

CRC REVIVALS

Insect Reproduction

Simon R. Leather, Jim Hardie

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Insect Reproduction

Edited by

Simon R. Leather and Jim Hardie

Imperial College of Science, Technology, and Medicine

University of London

Department of Biology

Imperial College at Silwood Park

Ascot, England



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INTRODUCTION

This book, consisting of ten review chapters contributed by leading workers in their respective fields, from around the world, covers the whole subject of insect reproduction. It begins with the basic physiological questions of insect reproduction, moves on to discuss the new advances seen in the fields of behavioral and ecological mechanisms, and culminates by examining the recent work on evolutionary biology and its application in the field.

Each chapter, although including a brief review of the basic seminal work, focuses mainly on the advances made within the last ten years and highlights those areas in which the respective authors see the greatest scope for further important advances. By allowing each author full rein to explore their chapter subject using their particular "hobby horse," it has been possible to make this not just a book of review chapters, but one in which exciting new ideas have been raised.

This book should be of general interest to all entomologists, whether they are in pure or applied fields, and should also be an important asset to any teaching program where entomology is taught at the undergraduate and post-graduate level.

SRL, JH.



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THE EDITORS

Dr. Simon R. Leather is presently Lecturer in Applied Ecology and Pest Management at Imperial College, London. He obtained his B.Sc. from the University of Leeds, England in 1977 with first class honors in Agricultural Zoology. After receiving his Ph.D. in 1980 from the University of East Anglia in Norwich, he embarked on further research in aphid ecology. Then followed a ten year spell with the British Forestry Commission where he worked in the Research Division, primarily on the population biology of forest pests with particular reference to their reproductive behavior. He started his current position in 1992. Dr. Leather is a Fellow of the Royal Entomological Society, a Member of the British Ecological Society, a Member of the Flora and Fauna Preservation Society, and a Member of the Institute of Biology. He sits on the Council of the Royal Entomological Society and edits their journal *Antenna*.

Dr. Jim Hardie is presently a Principal Research Fellow at Imperial College, London. He obtained a Ph.D. degree from the University of Birmingham, England in 1975 and a D.Sc. from London University in 1989. He has worked in the field of aphid physiology for more than twenty years and is regarded as one of the leading figures in this area. He is a Fellow of the Institute of Biology, the Royal Entomological Society, and the Royal Microscopical Society.



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CONTRIBUTORS

Roger L. Blackman, B.Sc., Ph.D.
Department of Entomology
The Natural History Museum
London, England

Carol L. Boggs, Ph.D.
Center for Conservation Biology
Department of Biological Sciences
Stanford University
Stanford, California
and Rocky Mountain Biological
Laboratory
Crested Butte, Colorado

A. F. G. Dixon, B.Sc., D. Phil.
School of Biological Sciences
University of East Anglia
Norwich, England

Cedric Gillott, B.Sc., Ph.D., D.Sc.
Department of Biology
University of Saskatchewan
Saskatoon, Canada

Jim Hardie, B.Tech., Ph.D., D.Sc.
Department of Biology
Imperial College of Science,
Technology, and Medicine
University of London
Silwood Park
Ascot, England

Klaus H. Hoffmann, Prof. Dr.
Department of Animal Ecology I
University of Bayreuth
Bayreuth, Germany

Simon R. Leather, B.Sc., Ph.D.
Department of Biology
Imperial College of Science,
Technology, and Medicine
University of London
Silwood Park
Ascot, England

Athol McLachlan, Ph.D., D.Sc.
Department of Agriculture and
Environmental Sciences
University of Newcastle-upon-Tyne
Newcastle-upon-Tyne, England

Rachel Neems, B.Sc., Ph.D.
Department of Genetics
University of Leeds
Leeds, England

Richard Wall, B.Sc., Ph.D.
School of Biological Sciences
University of Bristol
Bristol, England

Christer Wiklund, Ph.D.
Department of Zoology
University of Stockholm
Stockholm, Sweden



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DEDICATION

This book is dedicated to our families in recognition of the support given during the somewhat lengthy process that ensued once we had embarked upon this task. So, thank you Gill, Fern, John, James, Thomas, and Matthew from Simon, and thank you Ros, Sally, Nicola, and Robert from Jim.



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OOGENESIS AND THE FEMALE REPRODUCTIVE SYSTEM

Klaus H. Hoffmann

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I. INTRODUCTION

Insect reproduction results from a succession of interdependent steps which are often quite different in nature and take place at various stages of the insect life cycle. The main reproductive events in females are sex determination, gonial mitoses and meioses, differentiation of the

reproductive organs, previtellogenesis and vitellogenesis, accessory gland functioning, sexual behavior, mating, ovulation, and oviposition. It is the function of the female reproductive tract to produce the eggs and to deposit them at an appropriate time and in an appropriate place. In addition, the female reproductive tract must receive the spermatozoa from the male and transport them to the spermatheca where they are stored until they are used to fertilize the eggs as they are oviposited.

Insect reproduction strictly depends on environmental factors. Factors which may affect reproduction include temperature, humidity, photoperiod, nutritive conditions, and a suitable egg-laying substrate.

Regulation of insect reproduction involves numerous sensory receptors, nerve transmission, and integration in the brain, which regulates the synthesis of the two groups of insect developmental hormones, the juvenile hormones and the ecdysteroids, and produces its own neurohormones.¹ The mechanisms which regulate each reproductive step may vary with insect species (see Chapter by Hardie).

Present knowledge of oogenesis has progressed variously, depending on the event considered. The best-known field is that of vitellogenesis and its endocrine regulation, while the early events of ovarian development and its "fine-tuning" control by hormones are less well understood.

Sexual reproduction is the general rule in insects, although many exceptions and modifications are observed (see Chapter by Dixon). In the more highly evolved social insects (Hymenoptera), reproduction is limited to a small number of individuals, often one queen and a small number of males (drones).

