sperm maturation have not been clearly reported. Reger (1962, 1963) and Khalil (1969) assumed that this process occurs by evagination of the elongated spermatid, but did not demonstrate such. Most reported studies agree that each spermatozoon doubles in length during these final stages, however, there is no explanation of the event.
Comparative Sperm Morphology

Since the discovery of the 9+2 pattern of microtubules in cilia and flagella by Manton (1952) and Fawcett and Porter (1954), researchers have made numerous attempts to demonstrate the occurrence of this system in spermatozoa of various metazoan groups, usually efforts which involved ultrastructural and cytochemical studies. Such comparative studies of spermatozoa have demonstrated a great variety of sperm morphological structures. As a result, numerous attempts have been made to relate various spermatozoa on a morphological, functional, and phylogenetic basis (Tuzet, 1950; Franzen, 1956; Hughes, 1965; McFarlane, 1963; Tochimoto, 1967). Brown (1970) has emphasized the value of sperm morphology in determining phylogenetic relationships between taxa, based on the presence, absence, and modification of sperm structure.

Primitive metazoan sperm

Franzen (1956) has developed the concept of a primitive metazoan sperm based on comparative studies of sperm morphology, spermiogenesis and the biology of fertilization in different phyla of metazoa. Franzen (1956, 1970) stated that the variations in sperm morphology of living taxa are due to modifications of a primitive metazoan sperm during evolutionary processes.

The primitive type of spermatozoon, as described by
Franzén (1956, 1970) in the Metazoa is made up of a short, roundish or conical head, a middle piece and a vibratile tail or flagellum. The head is mainly composed of the nucleus and the anterior tip usually contains an acrosome of variable morphology. The middle piece contains the mitochondria which are collected in four to five spherical or oval-shaped aggregates, the proximal part of the flagellum, and the centriolar apparatus. The tail consists of a flagellum beginning at the centriolar apparatus and extending posteriorly. The ultrastructure of sperm flagellum is similar to cilia and flagella which consist of 9+2 pattern of microtubules. This primitive metazoan or typical sperm is exemplified in such diverse groups as the enteropneust, Saccoglossus (Colwin and Colwin, 1963a,b), the echiuroid worm, Urechis (Tyler, 1965), the urchin, Arbacia (Longo and Anderson, 1969), the serpulid fan worm, Hydroides (Colwin and Colwin, 1961a,b,c), the mussel, Mytilus (Niijima and Dan, 1965a,b; Longo and Dornfeld, 1967) and the horseshoe crab, Limulus (André, 1963a; Shoger and Brown, 1970).

**Modifications of primitive metazoan sperm**

Since spermatozoa of ticks diverge extremely from the "primitive metazoan sperm", it is necessary to examine the divergence of other metazoan sperm from the primitive sperm and relate this to the sperm morphology of arachnids.
and ticks in particular. In general, divergence from the primitive sperm morphology can be classified into five categories: 1) migration of the centrioles; 2) fusion or absence of mitochondria; 3) elongation of the nucleus; 4) lack of a distinct middle piece; and 5) absence of a structural acrosome.

The centrioles which usually are located in the sperm middle piece may migrate to unusual positions. For example, in the tiger beetle, *Cicindela* (Werner, 1965) the centrioles are positioned in front of the nucleus and in the silverfish, *Lepisma* (Werner, 1964) the centrioles have exchanged positions with the nucleus. The mitochondria which are usually grouped into four to five spherical or oval aggregates may fuse to form two spheres as in nemertians and bryozoans (Franzén, 1956) or the mitochondria may be absent as in the nematode, *Gastrothylax* (Dhingra, 1955), the dragonfly, *Symptrum hypomelas* (Nath and Rishi, 1953), and the spiders, *Plexipus, Paradosa* and *Pholocus* (Rosati et al., 1970). The nucleus which is usually spherical or oval may become elongated and thus the sperm head exhibits various shapes. For example, in the phoronid, *Phoronis ovalis*, the head is filiform and in the hirudinean, *Ottoniobdella burnnea*, the head is helical (Franzén, 1970). The middle piece, which usually forms a distinct region between the head and the flagellum, may be lacking as in octopods
and ascidians (Franzén, 1970). Finally, the acrosome which is usually located in front of the nucleus may be totally absent as in teleost fishes (Ginsburg, 1963).

In addition to the above mentioned divergences, some metazoan sperms are extremely different from the primitive sperm by lacking the flagellum and assuming various shapes. The nonflagellated spermatozoa can be divided into three types: 1) tubuliform, which acquires a tubular shape with a wider anterior end and a slender posterior end and is confined to ticks (cf. Nath, 1956); 2) vesiculiform, which acquires a cup shape or star shape and is found in millipedes (Nath, 1956) and decapod crustaceans (Brown, 1966; Pochon-Masson, 1965); and 3) ameboid, which is only found in some nematodes (Collier, 1936; Nath and Singh, 1956; Lee and Anaya, 1967).

**Sperm morphology of ticks and other arachnids**

Sperm morphology in the class Arachnida shows a conspicuous evolution. Baccetti (1970) stated that separate evolutionary lines might have arisen from the primitive sperm stock. These separate lines would be the encysted situation in spermatozoa of pseudoscorpions and spiders, the 9+2 pattern of microtubules in spermatozoa as found in pseudoscorpions and most scorpions and the 9+3 pattern of microtubules in spiders. In addition, spermatozoa of
harvestmen and some pseudoscorpions show progressive loss of the axial filament complex and spermatozoa of ticks totally lack the axial filament complex and have developed a different motile system.

**Ixodoidea**

Several investigators have shown interest in the strangely-shaped spermatozoa of ticks and have attempted to elucidate their structure. In general, mature spermatozoa of ticks are classified as aflagellate and tubuliform (for review see Nath, 1956). The mature spermatozoon is like an Indian club with a wider anterior end and varies in length from 350 μ in *Ornithodoros moubata* (Breucker and Horstmann, 1968) to 1050 μ in *Argas columbarum* (Oppermann, 1935). Sharma (1944) and Reger (1962) claimed the presence of a large centrosome at the tip of the anterior end. More recently, Breucker and Horstmann (1968, 1972) have shown that the centrosome in *Ornithodoros moubata* is located with the nucleus and acrosome at the posterior end and not at the anterior end. Many previous authors used the terms prosperms or spermiophores for the spermatozoon, believing that the elongated nucleus was the sperm proper. Samson (1909), Oppermann (1935), Tuzet and Millot (1937) and Stella (1938) made a false interpretation of a flagellum attached to the nucleus. Apparently this flagellum is the fine membranous tube described by Reger (1962), Khalil
(1969) and Breucker and Horstmann (1968).

Although spermatozoa of ticks are motile the process
of sperm motility is not known. Sharma (1944) reported
that motility takes place by the peculiar rotary movement
of the centrosome at the anterior end. Although the presence
of centrosome at this anterior end is no longer recognized,
this was a fairly accurate observation with regards to motil­
sidered the series of multiple cellular processes on the
surface of the spermatozoon as the source of the motile
mechanism. Rothschild (1961) thought a change in the form
of these processes passes from the front to the back end of
the spermatozoon and cause the movement. He was the first
to measure the speed of a tick spermatozoon, doing so with
*Ornithodoros moubata*. The presence of cellular processes
as means of motility is unique and is confined to ticks.

**Scorpiones**

Spermatozoa in most of the scorpions studied have a
filiform head with an elongated nucleus, an acrosome in
front and a long tail constituting the tail sheath and the
axial filament (Wilson, 1931; Bedi, 1962b). The tail sheath
consists mainly of mitochondria surrounding the axial fila­
ment. With the exception of spermatozoa of *Vejovis carolinianus*,
which exhibit a flagellum with 9+0 pattern of microtubules
(Hood *et al.*, 1972) and *Centruroides vitatus*, which exhibit
a 9+1 pattern (Hood et al., 1972), all scorpions studied have spermatozoa with a 9+2 pattern (Baccetti, 1970).

Wilson (1931) noted, during spermatogenesis, mitochondria undergo different arrangement in different species and thus he divided scorpions into two categories according to the arrangement of mitochondria: 1) in which the mitochondria form ball-like structures which have a definite number, e.g., Opisthocanthus, Hadrurus, Vejovis and Euscorpius; 2) in which mitochondria fuse to form a ring during the growth of spermatocytes, e.g., Buthus and Centrurus. André (1959, 1963) also observed that, during spermiogenesis, the mitochondria are fused to form large bodies which he called "culichondria" and the cristae anastomose to form a honeycomb-like structure. He followed Wilson's (1931) idea of classifying spermatozoa according to the arrangement of mitochondria and thus separated the two families of scorpions: 1) Buthidae, which have regular mitochondria in their spermatocytes, but with unusual beaded components in the helical sheath of the sperm; 2) Chartidae, which have spermatocytic mitochondria in a cup-shaped "culichondria".

**Pseudoscorpiones**

Spermatozoa of pseudoscorpions consist of an elongated head comprising a long, stout, tapering nucleus which is
wrapped with a thread-like structure. A large club-shaped acrosome is located at the anterior end of the nucleus. A centrosome is present and a short tail originates from it with a 9+2 pattern of microtubules (Tuzet et al., 1966; Boissin and Manier, 1966, 1967; Kanwar and Kanwar, 1968; Weygoldt, 1969). Spermatozoa of pseudoscorpions go through further development which is characterized by sperm encystment. This process involves coiling of the spermatozoon in a spiral-like manner which becomes enveloped in the cell membrane. Kanwar and Kanwar (1968) reported that during sperm encystment of *Diplotemnus* the flagellum is lost. After the sperm cysts reach the female genital tract and uncoil, the freshly emerged spermatozoa conspicuously lack their flagella. The development of a flagellate spermatozoan and its final transformation into a nonflagellate spermatozoan is very unique and is confined to pseudoscorpions (Kanwar and Kanwar, 1968).

**Araneae**

In addition of being filiform and encysted, mature spermatozoa of spiders present particular characteristics: the head of spermatozoon is rolled up in a spindle-shape, the mitochondria degenerate during maturation and a third central microtubule is present throughout the whole length of the tail giving it the pattern of 9+3 (Sharma, 1950;
Osaki, 1969; Rosati et al., 1970). Spermatozoa of the spider, *Pholcus phalangioides* (Rosati et al., 1970) contain a "central canal" which goes through the head region and becomes larger near the tail where the centriole is located. The upper part of the central canal goes through the nucleus and penetrates the acrosome. The tail starts immediately behind the nucleus and bears a 9+3 pattern throughout its length, but is devoid of the outer doublets at the extreme region. On the other hand, spermatozoa of the spider, *Heptathela kimurai* are immotile, in spite of the presence of flagella.

**Opiliones**

There are few references available in the literature dealing with spermatogenesis and sperm morphology of harvestmen. Bedi (1963) stated that spermatozoa of harvestman, *Melanopa* has a wide head containing a nucleus which is somewhat rectangular or discoidal in shape. The nucleus has marked depressions in the nuclear membrane at both the anterior and posterior ends. The acrosome lies in the anterior depression. The axial filament starts at the other depression and constitutes the relatively short tail. On the other hand, Sotelo and Trujillo-Cenóz (1954, 1958) have described the spermatozoa of *Heteropachyloidiellus robustus* as spindle-shaped and nonmotile. The centriole
occupies a depression on the nuclear membrane. Baccetti (1970) reported that spermatozoa of harvestmen lack flagella and are encysted like those of pseudoscorpions and spiders.

In conclusion, the class Arachnida shows a conspicuous evolution, in spite of the lack of reports on sperm morphology of some arachnid orders (Acari, Uropygi, Solifugae). In scorpions, the mitochondria fuse to form a honeycomb-like structure while in spiders the mitochondria degenerate during sperm maturation. In pseudoscorpions, although spermatozoa are flagellated and exhibit the 9+2 pattern, the flagellum is lost during maturation. If the concept of primitive metazoan sperm is accepted and that encysting of spermatozoa is considered a protection from drying, thus Arachnida, a terrestrial class, has three forms of spermatozoa: lower forms which include encysted spermatozoa with 9+2 pattern, higher forms which include encysted spermatozoa with a 9+3 pattern, and a highest form which include nonencysted and nonflagellated spermatozoa.

Sperm Transfer in Ticks and Other Arachnids

Spermatozoa of primitive terrestrial arthropods are often transferred from the male to the female in spermato- phores. During the evolutionary process of transmission from the aquatic to terrestrial life, at least three methods for sperm transfer have developed and are present in
living arachnid orders. These methods are: 1) copulatory organs for internal insemination, represented in the order Opiliones and few families of the order Acari; 2) spermatophores which are placed either directly into or at least on the female genital opening. This method is the most common among arachnids and is represented in the order Uropygi, Solifugae, the superfamily Ixodoidea and few families of the order Acari; 3) secondary genital appendages or gonopods represented in the order Araneae.

Ixodoidea

Sperm transfer in ticks occurs by means of spermatophore (cf. Arthur, 1962; Feldman-Muhsam, 1967b). Although copulation has been described in the argasid (Nuttall and Merrimann, 1911; Robinson, 1942; Feldman-Muhsam, 1967a,b) and ixodid ticks (Moorhouse, 1966; Feldman-Muhsam and Bourt, 1971), this is not a true copulation and no copulatory organs are present. Copulation behavior starts as the male creeps about on the female and brings his body in close apposition to her ventral surface. The male introduces his capitulum into the female genital aperture for various periods of time. When the capitulum is removed a spermatophore is extruded from the male genital aperture, carried by the chelicerae and deposited into the female genital opening (Feldman-Muhsam, 1967a). Argasid and ixodid ticks differ
slightly in this copulation process. For example, while argasids always copulate off the host (Feldman-Muhsam and Bourt, 1971) ixodids of the Metastriata group copulate on the host and ixodids of the Prostriata group may either copulate on or off the host (Balashov, 1967).

The structure of the spermatophore has been studied in few tick species. Robinson (1942) described the spermatophore of *Ornithodoros moubata* as an elongated structure with a slender neck connecting a bulb-like structure on one end and two capsules on the other end. Feldman-Muhsam (1967b) and Feldman-Muhsam and Bourt (1971) have proposed the term "ectospermatophore" for the bulb and "endospermatophore" for the capsules. The endospermatophore or the capsules are retained in the female genital tract after spermatophore transfer. These investigators have also shown that the spermatophore wall consists of three layers: an outer layer made of mucin, a middle proteinaceous layer, and an inner layer made of mucoprotein. They have also demonstrated that sperm are transferred from the ectospermatophore through the slender neck into the endospermatophore which is retained in the female genital tract. After copulation an empty ectospermatophore is always observed protruding from the female genital opening until it falls off (Robinson, 1942; Feldman-Muhsam, 1967a,b).

Although fertilization is the general rule, complete
and sporadic parthenogenesis has been reported in ticks. Complete parthenogenesis occurs in *Amblyomma agamum* where the males are infertile (Aragão, 1912) and in *Haemaphysalis bispinosa* (*longicornis*) where no males have been reported. Sporadic parthenogenesis has been reported for some species as *Ornithodoros moubata* (Davis, 1951), *Boophilus microplus* (Stone, 1963), *Hyaloma anatolicum* (Pervomaisky, 1949), some races of *Dermacentor variabilis* (Nagar, 1967; Gladney and Dawkins, 1971) and *Amblyomma dissimile* (Bodkin, 1918; Oliver, 1971) all of which normally have sexual reproduction. In these cases, although no copulation or sperm transfer occurs, some females after engorgement oviposit mature eggs which hatch and continue the life cycle.

**Scorpiones**

In scorpions, sperm transfer occurs only with spermatophores. Spermatophores are stalked and produced in special gland pockets (paraxial gland) of the males as two identical halves which are then joined together (Angermann and Schaller, 1956; Angermann, 1957). Before the transfer of the spermatophore, scorpions perform a long courtship. In general, the male holds the female by the chelae and in some species (*Euscorpius italicus*) the male stings the partner repeatedly in the joint proximal to the chela (Angermann, 1957; Angermann and Schaller, 1956). The male presses his
genital opening against the substrate to which he attaches the stalk of a spermatophore. The maneuver of the male to direct the female into position to pick up the spermatophore results in a dance which differs in various genera. During this dance, the male draws his partner to the spermatophore. The female then spins the spermatophore cover with a sudden movement which causes the release of spermatozoa into the genital opening (Schaller, 1971). The male immediately leaves his partner who subsequently consumes the empty spermatophore. Parthenogenesis is reported only in *Tityus serrulatus* (Matthiesen, 1962).

**Pseudoscorpiones**

As in scorpions, sperm transfer occurs with spermatophores. The spermatophore consists of a stalk and a sperm mass enclosed within a "sperm package" (cf. Kaestner, 1968). After the spermatophore is picked by a female, the sperm package swells until it bursts which causes the release of spermatozoa into the female genital system. In some chrenetid and cheliferid pseudoscorpions, the spermatophore contains a drop of fluid under the sperm package to trigger the swelling mechanism (Schaller, 1964, 1971).

Weygoldt (1969) classified sperm transfer in pseudoscorpions in two main categories: 1) sperm transfer without mating and 2) sperm transfer with mating. In the first
category, some pseudoscorpions (e.g., Chthonius, Neobisium) the male deposits spermatophores on the ground without the presence of a female. A receptive female investigates the spermatophore with the tips of her palpi, and then slowly steps over the spermatophore with extended legs and takes up the "sperm package" and leaves the emptied spermatophore behind. In other pseudoscorpions (e.g., Serianus) the spermatophores are formed without mating, but only in the presence of the female. In the second category, the spermatophore is extruded only when a receptive female is present and prepared to accept it. After a mating dance, the male stops and deposits a spermatophore on the ground and pulls the female over the spermatophore. She instantly takes up the sperm package and removes the empty spermatophore by rubbing her ventral side against the ground. The structure of spermatophores produced by pseudoscorpions without mating are simpler and more easily accessible than those produced during mating.

**Opiliones**

Sperm transfer in the harvestmen is direct, since copulatory organs are present (Tischler, 1967). When the male and female face each other, the male pushes his penis between the chelicerae of the female and into the genital opening. In the Trogulidae, the male climbs on the back of
female, moves laterally to her ventral side and inserts his penis into the genital opening for ten to 20 minutes (Tischler, 1967). Presumably during this copulatory process, spermatozoa are deposited into the female genital tract.

**Solifugae**

During mating, sperm transfer in sun spiders is accomplished by spermatophores or by direct deposition of seminal fluid in the genital tract (Cloudsley-Thompson, 1961; Muma, 1966). In the sun spider, *Galeodes caspius*, the male stops when approaching a female then leaps and grasps her abdomen with his chelicerae. If the female becomes completely passive, the male carries her and turns her on her back and pinches her genital region which cause the opening of her genital aperture. The male then extrudes a spermatophore on the substrate which he picks up by his chelicerae and pushes into the female genital opening. After spermatophore transfer, the male suddenly leaps off and moves away.

In the genus *Eremobates*, the male takes the female, turns her over and pushes the fixed cheliceral fingers deep into her genital opening. Then she is placed back in walking position with her abdomen bent upward (Muma, 1966) and a droplet of seminal fluid is transferred directly from the
male to the female genital opening. The male then forces the seminal fluid into the seminal receptacle by thrusting his chelicerae into the genital opening (Muma, 1966). After sperm transfer is completed, the male leaves and cleans his chelicerae.

**Uropygi**

The whipscorpions transfer their spermatozoa with spermatophores (McDonald and Hogue, 1957). The male usually follows the female until she stops and turns back. Then the male turns and the female hooks her distal cheliceral segments into the base of the male telson. The male moves forward followed by the female. Then the male stops and deposits a spermatophore, pulls the female forward enabling her to lift the tip of the spermatophore off and then the female is separated from the male (Schaller, 1971).