II. MORPHOLOGY OF THE FEMALE REPRODUCTIVE SYSTEM

The central site of egg production is the ovary. Ovaries are usually located dorsolateral to the gut and each comprises a number of tubular ovarioles ensheathed by a network of connective tissue (Figure 1). Each ovariole is composed of somatic and germ cell tissues. The number of ovarioles per ovary varies from one (e.g., in some viviparous aphids and in dung beetles) to about 3000 (in some higher termite queens).² Three basic types of ovary organization are found in insects (Figure 2). The panoistic ovarian, probably the most primitive one, is found in the oldest families of insects, such as in Archaeognatha, Zygentoma, Odonata, Plecoptera, and in most orthopteroid insects, but also in some Megaloptera (Corydalidae) and most Siphonaptera. In the panoistic ovary, specialized nutritive cells are absent and most of the informational resources of the oocyte are provided by the synthetic activity of the oocyte nucleus itself. The polytrophic meroistic ovary is found in most endopterygotes, and in Dermaptera, Psocoptera, and in Phthiraptera. In polytrophic ovaries, a number of nurse cells are enclosed in each follicle along with an oocyte. In the Hemiptera, polyphage Coleoptera, Megaloptera (Sialidae)/Raphidioptera, and also in the most "primitive" winged insects, the Ephemeroptera,³ telotrophic meroistic ovaries are found. In this ovarian type, a syncytium of nutritive cells is connected with each oocyte by means of a trophic cord. In the vast majority of insect species, the nutritive, or yolk, contribution is supplied largely by the fat body, but in some cases the follicle cells can also serve as additional source of yolk. Accumulation of yolk (vitellogenesis; Section IV.C) normally occurs only in the terminal oocyte, that is, the oocyte closest to the oviduct. Another follicle cell function is the formation of the protective layers of the egg. These include the vitelline membrane and the chorion (Section IV.D).

Besides the ovary, oviduct-associated secretory cells can have important contributions to egg production (Section II.C). The most significant of such structures are the spermathecal accessory glands and the female accessory glands, such as the colleterial glands. Last, secretory functions of the vagina may play a role in egg production. Like other terrestrial

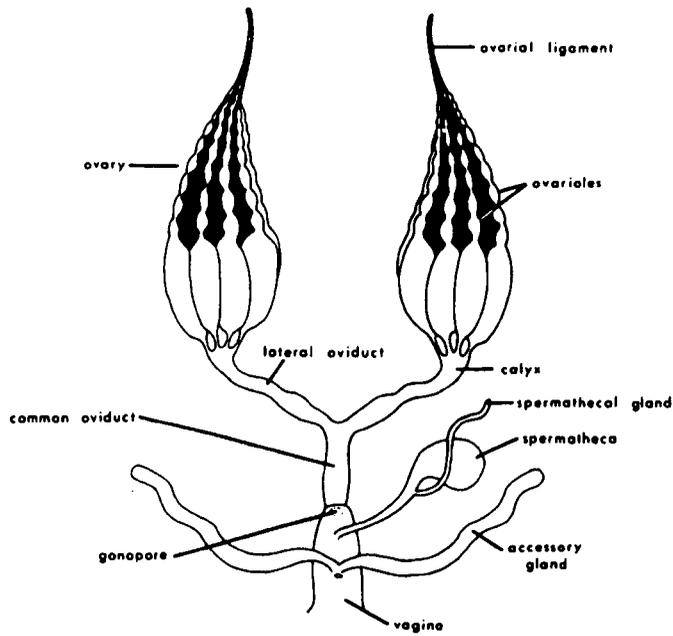


FIGURE 1. Female reproductive system: diagram of common type found in many insects. (From Gillott, C., *Entomology*, Plenum, New York, 1980, chap. 19. With permission.)

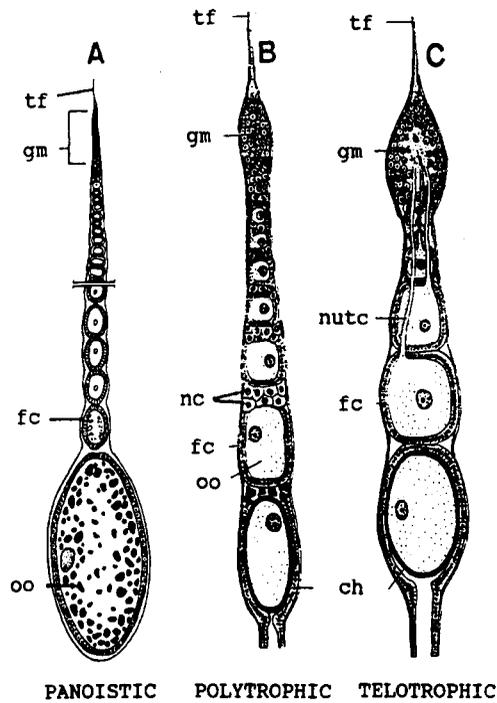


FIGURE 2. Schematic diagrams showing the types of ovarioles. Meroistic ovaries may be organized in the telotrophic and the polytrophic way, respectively.^{4,70} ch = chorion; fc = follicle cell; gm = germarium; nc = nurse cells; nutc = nutritive cord; oo = oocyte; tf = terminal filament. (From Gillott, C., *Entomology*, Plenum, New York, 1980, chap. 19. With permission.)

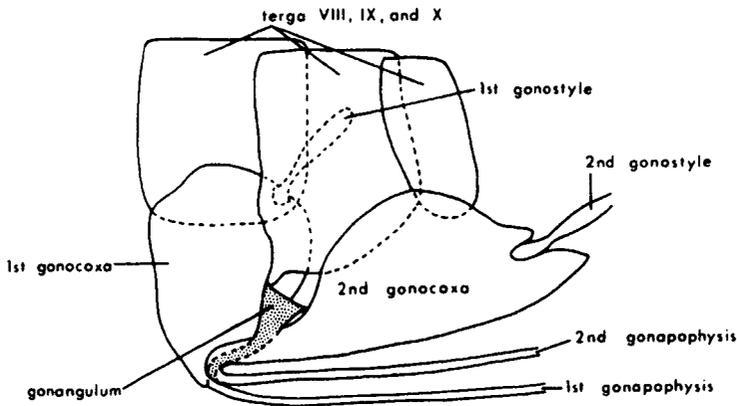


FIGURE 3. The primitive structure of the pterygote ovipositor in the thysanuran *Lepisma*. (From Gillott, C., *Entomology*, Plenum, New York, 1980, chap. 19. With permission.)

animals, insects have had to solve the problem of bringing together sperm and egg in the absence of surrounding water (internal fertilization, see Chapter by Wall). Almost all insects store the spermatozoa received from the male in a specialized organ, the spermatheca, until they are used to fertilize the mature eggs.

A. EXTERNAL GENITALIA

The morphology of the organs specialized for copulation and oviposition is highly varied. In the mayflies, the oviducts open directly to two genital pores behind the seventh abdominal segment. In most insects, the appendages of the genital segments (eighth and ninth abdominal segment) form an ovipositor. In the apterygotes and some of the winged insects, the ovipositor is a simple opening both for copulation and for the deposition of eggs. The primitive structure of the pterygote ovipositor can be seen already in the thysanuran *Lepisma* (Figure 3).⁴ Among Pterygota, an ovipositor is found in Notoptera (Grylloblattodea), Dictyoptera, Ensifera, Caelifera, and Hymenoptera, some Odonata and most Hemiptera, Thysanoptera, and Psocoptera. The structure and elaborateness of the ovipositor is determined by the site of egg deposition. The ovipositor of Hymenoptera may be considerably modified for boring, piercing, sawing, and stinging. In the stinging Hymenoptera, such as bees, the eggs are released at the base of the ovipositor, and the ovipositor is modified by the addition of poison glands and reservoirs that evacuate the venom through the hollow sting. The ovipositor of *Drosophila* has sharpened ends that penetrate the surface of fruit, while the ovipositors of some of the predatory wasps are long (up to 15 cm in length) and sharp to penetrate the body of the insect prey.⁵

B. STRUCTURE OF THE OVARY

Insects have become particularly adept in manufacturing large numbers of oocytes within the ovary. The fruit fly, *Drosophila melanogaster*, can produce, during a 10-week reproduction period, a quantity of eggs equivalent to 30 times her body weight.⁶ Her newly formed oocytes can undergo a 100,000-fold increase in volume within 3 days. Such reproductive feats are possible because of certain evolutionary adaptations: (1) Oocytes have developed methods for incorporating massive quantities of female-specific proteins (vitellogenins) which are transported in the hemolymph from the fat body to the ovary. (2) Mechanisms have evolved for loading unfertilized eggs with the ribosomes, tRNA, and long-lived mRNA that are required in early embryogenesis. (3) The ovarioles are supplied by tracheae of the aeriferous type, with a great diversity in the methods by which oxygen is delivered to the individual oocytes.⁷

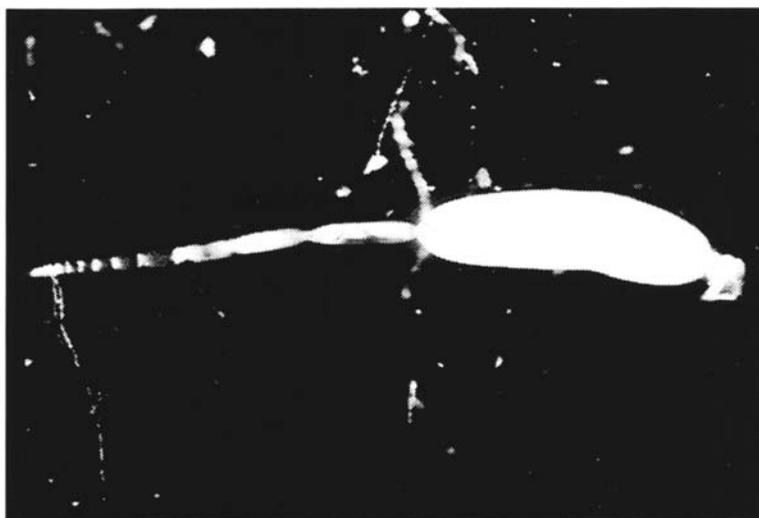


FIGURE 4. Panoistic ovariole of a cricket, *Gryllus bimaculatus* de Geer, with a mature egg in the terminal oocyte. Photograph courtesy of K. H. Hoffmann, Bayreuth.

Panoistic ovarioles can be developed by blocking germ cell cluster divisions totally, as is found in most “primitive” insects, or after germ cell cluster formation by final cleavage of cystocytes, all of which develop as oocytes as found in stone flies or thrips.⁸ In the panoistic ovary, each of the ovarioles is composed of a terminal filament, the germarium, a series of oocytes at the previtellogenic phase of development, one or more oocytes in the process of vitellogenesis, and last, the mature egg (Figure 2A; Figure 4). The terminal filament is made up of a group of flattened cells, surrounded by a basement lamina and an ovarian sheath, both of the latter surrounding the entire ovariole. The oogonia are located in the most anterior region of the germarium, followed by a zone of oocytes in the early stages of meiosis. At the posterior end of the germarium, the oocytes are beginning to be surrounded by a monolayer of follicle cells. The size increase of oocytes at the previtellogenic stage is accomplished by an expansion of the cytoplasmic volume. In many cases, a multilayered pad of interfollicular tissue is located between successive oocytes.⁹ In many insects with panoistic ovaries, vitellogenesis commences in the penultimate oocyte only after ovulation at the terminal one (e.g., in *Locusta migratoria* and *Schistocerca gregaria* [Caelifera]), or in the case of ovoviviparous cockroaches, after the loss of the egg case. The inhibitory effect is mediated by the interfollicular cells which pass an inhibitory substance from the anterior to the more posterior oocyte. In contrast, secretion from such cells located proximal to the oocyte stimulates vitellogenesis. In other species (e.g., in *Periplaneta americana* [Blattodea] and *Melanoplus sanguinipes* [Caelifera]), two or more oocytes may be vitellogenic at the same time, although at different stages of the yolk deposition cycle. All the above-mentioned insects produce eggs in batches. In the stick insect *Clitumnus extadentatus*, the different ovarioles can mature asynchronously, and the female lays a few eggs per day for several weeks. The number of ovarioles in panoistic ovaries can range from 4–3000 (5 in Acrididae, 15–30 in Tettigonioidea, 150–170 in Gryllidae, about 3000 in Isoptera queens).

A terminal filament and a germarium are also found in polytrophic meroistic ovaries (Figure 2B). In this case, the anterior region of the germarium contains one or more stem-line oogonia and a number of daughter cells or cystoblasts. The cystoblasts divide to give a cluster of cells remaining connected by structures called “ring canals” or “intercellular bridges.” The innovation of the polytrophic ovary is the differentiation of only one oocyte, which generates from one, central cell of the cluster, whereas all other siblings are transformed into nurse cells.

In many cases, clusters follow the 2^n -rule (1 oocyte + $2^n - 1$ nurse cells), in which n represents the number of cell cycles. Identical characters in polytrophic meroistic ovaries among hemimetabolous and holometabolous insects indicate a “basic type” of common origin.⁸ In *Drosophila*, where the number of cells in the cluster is 16, the clustering of the cells, as well as the formation of the ring canals, is mediated by structures called fusomes. The fusomes contain a random array of membranous vesicles and fibrils,^{10,11} but no mitochondria and few ribosomes and microtubules. In the central region of the germarium, prefollicular cells grow around the oocyte-nurse cell cluster, while in the posterior section of the germarium, typical egg chambers are detectable; which means the oocyte-nurse cell complex is completely surrounded by a unilayer of follicle cells. Previtellogenesis includes the enlargement of the oocyte, an increase in the number of follicle cells, and the polyploidization of the follicle cells and nurse cells. An epithelial sheath surrounds each ovariole and consists of a thin, acellular, inner membrane; a median cellular network of muscle and tracheal cells; and an outer epithelial membrane. The number of ovarioles in polytrophic ovaries can be highly variable (usually 4 in the Lepidoptera, 10–30 in *Drosophila*, and 70–100 in *Musca* or *Lucilia* [Diptera]). Commonly, a single oocyte per ovariole will become vitellogenic at one time.