**Araneae**

Sperm transfer in spiders is carried out by the complex organs of the male pedipalp (Abalos and Baez, 1963). Usually the male spider spins a "sperm web" on which he deposits the seminal fluid (Savory, 1928; Clyne, 1967). The male then dips his palps alternately into the seminal fluid for more than 90 minutes; a process referred to as "sperm induction" (Abalos and Baez, 1963; Rovner, 1967). After
sperm induction, the male goes in search of a mate. Abalos and Baez (1963) stated that the male spider recognizes the dragline and the web of a female of his own species. During courtship, the male may pluck rhythmically on the threads of the female web, approach the female with jerking movements, or stroke and pat her abdomen or whole body. Some males wrap the female in threads or hold her with long specially modified chelicerae, while others may present a fly to the female to induce courtship. After courtship, the male finally succeeds in inserting the tip of one pedipalp into the female genital opening and transfers the spermatozoa. In Dysdera, secretions from the palpal gland forces spermatozoa out of the pedipalp (Abalos and Baez, 1963).

Rovner (1967) stated that males of many species hurry off after sperm transfer, others may die soon after or during mating and may even be killed and eaten by the female. Parthenogenesis is reported only in Theotima and Steatoda triangulosa (cf. Kaestner, 1968).

**Acari**

Sperm transfer in different mites can occur in any of the three ways found among arachnid orders: by means of a penis, gonopods, or a stalked spermatophore (cf. Kaestner, 1968). The male of the acarid mite Caloglyphus mycophagus transfers spermatozoa to the female by an intromittent organ
or penis. He locates the female, crawls about her body and takes up a final position superimposing his caudal end upon that of the female (Rohde and Oemick, 1967).

Schaller (1971) reported that sperm transfer in gamasid mites is done by a tube-like appendage in the movable finger of the chelicerae. The male pushes his abdomen underneath that of the female and with his chelicerae lifts a spermatophore from his gonopore and introduces it into the female genital opening. Many mites have the third legs modified as gonopods (Schaller, 1971).

Sperm transfer by stalked spermatophores occurs in many oribatids and water mites (Woording and Cook, 1962; Böttger, 1962, 1965). The male of the water mite, Arrenurus globator, attaches the female to his abdomen with glandular secretions so that the long axis of the female is at right angles to that of the male. The male deposits the spermatophore on the ground and walks forward until the female genital opening is in position to pick up the spermatophore (Böttger, 1962). In many oribatids the male deposits the spermatophore with a penis, but has no contact with the female. The spermatophore will be picked up later by a female after careful touching with the first leg (Schuster, 1962, Böttger, 1965). Parthenogenesis has been reported only in Ophionyssus natricis (Schaller, 1971), where unfertilized eggs produce males and fertilized eggs produce females.
In conclusion, scorpions, pseudoscorpions, Uropygi, Solifugae and ticks transfer their spermatozoa by means of spermatophores. If the line of evidence presented by Petrunkevitch (1949, 1955) that scorpions are the most primitive arachnids, then the spermatophore would be the primitive method of sperm transfer in Arachnida. The other arachnids must have lost the spermatophore during the process of evolution and developed a different method for sperm transfer. Development of a copulatory organ in Opiliones would thus make them the most advance group of Arachnida. Since mites transfer spermatozoa by all methods there is a possibility that they are of polyphylatic origin, which agrees with the assumption made by Grandjean (1933) and Zakhvatkin (1952) based on fossil records.

Sperm Storage in Ticks and Other Arachnids

The ability to store spermatozoa in the female genital tract is a great advantage, especially where internal fertilization is involved. This advantage, as stated by Parker (1970) is very adequate presuming that all the stored spermatozoa are available to fertilize the average number of eggs which the female is likely to produce. Therefore maximum efficiency of sperm utilization is possible and most ova are certain of fertilization.

After copulation and spermatophore transfer, in both
argasid and ixodid ticks, spermatozoa within the endospermatophore are stored in the seminal receptacle (uterus). The time for sperm storage before fertilization has been reported only in a few species and is quite variable. For example, in *Ornithodoros moubata*, spermatozoa are stored for five days (Robinson, 1942), in *Ornithodoros tholozani* (Galun and Warburg, 1967) for several weeks, and in *Amblyomma americanum* they can be retained for several weeks (Gladney and Drumond, 1971). In all female ticks which have been examined the spermatozoa undergo further maturation (see spermatogenesis in the introduction).

The mechanism for rupturing the wall of the endospermatophore and the release of spermatozoa has not been ascertained yet. Robinson (1942), who suspected that sperm-egg interactions occur in the seminal receptacle, thought the rupturing of the endospermatophore wall occurs either during the female engorgement or when mature eggs enter the seminal receptacle during oviposition. This suggestion is improbable, since sperm-egg interactions occur before eggs enter the seminal receptacle and spermatozoa have to be released earlier. Khalil (1969, 1970), in her observations on argasid ticks, believes that secretions from the glandular epithelium of the uterus are responsible for rupturing the wall of the endospermatophore and freeing the spermatozoa.
Only a few reports about sperm storage in other arachnids are available and with the exception of the uncoiling of "sperm cysts" of pseudoscorpions (Kanwar and Kanwar, 1968) no other report of sperm maturation in the female animal exists. After spermatophore transfer in pseudoscorpions, the "sperm cysts" are retained in the spermatheca until uncoiling occurs and spermatozoa are released. Sperm storage is also reported in spiders (cf. Kaestner, 1968). Here the spermatozoa remain in the spermatheca until they pass into the fertilization duct which opens into the genital atrium. In mites, spermatozoa are either stored in a spermatheca (Dosse, 1958; Oldfield et al., 1972) or in a seminal receptacle (Rohde and Oemick, 1967). However, at the time of fertilization spermatozoa move into the ovaries.

Approaches to Fertilization in Ticks

As stated by Franklin (1970), the requirements for fertilization are: sperm migration to the egg, initial sperm-egg contact, acrosomal reaction, sperm penetration into the egg, pro-nuclear fusion and egg activation. These areas are totally unexplored in ticks. This neglect is due primarily to the difficulties in working with tick gametes. Spermatozoa in all reported tick species are nonflagellated and their motile mechanism is obscure (see the section on
Sperm Motility of Ticks in the introduction). The eggs also present various difficulties. They are yolky, non-transparent and usually covered with two or three egg coats which makes their fixation and sectioning a very difficult task.

No direct approach for studying fertilization in ticks has been reported. However, there are few sporadic reports about presumable sites for fertilization. Earlier in the literature, various investigators (Christophers, 1906; Samson, 1909; Lees and Beament, 1948; Till, 1961) reported that neither oviposited eggs nor eggs in the seminal receptacle have any micropyle. In considering these observations, various parts of the female genital tract, other than the seminal receptacle, were assumed to be a site for fertilization. For example, some authors (Lees and Beament, 1948; Till, 1961; Khalil, 1969; Brinton and Oliver, 1971b) suggested that oocyte fertilization occurs in the ovary since they observed spermatozoa inside the ovarian lumen. Other investigators (Bonnet, 1907; Robinson and Davidson, 1914; Dennis, 1932; Sokolov, 1956) believed that fertilization occurs in the oviduct since they observed some spermatozoa penetrating oviduct cells. Fertilization in the oviduct is rejected, since the micropyle-free egg coat forms a barrier for sperm penetration. Although not proven before, the ovary represents
the only probable site for fertilization in ticks.

Geigy and Wagner (1957), Wagner-Jevseenko (1958) and Geigy (1962) have proposed a different solution for the problem of fertilization in ticks. They claimed some spermatozoa are phagocytized by the oviduct cells and thus stated that "phagocytosis" results in liberation of the male DNA molecules which then diffuse into the eggs passing through the oviduct. This proposal is improbable and lacks any support. In addition, several authors reported that spermatozoa penetrated into the oviduct cells rather than being phagocytized by them.

Although the site of fertilization had not been ascertained before the present study, agamic reproduction has been observed in argasid and ixodid ticks. Sporadic, complete, and obligatory parthenogenesis occurs in a few tick species (cf. Oliver, 1971). For example, Stone (1963) reported sporadic parthenogenesis in Boophilus microplus, Oliver and Bremner (1968) showed complete parthenogenesis within Haemaphysalis longicormis which has infertile males, and Aragão (1912) demonstrated obligatory parthenogenesis in Amblyomma agamum and no males are found in this species.
MATERIAL AND METHODS

Culture Method

Most of the adult specimens of *Dermacentor* (D.) *variabilis* (Say) were obtained from Dr. C. M. Clifford who is associated with the Rocky Mountain Laboratory in Hamilton, Montana. Besides this source, some engorged nymphs and some newly molted nymphs were received from Dr. W. J. Gladney who is associated with the Agriculture Research Service in Kerrville, Texas. Other specimens of *D. variabilis* were collected locally in Iowa. For example, other adult specimens were obtained either from dogs living in the vicinity of Luther, a town approximately 10 miles west of Ames, or by flagging in prairie regions near Lake Okoboji. Adult specimens of *Dermacentor andersoni* (Stiles) were obtained only from Dr. R. K. Strikland of the United States Department of Agriculture at Beltsville, Maryland.

Ticks of all stages were maintained in the laboratory by keeping the specimens in glass vials 4" long and 1" diameter. These vials were filled 1" deep with a paste composed of a plaster of Paris and charcoal in the ratio of 6:1, plugged with fine mesh hollow stoppers, and were kept over damp sand in an incubator. Unfed adults were kept at 18°C and other stages particularly those specimens undergoing molting were kept at 25°C. Vials were cleaned
or changed every 4 to 5 weeks. A few drops of merthiolate solution of 1:10,000 concentration were deposited in the bottom of each vial as a fungal growth inhibitor.

Feeding

Larvae, nymphs and adults were allowed to engorge on white New Zealand rabbits. In order to prevent losing specimens, plastic capsules were used to enclose the ticks in a local shaved area on the rabbit. These plastic capsules (modified after Varma, 1964) were constructed from the necks of wide mouth plastic bottles. The caps were removed and replaced with a fine mesh nylon cloth and the rims of the capsules were coated with foam rubber. By attaching such a capsule to the sides or ears of the shaved rabbits with a wide adhesive tape, the feeding ticks were locally contained. As a further precaution, host rabbits were always kept in separate tick-proof cages.

The procedure of tick engorgement was dependent on the stage of development. For instance, juvenile stages of ticks were allowed to feed until fully engorged (dropping away from the site of attachment to the host), were then recovered, transferred to vials which were kept in a controlled incubator, and observed daily until molting occurred. Several days after molting, they were then placed on the rabbits and the procedure repeated. Since adults
were used for breeding and experimentation, the above procedure differed somewhat according to the treatment. In case of obtaining fertilized females, several specimens were placed first on the host and allowed to feed for 3 to 4 days. After this period, an equal number of males were added. Males and females were then recovered after full engorgement. Females needed for ovipositing were kept singly in vials in the controlled incubator. A week after molting these adult females were placed on the host. Other males and females were treated according to the different experiments as indicated in the text.

Insemination

Since copulation among ticks of the genus *Dermacentor* normally occurs on the host during engorgement, the following procedure was employed to induce tick copulation off the host. Female and male ticks of *D. variabilis* were allowed to engorge separately by placing them on separate rabbits or under separate capsules. After females had engorged for eight days and the males had engorged for four to five days, opposite sexes were paired in wax-plated dishes for observation of copulation. If this event did not occur within three hours, the ticks were again placed separately on the host for one or two more days and the process repeated. With such a procedure, copulation was observed
After copulation was completed, both male and female ticks were either immediately dissected in arachnid saline solution (after Rothschild, 1961 appendix) or were kept for varying periods of time. After dissection, the reproductive systems were prepared for light and electron microscopy.

Dissection Procedure

The dissection procedure was as follows. Ticks were placed in a wax dish, pinned (with the dorsal side up) by fine pins, and covered with arachnid solution. The legs were amputated to make dissection easier. An incision was made laterally on the tick body with microscissors and then extended laterally around the animal from the back to the front. The dorsal skin was lifted off exposing the internal organs. The hepatic caeca and tracheal branches were removed exposing the reproductive system. In males and females, these systems were removed completely from the body, were examined, and while some were fixed in Carnoy's formula A (Humason, 1967), stained with Feulgen's reagent (Davenport, 1960), dehydrated in ethanol, cleared in xylene and mounted in permount for morphological examination, others were prepared for electron microscopy.
Spermatophores and Spermatozoa

Spermatophores were obtained during and immediately after insemination. Intact spermatophores were obtained after or while exuding from the male genital opening and before implantation into the female genital tract. In cases of obtaining the spermatophore immediately after insemination, the ectospermatophore part of the spermatophore which was external to the female genital opening was obtained simply by dislodging with microforceps and placing in arachnid solution. The endospermatophore part of the spermatophore which was enclosed in the seminal receptacle was collected from the female only after the tick was dissected.

Immature spermatozoa were obtained from the testes and seminal vesicle while mature spermatozoa were obtained from the female system. Immature spermatozoa were removed from the male by dissecting out the male reproductive system, placing in fresh arachnid solution, and very gently with a microscissors opening the seminal vesicle on a microscopic slide. The spermatozoa and other contents of the seminal vesicle were then examined with phase-contrast and Nomarski interference light microscopy. Squash preparations of the seminal vesicle, testes or the whole genital system were also examined with these optics. For observation with the Nomarski microscope, the reproductive system was immersed
in acetocarmine solution for two minutes before a squash was prepared.

Live mature spermatozoa were obtained from the female seminal receptacle. In the case of D. variabilis, females were dissected and the seminal receptacle was removed. This was performed immediately after insemination and after varying periods of time (1 hour, 1 day, 2 days...up to 10 days and after oviposition) to determine the final stages of sperm maturation and to locate the site for sperm-egg interactions. In the case of female specimens which were fed in the laboratory but the event of insemination was not observed, these were usually collected and dissected after full engorgement. Other specimens were simply dissected at various stages of engorgement. Since no insemination event was observed in D. andersoni, females of this species were collected and dissected after complete engorgement.

After dissecting the female, each seminal receptacle was transferred to a depression slide and dissected in arachnid solution with microscissors. One portion of the mature spermatozoa was usually transferred to a slide, covered gently with a cover slip, and examined with phase-contrast optics or Zeiss Nomarski interference microscope. Other portions of this sperm suspension were mixed with a drop of 0.01 percent aqueous solution of acridine orange and examined with a fluorescent microscope or fixed and
stained in acetocarmine solution for 2 minutes to enhance the contrast before examining with the Nomarski microscope.

Sperm Motility

Various optical systems were applied to examine motility of mature spermatozoa. In order to clearly document this fact, cinephotomicrography was used. For this purpose, from the female seminal receptacle mature spermatozoa were obtained following the above procedure. Spermatozoa were then transferred to a microscopic slide and covered with a cover glass slightly raised to prevent any effect on the sperm movement. The sequence of sperm motility was pictured with phase-contrast optics on 16 mm film with an Arriflex movie camera.

Because of their stimulating effect on sperm motility in other taxa, solutions of ethylenediaminetetraacetic acid (EDTA) and benzethonium chloride (Hyamine) were used to treat the tick spermatozoon. Concentrations ranging from 0.1 to 1 percent were applied.

Sperm-Egg Interactions

To determine the time and site of sperm-egg interactions, females were dissected in arachnid solution and the whole reproductive system was removed, fixed, dehydrated, embedded, and sectioned in various regions of the seminal
receptacle, oviduct, and ovary (see Material and Methods, electron microscopy section). This was performed immediately after insemination and after various periods of time (1 hour, 1 day, 2 days...up to 10 days and after oviposition).

To observe sperm-egg interactions in vitro, females at different stages of engorgement were dissected and the ovary was removed. Single oocytes or strips of the ovary were then removed and transferred to a microscopic slide. Live, mature and motile spermatozoa were obtained by dissecting the seminal receptacle of inseminated females. Portions of these spermatozoa were then added and mixed gently with the oocytes and examined with a phase-contrast microscope. Spermatozoa were added to oocytes from the ovary of the same female or different females.

Electron Microscopy

Transmission electron microscopy

Tissues were prepared for electron microscopy as follows. The male and female genital systems were dissected out and immersed in 4 percent glutaraldehyde (pH 7.2, 0.1 M sodium cacodylate buffer) at 4°C for two hours. The tissues were then washed three times for a total of 15 minutes in 0.1 M sodium cacodylate buffer (pH 7.2) and post fixed in 1 percent osmium tetroxide (pH 7.2, 0.1 M sodium cacodylate
buffer) at room temperature (24°C) for 1.5 hours. After postfixation, the tissues were slowly dehydrated in a graded ethanol series and embedded in epon-araldite (Anderson and Ellis, 1965).

Sections were cut with glass knives on a Porter Blum MT II or LKB ultramicrotome, mounted on uncoated copper grids and stained with 5 percent uranyl acetate in 50 percent ethanol followed by lead citrate (Venable and Coggeshall, 1965). The grids were examined on a Hitachi HU-11 E-1 Electron Microscope.

Plastic sections (0.2 to 1.0 μ) were prepared for light microscopic orientation (Leeson and Leeson, 1970). Sections dried onto microslides were stained on a hot plate (80°C) with one of the following solutions:

1. 1 percent Toluidine Blue in 1 percent borax;
2. Azur II-Methylene Blue (Richardson et al., 1960);
3. 1 percent aqueous Basic Fuchsin, pH 10;
4. 2 percent aqueous Safranin O, pH 10.

The microslides were rinsed with distilled water, dried on the hot plate and mounted in immersion oil (refractive index 1.15). The coverslips were ringed with nail polish to make the microslides semipermanent.
**Scanning electron microscopy**

For examination with the scanning electron microscopy, the whole animal or mature eggs were prepared according to the particular treatment indicated in the text then attached to specimen holders with silver paint, coated with gold and palladium and then examined with a JSM-S1 scanning electron microscope.

To prepare mature eggs for this observation, the following techniques were applied:

1. Oviposited eggs were dry frozen over cold acetone and transferred to a freeze dryer for 24 hours or longer.

2. Eggs from the ovary were fixed in 4 percent gluteraldehyde for two hours and post fixed in 1 percent osmium tetroxide for 1.5 hours. Fixed eggs were washed in the buffer (sodium cacodylate, pH 7.2) for 5 minutes, dry frozen over cold acetone, transferred to a freeze dryer for 24 hours or longer.

3. Eggs from the ovary were fixed in gluteraldehyde and osmium tetroxide (as in 2), dehydrated in series of alcohol, passed through three changes of propylene oxide for total of 40 minutes, and placed in an oven (60°C) for 20 minutes.

Only the second and the third techniques gave satisfactory
results.

To examine the genital opening of the male and the female, specimens were fixed in 4 percent gluteraldehyde for 6 hours, washed in distilled water for 15 minutes and then postfixed in 2 percent osmium tetroxide (cacodylate buffer, pH, 7.2) for two hours. Specimens were then washed in the buffer, dehydrated in series of alcohol, and passed through three changes of propylene oxide (30 minutes each), transferred to aluminum weighing pans and kept in an oven 60°C for 20 minutes after which they were moved to a freeze dryer for 24 hours or longer.
OBSERVATIONS

Two species of ticks, the American dog tick, *Dermacentor variabilis* (Figures 1, 2) and the wood tick, *D. andersoni* (Figures 3, 4) were used in the present study. Observations were made on the male and female specimens in order to determine the typical morphology of the male and the female reproductive systems and the changes in morphological features during the attaching and feeding of ticks on the host. Reproductive biology was examined by studying the following events: copulation and insemination, spermatogenesis, sperm morphology, sperm motility, oogenesis and sperm-egg interactions. Observations refer to both species unless indicated otherwise.