Telotrophic ovaries differ from polytrophic ovaries by retention of all nurse cells in an anterior trophic chamber and by changing oocyte-nurse cell determination. This type of ovary developed independently three times (in Hemiptera, Raphidioptera/Megaloptera [Sialidae], and in polyphagous Coleoptera) from polytrophic ancestors and once directly from panoistic ancestors in mayflies.⁸ Despite fundamental differences between the subtypes of telotrophic ovarioles, they share some common characters. As the oocytes move towards the region of follicle formation, they become surrounded by prefollicular cells. The connection to the tropharium is retained via a cytoplasmic strand, the nurse strand or trophic cord. The young oocytes subsequently enlarge by incorporation of nurse cell material, transported through the nutritive cords (previtellogenesis). The vitellogenic growth phase begins when yolk spheres are observed to accumulate in the oocytes. The nutritive cords collapse during vitellogenesis. The follicular cells surround each growing oocyte to form a monolayered epithelium, and this tissue will secrete a vitellin membrane and the chorion. An interesting variant of the telotrophic ovary is found in the polyphagous Coleoptera. The number of ovarioles remains more or less constant in each species, but varies between species from 1 (some Scarabaeinae) to about 1000 in blister beetles (*Meloe proscarabaeus*).⁶ In *Creophilus maxillosus* (Staphylinidae), the differentiation of nurse cells and oocytes occurs within linear chains of sibling cells (linear chain model). Only the most basal member of the sibling cluster develops into an oocyte; the others differentiate into nurse cells.^{6,9}

C. STRUCTURE OF THE FEMALE ACCESSORY REPRODUCTIVE SYSTEM

The bottom of each ovariole forms a small duct or pedicel (Figure 1). The pedicels of each group unite to form a calyx, and each calyx opens into a lateral oviduct. Usually the oviducts of the two sides join to form a single median oviduct. The common oviduct is ectodermal in origin and typically it is heavily invested with muscles. The lateral oviducts can be of mesodermal or ectodermal origin. The presence or absence of a cuticular intima indicates the origin. In *Oncopeltus fasciatus* (Hemiptera), the lateral oviducts undergo a drastic larval-adult transformation during the last (fifth) larval stadium.¹² The long and thin larval oviducts shorten and become very wide. This transformation is ecdysteroid dependent in a dose-related manner and only takes place in the absence of juvenile hormone. The morphological transformation is accompanied by dramatic cytological changes. Whereas the cells of the anterior part of the oviduct commence with a strong secretory activity, the cells of the caudal part form a bizarre pattern of cell projections which deposit the cuticle. The secretory material of the ductal system may act as a lubricant for egg passage, as protective oothecal coverings, or as glues to attach eggs to various substrates or to hold batches of eggs together.⁹ The distal end of the common oviduct is called the gonopore, which serves for the discharge of eggs. In the

Dermaptera, the gonopore is the external opening, located on the posterior part of the seventh abdominal segment. In most insects, however, the gonopore opens into a genital chamber. The opening to the outside is the vulva. The genital chamber can be of variable complexity and is often associated with an ovipositor. Within some orders, an invagination of the primitive genital chamber forms a distinct intermediate structure, the vagina, between the external vulva and the gonopore. Generally, the vagina is not secretory and consists of a single layer of epithelial cells, covered by a cuticular intima and surrounded by muscle. In many species, the genital chamber has become modified to form a bursa copulatrix. An important function for this organ is to receive spermatophores or seminal fluid. In *Lepidoptera*, the bursa remains in the eighth abdominal segment, whereas the eggs are deposited through a separate opening, the oviporus, on segment IX. In this case, the bursa is connected with the vagina by the seminal duct. In other insects, however, there is no separation between the copulatory and egg-laying apertures, and both of them open as the vulva on segment IX. In the bursa of some butterflies (e.g., genus *Danaus*), tooth-like dentata are present and may function in tearing open the spermatophore. Some secretory activity may also be associated with the bursa copulatrix, since empty spermatophores are digested within the bursa of some insects. In ovoviviparous and viviparous species, a brood pouch or uterus forms as an expansion of the vagina wall.

Two ectodermal glands (ectademia) are connected with the genital chamber or vagina. One is the spermatheca in which spermatophores are stored and that has a gland attached to its duct. The other is a paired structure, the accessory glands or colleterial glands, with functions associated with egg deposition.

1. Spermatheca and Spermathecal Accessory Glands

The morphology and arrangement, as well as the number of spermathecae, vary from species to species. In most insects, the spermatheca is a single organ, spherical or ovoid in form. In some insects, such as cockroaches and mosquitoes, secretory cells are associated with the walls of the storage portion of the spermatheca, whereas in others, such as honeybees and many beetles, the distal end of the spermatheca is specialized for secretion and is referred to as the spermathecal accessory gland. In the genus *Rhodnius* (Heteroptera), the spermathecae are a pair of blind tubules which open into the common oviduct near its junction with the lateral oviducts.

In spite of the morphological diversity, the cellular elements of the secretory part of the spermatheca are rather constant. A generalized diagram of a spermathecal secretory cell is given in Figure 5. The cells form a cup-shaped cavity at their apical surface, and the membrane in this region is thrown into numerous microvilli. The central cavity communicates with the spermathecal lumen via a cuticle-lined ductule. In other forms, however, several ductules may open at a single pore. The cuticle lining the ductule and the spermathecal lumen is produced by separate duct cells which are interposed between the secretory cells and the lumen of the spermatheca. Ultrastructural studies showed that the secretory cells have a phenotype associated with cells which are specialized for export protein synthesis.⁹

The secretion produced by the gland cells is a mucoprotein or mucopolysaccharide and may be used by the spermatozoa for an energy source.¹³ Removal of the spermathecal accessory gland from females of *Anthonomus grandis* (Coleoptera) results in a gradual loss of motility among spermatozoa in the spermatheca. The epithelium of the spermatheca, particularly in the storage part, exhibits characteristics of ion-transporting epithelia and thus may be responsible for providing an ionic milieu within the lumen of the spermatheca different from that in the hemolymph. In some insects, the spermathecae may be sufficiently permeable to allow the passage of various male secretory products.