Male Reproductive Systems

**Gross anatomy**

The male reproductive system is located in the anterior half of the body cavity and anterior to the rectal sac. The major functional and histological divisions of this system (Figures 5, 6) include the testes, vasa deferentia, seminal vesicle, ejaculatory duct, and genital opening. In addition, well developed accessory glands are associated with the seminal vesicle.
**Testes**

Testes are paired. Each testis is an elongated tubular organ and is coiled in a particular pattern (Figures 5, 6). The testes are narrow at the anterior ends near the junction with the vasa deferentia and gradually widen towards the posterior ends where both organs are slightly bulbous and in close proximity. Each testis consists of groups of germinal cells arranged in cysts in the testicular wall. A cross section of the testis (Figure 7) reveals eight such germinal cysts arranged radially around a small lumen. This lumen extends the length of the testis and is continuous with the lumen of the vas deferens. Externally the testes are layered by thin covers of connective tissue (Figure 7).

The testes of unfed males are relatively small, approximately 1.5 mm long; however, after attaching to the host and the initiation of feeding, the testes gradually enlarge, a process related to the formation of new germinal cysts. As a result of this continuous enlargement, the testes increase considerably in length and diameter and become increasingly coiled. After four days of attachment to the host, the enlargement forces the anterior portion of the testes beyond the margin of the accessory glands (Figures 5, 6). After five days of attachment or at the time of full engorgement, the testes are approximately three times the original size. At this time numerous
elongated spermatids (immature spermatozoa) have formed and passed through the lumen of the testes to the vas deferens and have accumulated in the seminal vesicle and ejaculatory duct (Figure 13). This phenomenon will be discussed thoroughly in the section, Reproductive Biology of Ticks.

**Vasa deferentia** Near the anterior tip of each testis is the connection with the vas deferens, a narrow coiled tube (Figures 5, 6). The two vasa deferentia fuse into a common seminal vesicle anterior to the testes. In cross section, each vas deferens is lined with cuboidal epithelium and is covered with a thin layer of epithelial cells (Figure 15). In unfed males, the diameter of the vas deferens is approximately 12 μ. After attachment and feeding the diameter doubles in size as a result of cell enlargement.

**Seminal vesicle and ejaculatory duct** The seminal vesicle is a small triangular sac (Figures 5, 6) which is formed by the union of the two vasa deferentia and can only be recognized from the ventral side of the genital system since the posteriordorsal lobe of the accessory glands usually covers this structure. The seminal vesicle is difficult to observe in unfed males, but enlarges after attaching to the host and feeding. In cross sections, this organ contains a distinct lumen lined with a layer of
cuboidal epithelium and is coated externally with a thin layer of flat epithelial cells (Figure 14). The seminal vesicle serves two important physiological purposes: (1) a reservoir for the accumulation of immature spermatozoa (elongated spermatids) and (2) a recipient of various secretory products from the accessory glands. These products form the spermatophore and part of the seminal fluid.

The seminal vesicle opens into the ejaculatory duct which is a short narrow tube. The ejaculatory duct is lined with a thin chitinous intima which is covered with a single layer of small epithelial cells (Figure 16). The ejaculatory duct extends to the genital opening. This organ also enlarges after attaching and feeding, and stretches considerably during copulation with the passage of the spermatophore.

**Accessory glands**  Accessory glands of the male are well developed and constitute approximately one-third of the genital system. These glands consist of three major lobes: the anteriodorsal lobe, the ventromedial lobe, and the posterioventral lobe. The anteriodorsal lobe is a single and relatively large lobe which extends anteriorly beyond the ejaculatory duct. The anterior end of this lobe is slightly wider than the posterior end and forms two lateral projections (Figures 5, 6). The ventromedial lobe
which is the largest of the three major lobes is an oval structure close to the tick ventral body wall. This lobe gives rise to three paired lateral lobules: the dorso-laterals, the lateroventrals, and the posteriolaterals. The posteriorventral lobe is elongated and curves inwardly beyond the posteriolateral lobules. As in other male organs, the accessory glands enlarge during attaching and feeding on the host and by the fourth day of attachment to the host will double in size.

Each lobe of the accessory glands contains a lumen in which the accessory products accumulate (Figure 17) and pass to a duct connected to the seminal vesicle. These lumens are lined with elongated epithelial cells averaging 17 μ long and containing large oval nuclei (Figures 17, 18, 19). The cytoplasm of these cells contain numerous secretory droplets of various sizes which are used to build the spermatophore during insemination. Externally, the secretory glands are coated by a thin sheath of connective tissue which is covered with epithelial cells (Figures 17, 18, 19).

Female Reproductive System

Although the anatomy of the female genital tract was studied in both species, more emphasis was placed on D. variabilis. Since adequate observations (Brinton and Oliver, 1971a) were made on ovarian development in D. andersoni,
this species was only superficially examined in the present study. Observations of gross anatomy and histology aid in determining the general features of the reproductive system before and after attachment to the host.

Gross anatomy

The major regions of the female reproductive system (Figures 20, 31) include a single ovary, two oviducts, a seminal receptacle, a vagina, and a genital opening. Two small tubular accessory glands are associated with the vagina.

Ovary In unfed females the single U-shaped ovary is very small and only becomes greatly enlarged after engorgement. The arms of the U-shaped ovary extend posteriorly on both sides of the body cavity while the base or central part is anterior to the rectal sac. In cross sections, the ovary consists of a distinct lumen which extends the entire length of the ovary and is continuous with the lumen of the oviduct (Figure 33). The ovarian wall consists of epithelial cells, interstitial cells, funicular cells, oocytes, a membranous layer of tunica propria (Figure 23), and externally a few scattered epithelial and muscle cells attached to the ovarian wall (Figures 21, 22, 23). Lining the ovarian lumen are epithelial cells containing relatively large nuclei (Figure 21). The interstitial cells are between and above these epithelial
cells and have numerous projections and intercellular connections (Figure 23). The funicular cells are pedicle-like and attach the oocytes to the ovarian wall (Figures 21, 22, 24).

After the female attaches to the host and starts feeding, the ovary undergoes distinct changes in shape and morphology. The regional differences occurring in the ovarian wall shortly after attachment are primarily due to the growth of oocytes. Forty-eight hours after attachment to the host, the ovary shows a progressive increase to thrice the size (both in diameter and length) of the unfed female ovary. Oocytes are classified into two sizes which are related to locations. The "longitudinal groove" which is evident and extends most of the ovarian length contains oocytes approximately half the size of oocytes comprising the remaining ovarian wall. After four to eight days of feeding, the oocytes on the ovarian wall increase considerably in size and eventually protrude into the haemocoel (Figure 28). Also at this time, the longitudinal groove is more evident and becomes convoluted giving the ovary a slightly contorted and collapsed appearance. Approximately four to six days after attachment to the host, the enlargement of the ovary has slowly increased to a maximum size. Usually mating occurs at this time and a further rapid increase in oocyte growth is stimulated. Within
three to four days after mating, the female attains full engorgement and drops away from the attachment site. By this time the ovary has increased in length and diameter to about five to six times the original size and oocyte maturation has been greatly accelerated, although variation of sizes is still prevalent. For example, the oocytes at the longitudinal groove and the termini of the ovary are always smaller than those occupying the rest of the ovarian wall.

One or two days after engorgement, most eggs have presumably been fertilized and their development proceeds with the formation of numerous yolk bodies (Figures 26, 27). Four to five days after engorgement, mature fertilized eggs pass to the ovarian lumen which becomes extended and subsequently the eggs pass to the oviduct where they are linearly arranged. One week after engorgement (Table 2) oviposition starts and continues for four to five days. Oviposited eggs range from 350-450 μ in diameter. Although some eggs remain in the ovarian wall after oviposition, most of the ovarian wall is now occupied with a large number of convolutions and collapsed areas which consist of the remaining tunica propria and degenerating funicular cells (Figures 29, 30).

Ovaries of unmated but engorged females are always less developed than the ovaries of engorged females which have
been mated. In addition, comparison with the mated condition, unmated females needed a longer time for engorgement. Oocytes develop slower, mature eggs were not observed in the ovary, and oviposition did not occur.

**Oviduct** The oviducts extend anteriorly from each end of the U-shaped ovary. These organs are narrow convoluted tubes which slightly widen and coil several times in close proximity before joining the seminal receptacle on the tick ventral side. In cross sections, the oviduct consists of a lumen which is continuous with the lumen of the ovary and is lined with cuboidal epithelium that gradually becomes columnar near the junction of the oviduct with the seminal receptacle. Externally, few scattered epithelial and muscular cells are attached to the outer wall of the oviduct (Figures 32, 33).

The oviducts of unfed females are very narrow and average 65 μ in diameter. After attachment of the female to the host and the initiation of feeding, the luminal epithelial cells multiply, elongate, and exhibit club-shaped tips (Figure 32). These epithelial cells also form folds which fuse together to form a network (Figure 32). The diameter of the oviducts progressively increases and after four days of attachment to the host the diameter is approximately three times the original size. The oviducts continue to enlarge until full engorgement occurs.
One or two days after full engorgement or four to five days after insemination, spermatozoa are seen in the lumen of the oviducts (Figures 34, 35) migrating towards the ovary. The eggs are fertilized in the ovary, then pass into the ovarian lumen, and shortly afterwards into the oviducts. Passage of these eggs causes considerable stretching of the oviducts. After oviposition, the oviducts still contain a large number of eggs which are packed along the lumen (Figure 31).

**Seminal receptacle** The seminal receptacle is a sac-like trilobed structure lying ventromedially in the anterior part of the body cavity. This organ receives the two oviducts on the ventral side at the level of the wide region (Figures 20, 31). In unfed females, the seminal receptacle is a very narrow structure (Table 1) but enlarges considerably during attachment to the host. In cross sections, the lumen of the seminal receptacle is lined with columnar epithelium which becomes highly folded during engorgement (Figures 36, 39), and is coated on the outside by a thin layer of connective tissue and muscular cells. After mating, the seminal receptacle increases to about six times the original size (Figure 39) and becomes easily recognized in dissected animals. At this time, at least one endospermatophore (Figure 37) is present inside the seminal receptacle, but as many as three have been observed. For
example, in most females collected locally from natural hosts, the seminal receptacle contained two or three endo-spermatophores. The immature spermatozoa within the endo-spermatophore remain in the seminal receptacle for approximately three to four days before they reach maturity and become freely motile. At this time, the wall of the endo-spermatophore dissociates and the now mature spermatozoa migrate through the oviduct (Figures 34, 35) towards the ovary.

After oviposition, some spermatozoa are still present in the seminal receptacle of *D. andersoni*. Also numerous macrophage-like cells which contain large pigmented granules (Figures 38, 40) are present and appear to be engulfing these remaining spermatozoa. In *D. variabilis*, no spermatozoa were seen in the seminal receptacle after oviposition.

**Vagina and accessory glands**  
The vagina in both species is a short tubular structure which is very narrow in unfed females (Table 1), but increases considerably during feeding and stretches maximally during oviposition (Table 1). The posterior end of the vagina is connected to the narrow anterior end of the seminal receptacle and the anterior end of the vagina is connected to the genital aperture (Figure 20). The lumen of the vagina is lined with a thick layer of cuticle and covered with a layer of columnar epithelium (Figure 43). Externally to the
columnar epithelium is a well developed muscular layer.

Associated with the vagina at the junction with the seminal receptacle is a pair of tubular accessory glands which also increase in size during feeding (Table 1). These accessory glands consist of columnar epithelium containing secretory products (Figure 42) which pass into the vagina during oviposition. Externally, the accessory glands are coated with a layer of flat epithelial cells.

Reproductive Biology of Ticks

The events associated with copulation and insemination, spermatogenesis, sperm morphology, sperm motility, oogenesis and sperm-egg interactions have been examined and a general pattern of tick reproduction involving these events has been determined. A special interest in this study was to determine the site of sperm-egg interactions. The chronology of reproductive events in the female of D. variabilis is given in Table 3. A summary of life cycles for both species is given in Table 2. In general all the following statements relate to both species unless otherwise stated.

Copulation and insemination

After feeding on the host, several observations were made of ticks in the copulatory position; a male and a female tick attached with their ventral sides facing each
other. After allowing ticks to feed separately on the host, opposite sexes were placed together in wax plated dishes after removal from the host and the following observations were made. Within a few minutes, the male creeps onto the back and crawls down to the ventral side of the female. The female reacts with rhythmic contractions of the surrounding area of the genital aperture. As the male crawls back and forth, he is constantly moved towards the female genital aperture by movement of the female legs. This process continues for 10 to 15 minutes ending with the male being directed to the genital opening with his anterior end facing forward. In this position, the ticks clasp from four to five minutes to 60 minutes. However, during this period, the ticks frequently separate for short durations. If they separate permanently, the procedure is repeated by returning the animals to the host for one or two more days. Eventually, this whole process culminates in the transferring of the spermatophore and insemination.

Generally during the clasping period, the first legs of the male are between the first and the second legs of the female, and so on. This body holding and back and forth movement represents some courtship pattern. After this courtship, the insemination process starts when the male bends his capitulum and introduces his mouth parts in the female genital opening. The pair remains in this position
from five to 15 minutes, during which time the male raises his body slightly and extrudes from his genital opening a dumb-bell shaped spermatophore 3 mm long (Figure 44). The spermatophore is quite stout with a smooth glistening texture and consists of an ectospermatophore (which contains the immature spermatozoa) connected to an endospermatophore by a slender neck. After this extrusion, the male withdraws his mouthparts, carries the spermatophore with his chelicerae, and implants the spermatophore neck into the female genital opening. Immediately after this implantation, the endospermatophore passes through the vagina and into the seminal receptacle. The completion of the spermatophore transfer from the male to the female constitutes insemination.

After insemination, the male usually left the female five to 10 minutes after the process was completed, an observation made in 11 separate cases. However, in one single observation, the male remained for three hours. In this latter case there was no reoccurrence of insemination. In three separate cases of observed copulation of ticks on a dog, two or three males were observed attached to one female. In each case then the female was dissected, two to three endospermatophores were found in the seminal receptacle. Whether only one male or each male copulated with the female is not known. However, in view of the above
observations and the fact that many engorged females collected from dogs contained more than one endospermatophore in the seminal receptacle, it is strongly suggested that each male mates once during a courtship.

During or shortly after spermatophore transfer, spermatozoa move from the ectospermatophore to the endospermatophore. Once this has occurred, the now empty ectospermatophore (Figure 45) extends from the female genital opening for approximately four hours before dropping off.

Spermatogenesis

For study of spermatogenesis, observations were made on spermatocytes, spermatids, and immature spermatozoa from the male reproductive system. Mature spermatozoa were examined from the female seminal receptacle. The time of events as well as the morphological aspects were correlated.

Spermatocytes of unfed adult males are spherical and contain relatively large nuclei (Figure 7). After attachment of those males to the host and the initiation of feeding, these spermatocytes progressively enlarge (Figures 8, 9). Simultaneously with this enlargement, Golgi bodies, multivesicular bodies and tubular-shaped endoplasmic reticula become evident (Figure 46). In particular, after two to three days of feeding, a narrow striated border
appears around the periphery of the spermatocyte (Figure 10). This unique border consists of cup-shaped or circular-shaped subsurface cisternae containing cytoplasmic materials which in many cases are less dense than the rest of the spermatid, but are similar to those of the nutritive somatic cells (Figure 46). As the spermatocytes continue to enlarge, the subsurface cisternae elongate in shape, increase in size and number and thus become considerably crowded (Figures 48, 49).

Three days after attachment of the male tick to the host, the spermatocytes undergo differentiation, enlarge considerably and form early spermatids. By the fourth day of attachment, these spermatids are prominent in the testes. They are spherical and are usually surrounded by nutritive somatic cells. Frequently between the spermatids, intercellular junctions are observed (Figures 47, 48). In addition to the previously mentioned subsurface cisternae which are continuously increasing in number, the spermatid contains relatively large numbers of Golgi complexes, mitochondria and multivesicuclar bodies (Figures 49, 50).

During spermiogenesis or sperm maturation, the spermatid passes through two important morphological transformations: "polarization" and "invagination". Polarization consists of the movement of the nucleus to one pole and the accumulation of subsurface cisternae at the other pole of the spermatid
After polarization is completed, the subsurface cisternae undergo multiple fusion at their distal tips to form a membrane which encloses the cisternae around one cisternal cavity (Figures 52, 53, 54, 55). The subsurface cisternae then fuse longitudinally to form long rod-like structures which project into the cisternal cavity (Figures 54, 55, 56a). These structures are the "cellular processes" which are instrumental in sperm motility.

After the formation of the cisternal cavity (Figure 56a), the invagination process begins. Adjacent to the nuclear pole, the inner wall of the cisternal cavity invaginates into the cavity in a unique process which leads to the formation of an inner tube (or invagination tube, Figure 56b). The cellular processes now extend from the outer sides of the inner tube and from the inner side of the outer tube (Figures 56c, 57). During this invagination process most of the cytoplasm including mitochondria are incorporated into the invagination tube (Figures 56c, 57). As a result, the inner tube becomes enveloped by the outer tube which is enclosed externally by the cell plasma membrane (Figures 56c, 57, 58). During these events the spermatid gradually elongates (Figures 56b,c, 57). The nucleus elongates and migrates between the outer tube and this cell membrane and moves towards the anterior part of the elongated spermatid (Figures 58, 59). The nucleus eventually
becomes smaller, more compact, and spatula-like. After the inner tube has extended most of the spermatid length and has approached the tip of the outer tube, the spermatids are released from the testes and pass through the testicular lumen to the seminal vesicle (Figure 13).

In the seminal vesicle, the elongated tube-like spermatids do not undergo any obvious morphological changes. At this stage of development, the elongated spermatids (immature spermatozoa) of *D. variabilis* are 75 μ to 80 μ long and 4 μ to 5 μ in diameter while those of *D. andersoni* are 100 μ to 110 μ long and 4 μ to 5.3 μ in diameter. In general, the elongated spermatid has a pointed anterior end and a broad posterior end (Figures 60, 61). The broad end appears open due to the presence of a groove-like depression running from the broad end laterally along the spermatid (Figure 60). In cross section, the inner tube contains an inward projection from the side of the inner tube which resembles the typhlosole of the earthworm gut (Figures 63, 64). During or shortly before copulation, the immature spermatozoa are packed in a spermatophore which is transferred to the female genital opening. The immature spermatozoa or late spermatids are then introduced enclosed in the endospermatoaphore into the female seminal receptacle.

In the female seminal receptacle, the last stage of spermiogenesis is resumed. Three to four days after
insemination, the development of mature spermatozoa is completed (Figures 56d, 61, 62). This final development begins when the plasma membrane overlying the anterior end of the spermatid ruptures (Figures 56d, 61, 62) and the tip of the inner tube penetrates through the outer tube. This penetration of the outer tube constitutes the process of "evagination" (Figure 56d) and allows the inner tube to become exposed. The inward projection of the inner tube is expanded and the newly exposed anterior end (club region) of the spermatozoon is considerably wider than in the spermatid. The ruptured plasma membrane of the immature spermatozoa retracts and is gradually brought to the middle of the posterior cytoplasm and appears later as a long "filament canal" (Figures 56d, 81, 82). After the process of evagination and the exposure of the inner and outer tube is completed, the previous anterior end of the outer tube is now the sperm posterior end which is located at the end of the filament canal and is represented in the form of a "plug-like structure" which contains numerous tubules of various sizes (Figures 56e, 84, 85). The nucleus elongates further and becomes located lateral to the filament canal. Due to this final maturation change, the spermatozoon doubles in length and acquires a narrow posterior end and a wide anterior club region. At this stage, the spermatozoa are motile and when the wall of the endospermatophore
dissociates (four days after insemination) they migrate through the oviduct (Figures 34, 35) into the ovary where sperm–egg interactions take place.

Sperm Morphology

Spermatozoa of both species are similar in all aspects except the size. The mature spermatozoon of *D. andersoni* is 220 µ long, while that of *D. variabilis* is only 140 µ long. The spermatozoa are aflagellate and tubuliform and are shaped like a flat Indian club (Figures 65, 66, 67, 68). The anterior end is wider than the posterior end and a unique motile organelle is located at the tip. The club becomes thinner towards the middle part and joins the slender posterior region which contains an elongated nucleus, an acrosome, and a small plug-like structure.