Information on the control of spermatheca differentiation is rather limited. In the beetle *Tenebrio molitor*, the differentiation of the spermatheca occurs in the pupal stage prior to the eclosion to the adult. The differentiation process can be divided into three phases: (1) cellular proliferation, (2) cellular morphogenesis, and (3) cuticulogenesis. From *in vitro* culture

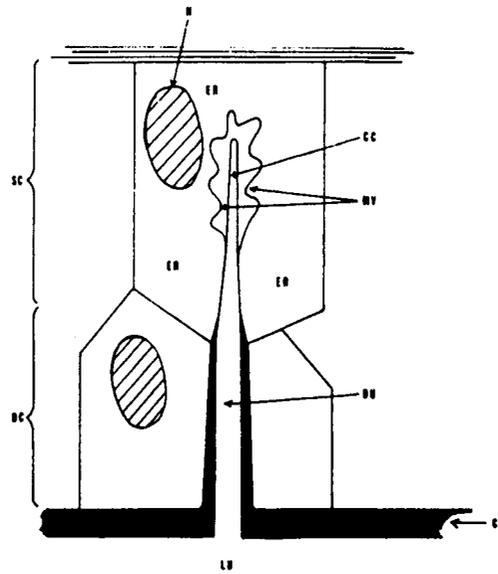


FIGURE 5. Diagrammatic representation of a spermathecal secretory cell and its spatial relationship to the duct cell and basement membrane. CC, central cavity; CU, cuticle; DC, duct or canal cell; DU, ductule; ER, rough endoplasmic reticulum; LU, lumen; MV, microvilli; N, nucleus; SC, secretory cell. (From Kaulenas, M. S., *Insect Accessory Reproductive Structures. Function, Structure, and Development*, Springer-Verlag, Berlin, 1992. With permission.)

experiments, it was concluded that cuticulogenesis is under control of 20-hydroxyecdysone; the hormone is necessary to initiate cuticle deposition.¹⁴ With regard to the control of the acquisition of definitive levels of differentiated functions by the spermatheca, some information is available for a number of Orthopterans. In *Chorthippus curtipennis* (Caelifera, Acridinae), allatectomy leads to degeneration of the spermatheca, while in *Gomphocerus rufus* (Caelifera, Acridinae), allatectomy resulted in the inability of females to dissolve transferred spermatophores, suggesting a failure in the production of spermathecal proteolytic enzymes.⁹ The results suggest that juvenile hormone is necessary to initiate and possibly maintain differentiative secretory function in these grasshoppers. On the other hand, allatectomy in *L. migratoria* (Caelifera, Locustinae) does not alter the histology of the spermatheca. In *Rhodnius prolixus* (Heteroptera), removal of the neurosecretory cells in the pars intercerebralis results in a failure of the secretion to appear in the lumen of the gland as a result of the absence of a myotropin from the neurosecretory cells. The myotropin acts on the muscles of the spermatheca to squeeze the secretion from the reservoir beneath the intima into the lumen.¹³

2. Colleterial Glands and Mesodermal Accessory Glands

Many insects produce protective coatings for the newly laid eggs. The Lepidoptera coat individual eggs with a proteinaceous glue that hardens on contact with air and attaches the eggs onto appropriate substrates. Accessory glands in the genus *Musca* (Diptera) contribute in aiding in the fertilization process by providing secretions which assist in liberating sperm acrosomal contents.¹⁵ Aquatic insects often produce egg cases of a gelled substance, but the most complex of these structures may be the tough ootheca of cockroaches and mantids. In mantids, the glands produce a polymerized protein foam. In cockroaches, several layers of tanned protein form a complicated egg chamber with elaborate respiratory apparatus and release valves. The sources of these protective devices are the colleterial glands which branch off the vagina or genital chamber. In most cases, the colleterial glands are a pair of organs composed of a number of multibranching tubules and are formed from invagination of the epidermis (Figure 1). The morphology of the colleterial glands has been well documented. Five cell types compose the left colleterial gland; four types of glandular cells are homologous

with the dermal glands of the integument, and a chitinogenic cell type is homologous with the epidermal cell of the integument. The latter cell type is found interspaced between the glandular cells and secretes the protective intima which lines the lumen of the tubule. Each of the gland cell types is distinct from the other, and is located in separate regions of the tubule. Type 1 cells are found in the most posterior region of the tubule and are in a presecretory stage. Type 2 gland cells are found anterior to type 1 cells and lack a well-defined rough endoplasmic reticulum (ER), but are packed with mitochondria. The morphology of the type 3 cells resembles that of both type 2 and type 4, and appears to be a transient intermediate form. The type 4 cells dominate the anterior ends of the tubules and are packed with rough ER and with mitochondria.¹⁶

The colleterial gland of *P. americana* has been studied most extensively.^{9,13} The left side tubules (type 4 cells) secrete the proteins (oothecins) which make up the structure of the ootheca, together with a polyphenol oxidase (type 2 cells) as well as the precursor of the tanning agent, the 4-O- β -glucoside of protocatechine acid. The right colleterial gland secretes the enzyme β -glucosidase. The opening for the duct from the right gland is more anterior than the opening for the duct from the left gland. At the time of ovulation (see Section VI), the chorionated oocytes migrate down the oviduct to the genital atrium for fertilization. After fertilization, the eggs are transported more posteriorly and pass the duct of the right gland where the contents of the gland (β -glucosidase) are secreted onto the fertilized egg. Thereafter, as the egg moves more posteriorly, it encounters the secretions of the left colleterial gland. The subsequent mixing of the secretions from both glands results in the hardened ootheca.¹⁶

Apparently, juvenile hormone affects the synthesis of oothecins in left colleterial glands.^{9,13,16,17} Analyses of the *Periplaneta* oothecin cDNA sequences and of the resultant predicted amino acid sequences have confirmed the existence of 11 major glycine-rich oothecins which represent six size classes with molecular weights of 14.5, 15.5, 17–18.5, 23.5–26, 28.0, and 37–39 kDa, respectively.¹⁸ The oothecin sequences have numerous similarities to silkworm chorion proteins. In mantids, the chemistry of the ootheca proteins shows some unique features. The glucosides identified in five mantid species are 3-O- β -glucosides of *N*- β -alanyldopamine and *N*-(*N*-malonyl- β -alanyl)dopamine. The light color of the ootheca and the occurrence of phenolic compounds modified at the β position of the parent compounds suggest that β -sclerotization occurs in mantid oothecae.

In *S. gregaria* and other Orthopterans, the foamy ootheca is produced, at least in part, by mesodermal accessory glands (mesadenes) which consist of convoluted blind tubules opening into the proximal end of the lateral oviducts. In spite of the great structural differences between glands responsible for oothecal formation in cockroaches and locusts, similar mechanisms may operate to harden the ootheca.