The following description refers to spermatozoa of both species unless indicated otherwise. The mature spermatozoon can be divided into four regions:

1. the motile organelle at the anterior tip;
2. the club region;
3. the middle region;
4. the posterior region.

The motile organelle

The "motile organelle" is a narrow projection-like structure (Figures 66, 67, 68) located at the anterior tip
of the spermatozoon. This organelle is 3.0 to 3.5 μ in diameter and 3 μ high and acquires different shapes during movement of the spermatozoon. In fixed or dead specimens, this organelle is always a ring-like structure (Figures 69, 70). The cytoplasm of the motile organelle is not as dense as the rest of the spermatozoon, has no mitochondria, and contains numerous microtubules (Figures 71, 72, 73). The surface of the motile organelle (as is true with all of the sperm surface) is covered with cellular processes. However, a distinct constriction which occurs at the junction between this organelle and the club region (Figure 72) is devoid of cellular processes.

**Club region**

This is the widest region of the spermatozoon (Figures 66, 67, 68) and is connected anteriorly to the motile organelle and posteriorly to the middle region. This region averages 47 μ long in *D. andersoni* and 30 μ in *D. variabilis*. The diameter ranges from 5 μ to 8 μ, but becomes gradually thinner towards the middle region. In addition of being the widest part, this region is also characteristic of having large numbers of mitochondria arranged in a particular pattern. Near the motile organelle, the mitochondria are round in shape and form numerous aggregates (Figure 74) but through the main body of the club
region, the mitochondria are elongated, less numerous, and do not form aggregates (Figure 75). Near the middle region, the mitochondria are mainly located near the periphery (Figure 76).

As mentioned, the surface of this region is covered with cellular processes, and closely associated with these processes are numerous subsurface filaments which range from 130 Å to 160 Å in diameter (Figure 80). Large numbers of small endoplasmic reticula are equally distributed in this region.

**Middle region**

Immediately posterior is the middle region which is narrower in diameter than the anterior club region. The middle region is 55 µ long and 2.5 µ in diameter in spermatozoa of *D. andersoni* and 32 µ long and 2.0 µ in diameter in *D. variabilis*. This region is also covered with cellular processes and contains numerous subsurface filaments associated with the cellular processes (Figure 80). The cytoplasm in this region is denser than the club region and contains fewer mitochondria. Similar to the posterior portion of the club region, the mitochondria are primarily distributed peripherally (Figure 78). During sperm motility, this region and the club region twist in a spiral manner and in some cases the spermatozoon folds backwards in the
middle region (Figure 79).

Posterior region

The final or posterior region is a continuation of the middle region and gradually tapers to the sperm posterior tip. This region is 95 \( \mu \) long in spermatozoa of \emph{D. andersoni} and 70 \( \mu \) long in spermatozoa of \emph{D. variabilis}. The diameter ranges from 1.3 \( \mu \) to 2.4 \( \mu \). Throughout most of this region is the unique filament canal (Figures 81, 82) which has formed as a result of the unusual spermiogenetic process.

The posterior region amazingly contains most of the sperm structures which are equivalent to the head and middle piece of a typical spermatozoon. These structures include the nucleus, acrosome, and centrioles (Figures 65, 84, 85, 86). The nucleus is a thin, dense and elongated structure located laterally to the filament canal and is 84 \( \mu \) long in spermatozoa of \emph{D. andersoni} and 59 \( \mu \) in those of \emph{D. variabilis}. Lateral to the nucleus and next to the sperm membrane is an oval-shaped acrosome which is 5 \( \mu \) long (Figures 56e, 67) and is connected to the nucleus by a rod which continues through the nucleus (Figure 83). When examined by fluorescent microscopy using acridine orange, the nucleus stains green and the acrosome stains red, a criterion for the presence of an acrosome (Figure 67). Two centrioles are located near the end of the posterior region (Figure 86) but are quite difficult to demonstrate adequately.
Mitochondria in this region are relatively few in number, round in shape, and located laterally to the filament canal. At the posterior tip of the posterior region, a membrane bounded plug-like structure is demonstrated (Figures 84, 85) which is associated with the posterior end of the nucleus. This plug-like structure which is the remaining part of the outer tube after the completion of the evagination process during sperm maturation, is 2.1 μ long and 0.9 μ in diameter, and contains numerous tubules of various diameter ranging from 0.1 μ to 0.6 μ in diameter (Figure 85).

**Surface structure**

The surface of the mature spermatozoon is covered with cellular processes which develop as infoldings from the cell membrane during spermatogenesis. These cellular processes are dense plate-like structures which average 0.17 μ thick, 0.65 μ high, and 7.0 μ long and are arranged in rows along the surface of the spermatozoon (Figures 87, 88). Each dense plate-like structure is enclosed in a loose and corrugated membrane. The cellular processes are so patterned in their distribution that usually when one process ends another starts. However, in some cases the processes are not continuous.

The cellular processes are attached to the sperm surface by small narrow, connections about 500 to 700 Å wide.
In cross sections, these cellular processes appear as projections which are narrow at the surface of the spermatozoon and widen at the top (Figures 74, 75, 78, 79, 89). These processes are not of the same height throughout the sperm surface. For example, in cross sections of the club and middle regions, these processes are found at two separate heights. The arrangement is such that every third, fourth or fifth cellular process is the same height (0.83 μ) while those located between have a lower height (0.56 μ) (Figures 78, 79, 89). In the posterior region of the spermatozoon, the cellular processes are the same height and average 0.2 μ high.

Sperm Motility

The present investigation has demonstrated some previously unknown phenomena in regards to tick sperm motility. Sperm movement has been casually observed by several investigators, but only recently has this motility been considered unique in the animal kingdom. In order to describe this unusual process, mature spermatozoa from dissected seminal receptacles of both species were examined with various optical systems and cinemicroscopy.

During motility, spermatozoa of both species follow a certain sequence of changes which involve the motile organelle and the cellular processes on the surface of the spermatozoon.
In reference to the former, the following five phases (Figures 90-94) or steps of sequential changes are involved:

1. The motile organelle revolves slowly to the right,
2. elongates and frequently acquires a distinct projection-like structure,
3. bends backwards either slightly or sharply,
4. suddenly stretches, and
5. finally retracts slowly to acquire a ring-like shape (Figures 95-99). This sequence is repeated as long as the spermatozoon is moving.

In addition to the movement of the motile organelle, the cellular processes also take place in the process of motility. The cellular processes cannot be resolved by the light microscope, but in reference to their ultrastructure, arrangement and attachment to the sperm surface, the sperm forward movement is directly correlated to these processes. A combination of the motile organelle movements and the cellular processes move the spermatozoon forward in a gliding and slightly twisting manner. An unknown phenomenon is the frequent bending backwards occurring in the club and middle regions (Figures 67, 79).

The spermatozoon of *D. variabilis* moves about 4 to 7 µ/sec while that of *D. andersoni* moves about 6 to 10 µ/sec. By using the club region for observation, the spermatozoon completes one revolution in a distance of 15 µ. During motility, with the exception of the five described stages of the motile organelle and the bending of the club and
middle regions, no other movements of the spermatozoon are observed. However, if small particles come in contact with the club region, they slide rapidly to the posterior end of the club region indicating a surface movement which must be correlated to the cellular processes.

Oogenesis

Oocyte maturation was studied primarily in D. variabilis since an adequate recent study of D. andersoni has been performed (Brinton and Oliver, 1971b). Observations of oogenesis were made on adult females from the period before attachment to the host, during attachment to the host, after full engorgement, and shortly before oviposition.

The day of attachment to one day after attachment to the host

As mentioned previously in the description of the female genital tract, the ovary of adult unfed females consists of luminal epithelium, interstitial cells and oocytes which are attached to the ovarian wall by funicular cells. At this time, before attachment to the host, the oocytes are relatively small, average 20 to 25 μ in diameter, and contain large nuclei which are elliptical to spherical in shape. After attachment to the host and the initiation of feeding, the oocytes gradually enlarge until mature eggs are developed, a process which is not completed until a few days after the female leaves the host. After
one day of attachment to the host, some dense, particulate materials begin migrating through the nuclear membrane to the ooplasm (Figures 100, 101). A considerable number of well differentiated, round to elongated mitochondria and a few endoplasmic reticula are distributed in the ooplasm.

Two days after attachment to the host  At this stage oocytes are of two different sizes: small oocytes in the longitudinal groove varying from 25\(\mu\) to 30\(\mu\) in diameter and larger oocytes which are outside the longitudinal groove and range from 45\(\mu\) to 60\(\mu\) in diameter. At this time, the larger oocytes are developing new structures and undergoing changes. For example, small groups of projecting microvilli appear on the surface of the oocyte and bend in different directions forming a thin border under the tunica propria (Figures 102, 103). Present for the first time in different areas of the ooplasm are multivesicular, membrane-bounded bodies which are formed by the fusion of ribosomes and Golgi complexes (Figure 103). Mitochondria are distributed throughout the ooplasm and at various times form aggregates around the nucleus. Sparsely diffused endoplasmic reticula are also present (Figure 102). Emissions of dense granular material via the pores of the nuclear membrane are observed frequently and result in the formation of extranuclear granular bodies (Figure 103) which are often associated with mitochondria.
Four days after attachment to the host Oocytes are continuing to enlarge and measure 83 μ in diameter. Accompanying this enlargement is additional development of the microvilli forming a distinct brush border under the tunica propria (Figure 105). Although this border of microvilli measures 3 μ wide in some oocytes, many different stages of microvillar development are observed in other oocytes. As after two days of attachment to the host, dense granular materials are continuously passing through the nuclear membrane (Figure 104). These dense materials are accumulating in juxtaposition to the outer periphery of the nucleus. As with many nuclei during high synthesis periods, these oocyte nuclei at this time contain well developed nucleoli (Figure 104). Mitochondria are well distributed in the ooplasm, but are more abundant around the nucleus.

Six to eight days after attachment to the host Most of the oocytes at this stage exhibit a distinct irregular brush border of microvilli (Figure 106). Ribosomal particles are abundant and multivesicular bodies continue to be present.

In addition to the formation of numerous microvilli, a process of micropinocytosis occurs around the entire periphery of the oocytes which suggests that oocytes at this stage are active in obtaining extra-oocytic nutritive material. The micropinocytotic pits vary in diameter from
77 to 130 μ⁣ and are observed around the periphery of the oocyte. Amorphous materials are present along the plasmalemma and are often concentrated in these micropinocytotic pits. Also micropinocytotic tubes containing amorphous materials extend from the oocyte periphery through the ooplasm and are associated with irregular membrane-limited reservoirs located in the peripheral regions of the ooplasm and contain amorphous materials (Figures 107, 108). Evidently, the micropinocytotic tubes pass materials from the periphery of the oocytes to accumulate in the reservoirs. In addition to this extra-oocytic source of nutritive materials, the funicular cells quite possibly also function in this category besides attaching the oocyte to the ovarian wall. Some microvilli of the oocyte are in intimate contact with the microvilli from funicular cells. At the site of the contact with funicular cells, the oocyte has a micropyle which is 5 to 8 μ⁣ in diameter and is directed towards the ovarian lumen (Figure 24). This micropyle is the site for sperm-egg interactions.

In this stage also, oocytes are engaged in a process of vitellogenesis. This proceeds with the formation of small spheres or yolk bodies (Figures 107, 108) which enlarge, become crowded and compressed (Figure 108) and after eight days of attachment to the host are quite obvious with diameters measuring approximately .4 μ⁣. Mitochondria,
ribosomes, and membrane-bounded reservoirs filled with amorphous material are in close associated with these vitelline spheres.

During this attachment period of the female ticks to the host, insemination occurs. While oocytes are still progressing in development and maturation, spermatozoa also are undergoing maturation in the seminal receptacle and upon completion migrate to the ovary through the oviduct (Figures 34, 35). Developing oocytes are fertilized in the ovary shortly before the time of full engorgement which is equivalent to the fifth or sixth day after insemination.

**Two days after engorgement** After the eggs are fertilized, they enlarge considerably and by this stage measure approximately 200 to 240 μ in diameter. Numerous yolk bodies and lipid droplets are present in the ooplasm (Figure 109) and presumably are fusing to form larger yolk bodies. These then migrate from the peripheral region towards the middle of the oocyte. Many mitochondria have become trapped between the enlarging yolk bodies and eventually are absorbed (Figure 110). Also numerous ribosomes and endoplasmic reticula are crowded between the yolk bodies. At this stage, the fertilized eggs are ready to be released in the ovarian lumen in one or two days.
**Vitelline envelope formation**  
Elaboration of the vitelline or the cuticular envelope which surrounds the eggs is initiated six to seven days after attachment to the host. Deposition of cuticle is first observed between the bases of the microvilli as small darkly stained units on the plasmalemma, indicating that the deposition of cuticle is from an intra-oocytic source and not from an extra-oocytic source. The external margin of the cuticle is relatively smooth while the internal surface is more irregular and contains many depressions. This is due to irregular deposition of cuticle and suggests that it occurs through certain areas of the plasmalemma.

During elaboration and deposition, the cuticle is a sieve-like structure with many pores through which one or more microvilli extend (Figure 109). As cuticular deposition progresses and enters the terminal stage, microvilli become fewer in number, smaller in diameter, and appear to shrivel and retract from the cuticle. The pores in the cuticle which contained microvilli eventually close.

Before considerable deposition of cuticle occurs, the oocytes are fertilized, sometime between the fifth or sixth day after insemination, which is approximately equivalent to the time of full engorgement. After fertilization, continuous deposition of cuticle subsequently seals the egg micropyle. As previously mentioned, no fully mature eggs
were observed in unmated engorged females.

**Fertilized eggs** After fertilization, fertilized eggs or zygotes progress in development until maturation is completed. These fertilized eggs on the ovarian wall range from 280 to 420 μ in diameter. At this stage, when the ovary is dissected in arachnid solution, the tunica propria swells and appears as a loose outer layer (Figure 28) which resembles the jelly coats of frog eggs. The loose tunica propria can be separated easily to expose the cuticle. When fertilized eggs are released from the ovarian wall into the ovarian lumen, no micropylles are present and the tunica propria and funicular cells are left on the ovarian wall appearing as collapsed areas (Figures 29, 30). After the fertilized eggs are in the lumen, they move into the oviduct in a linear arrangement (Figure 31). From observations with the scanning electron microscope, the cuticle exhibits an uneven surface which consists of spherule-like structures (Figures 112, 113) ranging from 0.6 μ to 1.1 μ in diameter. In addition, small pits or pores averaging 0.3 μ in diameter are observed between these spherules (Figure 113) and are presumably due to the previous presence of microvilli in the region of cuticular deposition.

Oviposited eggs (zygotes) have an amber color and sticky texture due to the waxy coating from the Gene's organ (see female accessory glands in the Introduction).
During oviposition, the fertilized eggs pass through the genital opening and aggregate into clusters on the female skin near the scutum. Oviposited eggs are slightly oval and range from 370 to 510 μ in diameter. They are quite impermeable and very difficult to fix or section.

**Sperm-Egg Interactions**

Previous to the present study, neither sperm-egg interactions nor the site of these events had been observed. The method of dissecting engorged females at various periods of time after insemination was designed to observe this phenomenon. After four to six days of insemination, initial sperm-egg contacts were observed six times. The spermatozoon was observed extending into the ovarian lumen at the site of the micropyle with the motile organelle towards the egg proper (Figure 114), a position suggesting that the motile organelle is the first sperm-structure to interact with the oocyte. However, actual contact and penetration of the spermatozoon into the oocyte was not observed. Before sperm-egg interactions occur, the egg micropyle is open to the ovarian lumen (Figure 114) and presumably shortly after sperm-egg interactions the micropyle is sealed by deposition of cuticle (Table 3). This closure of the micropyle correlates with the time of full engorgement or seven days after insemination off the host.
Observations of sperm-egg interactions in vitro were also attempted. Mature and motile spermatozoa from the female seminal receptacle were mixed with oocytes or strips of ovary from the same or different female. Spermatozoa were observed to move towards the oocyte (Figure 115) and in a few cases a slight increase in the sperm speed was noticed. Generally, several spermatozoa were observed to move towards the oocyte. One phenomenon observed was the stopping of several spermatozoa approximately 20 μ from the oocyte. However, in most cases, each spermatozoon made contact with the oocyte and maintained contact (Figure 116) from one to approximately 15 minutes before moving away. In exceptional cases, spermatozoa stayed in contact with the oocyte for the whole period of observation (two hours). No actual penetration was observed. On the other hand, some spermatozoa moved above or under the oocytes without showing any signs of contact. When fertilized eggs or zygotes were used, the spermatozoa did not significantly move towards them. In all these observations, when the spermatozoon moved towards the oocyte, the motile organelle was the first sperm structure to come into contact with the surface of the oocyte.
DISCUSSION

The major observations made in the present study on two species of ticks, *D. variabilis* and *D. andersoni* are concerned with the processes of gametogenesis, sperm transfer, sperm storage, sperm morphology, sperm motility, and sperm-egg interactions. Since, in various animal groups, a definite relationship exists between sperm morphology and the biology of fertilization, functional implications of the various organelles in this unusual spermatozoon described and the sperm structure is discussed in relation to the process of fertilization. The sperm structure is also discussed in relationship to the processes of copulation and insemination, spermatophore production, sperm storage, and sperm maturation. In addition, the implications of sperm morphology, sperm structure, and sperm-egg interactions are discussed in relation to arachnid phylogeny, and finally an attempt is made to determine the origin of the unique reproductive features in ticks.

Typical Tick Spermatozoon

From the foregoing descriptions of sperm morphology and a comparison with studies in the literature emerges a picture of a tick spermatozoon which is probably representative for all tick species. This spermatozoon (Figures 65, 66, 67, 68) consists primarily of: 1) a motile organelle
at the anterior tip, 2) a wide club-shaped region with large number of mitochondria which aggregate towards the motile organelle and tapers toward the end, 3) a middle region with mitochondria mainly towards the periphery, and 4) a posterior region which contains a filament canal, an elongated nucleus lateral to the filament canal, an acrosome connected to the nucleus, two centrioles, and a plug-like structure at the posterior tip. The surface of this spermatozoon is covered with cellular processes which are arranged in longitudinal rows and are involved with sperm motility. The general shape of such a typical tick spermatozoon has been reported in various tick species (for review see Nath, 1956). However, a configuration of sperm ultrastructural components which agrees with the present description has only been reported in Ornithodoros moubata (Breucker and Horstmann, 1968, 1972).

Comparative Morphology of Tick Spermatozoa

The general morphology of tick spermatozoa has been described by a number of investigators: Christophers (1906, Ornithodoros savignyi, Rhipicephalus annulatus, and Hyalomma aegyptium); Nordenskiold (1909b, Ixodes reduvius); Oppermann (1935, Argas columbarum); Tuzet and Millot (1937, various species of Ixodes); Sharma (1944, Hyalomma aegyptium, Rhipicephalus sanguineus and Argas persicus); Rothschild
(1961, Ornithodoros moubata); Reger (1962, Amblyomma dissimili) and Breucker and Horstmann (1968, Ornithodoros moubata). Most of the reports of these investigators agreed that the shape of the tick spermatozoon is as an Indian club, however, considerable contradiction exists on the details of sperm structure.

Sharma (1944) and other investigators referred to a conspicuous centrosome at the anterior tip of the spermatozoon and described this centrosome as a ring-like structure and related to sperm motility. Rothschild (1961) later questioned the presence of such a centriole but did not prove otherwise. In considering the observations in the present study and those made by Breucker and Horstmann (1968), no centrosome or centriole exists at the sperm anterior tip, in fact contrary to Sharma's observations, two centrioles have been observed in the posterior region next to the nucleus. The "centrosome" of Sharma is definitely the unique motile organelle described in the present study.