3. Milk Glands

In tsetse flies, members of the genus *Glossina*, the accessory glands of the female are transformed into milk glands and supply a secretion upon which the developing larva feeds (adenotrophic vivipary). In these flies, the female ovulates a single egg into a uterus, where it hatches. The growth of the larva is rapid under such circumstances. In *Glossina austeni* for example, development from an egg to a fully grown larva weighing some 30 mg requires only 9–10 days. The fully mature larva is then “larviposited.” The milk gland in *Glossina* is a branched tubular structure ramifying throughout the abdomen and emptying into the uterus via a single muscular collecting duct which contains two channels.¹³ The tubules consist of a single layer of secretory cells similar to those in other accessory glands. The tubules undergo cyclic changes in diameter, largely as a result of changes in the volume of secretory reservoirs. The secretory reservoir is an extracellular structure, formed by a cup-shaped invagination of the apical membrane of each of the secretory cells. In *G. austeni*, the tubular diameter reaches a peak of about 100 μ m 3 days before each larviposition, and a diameter of about 30 μ m at

each larviposition, followed by a resynthesis of the "synthetic machinery." Removal of the corpora allata greatly reduces the production of milk in tsetse flies.

Milk glands also occur in some cockroaches. In the viviparous cockroach *Diploptera punctata*, nutrient is supplied to the developing embryos, which increase in weight by a factor of about 50 before they hatch. The developing embryos receive this nutrition by ingesting a fluid secretion ("milk") in the brood pouch. The brood pouch is a part of the genital chamber. The intima of this cuticle-lined chamber is penetrated by pores, each of which is the opening of a ductule leading from a secretory cell. In *Diploptera*, juvenile hormone is necessary to allow the decline of milk gland synthesis at the termination of pregnancy.¹⁹

D. ENDOCRINE CONTROL OF DIFFERENTIATION OF ACCESSORY GLANDS AND DUCTS

The dependency on ecdysone and 20-hydroxyecdysone of the organogenesis of accessory glands and genital ducts has been demonstrated in several insect species.^{1,20} The development of the colleterial glands of *P. americana*, which occurs at the end of the last larval instar, requires ecdysone. In young females of *L. migratoria* and *S. gregaria*, implantation of additional corpora allata accelerated the development of both the oviducts and colleterial glands. In *T. molitor*, *Ephestia kühniella* and *Samia cynthia*, *L. migratoria*, and *O. fasciatus*, duct differentiation only occurs in the presence of ecdysone *in vivo* and *in vitro*. However, other tissues of epidermic origin, such as the spermatheca, escape mitotic stimulation by ecdysone.

Besides the effects exerted by hormones upon the reproductive organs, humoral relationships between gonads and ducts have been demonstrated. In *Drosophila*, female ducts were sometimes found to be attached to the gonads, thus causing degeneration. In the bugs *Dysdercus fasciatus* and *Triatoma infestans*, atrophy of one ovary was observed together with the regression of the upper part of the corresponding lateral oviduct, and the experimental section of the oviduct had the same effect in both species.¹

III. ORIGIN AND FORMATION OF THE GERM CELLS

Both sperm and eggs are derived from primordial germ cells set aside very early in the development of the embryo. Among the orthopteroid insects, in species where the germ cells appear early in development, such as in the house cricket *Acheta domesticus* and the grasshopper *Melanoplus differentialis*, the germ cells appear to be of ectodermal origin, forming at the posterior pole of the egg at the time of mesoderm segregation.⁹ Later in development in *A. domesticus*, they become associated with the mesoderm of the second and third abdominal segments; in *M. differentialis* they migrate into the coelomic cavities of the first to eighth abdominal segments, where they associate with the splanchnic wall and form a genital strand, from which the gonad differentiates later in embryogenesis. In species where the germ cells are first recognizable slightly later in embryonic development, they are associated with the median walls of the dorsal cavities in the abdominal segments (e.g., *L. migratoria*, *Blattella germanica*, *P. americana*). In each of these species, a genital ridge containing the germ cells is formed on each side of the embryo. The typical genital rudiment in these insects, during or just after involution of the germ band (anatrepsis), consists of a terminal filament membrane; a mesodermal dorsal cell mass, ventral to the filament membrane; a central cell mass, composed of primordial germ cells and mesodermal cells; a ventral cell strand of mesodermal cells which are the primordia of the gonadal portion of the genital ducts; and a surrounding epithelial membrane which envelops all of the above (Figure 6).

Among the endopterygotes, germ cell formation is well understood in the Diptera. The germ cells are formed from pole cells, which are established very early in development at the posterior pole of the embryo. In *Drosophila*, about 18 energids (cleavage nuclei) enter the posterior pole plasma and are pinched off as pole cells. The pole cells continue to divide, to

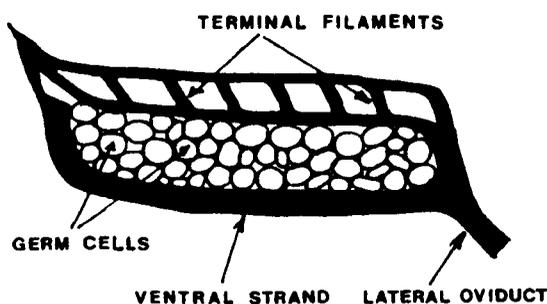


FIGURE 6. Diagrammatic representation of the early development (late embryonic stage) of the female reproductive system in an orthopteroid insect. (From Kaulenas, M. S., *Insect Accessory Reproductive Structures. Function, Structure, and Development*, Springer-Verlag, Berlin, 1992. With permission.)

produce eventually between 37 and 71 cells.⁹ Only some of the pole cells migrate to the presumptive gonads, which lie on either side of the gut and are mesodermally derived. The final number of pole cells in the gonad has an upper limit of about 13 pole cells per gonad at about stage 16 of embryogenesis.²¹ The pole cells which fail to reach the gonad probably degenerate later on. The determination of the pole cells as presumptive germ cells depends upon the interaction of the entering energids with the cytoplasm of the posterior pole cells (polar plasm). Likely candidates for cytoplasmic elements important for germ cell determination are polar granules, which are concentrated at the posterior pole of late stage oocytes and early embryos.²² Functionally similar posterior pole plasmas, which determine germ cell differentiation, occur in the Coleoptera and the Hymenoptera. In the Coleoptera, germ cells become distinguishable at the time of blastoderm formation, at the posterior end of the egg. In most Hymenoptera, germ cells first become recognizable during gastrulation or later, forming from the mesodermal tube. In many Lepidoptera, germ cells appear at the posterior pole just after blastoderm formation.

Germ cells (oogonia) are the only cells that normally exhibit genetic programs that lead to the construction of eggs.

IV. OOGENESIS

The first events in oogenesis include mitosis, the onset of meiosis, and ovariole differentiation. In the panoistic ovary, all oogonia (except stem line oogonia) are transformed to oocytes, whereas in the meroistic type oogonia generate both oocytes and nurse cells.