The club-shaped region which is described in the present study has been referred to by some authors (Casteel, 1917; Sharma, 1944) as the anterior region of the spermatozoon and by others (Rothschild, 1961) as the posterior region. Although this discrepancy exists, most investigators agree that large groups of mitochondria are contained in this region. Sharma (1944) reported the following strange
observation about this region: "during the movement of the sperm, the mitochondria granules seem to be in state of commotion...". In the present study, aggregation of mitochondria was observed, particularly towards the motile organelle, an observation also reported in *Amblyomma dissimili* (Reger, 1961, 1962) and *Ornithodoros moubata* (Breucker and Horstmann, 1968, 1972).

In reference to the posterior region (as described in the present study) of the spermatozoon, the presence of an acrosome was first observed by Sharma (1944). Other investigators failed to describe an acrosome, but did observe the region where the acrosome is located. Casteel (1917) referred to this region as the "flagellar process" and Warren (1931) designated it the "finger-like process". Tuzet and Millot (1937) falsely interpreted this region as the whole spermatozoon and the anterior portion (club shaped region) as the "spermiophore". Formation of the acrosome was described by Sharma (1944), Reger (1963) and Breucker and Horstmann (1968, 1972) as aggregation of materials from the Golgi complexes. These aggregations form an acrosomal vesicle and eventually develop into an acrosome. However, the shape of the acrosome as described by Reger (1961) in *Amblyomma dissimili* is totally different from the shape of the sperm acrosome observed in the present study in the two *Dermacentor* species and in *Ornithodoros moubata* (Breucker...
and Horstmann, 1968, 1972). In reference to these latter studies, the acrosome is oval or round as compared to a thin elongated U-shaped structure shown by Reger. This difference is due to the fact that Reger examined immature spermatozoa. In general, the acrosome is a relatively small roundish structure connected to the nucleus by a supposed acrosomal rod. In addition to the mentioned structures, the posterior region contains a slender filament canal which has also been reported in *Amblyomma dissimili* (Reger, 1961, 1962) and *Ornithodoros moubata* (Breucker and Horstmann, 1968, 1972). The unusual plug-like structure which has been described for the first time in the present study is at the posterior tip of the spermatozoon and is the result of an unusual spermiogenetic process. The presence of this structure in spermatozoa of other tick species is highly suspected.

The cellular processes which cover the entire surface of the spermatozoa of both *Dermacentor* species represents another area of conflicting reports in sperm morphology of ticks. Warren (1931) adequately reported the occurrence of cellular processes in the elongated spermatid, but certainly erred when he interpreted these as individual spermatozoa "budding" from an elongated spermatid. Reger (1961) and Rothschild (1961) were the first to describe these cellular processes correctly and to relate them to sperm motility.
However, while Rothschild considered them "motile ridges" which pass through a periodicity of straight and wavy configuration, Reger attempted a homology to cilia or flagella. In the present study, cellular processes are unquestionably related to sperm motility and unquestionably not homologous to cilia or flagella.

Functional Implications of Tick Spermatozoa

As stated by Afzelius (1970), the basic necessary functions of a spermatozoon are to have proximity to the eggs by some means of motility or by other device and to have the proper size and form to move in the female ducts and eventually to the egg. In considering ticks, these functions have not been previously adequately studied. For example, sperm motility has not been clearly demonstrated, sperm-egg interactions have not been observed before, and the sperm structure has been poorly understood. Thus to ascribe functions to the various parts and organelles of the spermatozoon in the present study is a difficult task. However, in considering the observations on the morphology of immature and mature spermatozoa, sperm motility, and the initial sperm-egg interactions in vivo and in vitro, substantial attempts and strong speculations can be made to determine the functional roles of the various organelles.
The motile organelle

At the anterior tip of the spermatozoon is the motile organelle believed by the author to be the first structure to interact with the egg since the spermatozoon moves only in the direction of this organelle and in vitro the spermatozoon moves towards the egg and contacts first by this organelle. No similar structure has been shown in spermatozoa of any other taxon. The motility of the motile organelle must be a direct result of the contractile property of microtubules (Fawcett, 1962) which are the main constituents of this organelle. Whether this organelle contains acrosomal-like enzymes necessary for sperm penetration into the egg is not known, but since the sperm-egg contact occurs through an egg micropyle such enzymatic action may be minimal or totally lacking. A similar case is found in the spermatozoa of teleost fish which lack acrosomes and also enter through a micropyle (Nicander, 1970). Due to the constant change in shape, the motile organelle may have the ability to penetrate the egg mechanically. For argument sake, this organelle could have a similar penetration ability as the parasitic cercaria larva which acts mechanically in addition to lytic enzymes during penetration (Standen, 1953; Hunter, 1960).
The club and middle regions

The club region is located behind the motile organelle and is joined posteriorly to the middle region. The club region is unique to the sperm in having large numbers of mitochondria. The club and middle region (as the rest of the spermatozoon) is covered with cellular processes underneath which are subsurface filaments. The possible flexibility of these cellular processes and contractility of the subsurface filaments would require a source of energy. In most spermatozoa throughout the animal kingdom, mitochondria are associated with the motile tail (Gibbons, 1968). Since the motile organelle and the cellular processes are the motile elements in this spermatozoon, mitochondria are in close association with these structures. This explains why the mitochondria aggregate in large numbers near the motile organelle and spread towards the periphery throughout the rest of the spermatozoon.

The posterior region

Since the nucleus and the acrosome are contained in the posterior region, previous investigators (Sharma, 1944; Rothschild, 1961) considered this region as the head of the spermatozoon. Although the structures included in this region are equivalent to the head and the middle piece of a typical spermatozoon, this region should not be called a
head since the first contact with the oocyte and the sperm movement is with the anterior end. Also the tick spermatozoon is so highly complex and specialized that close comparisons to a typical sperm are not deemed advisable. While the posterior region of the tick spermatozoon is still under investigation, the questions to be asked are: 1) why does the nucleus have an elongated shape and 2) what is the function of the acrosome in the posterior region of the spermatozoon? With regard to the first question, the elongation of the nucleus conforms to the narrow space lateral to the filament canal. With regard to the acrosome, neither the nature of the acrosomal lytic enzymes nor the role of this acrosome in sperm-egg contact is known. One possible function because of the relationship of the acrosome to the nucleus is a lytic action on the cell membrane releasing the nucleus during sperm-egg interactions.

The posterior region also contains two unique structures: a filament canal which runs through most of the posterior region, and a plug-like structure which is located at the tip end of the posterior region. Although the function of the filament canal is uncertain, it may act as support for the sperm form. The plug-like structure may simply function to close the end of the posterior region after the completion of the spermiogenetic process.
Motility of Tick Spermatozoa

The motility of tick spermatozoa has long puzzled investigators and different ideas to explain this process have been proposed. Christophers (1906) reported that tick spermatozoa exhibit a steady gliding movement together with vermicular contortions of the anterior portion. Although he did not explain any further, his observations about the gliding nature of sperm motility agree with the observations in the present study. Sharma (1944) stated that movement of the mature spermatozoon takes place by the peculiar rotary movement of the centrosome at the anterior portion. Since the absence of any centriole or centrosome in this region has been confirmed in the present study and in studies by Breucker and Horstmann (1968), the rotation centrosome described by Sharma (1944) and others (cf. Nath, 1956) definitely corresponds to the motile organelle at the anterior tip of the spermatozoa of both Demacentor species.

The presence of a motile organelle other than a tail or flagellum in a spermatozoon is unique and has not been reported before. The active movement and the constant changes in the shape of the motile organelle are due to the nature of its attachment to the club region and the presence of numerous microtubules in this organelle. Microtubules have been associated with protoplasmic movements (Burgos and Fawcett, 1955) and contractility (Fawcett, 1962). In
addition to the role of microtubules, the constriction or attachment area between the motile organelle and the club region allow this organelle to stretch and retract. Thus the microtubular function and the adjustable constriction are directly connected to the motility of this organelle. Accumulation of large aggregates of mitochondria in the club region near the attachment to the motile organelle serves as an energy supply for the motile organelle.

Rothschild (1961) was the first to study sperm motility in ticks and to measure the speed of spermatozoa in *Ornithodoros moubata*. He interpreted sperm motility as a deformation of cellular processes which he called "ridges". He did not indicate what or how these deformations took place, but implied the existence of a contractile system associated with the sperm surface. In experiments using polystyrene balls sticking to the surface of the spermatozoon, he observed a backward movement of these balls. This observation agrees with the present study where particles coming into contact with the surface of the spermatozoon, moved rapidly backwards. However, this surface phenomena is restricted to the club and middle regions of the spermatozoon and presumably is due to the arrangement of the cellular processes in these regions.

The cellular processes are found over the entire surface of the spermatozoon in a longitudinal pattern and are
connected to the sperm surface by narrow connections which are presumably flexible and allow the cellular processes to move in a wave manner. This movement is believed to move the spermatozoon forward. The cellular processes are also variable in height at different regions of the spermatozoon. For example, at the club and the middle regions, the cellular processes are arranged in a manner where every third, fourth or fifth cellular process is higher than the intermittent ones. The higher cellular processes presumably have more waving power than the shorter ones and may explain the twisting ability of the spermatozoon at the club and middle regions and the rapid sliding behavior of particles if they come in contact with these regions. In the posterior region the same height prevails for all processes and no similar movement of particles in contact with this region was observed.

Although Rothschild (1961) indicated that cellular processes on the sperm surface of Ornithodoros moubata had variable heights, he did not specify if this arrangement applies to the whole spermatozoon. Breucker and Horstmann (1968) also described variable heights in cellular processes and indicated that these processes are longitudinally arranged in two ways: regular arrangement in which one cellular process ends at the beginning of another one, and irregular arrangement in which one cellular process ends
and there is no continuation of another. These authors believed that the irregular arrangement was confined to the corresponding club region (as described in the present study). However, in the present study this arrangement was also observed in the middle region.

In close association with the cellular processes, a series of subsurface filaments occur under the cell membrane throughout the spermatozoon. These subsurface filaments may contain contractile protein and thus contribute to the movement of cellular processes. In conclusion, a combination of the motile organelle movements and the cellular processes move the spermatozoon forward in a slightly twisting manner.

Although the tick spermatozoon as observed in the present study is slow (4 to 11 μ per second) in comparison with other spermatozoa, e.g., bull (97 ± 6 μ per second, Rikmenspoel, 1962), the size of the tick spermatozoon is considerably larger and the present mechanism of motility is quite efficient.

All motile spermatozoa described in the literature move either by amoeboid motion like immature *Ascaris* spermatozoa (Panigel, 1951) or by propagating waves along their tails (flagella) which invariably contain a variable pattern of microtubules (Hood *et al.*, 1972; Fawcett and Phillips, 1970; Rosati *et al.*, 1970). These sperm motile systems are absent in tick spermatozoa which have unique motility and must be
Gametogenesis in Ticks

Problems of gametogenesis in ticks concern the cellular transformations by which the spermatocytes and oocytes develop into spermatozoa and eggs. In case of the egg, the enlargement during development raises special questions concerning the mechanism of the growth process and the extent which this growth is accomplished by intrinsic synthetic activity or by nutritional materials manufactured by other cells. In the case of the spermatozoon, the appearance and nature of subsurface cisternae in the spermatid and the mechanism of spermatid elongation and invagination are considered. Gametogenesis also poses other problems concerning the timing of various developmental stages and the mechanism of the release of gametes from the reproductive system.

Spermatogenesis

Spermatogenesis in ticks is atypical in comparison with other taxa and results in spermatozoa with unusual morphology. The formation of subsurface cisternae in spermatids and the sequential changes involving polarization, fusion, and invagination to form cellular processes on the surface of mature spermatozoa are unique and are confined to ticks.

The presence of subsurface cisternae which were previously referred to as a "striated border" at the periphery
of spermatocytes and spermatids was reported by other in-
vestigators, but the nature of this striated border has been
controversial. For example, Christophers (1906) mistakenly
considered this as an abnormal degeneration at the periphery
of some spermatocytes and Bonnet (1907) definitely erred
when he considered these striations to be the future spermat-
оза. Casteel (1917), although he gave an accurate descrip-
tion of spermatid maturation, misinterpreted the striated
border as cilia formation. Others referred to this border
as a "thick ectoplasm" (Nordenskiold, 1920) or "lipoplasm"
(Tuzet and Millot, 1937; Till, 1961). More recently, Khalil
(1969) referred to these striations as membrane folds which
were necessary to retain an appropriate ratio between size
and surface area of the rapidly growing spermatocytes.

Reger (1961) was the first to study the development
of tick spermatids by electron microscooy and described the
striated border as subsurface cisternae. However, he (1962)
claimed that these cisternae originated from the endoplasmic
reticulum. Although the origin of these cisternae is not
totally demonstrated in the present study, the relationship
to the endoplasmic reticulum cannot be accepted. Instead,
the strongly suggestive evidence in the present study of an
origin of the subsurface cisternae from the cell membrane is
consistent with the evidence presented by Breucker and
Horstmann (1972). Contributions from somatic cells to the
formation of these cisternae is also possible since cytoplasmic contents of the cisternae are less dense than the rest of the spermatid, but similar to that of somatic cells.

During early spermiogenesis, the spermatids undergo two important events which involve the process of polarization and invagination. The process of polarization was first reported by Casteel (1917) who observed during development the disappearance of the striated border of the spermatid at one end of the cell. Recently, an accurate description of this polarization process has been reported (Reger, 1962; Breucker and Horstmann, 1972). In reference to the present study, the subsurface cisternae immediately after polarization at one pole of the cell undergo multiple fusion at their tips to form a membrane enclosing the subsurface cisternae. Further modification of these cisternae lead to the formation of elongated processes which are enclosed in a cisternal cavity (Figures 55, 56). Although the occurrence of a cisternal cavity was reported by Reger (1962) in *Amblyomma dissimili* and by Breucker and Horstmann (1972) in *Ornithodoros moubata*, no elaborate description of this event was described.

After the formation of the cisternal cavity, the walls of this cavity invaginate in such a unique process that an inner and an outer tube are formed. This invagination process also rearranges the cellular processes to the
outside of the inner tube (which is the future club region) and to the inner surface of the outer tube (which is the future middle and posterior regions of the mature spermatozoon). Since the mature spermatozoa have the cellular processes on the outer surface, this invagination process of the spermatid is only an additional step in spermogenesis.

The process of invagination is accompanied by migration of cytoplasmic components, especially mitochondria into the inner tube. This migration explains how most of the mitochondria in the mature spermatozoon are located in the club region near the motile organelle. These observations are consistent with the observations reported by Breucker and Horstmann (1972). During invagination the nucleus elongates and moves slightly to become situated in the outer tube adjacent to the cell membrane of the spermatid. This process of nuclear elongation is essential assuming that the nucleus must fit in the narrow space of the posterior region of the mature spermatozoon. As a result of these morphological transformations, the spermatid elongates and becomes tubular in shape with one pointed end and one broad end.

The elongated spermatids (immature spermatozoa) are transferred to the female during insemination and the final stages of sperm maturation take place in the female seminal
receptacle. The process of sperm maturation is initiated by rupturing of the sperm cell membrane (pointed end of spermatozoon) and evagination of the inner tube (Figures 56d, 61, 62) gradually exposing the motile organelle, the inner tube, and the outer tube. During the exposure of the inner tube, the fold in its wall unfolds resulting in the formation of the wide club region of the mature spermatozoon. This process of sperm maturation explains why motile spermatozoa are almost double the length of the immature ones and are only found in the female genital system. Although other investigators (Reger, 1963; Khalil, 1970; Breucker and Horstmann, 1972) referred to this final process of sperm maturation or evagination, no satisfactory explanation was offered. With the exception of the genus *Ixodes* (Tuzet and Millot, 1937; Balashov, 1956), this final process of evagination is probably universal in ticks.

**Oogenesis**

Since adequate observations were made of oogenesis in *D. andersoni* (Brinton and Oliver, 1971b), this species was not examined in the present study. The following discussion of oocyte development mainly concerns the observations made on *D. variabilis*.

During early development of the oocyte of *D. variabilis*, considerable electron-dense material migrates from the nucleus
into the ooplasm via prominent pores in the nuclear membrane. Such emissions containing RNA and proteins have been previously reported by Sareen (1965b) in *Hyalomma aegyptium* and *Argas persicus*. Since similar nuclear emissions have also been reported in *Ornithodoros moubata* (Aeschlimann and Hecker, 1967, 1969) and *D. andersoni* (Brinton and Oliver, 1971b), the nucleus definitely plays a major role in oocytic protein synthesis in ticks. The phenomenon of nuclear emissions during oogenesis is also common in other arachnids such as scorpions (André, 1959), harvestman (Sareen, 1963) and scorpions (Sareen, 1961) and in other taxa such as ascidians, *Ciona* (Kessel, 1966a), *Boltenia villosa* (Hsu, 1967) and the lung-fish, *Protopterus* (Sharrer and Warzelmann, 1969).

During protein synthesis and yolk formation in the oocytes, two main sources are involved: intra-oocytic and extra-oocytic. Intra-oocytic formation of yolk protein is apparently initiated by the endoplasmic reticula and the Golgi complexes. During the past decade the role of Golgi complexes in the formation of yolk protein granules has been illustrated in other ticks (Sareen, 1965b) and a number of other species including several arachnids (Sareen, 1967; Anderson and Huebner, 1968; Dumont, 1969; Tayler and Anderson, 1969). In addition, the Golgi complex has recently been implicated (Neutra and Leblond, 1966; Tayler
and Anderson, 1969) in formation of the carbohydrate portion of yolk. The endoplasmic reticulum has also been shown to have a supportive role in formation of yolk bodies (Beams and Kessel, 1962; Kessel, 1966a,b; Tayler and Anderson, 1969).

The presence of mitochondria in association with yolk bodies and their possible fusion with the granular emissions from the oocyte nucleus is further support for mitochondrial synthetic roles. This fact agrees with the observations on D. andersoni (Brinton and Oliver, 1971b) and Ornithodoros moubata (Aeschlimann and Hecker, 1967). Since follicle or nurse cells are nearly or completely absent in arachnids and insects with panoistic ovarioles (King, 1960) mitochondria in oocytes are instrumental in the formation of yolk bodies. Besides this function and in addition to providing ATP and oxidative enzymes, mitochondria may also play an important role in lipid synthesis (Favard and Carasso, 1958). Golden and Keith (1968) demonstrated that isolated mitochondria of Drosophila melanogaster can synthesize fatty acids \textit{de novo} and incorporate them into complex lipids. Thus, some oocyte mitochondria are likely to be essential depositories for early products of vitellogenesis.

The extra-oocytic source for yolk formation, although probably minor, involves the passage of material from the
haemolymph to the oocyte. The extra-oocytic material is probably derived from ingested blood, since the time of oocyte maturation is directly related to the amount of ingested blood. In *D. variabilis*, the passage of such material occurs by micropinocytosis via the region of the microvillar brush border. The micropinocytotic pits around the oocyte periphery obtain the extra-oocytic material which pass into the ooplasm via micropinocytotic tubes. Similar observations have been reported in *D. andersoni* (Brinton and Oliver, 1971b), in other tick species (Aeschlimann and Hecker, 1967), the closely related horseshoe crab, *Limulus polyphemus* (Dumont and Anderson, 1967), and in various other invertebrates (Telfer, 1961; Anderson, 1964, 1969; Roth and Porter, 1964) as well as vertebrates (Brambell, 1926; Balinsky and Davis, 1963; Droller and Roth, 1966; Van Gansen, 1966a,b; Fawcett, 1965).

From observations made in the present study and by other investigators, the funicular cells by which oocytes are attached to the ovarian wall also play a role in supplying extra-oocytic material for yolk formation. Aeschlimann and Hecker (1967) concluded that funicular cells in *Ornithodoros moubata* are one of the major sources for extra-oocytic nutrient material. Although the main function of funicular cells in *D. variabilis* is support and attachment of oocyte to the ovarian wall, the possible nutritional
function cannot be neglected. The intimate contact of microvilli of oocyte and funicular cells support this hypothesis. However, attachment of oocytes to the ovarian wall by a special group of cells is not unique with ticks. A similar situation exists in Limulus (Dumont and Anderson, 1967) where oocytes are attached by elongated cells and whose junctional area also acquires microvilli during vitellogenesis.

During oogenesis, a cuticular envelope is deposited on the surface of the oocyte. The mechanism and the time of cuticular deposition in ticks has not received adequate attention by other investigators. In the present study, cuticular deposition around the oocyte of D. variabilis is initiated six days after attachment of the ticks to the host and continues until two to three days after full engorgement. Since the ovaries of D. variabilis and ticks in general lack any follicle cells, deposition of the cuticle is suspected to be from intra-oocytic materials. This is supported since the accumulation of cuticle starts between the microvillar bases on the oolema at different times for each oocyte. Also the presence of pores at the site of microvilli after considerable deposition starts on the oolema and accumulates towards the outside (Figure 108). Intra-oocytic deposition of cuticle was also proposed by Brinton and Oliver (1971b) in oocytes of D. andersoni and
by Aeschlimann and Hecker (1967) in oocytes of O. moubata.

The process of oogenesis has been divided into various stages by several investigators. For example, Balashov (1956, 1964, 1967) reported five stages. Khalil (1969) reported only two stages: the first stage occurs during the feeding of the female on the host and the second stage occurs after fertilization and full engorgement. On the other hand, Brinton and Oliver (1971b) have reported two different stages: the intra-oocytic stage which involves the activity of Golgi complexes, mitochondria and nuclear emissions and the extra-oocytic stage which involves micropinocytosis. The present study has shown that intra-oocytic and extra-oocytic stages of oogenesis in D. variabilis are similar to those in D. andersoni, thus is in agreement with the two stages of oogenesis as described by Brinton and Oliver (1971b). Also a definite relationship exists between the time of insemination and oocyte maturation. For example, in the present study no eggs attained maturity and no oviposition was observed in unmated females. The strange notion by Nagar (1967) about parthenogenesis in D. variabilis is questionable. His brief note has never been elaborated and even if his observations are correct, the race of the species which he used must be different from the one used in the present study.

The occurrence of the egg micropyle as the site for
sperm-egg interactions in *D. variabilis* is reported for the first time. The observation of spermatozoa at the site of this micropyle definitely proves this. The diameter of the spermatozoon at the club region is also comparable to that of the micropyle. Although Brinton and Oliver (1971b) have demonstrated the presence of a micropyle in *D. andersoni*, they believe this structure is too small to permit sperm entry into the oocyte. Instead, these authors consider the micropyle as an attachment site for the posterior part of the spermatozoon. This suggestion is unlikely since spermatozoa of *D. variabilis* and *D. andersoni* move only in the direction of the motile organelle and also they did not observe any attachment of spermatozoa by the posterior end to the oocyte. In addition, the diameter of the spermatozoon of *D. andersoni* is 7 μ which is the diameter of the micropyle reported by these workers. This micropyle is subsequently sealed between 6 to 8 days after insemination by the deposition of cuticle.

**Biology of Fertilization in Ticks**

Franzén (1956, 1970) emphasized that a definite relationship exists between sperm morphology and the biology of fertilization. Thus the function of a spermatozoon cannot only be based on detailed morphological and cytochemical descriptions alone, but also must include the roles of
spermatozoa in interacting with the egg. If the morphology of a spermatozoan diverges from the "primitive metazoan sperm-type" (Franzén, 1956) this might be explained as an adaptation to the specific biology of fertilization in a taxon of animals. These morphological adaptations might be in response to the exigencies of 1) copulation and insemination, 2) spermatophore production, 3) sperm storage and maturation, and 4) sperm-egg interactions.

Copulation and insemination

The copulation behavior observed in the present study and which could last for 30 minutes is common for ticks. During copulation, the male usually seeks the female, an observation agreeing with other reports (Sacktor et al., 1948), and is the aggressive partner in copulation. This notion is reasonable to believe since the presence of sex pheromones in ticks has been recently demonstrated to occur in the female reproductive organs of D. variabilis, Amblyomma americanum, and A. maculatum (Berger et al., 1971).

From the present observations and other reports in literature, similarities and differences in the pattern of copulation and sperm transfer exist in argasid and ixodid ticks. In considering the argasid ticks, copulation always occurs off the host and the female will mate again after oviposition. In addition, during copulation the male
introduces the whole capitulum including the palpi into the female genital opening (Nuttall and Merriman, 1911; Feldman-Muhsam, 1967a,b). In the ixodid ticks of the Metastriata group, copulation occurs on the host after being fed for several days (Balashov, 1967). However, by proper monitoring of feeding time for D. variabilis and then placing males and females in wax plated dishes copulation will occur off the host. No mating occurs after oviposition since females usually die. During copulation, only the male chelicerae are introduced into the female genital opening. Ixodid ticks of the Prostriata group can copulate, even without feeding, both on or off the host. During copulation the hypostome and chelicerae are introduced in the female genital opening while the palpi remain outside (Feldman-Muhsam and Bourt, 1971). The male of all studied ticks during copulation uses the chelicerae to transfer the spermatophore to the female genital opening (Nuttall and Merriman, 1911; Robinson, 1942; Feldman-Muhsam, 1967a; Feldman-Muhsam and Bourt, 1971). Thus, this form of copulation (with no copulatory organs) is universal in ticks and represents the first event of fertilization.

**Spermatophores**

All ticks package their spermatozoa in spermatophores. Although the spermatophore is used to transfer spermatozoa
to the female system, evidence of this structure is not present in the male genital tract but in both examined species is formed during copulation or probably shortly before extrusion from the male genital opening. This is consistent with the observations made by Feldman-Muhsam (1967b) who even believes that the spermatophore is completed outside the male.

The structure of spermatophore is slightly different in various tick species. While the endospermatophore (Figure 5) of both examined species in the present study consists of a single capsule, the endospermatophore of other ticks, especially argasids, consists of two capsules (Robinson, 1942; Feldman-Muhsam, 1967a). The occurrence of two capsules has led some investigators (Feldman-Muhsam, 1967b 1969) to assume that each capsule, once in the seminal receptacle is directed towards an oviduct. In ticks with a large seminal receptacle or uterus, this would enhance sperm transfer into the oviduct, however, in both examined species in the present study, the rupture of the endospermatophore wall allows spermatozoa to enter either oviduct. Although the chemical structure of the spermatophore was not investigated in the present study, Feldman-Muhsam (1967a) reported the spermatophore wall of several ticks consists of an outer mucin layer, inner mucoproteinaceous layer, and a proteinaceous intermediate layer.
The massive accessory glands of the male are the site for producing the substances needed for spermatophore formation. The numerous secretory products pass from the accessory gland cells through ducts to the seminal vesicle. In addition, the secretory products from the accessory glands probably contain other substances which are used in the seminal receptacle during sperm maturation.

**Sperm storage and maturation**

With the exception of ticks of the genus *Ixodes* (Tuzet and Millot, 1937), all ticks store spermatozoa for various periods of time. The descriptive aspects of sperm storage in the present study involve retention of the endospermatophore, which is received at the time of insemination, for three to four days in the seminal receptacle. This organ is a very distinct region of the female reproductive tract of both examined species in this study and is similar to the seminal receptacle of other tick species (cf. Arthur, 1962). On the other hand, the uterus as described by Roshdy (1961, 1962, 1963) and Khalil (1969) is restricted to argasids and is much larger than the seminal receptacle of ixodids. However, the time of endospermatophore retention in the seminal receptacle or uterus varies from four days in *Ornithodoros moubata* (Robinson, 1942) to several weeks in *Amblyomma americanum* (Gladney and Drumont,
During retention of the endospermatophore in the seminal receptacle, spermiogenesis is resumed. The rupture of the cell membrane and the exposure of the inner and outer tubes of each immature spermatozoon leads to a doubling in the length of the sperm and an exposure of the motile organelle which is necessary for sperm motility. The major questions arising from these observations are: 1) what are the features of the seminal receptacle which stimulate the resumption of spermiogenesis or sperm maturation, 2) what are the features of the seminal receptacle which cause the rupture or dissociation of the endospermatophore wall, 3) what promotes the movement of spermatozoa into the oviduct, and 4) what are the contents of the seminal fluid or spermatophore which trigger the continuation of the oocyte development?

In an attempt to answer these questions, comparison with the few available reports of these aspects and strong speculations must be made. In regard to the resumption of spermiogenesis, the seminal receptacle probably contains certain secretions which trigger the rupture of the cell membrane of immature spermatozoa. The possibility of the presence of certain substances in the seminal fluid which could also cause rupture of the cell membrane should not be excluded. However, Khalil (1969) suggested that secretions
from the seminal receptacle cause the dissociation of endo-
spermatophore wall. Dissociation of the endospermatophore
wall might also be due to the pressure caused by spermatozoa
after maturation and length duplication. As to what pro-
motes movement of spermatozoa into the oviduct, Tatchell
(1962) showed that the accessory glands of male *Argas*
*persicus* contain a pharmacological agent which is carried
in the seminal fluid and causes contraction of the oviduct
promoting spermatozoa to move to the ovary. Since the
seminal receptacle and oviducts are coated with a muscular
layer, contraction of the seminal receptacle and oviducts
in certain sequence could also force spermatozoa into the
oviduct where the sperm motility is stimulated. As for the
stimulative effect of seminal fluid (or spermatophore) on
the continuation of oocyte development, Gregson (1944, 1947)
showed that mating is essential for oocyte maturation of
*D. andersoni*. Similar results were reported for
*Ornithodoros tholozani* (Galun and Warburg, 1967) and *D.*
*variabilis* (Papas et al., 1971). Galun and Warburg (1967)
have demonstrated that catecholamines in the seminal fluid
when transferred to the female after mating produce an
afferent stimulus which is transmitted to the brain and
induces the secretion of a gonadotropic hormone (from un-
known organ) necessary for normal egg development. A sim-
ilar method of induction was shown by Roth (1964) and
Davis (1965) to take place in insects. These authors believed that insertion of the spermatophore into the bursa releases stimuli via the nerve cord to the brain which increases the activity of the corpora allata.

**Sperm-egg interactions**

Although fertilization in ticks is internal, the actual site and time has not been previously demonstrated. From the observations in the present study, sperm-egg interactions in *D. variabilis* occur in the ovary (Figure 114) and are based on the presence of spermatozoa in the ovarian lumen at the site of the egg micropyle. The motile organelle is the first sperm structure approaching the oocyte and presumably makes contact with it. Although some authors (Christophers, 1906; Samoson, 1909; Lees and Beament, 1948; Till, 1961; Khalil, 1969) have suggested ovarian fertilization, others believe that this process takes place in the oviduct (Bonnet, 1907; Robinson and Davidson, 1914; Dennis, 1932; Sokolov, 1956). In considering the results in this study, fertilization in the oviduct is improbable since all the eggs observed in the oviduct had micropyles which were sealed with a layer of cuticle forming a barrier for sperm penetration. However, Geigy and Wagner (1957), Wagner-Jevesenko (1958) and Geigy (1962) suggested that "phagocytosis" of spermatozoa by the oviduct cells results in
liberation of the male DNA molecules which diffuse into the egg through the egg coat. This is very unlikely and lacks any support. Sperm-egg interaction in the ovary is probably universal in ticks (except parthenogenetic species). The requirements for this form of fertilization may provide a functional interpretation of sperm morphology in ticks.

The events preceding sperm-egg interactions include copulation, spermatophore transfer, sperm storage, sperm maturation, and sperm migration through the oviduct to the ovary. Such a sequence of fertilization events indicates the requirements for internal fertilization. Since spermatozoa are transferred to the female in an immature stage they have to be retained in the female until maturation. During the time for sperm maturation and migration through the oviduct, the oocytes have developed to a certain stage just prior to the initial deposition of cuticle. At this time the egg micropyle is accessible and sperm-egg interaction can occur between six to eight days after attachment of the tick to the host or five to six days after insemination (Table 3). Also during sperm maturation the motile organelle is exposed. With the development of such a motile organelle at the anterior tip of the spermatozoon and motile processes on the surface, there is no longer a need for a flagellum or tail. In addition, since the spermatozoon moves in direction of the motile organelle, it is advantageous if
this organelle other than the posterior region (where the nucleus is located) is capable of initial contact with the oocyte. Thus, this system of sperm-egg interactions in ticks is synchronus, very well arranged, and the spermatozoon is adapted to the biology of fertilization.

Tick Reproduction and Phylogeny

A discussion of tick reproduction will not be complete unless it is compared to other arachnids. A fundamental approach to morphology, structure and mechanism is to determine the origin of the structure, evolution of the mechanism and how the present form evolved. In other words, 1) what are the implications of sperm morphology, sperm structure and mechanism of sperm transfer to arachnid phylogeny and 2) what is the origin of the unique reproductive features in ticks?

Arachnid relationship

An attempt to analyze the reproductive aspects of arachnid phylogeny must be recognizant of the relationships between arachnids. Only after the year 1801 (cf. Petrunkevitch, 1955) was a separate class assigned to arachnids which previously were treated as insects. Presumably the arachnid orders which have book lungs (Scorpions, Uropygi, Amblypygi and Spiders) can be grouped together (cf.
Kaestner, 1968). The Amblypygi resemble spiders in body shape, segmentation of organs and in having two pumping stomachs. Though the evolution may be convergent, the organization of the Amblypygi could lead to that of the spiders (cf. Kaestner, 1968). Pseudoscorpions and mites in their transformation of pedipalpal coxae resemble Uropygi. Harvestman (in external genitalia) resemble some groups of mites, thus the groups are related.

Snodgrass (1938) and Petrunkievitch (1949, 1955) postulated that ancestral arachnids arose in the Cambrian period and changed essentially to chelicerate forms with a ventral mouth which resemble eurypterids. Scorpions are the most primitive arachnids since they retained ancestral segmentation. Schultz (1937) derived ticks directly from the Anthrachomartida which became extinct in the Carboniferous. Thor (1928) proposed a possible common ancestor for mesostigmatic mites and ticks. More recently, Wooley (1961) proposed that ticks have developed from a mesostigmatid mite type of ancestor and argasids are more primitive since they are closer to mesostigmatids. According to Woolley (1961) several authors believed that argasid ticks were derived directly from Uropodids (parastigmatid mites) and the ixodid ticks from a gamasid mite ancestor. Snodgrass (1952) indicated that ticks and mites have developed a highly specialized type of structure correlated with
parasitic habits. In spite of the lack of enough evidence about the evolutionary background of ticks, they are strongly believed to have evolved from a mite ancestor. However, authors agree that argasid ticks are more primitive than ixodid ticks.

**Implications of sperm morphology and sperm structure of ticks to arachnid phylogeny**

Although great specialization is present in the animal kingdom, in general, the evolution of sperm morphology can be considered conservative (Franzén, 1956, 1970) and therefore a good indicator for evolutionary relationships (Mayr, 1969). Considering this aspect and the available reports on sperm morphology for arachnid groups, a hypothetical picture of an arachnid spermatozoon can be constructed. This hypothetical spermatozoon containing all the elements present in the various modern arachnid spermatozoa would be filiform or elongated in shape and consists of an elongated head (with nucleus, acrosome and acrosomal rod) and a tail or flagellum.

A picture which is relatively similar to that of the hypothetical spermatozoon is found in scorpions and pseudoscorpions which represent the phylogenetically lowest stock of arachnids (Petrunkevitch, 1955; Kaestner, 1968). Representative spermatozoa of these groups are filiform and
flagellated. With the exception of *Veiovis carolinianus* which exhibits a flagellum with 9+0 microtubules pattern (Hood *et al*., 1972) and *Centruroides vitatus* which exhibit a 9+1 pattern (Hood *et al*., 1972), all other studied scorpions and pseudoscorpions have spermatozoa with 9+2 pattern (Baccetti, 1970; Kanwar and Kanwar, 1968). Spiders which represent one of the middle orders of arachnids also have flagellated spermatozoa but the flagellum has a 9+3 pattern and the head is of a spiral shape rather than filiform (Rosati *et al*., 1970). Opiliones and ticks which are among the highest orders of Arachnida have nonflagellate spermatozoa. Spermatozoa of Opiliones are of a spindle shape, but those of ticks are more of a tubular shape. Although spermatozoa of Opiliones have lost their flagella and have become nonmotile (Baccetti, 1970), spermatozoa of ticks have developed a new device for motility. Thus sperm morphology during evolution has been modified gradually from a filiform flagellated spermatozoon to a slightly tubular nonflagellated one.

If the spermatozoa of ticks are considered highly evolved among arachnids, their structure needs explanation. Since tick spermatozoa are nonflagellated, the development of a new device for motility is necessary to accomplish their migration from the seminal receptacle through the oviduct to the ovary where fertilization occurs. The
motile organelle at the anterior tip and the cellular processes covering the whole surface are unique and not present in other arachnids. The long filament canal next to the nucleus in the posterior part of the tick spermatozoon may be similar to the "central canal" of the spider, *Pholcus phalangioides* (Rosati et al., 1970). This central canal differs from the filament canal in being connected to the nucleus, but may be homologous to that in spermatozoa of ticks. Another unique characteristic of tick spermatozoa is the presence of large amounts of mitochondria. This characteristic agrees with the suggestion made by Favard and André (1970) that mitochondrial number and arrangement are related to the mode of fertilization. These authors believed that with internal fertilization an increase in the ratio of the volume of mitochondria to the volume of the sperm cell occurs. Although spermatozoa of ticks are highly modified from the hypothetical spermatozoon, they are highly efficient in structure. By developing a new mechanism for motility and accumulating enough mitochondria to supply it with energy, the spermatozoon is able to reach the egg and interact with it. Thus in spite of the divergence from the "primitive-type sperm", spermatozoa of ticks are still adapted to the mode of internal fertilization.
Origin of tick reproductive features

The assessments of ancestral arachnid reproductive patterns provide a basis with which to compare the tick reproductive features. Specifically, 1) what are the origins of patterns of insemination, sperm storage and ovarian fertilization and 2) what do these features contribute to the tremendous tick radiation and their parasitic life.

Insemination and ovarian fertilization

Since the ancestral arachnid (eurypterid) was an aquatic creature, form and the genital appendages of the male and female were similar to those of *Limulus* (Petrunkevitch, 1955), and the sexes had a very simple mode of mating. The male probably clasped the female and spermatozoa were released in the medium in close vicinity to the eggs. Fertilization was external. Thus, mating without a copulatory organ is an ancestral characteristic in ticks. Since ticks are totally terrestrial, the formation of spermatophores is an obvious necessity to avoid desiccation. However, the transfer of immature spermatozoa is different although slightly similar to the transfer of encysted spermatozoa in pseudoscorpions, spiders and Opiliones (Kanwar and Kanwar, 1968; Rosati *et al.*, 1970; Baccetti, 1970).
Although no other reports about fertilization in ticks are available, ovarian fertilization as observed in ticks in this study is a very unique reproductive feature in arthropods. Among other arthropods very few examples of ovarian fertilization exists, i.e., polycatenid insects (Hagan, 1931), the coccid insects *Aspiciotus astreolormis* (Pesson, 1950), the bed bugs (Davis, 1956) and the isopod *Trachelipus rathkei* (Kutish, 1972). Although ovarian fertilization is advantageous in assuring fertilization of all potential eggs, the entire process is dependent on sperm migration into the oviduct and the ovary. No indication exists about how or why this pattern of sperm migration started in the ancestral tick.