A. EARLY EVENTS IN OOGENESIS

Since more information is available concerning oogenesis in *D. melanogaster* than for any other insect, this fruit fly will be used to illustrate the early events of ovarian development that are shared by many evolutionary advanced insects with a polytrophic meroistic ovary.⁶ Each *Drosophila* ovariole contains a collection of egg chambers in which each oocyte is one member of a clone of interconnected cells. In brief, four consecutive mitoses of the cystoblast and its daughter cells in region 1 of each germarium give rise to the 16 germ-line cells (cystocytes), which remain connected via intercellular bridges due to incomplete cytokinesis (Figure 7). King et al.¹¹ suggest that the interactions of the centrioles and fusomes during the cystocyte divisions are responsible for the multiple-branched canal system that results. In region 2, the individual 16-cell clusters become separated by invading somatic follicle cells. One cell with four intercellular bridges (ring canals), the prospective oocyte, moves from a central position in the cell cluster to a posterior location between regions 2 and 3 of the germarium. This spatial reorganization of the 16 germ-line cells results in a polarization of the cluster: the prospective oocyte becomes positioned posterior to the remaining 15 cells, which differentiate into polyploid nurse cells. The forming follicle has established an anteroposterior

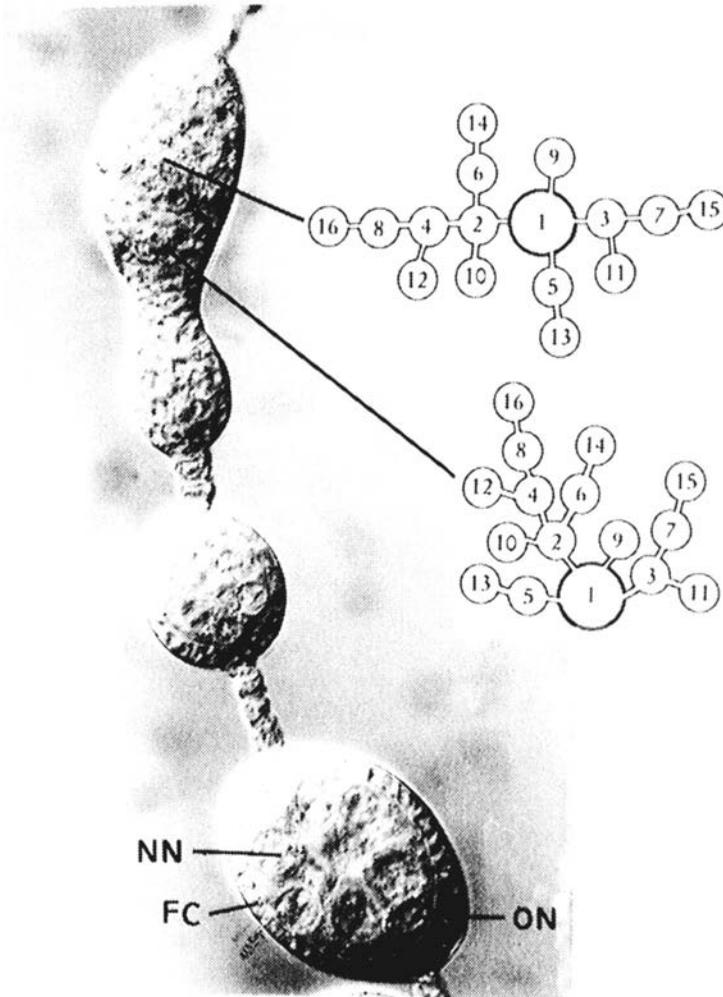


FIGURE 7. Germarium and young follicles in *Drosophila* oogenesis. The insert shows a diagram of the steps in the production of a clone of 16 cystocytes. By a series of four mitoses, each followed by incomplete cytokinesis, a branching chain of 16 interconnected cells is produced. Cell 1 represents the later oocyte which moves from a central position in the cell cluster to a posterior location. FC, follicle cell; NN, nurse cell nucleus; ON oocyte nucleus. Photograph courtesy of H. O. Gutzeit, Dresden.

axis. The nurse cells grow and simultaneously transfer cytoplasmic macromolecules to the oocyte (see Section IV.C). The follicle cells begin to form a monolayered epithelium around the germ-line cells in region 3 of the germarium. This process starts at the posterior end of the follicle. At the anterior end, a special group of follicle cells forms a stalk (“stalk cells”), thus separating the follicle from the germarium and releasing it into the vitellarium.²³

In the silkworm *Bombyx mori*, there are only three cystocyte divisions resulting into eight germ-line cells ($n = 2^3$). In the braconid wasp *Habrobracon juglandis*, the final number of cystocytes per cluster is 32; in other wasps, the number is not fixed but varies from 20–80.²⁴ Fleas with polytrophic meroistic ovarioles (some species of the Hystrichopsylloidea) have germ cell clusters consisting of 32 cells (2^5) which are generated by five mitotic cycles during the pupal stage. One of the cells containing five intercellular bridges becomes the oocyte; the others serve as nurse cells. However, nurse cells remain small and show the same ultrastructural characters as the oocyte.²⁵ Certain species of lacewings do not obey the $N = 2^n$ rule.⁶ For example, in *Chrysopa perla*, egg chambers contain 12–14 cystocytes. In this case, first- and second-generation cystocytes divide in synchrony, whereas at M_4 (see Figure 7), cells 3, 4, 7, and 8 divide; the rest do not. In the earwigs (Dermaptera), each follicle in the vitellarium