**Sperm storage**

Obviously sperm storage offers a very real advantage so that from one insemination the tick has enough spermatozoa to fertilize all potential eggs in the ovary. Although few reports of sperm storage in other arachnids are available, this phenomenon may be common in arachnids. There are no great problems involved in achieving internal sperm storage when the ancestral tick already had developed spermatophores and ovarian fertilization was established.

Insemination, sperm storage and ovarian fertilization coupled with high fecundity obviously offer the greatest
adaptive advantage to reproductive success in terrestrial parasitic life. Only one insemination is needed and from then on the tick is a self sufficient reproductive machine. This is a very efficient form of reproduction in the harsh terrestrial environment.
SUMMARY

1. Morphological and ultrastructural studies were conducted on the genital systems of two tick species, *Dermacentor variabilis* and *Dermacentor andersoni*. The processes of gametogenesis and structure of mature gametes were examined by light microscopy, scanning and transmission electron microscopy. Sperm motility was demonstrated by various optical systems and cinephotomicrography and sperm-egg interactions were observed in sections of fixed female ovarian tissue and in vitro.

2. The male reproductive system in both species is located in the anterior half of the body cavity in front of the rectal sac and consists of: two tubular and coiled testes, a narrow vas deferens at the anterior end of each testis, a small sac-like seminal vesicle, an ejaculatory duct and a genital opening. Well developed accessory glands which consist of several lobes are associated with the genital system. The secretions of the accessory glands are released in the seminal vesicle and are used to build the spermatophore during insemination.

3. The female reproductive system in both species consists of a single U-shaped ovary which extends posteriorly on both sides of the body cavity and loops in front of the rectal sac, an oviduct at each anterior end of the
ovary, a trilobed sac-like seminal receptacle, a vagina, and a genital opening. Two small tubular accessory glands are associated with the genital system.

4. Copulation in both species occurs during feeding on the host and the seminal receptacle of engorged females contain from one to three endospermatophores. By developing a method of separate feeding and monitoring the time of attachment to the host, copulation and insemination off the host was demonstrated.

5. Spermatogenesis is accomplished by two main stages. The first stage takes place in the male gonads and involves the following events: (1) appearance of subsurface cisternae as infoldings of the cell membrane at the periphery of the spermatocytes, (2) increase in number of subsurface cisternae in the spermatid, (3) polarization of the subsurface cisternae by accumulation at one pole of the spermatid, (4) multiple fusion at the tips of the subsurface cisternae to form a membrane which encloses the cisternae in one cisternal cavity, (5) invagination of the inner wall of the cisternal cavity into the cavity and formation of an inner tube and an outer tube, (6) migration of most of the cytoplasm including mitochondria into the inner tube, (7) elongation of the nucleus which migrates anteriorly in the outer tube, and (8) elongation of the spermatid as a result
of these changes, and when the spermatids reach a certain stage of elongation they pass through the testicular lumen and accumulate in the seminal vesicle until they are transferred to the female during insemination. The second stage of spermatogenesis or sperm-maturation takes place in the female seminal receptacle and involves the following events: (1) rupture of the cell membrane, (2) evagination of the immature spermatozoa (elongated spermatids) and exposure of the motile organelle, inner tube and outer tube, (3) retraction of the cell membrane to become located in the cytoplasm and form a filament canal, and (4) migration of the elongated nucleus to be located lateral to the filament canal. Due to all these changes, the spermatozoa double in length and become freely motile.

6. Oogenesis is related to the time of the female attachment to the host. The main events which occur during oocyte development include: nuclear emissions, formation of microvilli which project from the oolema, sperm-egg interactions which occur five to six days after insemination, formation of cuticular envelope, and formation of yolk bodies. Deposition of cuticle around the oocyte begins about six days after attachment of the female to the host. This deposition of cuticular envelope takes place from within the oocyte and in the mature egg this envelope averages from 3 to 5 μ in thickness. Formation of the
cuticular envelope subsequently seals the micropyle approximately seven days after insemination. Two sources are involved in the formation of yolk bodies: 1) intra-oocytic which includes nuclear emissions, endoplasmic reticulum and mitochondrial activities and 2) extra-oocytic which includes micropinocytosis and presumably funicle cells.
CONCLUSIONS

1. Spermatozoa are transferred from the male to the female in an immature stage within a spermatophore which is formed during insemination. The spermatophore consists of an ectospermatophore connected to an endospermatophore by a slender neck. After insemination the endospermatophore (which contains the immature spermatozoa) is retained in the female seminal receptacle for a period of three to four days during which spermatozoa attain maturity and become freely motile.

2. Mature spermatozoa of both species are nonflagellated and tubular in shape with a club-shaped anterior region and a slender posterior region. The spermatozoa of these species differ only in size. The spermatozoon is divided into four regions: (1) the motile organelle which consists of numerous microtubules, (2) the club region which contains aggregates of mitochondria, (3) the middle region which contains few mitochondria distributed towards the periphery, and (4) the posterior region which contains the filament canal, nucleus, acrosome, centrioles, and a unique plug-like structure at the end of the posterior region.

3. The surface of the mature spermatozoon is covered with cellular processes which are of the same height at the posterior region and of two different heights in the other
regions.

4. After the rupture of the endospermatophore wall, mature spermatozoa migrate from the seminal receptacle through the oviduct to the ovary.

5. Spermatozoa of both species move in a gliding and a slightly twisting manner. This sperm motility has been clearly documented to take place by a combination of movements of the motile organelle and the cellular processes. The motile organelle goes through five steps of sequential changes which involve slow rotation, elongation, bending, sudden stretching, and retraction. The cellular processes presumably move in a wave-like manner.

6. The oocyte has a micropyle of 5 to 7 µ in diameter and opens towards the ovarian lumen.

7. Sperm-egg interactions definitely take place in the ovary through the egg micropyle after five to six days of insemination. The egg micropyle is subsequently sealed by cuticular deposition and mature eggs lack any micropyle.
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APPENDIX A. TABLES AND FORMULAS
Table 1. Dimensions\(^a\) of male and female reproductive organs of *D. variabilis* and *D. andersoni* before attachment to the host and at various times during attachment to the host

<table>
<thead>
<tr>
<th>Organ</th>
<th>Time</th>
<th>Dimension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testes</td>
<td>before attachment</td>
<td>0.13 mm diameter</td>
</tr>
<tr>
<td></td>
<td>four days after attachment</td>
<td>0.5 mm diameter</td>
</tr>
<tr>
<td>Vas deferens</td>
<td>before attachment</td>
<td>12 (\mu) diameter</td>
</tr>
<tr>
<td></td>
<td>four days after attachment</td>
<td>21 (\mu) diameter</td>
</tr>
<tr>
<td>Seminal vesicle</td>
<td>before attachment</td>
<td>22.4 (\mu) diameter</td>
</tr>
<tr>
<td></td>
<td>four days after attachment</td>
<td>43.2 (\mu) diameter</td>
</tr>
<tr>
<td>Ovary</td>
<td>before attachment</td>
<td>0.21 mm diameter</td>
</tr>
<tr>
<td></td>
<td>two days after attachment</td>
<td>11 mm long</td>
</tr>
<tr>
<td></td>
<td>six days after attachment</td>
<td>0.5 mm diameter</td>
</tr>
<tr>
<td></td>
<td>eight days after attachment</td>
<td>24 mm long</td>
</tr>
<tr>
<td></td>
<td>five days after insemination</td>
<td>0.7 mm diameter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34 mm long</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 mm diameter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>47 mm long</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3 mm diameter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>47 mm long</td>
</tr>
<tr>
<td>Oviduct</td>
<td>before attachment</td>
<td>62.2 (\mu) diameter</td>
</tr>
<tr>
<td></td>
<td>four days after attachment</td>
<td>191 (\mu) diameter</td>
</tr>
<tr>
<td>Seminal receptacle</td>
<td>before attachment</td>
<td>0.3 mm diameter</td>
</tr>
<tr>
<td></td>
<td>four days after mating</td>
<td>1.4 mm diameter</td>
</tr>
<tr>
<td>Vagina</td>
<td>before attachment</td>
<td>65 (\mu) diameter</td>
</tr>
<tr>
<td></td>
<td>eight days after attachment</td>
<td>220 (\mu) diameter</td>
</tr>
<tr>
<td>Female accessory glands</td>
<td>before attachment</td>
<td>170 (\mu) diameter</td>
</tr>
<tr>
<td></td>
<td>eight days after attachment</td>
<td>280 (\mu) diameter</td>
</tr>
</tbody>
</table>

\(^a\)Mean of 24 observations.
Table 2. Summary of life cycles of *D. variabilis* and *D. andersoni*

<table>
<thead>
<tr>
<th>Species</th>
<th>Event</th>
<th>Temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>°C</td>
<td>(days)</td>
</tr>
<tr>
<td><strong>D. variabilis</strong></td>
<td>engorgement of adult male</td>
<td>22</td>
<td>5-9</td>
</tr>
<tr>
<td></td>
<td>engorgement of adult female</td>
<td>22</td>
<td>8-13</td>
</tr>
<tr>
<td></td>
<td>oviposition</td>
<td>22</td>
<td>10-16</td>
</tr>
<tr>
<td></td>
<td>hatching</td>
<td>22</td>
<td>17-32</td>
</tr>
<tr>
<td></td>
<td>engorgement of larva</td>
<td>22</td>
<td>4-7</td>
</tr>
<tr>
<td></td>
<td>molting of larva</td>
<td>25</td>
<td>9-18</td>
</tr>
<tr>
<td></td>
<td>engorgement of nymph</td>
<td>22</td>
<td>4-8</td>
</tr>
<tr>
<td></td>
<td>molting of nymph</td>
<td>25</td>
<td>15-25</td>
</tr>
<tr>
<td><strong>D. andersoni</strong></td>
<td>engorgement of adult male</td>
<td>22</td>
<td>4-8</td>
</tr>
<tr>
<td></td>
<td>engorgement of adult female</td>
<td>22</td>
<td>8-14</td>
</tr>
<tr>
<td></td>
<td>oviposition</td>
<td>22</td>
<td>9-15</td>
</tr>
<tr>
<td></td>
<td>hatching</td>
<td>22</td>
<td>18-27</td>
</tr>
<tr>
<td></td>
<td>engorgement of larva</td>
<td>22</td>
<td>4-7</td>
</tr>
<tr>
<td></td>
<td>molting of larva</td>
<td>25</td>
<td>16-28</td>
</tr>
<tr>
<td></td>
<td>engorgement of nymph</td>
<td>22</td>
<td>3-8</td>
</tr>
<tr>
<td></td>
<td>molting of nymph</td>
<td>25</td>
<td>16-24</td>
</tr>
</tbody>
</table>
Table 3. Chronology of reproductive events in the female *D. variabilis* after insemination off the host

<table>
<thead>
<tr>
<th>Event</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spermatozoa within endospermatophore in the seminal receptacle</td>
<td>10 min - 1 hour</td>
</tr>
<tr>
<td>Sperm maturation begins</td>
<td>1 - 2 days</td>
</tr>
<tr>
<td>Endospermatophore dissociation and migration of spermatozoa through the oviduct</td>
<td>4 days</td>
</tr>
<tr>
<td>Migration of spermatozoa into the ovary</td>
<td>4 - 5 days</td>
</tr>
<tr>
<td>Sperm-egg interactions</td>
<td>5 - 6 days</td>
</tr>
<tr>
<td>Closure of egg micropyle</td>
<td>7 - 8 days</td>
</tr>
</tbody>
</table>
Formula of Arachnid Solution

Arachnid solution for dissection of ticks, genital systems, and spermatophores was prepared (after Rothschild, 1961) in the following method:

- 9.1 gm KH₂PO₄ per liter
- 9.5 gm Na₂HPO₄ per liter
- 31.6 gm NaCl per liter
- 52.9 gm CaCl₂ · 6H₂O per liter
- 73.1 gm MgCl₂ · 6H₂O per liter
APPENDIX B. FIGURES AND ABBREVIATIONS
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac</td>
<td>Acrosome</td>
</tr>
<tr>
<td>AdL</td>
<td>Anteriodorsal lobe</td>
</tr>
<tr>
<td>AG</td>
<td>Accessory glands</td>
</tr>
<tr>
<td>AR</td>
<td>Acrosomal rod</td>
</tr>
<tr>
<td>BC</td>
<td>Basis capitulum</td>
</tr>
<tr>
<td>C</td>
<td>Subsurface cisternae</td>
</tr>
<tr>
<td>CB</td>
<td>Cellular bridge</td>
</tr>
<tr>
<td>CC</td>
<td>Cisternal cavity</td>
</tr>
<tr>
<td>Ch</td>
<td>Chelicerae</td>
</tr>
<tr>
<td>CM</td>
<td>Cell membrane</td>
</tr>
<tr>
<td>Cn</td>
<td>Centrioles</td>
</tr>
<tr>
<td>CO</td>
<td>Constriction between the club region and motile organelle</td>
</tr>
<tr>
<td>CP</td>
<td>Cellular processes</td>
</tr>
<tr>
<td>CR</td>
<td>Club region</td>
</tr>
<tr>
<td>D</td>
<td>Duct</td>
</tr>
<tr>
<td>D1L</td>
<td>Dorsolateral lobule</td>
</tr>
<tr>
<td>DM</td>
<td>Dense materials</td>
</tr>
<tr>
<td>Doc</td>
<td>Developing oocytes</td>
</tr>
<tr>
<td>Dr</td>
<td>Droplets</td>
</tr>
<tr>
<td>EcS</td>
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Figure 1. D. variabilis. Dorsal view of mature unfed male. The body is covered with a dorsal shield or scutum (S) and the mouth parts or capitulum consists of a basis capitulum (BC), two chelicerae which surround the hypostome, and a pedipalp on each side of the basis capitulum.

Figure 2. D. variabilis. Dorsal view of mature unfed female. The major parts of the body are the same as the male except the scutum (S) covers only a portion of the body.
Figure 3. *D. andersoni*. Dorsal view of mature unfed male. The body is covered with a dorsal shield or scutum (S) and the mouth parts or capitulum consists of a basis capitulum (BC), two chelicerae which surround the hypostome, and a pedipalp on each side of the basis capitulum.

Figure 4. *D. andersoni*. Dorsal view of mature unfed female. The major parts of the body are the same as the male except the scutum (S) covers only a portion of the body.
Figure 5. *D. variabilis*. Ventral view of a male reproductive system four days after feeding. The reproductive system consists of two testes (T) each connected to a vas deferens (VD) which meet to form a small seminal vesicle (SV) and lead to an ejaculatory duct (ED). The accessory glands consist of 3 main lobes: anteriodorsal lobe (AdL), posterioventral lobe (PvL), and ventromedial lobe (VmL) which also consists of three paired lobules: dorsolaterals (DIL), lateroventrals (LvL) and posteriolaterals (PlL).

Figure 6. *D. andersoni*. Dorsal view of a male reproductive system four days after feeding, showing the same structures as in Figure 5. The posterioventral lobe (PvL) of the accessory glands is less curved than that of *D. variabilis*. 
Figure 7. *D. variabilis*. Section of a testis of unfed male. The testis consists of germinal cysts (Gy) which contain spermatocytes (Sp). The testis is enclosed in a thin layer of connective tissue. X300.

Figure 8. *D. variabilis*. Section of a testis of a male one day after attachment to the host, showing growing spermatocytes (Sp) and somatic cells (SC). X400.

Figure 9. *D. variabilis*. Section of a testis of a male two days after attachment to the host showing enlarging spermatocytes (Sp). X400.

Figure 10. *D. variabilis*. A spermatocyte from a section of testis of a male three days after attachment to the host, showing early appearance of subsurface cisternae (C). X10000.
Figure 11. *D. variabilis*. A spermatocyte from a section of testis of a male three days after attachment to the host, showing early formation of subsurface cisternae (C) from the cell membrane (arrow). X10000.

Figure 12. *D. variabilis*. A section of testis of a male three days after attachment to the host, showing spermatids with subsurface cisternae (C) which give the spermatid a striated periphery. The spermatid is surrounded with a somatic cell (SC) and in some cases spermatids are connected to each other by intercellular junctions (arrow). X3200.

Figure 13. *D. variabilis*. Elongated spermatids (immature spermatozoa) accumulating in the male seminal vesicle. X1000.
Figure 14. *D. variabilis*. An oblique section of seminal vesicle of a male three days after attachment to the host. The seminal vesicle is lined with cuboidal epithelium on the inside and coated on the outside with a thin layer of flat epithelium. X400.

Figure 15. *D. variabilis*. A section of vas deferens of a male three days after attachment to the host. As in the seminal vesicle, the vas deferens is coated with a thin layer of flat epithelium and lined on the inside with cuboidal epithelium. X400.

Figure 16. *D. variabilis*. A section of ejaculatory duct of a male three days after attachment to the host. The duct is lined with a thin layer of cuticle (Cu) which is continuous with the external cuticle through the genital opening. A thin layer of flat epithelium rests on the cuticle. X400.

Figure 17. *D. variabilis*. A section of accessory glands of a male three days after attachment to the host. The accessory glands consist of cuboidal epithelium with secretory products which are shown accumulating in the lumen (arrow). On the outside the accessory glands are coated with a thin layer of flat epithelium. X400.

Figure 18. *D. variabilis*. A longitudinal section of accessory glands of a male after three days of attachment to the host. The epithelial cells contain numerous secretory droplets which will pass through a duct (D) to the seminal vesicle (SV). X400.

Figure 19. *D. variabilis*. A longitudinal section of accessory glands of a male after three days of attachment to the host. The epithelial cells, which consist of the accessory glands, contain secretory droplets moving towards the lumen (L). The accessory glands are coated with a thin layer of flat epithelium. X400.
Figure 20. *D. variabilis*. A diagram of female reproductive system eight days after attachment to the host. The reproductive system consists of a single U-shaped convoluted ovary (Ov) which shows regional variations with oocytes of small size at the longitudinal groove (LG) and oocytes (Oc) of larger size outside the longitudinal groove. The ovary also contains a few developing oocytes (DOc) which are approaching maturity. From each side of the ovary extends an oviduct (OD) which joins the seminal receptacle (SR) anteriorly. The trilobed seminal receptacle is connected to the genital opening by a vagina (V). At the junction of the vagina and the seminal receptacle a pair of small tubular accessory glands (AG) is associated.
Figure 21. *D. variabilis*. Cross section of ovary six days after attachment to the host. The ovarian wall consists of luminal epithelial cells (LE) around the lumen (L) which is crescent shape. The oocytes are attached to the ovarian wall by funicular cells (F). External epithelium (EE) and occasional muscle cells are distributed on the outside of the ovarian wall. X400.

Figure 22. *D. andersoni*. Cross section of ovary eight days after attachment to the host, showing luminal epithelium (LE), oocytes (Oc) at different stages of maturation and funicular cells (F) which form a pedicle attaching oocytes to the ovarian wall. X400.
Figure 23. *D. variabilis*. Relation of oocyte to the ovarian wall. Showing oocyte (Oc), funicle cell (F), interstitial cell (IC), and external epithelium (EE). The tunica propria (TP) of funicle cells is continuous with that of the oocyte. X15000.
Figure 24. *D. variabilis*. Relation between oocyte and funicular cells. Oocytes (Oc) are attached to the ovarian wall by a group of funicular cells (F) which contact the oocyte at the micropyle side (My). The funicular cells function mainly as supportive and probably nourishing too. The egg-micropyle opens to the ovarian lumen and represents the site for sperm interaction. X5000.

Figure 25. *D. variabilis*. A section of an ovary eight days after attachment to the host. Developing oocytes (Oc) are at different stages of maturation with the nuclei (N) the base. X250.

Figure 26. *D. variabilis*. Developing oocyte from an ovary one day after engorgement, with numerous vitelline bodies. X350.
Plate 11

Figure 27. *D. variabilis*. Developing oocyte from an ovary two days after engorgement showing enlarging vitelline bodies. X400.

Figure 28. *D. variabilis*. Maturing oocyte at the ovarian wall eight days after attachment to the host. As oocytes advance in developing they protrude in the haemocoel. When the ovary is dissected in saline solution, the tunica propria (TP) swells like a jelly coat. X350.

Figure 29. *D. variabilis*. A section of the ovary after oviposition. Only a few oocytes (Oc) are left on the ovarian wall. Collapsed tunica propria (TP) and dead funicle cells (F) are left after oviposition. The luminal epithelium (LE) shrink considerably. X250.

Figure 30. *D. variabilis*. Magnified tunica propria which collapses after oocytes pass to the ovary. X10000.
Figure 31. *D. andersoni*. Female reproductive system during oviposition. Few eggs of various stages of development are still attached to the ovarian wall. Fertilized mature eggs pass through the ovarian wall to the oviduct (OD) where they become linearly arranged. Mature eggs then pass to the seminal receptacle (SR) and through the vagina to the external genital opening. X4.
Figure 32. *D. variabilis*. Section of oviduct. The oviduct wall consists of columnar epithelial cells which become highly folded towards the lumen (L). On the outside, it is covered with a thin muscular layer and external epithelium (EE) cells. X600.

Figure 33. *D. variabilis*. Relation of oviduct with the ovary. The oviduct is continuous with the ovary and the lumen of the oviduct continues with that of the ovary. At this region, the oviduct is lined with cuboidal epithelium and on the outer surface, both are covered with muscular (Ms) and external epithelial cells (EE). X400.

Figures 34-35. *D. variabilis*. Spermatozoa migrating through the oviduct. After dissociation of the endospermatophore wall, mature spermatozoa leave the seminal receptacle and migrate through the oviduct towards the ovary. Fewer spermatozoa are seen in the oviduct closer to the ovary (35). X1000.
Plate 14

Figure 36. *D. variabilis*. Section of a female seminal receptacle. The wall of the seminal receptacle consists of columnar epithelium with folded tips (arrow). The outer surface is covered with a thin layer of connective tissue, external epithelial cells (EE) and muscular layer (Ms). X600.

Figure 37. *D. andersoni*. Endospermatophore in the female seminal receptacle after insemination. The endospermatophore is full of immature spermatozoa. X800.

Figure 38. *D. andersoni*. Section of seminal receptacle after oviposition. Few spermatozoa (Sp) are still present, but large numbers of macrophage-like cells (Mg) are also present which presumably engulf the remaining spermatozoa. X800.

Figure 39. *D. andersoni*. Section of a female seminal receptacle after complete engorgement. The seminal receptacle is extended considerably at this time and the tips of the columnar cells are highly folded (arrow) and contain large nuclei (N). The surface of the seminal receptacle is covered with a thin layer of connective tissue and few external epithelial cells. X600.
Plate 15

Figure 40. *D. andersoni*. Macrophage-like cells (Mg) in the seminal receptacle, which appears to engulf a spermatozoon (Sp) after oviposition. These cells contain many granules (Gr) which probably help in destruction of spermatozoa. X10000.

Figure 41. *D. andersoni*. Section of a female seminal receptacle with spermatozoa (Sp) penetrated or engulfed by the seminal receptacle cell. X8000.

Figure 42. *D. variabilis*. A section of the accessory glands of a female after six days of attachment to the host. The accessory glands consist of elongated epithelial cells which contain secretory products that pass to the vagina during oviposition. The accessory glands are coated on the outside with a thin layer of flat epithelial cells. X300.

Figure 43. *D. variabilis*. Section of a female vagina eight days after attachment to the host. The vagina is lined with a layer of cuticular intima (Cu) which sets on a layer of columnar epithelium. The vagina is surrounded with a well developed muscular layer (Ms) which aids during oviposition. X500.
Figure 44. *D. variabilis*. Diagram of a spermatophore after extrusion from the male during insemination process. The spermatophore consists of an endospermatophore (EnS) and an ectospermatophore (EcS) connected by a slender neck (Ne).

Figure 45. *D. variabilis*. Diagram of an ectospermatophore which protrudes from the female genital opening after spermatophore transfer and drops off about four hours later.
Figure 46. *D. variabilis*. Spermatocytes from a section of a male three days after attachment to the host. The subsurface cisternae (C) increase in number and size and become elongated. The spermatocyte contains a large number of Golgi complexes (G) and endoplasmic reticula (ER). Next to the spermatocyte is a somatic cell (SC) which probably has a nourishing function. X30000.
Figure 47. *D. variabilis*. Spermatids from a section of testis of a male four days after attachment to the host. The spermatids, in many cases, are connected to each other by intercellular junctions (arrow). X1000.

Figure 48. *D. variabilis*. Spermatids from a section of testis of a male four days after attachment to the host, showing an intercellular junction (arrow). X12000.
Figure 49. *D. variabilis*. Spermatids from a section of testis of a male four days after attachment to the host. The spermatids have a large number of subsurface cisternae (C) which develop as infolding of the cell membrane and, at this stage, become crowded at the periphery. The cytoplasm contains a large number of mitochondria (M), multivesicular bodies (MB), few endoplasmic reticula (ER) and Golgi complexes (G). X15000.

Figure 50. *D. variabilis*. A spermatid from a section of testis of a male four days after attachment to the host. The spermatid is at the beginning of the polarization process which consists of movement of the nucleus (N) to one pole and accumulation of the subsurface cisternae (C) at the opposite pole of the spermatid. X15000.
Figures 51-53. *D. variabilis*. Spermatocytes from testis of a male four days after attachment to the host. The subsurface cisternae are in progressive multiple fusion. The subsurface cisternae became narrow and started to fuse longitudinally to form elongated processes. The tips of the subsurface cisternae are slightly thickened and started to fuse (arrow). X20000; X40000.
Figures 54-55. *D. variabilis*. Spermatids from a section of a testis of a male four days after attachment to the host. The tips of the subsurface cisternae are fusing to form a membrane (arrow) which enclose the subsurface cisternae in a cisternal cavity (CC). The subsurface cisternae become modified into elongated processes (Pr) which extend in the cisternal cavity. X20000.
Figure 56. Schematic diagrams representing stages of spermiogenesis.

A. Formation of the cisternal cavity (CC) after fusion of the subsurface cisternae which now extend as cellular processes. The cytoplasm contains the nucleus (N), Golgi complexes (G), mitochondria (M), and endoplasmic reticula (ER).

B. The invagination process and the beginning of the inner tube (IT) formation. The mitochondria flow in this tube and the nucleus is slightly elongated.

C. Completion of the invagination process and the formation of elongated spermatids (im- mature spermatozoa) which consist of an inner tube (IT) and outer tube (OT) enclosed in the cell membrane (CM). The tip of the inner tube does not completely touch the outer tube. Most of the mitochondria are enclosed in the inner tube and the nucleus (N) is elongated and migrated anteriorly between the outer tube and the cell membrane. At this stage, elongated spermatids accumulate in the seminal receptacle and are transferred to the female within the endospermatophore during insemination.

D. Evagination process during sperm maturation in the female seminal receptacle. The cell membrane (CM) ruptures and the inner tube (IT) penetrates the outer tube (OT) and results in the exposure of the motile organelle (MO) and separation of the outer and inner tubes. The cell membrane retracts into the cytoplasm and starts the formation of the filament canal (FC). The cellular processes (CP) are now on the outer surface of the maturing spermatozoon.

E. Mature spermatozoon after completion of the evagination process. The spermatozoon consists of 1) motile organelle (MO), 2) club region (CR), 3) middle region (MR), and posterior region (PR) which contains the elongated nucleus (N), acrosome (Ac), filament canal (FC) and a unique plug-like structure (Pg). X2200.
Figure 57. *D. variabilis.* Elongated spermatid from a section of seminal vesicle of a male four days after attachment to the host. Showing are the inner or invagination tube (IT) with mitochondria (M), outer tube (OT) and cell membrane (CM). X10000.

Figure 58. *D. variabilis.* Oblique section of elongated spermatids (immature spermatozoon) from a section of seminal vesicle of a male after five days of attachment to the host (or after full engorgement). The spermatid consists of an inner tube (IT), outer tube (OT) and cell membrane (CM). The nucleus (N) is situated between the cell membrane and the outer tube. X10000.

Figure 59. *D. variabilis.* Longitudinal section of elongated spermatid (immature spermatozoon) from the seminal vesicle of a male five days after attachment to the host. The immature spermatozoon also consists of an inner tube (IT), outer tube (OT) and the elongated nucleus (N) between the cell membrane (CM) and the outer tube. X10000.
Figure 60. *D. variabilis*. Immature spermatozoon (examined by Nomarski interference microscope) from the female seminal receptacle one day after insemination. The spermatozoon has a pointed end and a broad end which appears open and a groove-like depression (Gv) runs along the side of the spermatozoon. X1300.

Figure 61. *D. variabilis*. Immature spermatozoon (examined by Nomarski interference microscope) from the seminal receptacle of a female two days after insemination. The spermatozoon also appears as a tubule inside another, but the spermiogenesis (sperm maturation) presumably is resumed as indicated by the ruptured cell membrane (arrow). X1300.

Figure 62. *D. andersoni*. Immature spermatozoon from the seminal receptacle of inseminated female. This also consists of an inner tube, outer tube and cell membrane on the outside and sperm maturation is resumed. X1300.
Figure 63. *D. andersoni*. Sections of immature spermatozoa from the seminal receptacle of an inseminated female. The spermatozoa consist of an inner tube (IT) with mitochondria (M), outer tube (OT) and cell membrane (CM). The nucleus is situated between the cell membrane and the outer tube. The invaginated part of the inner tube (arrow) represents the groove along the side of the spermatozoon (shown in Figure 60). X10000.

Figure 64. *D. andersoni*. Arrangement of cellular processes in immature spermatozoa. Magnified section of an immature spermatozoon from the seminal receptacle of an inseminated female. The outer tube contains the processes on the inner side while the inner tube contains the processes on the outer side. X25000.
Figure 65. D. variabilis. Mature spermatozoa from a female seminal receptacle and examined by fluorescent microscope. The nucleus (N) which is an elongated filiform green structure and the acrosome (Ac) which is an oval red structure are located in the posterior region of the spermatozoon.
Figure 66. *D. variabilis*. Mature spermatozoon from a dissected female seminal receptacle. X600.

Figure 67. *D. andersoni*. Mature spermatozoa from a dissected female seminal receptacle. The spermatozoon of both species is divided into four regions: 1) motile organelle (MO), 2) club region (CR), 3) middle region (MR), and 4) posterior region (PR). X500.
Figure 68. *D. andersoni*. Mature spermatozoa examined with a Nomarski interference microscope. The motile organelle (MO) forms a lid-like structure at the anterior tip of the club region (CR) with a constriction at the junction between the two structures. X2000.

Figure 69. *D. andersoni*. Mature spermatozoa examined with a Nomarski interference microscope. The motile organelle (MO) is retracted forming a ring-like structure. X2000.

Figure 70. *D. variabilis*. Mature spermatozoon examined the same way as above. X1200.
Figures 71-73. *D. variabilis*. Cross sections of the mature spermatozoa through the motile organelle. The motile organelle consists of numerous microtubules (Mt) of which some are connected to the surface (arrow). The surface of the motile organelle is covered with cellular processes except at the constriction (Co) which is the junction between this organelle and the club region. X10000 (71,72); X40000 (73).
Figure 74. *D. andersoni*. Cross section of mature spermatozoon through the anterior part of the club region near the motile organelle, showing large aggregates of mitochondria (M). The surface is covered with cellular processes (CP) of two different heights. X10000.

Figure 75. *D. andersoni*. Cross section of the mature spermatozoon through the middle part of the club region, showing also a large number of mitochondria (M) which become spread out towards the periphery. Endoplasmic reticulum (ER) is present. X10000.

Figure 76. *D. andersoni*. Cross section of mature spermatozoon through the posterior part of the club region, showing few mitochondria (M) which become elongated. The cellular processes (CP) are also of two heights. X15000.
Plate 31

Figure 78. *D. andersoni*. Section of mature spermatozoon at the middle region. The surface is also covered with cellular processes (CP) which are of two heights. X20000.

Figure 79. *D. andersoni*. Longitudinal section of mature spermatozoon at the middle region which in some cases could fold during motility. Mitochondria (M) tend to be arranged at the periphery. Subsurface filaments (SF) and endoplasmic reticula (ER) are present. X8000.

Figure 80. *D. variabilis*. Oblique section of the surface of mature spermatozoon at the middle region. Numerous subsurface filaments (SF) are present which are presumably contractile and associated with the cellular processes. X5000.
Figure 81. *D. andersoni*. Longitudinal section of mature spermatozoon at the posterior region. Most of this region is occupied with the filament canal (FC) and also contains the nucleus (N). X10000.

Figure 82. *D. andersoni*. Longitudinal section of mature spermatozoon at the posterior region. Mitochondria (M) occur at the periphery and along the filament canal. The surface is covered with cell processes (CP) but all are of the same height and subsurface filaments (SF) are abundant. X10000.

Figure 83. *D. andersoni*. Cross section of mature spermatozoon at the posterior region. The nucleus (N) is connected to the acrosome by a rod (R). X15000.
Plate 33

Figure 84. *D. andersoni*. Longitudinal section of mature spermatozoon at the posterior region. In addition to the elongated nucleus (N) this region also contains a plug-like structure (Pg) at the end. X12000.

Figure 85. *D. andersoni*. Longitudinal section of mature spermatozoon at the posterior region. The plug-like structure (Pg) contains numerous tubular structures (arrow) of various sizes. The nuclear membrane contains many constrictions. X24000.

Figure 86. *D. andersoni*. Section of mature spermatozoon at the posterior region. Two centrioles (Cn) occur next to the nucleus (N). These centrioles only occur in this region of the sperm, but they do not give a clear contrast like in other organisms. X24000.
Figure 87. *D. andersoni*. Longitudinal section of a mature spermatozoon. The surface of spermatozoon is covered with cellular processes (CP) arranged in rows and run the whole length of the spermatozoon and when one process ends another starts (arrow). The process is made of a plate of dense material enclosed in a loose and corrugated envelope of membrane. Numerous subsurface filament (SF) are associated with the cellular processes. X25000.

Figure 88. *D. andersoni*. Oblique section of mature spermatozoon at the posterior end. The cell processes (CP) also cover the surface and are arranged in rows, but in this region are of the same height. X25000.

Figure 89. *D. andersoni*. Cross section of mature spermatozoon at the middle region. Cell processes (CP) which cover the surface are attached by narrow connections (arrow). This connection to the surface is presumably flexible and allow the movement of the cell processes. X25000.
Figures 90-94. *D. variabilis*. Live mature and motile spermatozoa from the female seminal receptacle and examined with a Nomarski interference microscope. During motility, the motile organelle (MO) goes through several phases of sequential changes involving rotation (90), bending (91, 93) and retraction (92, 94). In addition, the club region (CR) and the rest of the spermatozoon twist slowly which give the sperm a mode of spiral movement. X650.
Figures 95-99. *D. andersoni*. Live mature and motile spermatozoa from the female seminal receptacle and examined with phase-contrast optics. During motility, the motile organelle goes through a series of sequential changes of: rotation (95), stretching (96), bending (97, 98), and retraction (99). X500 (95, 98); X350 (96, 97, 99).
Figure 100. *D. variabilis*. A section of oocyte from an ovary of a female after one day of attachment to the host. Showing are the nucleus (N) with few dense material (arrow) passed through the nuclear membrane. The cytoplasm contains round and elongated mitochondria (M) and very few endoplasmic reticulum (ER). X22000

Figure 101. *D. variabilis*. Section of an oocyte from an ovary of a female one day after attachment to the host. The nucleus (N) with dense material (arrow) near the nuclear membrane and also associated with the mitochondria (M). X20000.
Figure 102. *D. variabilis*. Section of an oocyte from an ovary of a female two days after attachment to the host. The oocyte starts to develop microvilli (Mv) on the surface underneath the tunica propria (TP). X10000.

Figure 103. *D. variabilis*. Section of an oocyte from an ovary of a female two days after attachment to the host. In addition to the beginning of microvilli formation, multivesicular bodies (MB) begin to appear. More dense materials (arrow) have migrated to the cytoplasm through the nuclear membrane. X10000.
Figure 104. *D. variabilis.* Section of an oocyte from an ovary of a female four days after attachment to the host. The nucleus (N) contains a well developed nucleolus (Nu) with a porous side (arrow). The nuclear membrane has definite and well recognized pores (NP). Numerous dense materials are extruded through the nuclear membrane to the cytoplasm. Mitochondria (M) are abundant near the nucleus. X24000.

Figure 105. *D. variabilis.* Section of an oocyte from an ovary of a female four days after attachment to the host. Many more microvilli (Mv) have been formed which obtain a border underneath the tunica propria (TP). Micropinocytotic pits (MP) also start to appear at the periphery. X24000.
Figure 106. *D. variabilis*. Section of an oocyte from the ovary of a female six days after attachment to the host. Numerous microvilli (Mv) have developed which form a brush border on the surface underneath the tunica propria (TP). Multivesicular bodies (MB) are also abundant. X13000.

Figure 107. *D. variabilis*. Section of an oocyte from the ovary of a female six days after attachment to the host. Deposition of cuticle has started on the surface of the oocyte between the microvilli. Deposition of cuticle starts at different times in different oocytes. Micropinocytotic pits (MP) and micropinocytotic tubes (Mc) are abundant and associated with reservoirs (R) which presumably contain amorphous material as a result of micropinocytosis. Small yolk bodies (YB), lipid droplets (LD) and mitochondria (M) are also present and associated with the reservoirs in some cases. X17000.
Figure 108. *D. variabilis*. A section of an oocyte from an ovary of a female eight days after attachment to the host. Microvilli (Mv) are still present, but cuticle (Cu) begins to appear as a continuous layer. Numerous micropinocytotic pits and micropinocytotic tubes are present. More reservoirs (R) are present and become associated with mitochondria (M), yolk bodies (YB) and lipid droplets (LD). X20000.

Figure 109. *D. variabilis*. A section of an egg from an ovary of a female two days after engorgement. Considerable amount of cuticle (Cu) has been deposited which forms a continuous layer under the tunica propria but very few microvilli (Mv) are present. The cuticular layer has pores (arrow) which represent the basis of the microvilli. Yolk bodies (YB) and lipid droplets (LD) are abundant with interspersed mitochondria (M). Presumably, the oocyte has been fertilized before the complete deposition of cuticle. X10000.
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Figure 110. *D. variabilis*. A section of an egg from a female two days after engorgement. Yolk bodies (YB) are enlarging considerably. Mitochondria (M) are trapped between the yolk bodies and numerous endoplasmic reticula (ER) and ribosomes (r) are also associated with them. X17000.

Figure 111. *D. variabilis*. A section of an egg from a female two days after engorgement. Yolk bodies (YB) are fully developed with few endoplasmic reticula (ER) and mitochondria (M) associated with them. X17000.
Figures 112-113. *D. variabilis*. Scanning electron micrographs of fertilized eggs from the ovary of a female during oviposition. The cuticular envelope consists of spherules of different sizes with few small pores (arrow) which presumably represent the previous place of microvilli. X10000.
Figure 114. *D. variabilis*. Sperm-egg interactions. A section of an ovary of a female five days after insemination. The spermatozoon (Sp) is observed through the ovarian lumen (L) at the site of the egg micropyle (My) with the anterior reaching the micropyle first and the motile organelle is presumably the first sperm-structure to contact the egg proper. X1200.
Figure 115. *D. variabilis*. *In vitro* sperm-egg interactions, showing the spermatozoon moving towards the oocyte (Oc). In some cases the spermatozoon accelerated when it reaches a distance of approximately 20 μ from the oocyte.

Figure 116. *D. variabilis*. *In vitro* sperm-egg interactions. Spermatozoon has contacted the oocyte and in some cases stay in this position for a few minutes. No penetration was observed. X300.