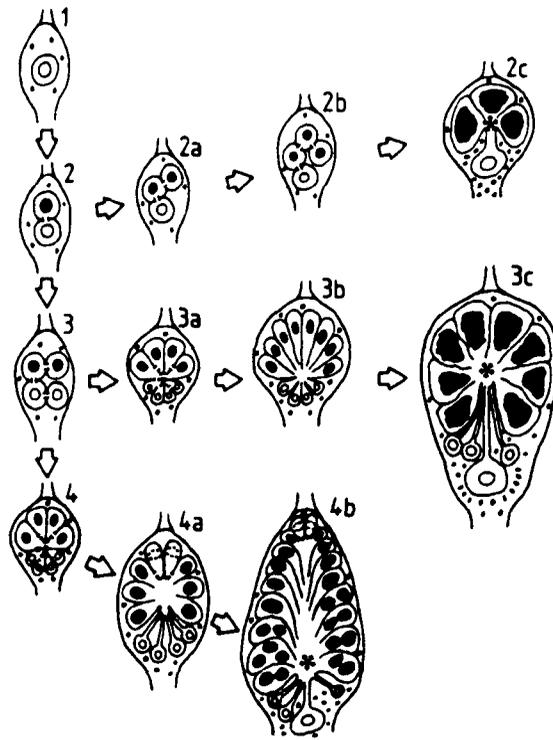


FIGURE 8. Hypothetical diagrammatic representation of germ cell cluster formation in subgroups of Hemiptera: (1) development begins in one persisting germ cell; (2) the germ cell divides by mitosis, followed by incomplete cytokinesis. One of the daughter cells is determined as a presumptive nurse cell (black nucleus), the other will be a presumptive oocyte (white nucleus); (2 a-c) germ cell division in scale insects (*Coccinea*); (3) in other groups, the presumptive nurse cell and the presumptive oocyte divide, giving rise to a cluster of four germ cells arranged in a rosette configuration; (3a-c) germ cell division in aphids (*Aphidina*); (4) further divisions of presumptive nurse cells and oocytes; (4 a-b) germ cell division in bugs (*Heteroptera*), where the oocyte subclone has its divisions limited. Asterisk = region of microtubule matter. For further details see text. (Reprinted from King, R. C. and Büning J., *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, Vol. 1, Kerkut, G. A. and Gilbert, L. I., Eds., Copyright 1985, ch. 3. With permission from Pergamon Press Ltd., Headington Hill Hall, Oxford OX3. OBW, UK.)

consists of one oocyte and one nurse cell, surrounded by a single layer of follicle cells. Formerly, the polytrophic meroistic ovary of earwigs has been looked at as a parallel development, but new findings indicate only one origin of the polytrophic meroistic ovary (see Section II.B).⁸

In all hemipteran species with telotrophic ovarioles, the germ cells are also clearly separated into nurse cells and oocytes. The oocytes are found at the base of the tropharium (see Figure 2C), whereas the nurse cells occupy its upper parts. Two models of germ cell cluster formation have been proposed. Both assume that independent stem cells in the apical region of the tropharium fuse to form germ cell clusters in which basally located germ cells are subsequently determined as oocytes. Based on data derived from several groups of Hemiptera, a new model has been advanced which assumes that cluster formation begins with a single cell. The first division is a differential mitosis, leading to a presumptive oocyte and a presumptive nurse cell. In scale insects (*Coccinea*), the presumptive nurse cell divides and its apical descendant will divide again. The final configuration is a rosette of four cells in which the intercellular bridges stay close together. During rosette formation, the intercellular bridges then vanish, and the trophic core forms (Figure 8). The last step in cluster formation is the polyploidization of nurse cells. In other groups, the presumptive nurse cell and the presumptive oocyte divide, giving rise to a cluster of four germ cells arranged in a rosette configuration. In aphids, germ cells continue to divide, and subclones of 2ⁿ oocytes and 2ⁿ nurse cells

arise. Rosette formation, formation of the trophic core, and polyploidization of nurse cell nuclei are the same as proposed for scale insects. Further divisions of presumptive nurse cells and oocytes lead to the situation in bugs (Heteroptera) with a constant number of oocytes, but an increasing number of nurse cells.

In snakeflies (Raphidioptera) and alderflies (Megaloptera, Sialidae), the same type of telotrophic ovary occurs. Cluster formation starts with germ cell migration into the ovariole anlage. The number of germ cells increases as more germ cells enter the anlage and as those already there divide. Dividing stem cells undergo complete cytokinesis next to the terminal filament (apical region), whereas germ cell clusters arise by incomplete cytokinesis more basally. The clusters are disc-shaped and oriented at right angles to the long axis of the ovariole. Each cluster presumably contains 2^n cells, with five as the maximum number of divisions.⁶ Prospective oocytes do not differ from nurse cells in their ultrastructure, except for one fact: nurse cells lose their cell membranes totally to form a syncytium, whereas prospective oocytes remain in their original status, which they acquired at the end of cluster divisions.⁸

In polyphage Coleoptera, germ cells migrate in the ovariole anlagen, and cluster formation is started by mitosis of each germ cell. Each germ cell can undergo only a limited number of mitoses, each followed by incomplete cytokinesis. Later mitoses are highly synchronized within each cluster, but not between different clusters. In most species, the clusters are oriented parallel to the long axis of the tropharium. A three-dimensional network of interstitial cells keeps the nurse cell nuclei in place, when nurse cell-nurse cell membranes are reduced.⁶ Oocytes develop at the base of the tropharium, primarily connected to nurse cells by an intercellular bridge. However, recent investigations have shown that cluster formation is more complex than has been assumed before, and that ramifications of clusters occur, even between oocytes.

1. Oocyte Differentiation

Immediately after the cluster of 16 cystocytes is found in a *Drosophila* germarium, both four-canal cells (cells 1 and 2) form synaptonemal complexes in their nuclei during the time they pass through germarial region 2. The synaptonemal complexes form during zygonema, are completed during pachynema, and are responsible for the synapsis of homologous chromosomes during meiotic prophase.⁶ Since cells 1 and 2 start meiosis, they are called pro-oocytes. Sometimes cells 3 and 4 also form synaptonemal complexes and enter meiosis, whereas cells 5–16 fail to enter meiotic prophase. In the posterior region 2, one of the two four-canal cells loses its synaptonemal complexes and enters the cycle of endomitosis characteristic of nurse cells. The other cell continues to develop as an oocyte and retains its synaptonemal complexes during the previtellogenic stage of oogenesis in the vitellarium (see Section IV.B). The divergence of the two pro-oocytes takes place in the region where follicle cells first surround the 16-cell clone. It is suggested that the first pro-oocyte to come into contact with a follicle cell is the one that receives the critical stimulus that causes it to continue on the oocyte developmental pathway. Such cellular interactions between the germ-line cells and the somatic follicle cells have been studied for a long time.^{26,27} More recent data show that the correct cellular organization and determination of cells in the germarium seem to depend on the activity of several genes. For example, in *Drosophila*, genes like *egalitarian* and *Bicaudal-D* are apparently involved in cystocyte diversification, since in mutant follicles a nurse cell differentiates instead of the oocyte. The genes *dicephalic* and *spindle-C* are required for the correct spatial arrangement of the cystocytes. Moreover, the mutant *dicephalic* illustrates the importance of early cellular interactions between somatic follicle cells and germ-line cells.²⁸ However, it remains to be analyzed which kind of specific signals the pro-oocytes receive in the germarium.

In some carabid beetles, all cystocytes of a clone form synaptonemal complexes, and consequently all nurse cells enter meiotic prophase together with the presumptive oocyte. In some telotrophic ovaries, the cell which differentiates as an oocyte also seems to depend upon