

Developmental Systems:

Insects

Vol. I



DEVELOPMENTAL SYSTEMS: INSECTS

Edited by

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PREFACE

Insects have been utilized for experimental studies of development for more than seventy years. Yet much significant work of general interest remains virtually unknown to biologists outside the field itself. The aim of these volumes is to provide, for both specialist and neophyte, detailed and critical analyses of several aspects of the developmental biology of insects. Because an understanding of normal development is essential to the appreciation of experimental studies, and, more importantly, to the identification of those species uniquely or especially well suited as material for further investigation of particular problems of interest to developmentalists, Volume 1 deals with oogenesis and normal embryogenesis in the major insect groups, followed by a chapter on development in polyembryonic species. Chapter 1 in Volume 2 reviews causal analyses of the developmental mechanics of insect embryos and serves as a bridge to chapters dealing with two special systems, the hypodermis and imaginal discs, which are being utilized to study such general topics as the origins of pattern and form, the stability of the differentiated state, and the control of gene action in development. There follows a broad consideration of hormonal action in insect development, and the volume concludes with a general discussion and historical appreciation of studies on gene action and pattern formation.

To keep the volumes within a manageable size and yet provide each contributor with the opportunity to examine in breadth and depth the subjects chosen for inclusion, some areas of interest such as regeneration, metamorphosis as a developmental phenomenon, developmental cytology and genetics, and the biochemistry of development are not dealt with separately, although each is considered in several chapters. It has not been possible to provide detailed technical information, desirable as it would be in a volume with the aims of this one, but appropriate literature citations in each chapter will enable the interested reader quickly to find such information.

This book has been made possible by the generosity and good will of those who have contributed to it at the expense of their own research efforts, and by the larger body of colleagues who have provided suggestions, illustrative material, and access to much unpublished data. My own long-standing intellectual debt to three friends and teachers—Professors Edwin R. Helwig, C. H. Waddington, and D. F. Poulson—is a pleasure to acknowledge. Finally,

grateful acknowledgement is made of the patient support provided during the long gestational period of this volume by my departmental chairman, Professor J. David Robertson, and my husband, Professor R. Bruce Nicklas.

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July, 1972
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The Development of Spatial Patterns in the Integument of Insects. PETER A.
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The Imaginal Discs of *Drosophila*. W. J. GEHRING and R. NÖTHIGER

Role of Hormones in Insect Development. W. W. DOANE

The Morphogenesis of Patterns in *Drosophila*. C. H. WADDINGTON

INTRODUCTION

The scientific study of animal development has enjoyed two great times of blossoming in the last half-century. The first began with Spemann's discovery of "the organiser" around 1920, the second with the acceptance of the "DNA makes RNA makes protein" doctrine in the 1950's. The character of the science, and the special skills of its leading workers, have been rather different in the two periods. In the earlier, the advances were mainly in the analysis of causal processes affecting cells or tissues; the traditions were those of anatomy combined with skill in manual manipulation. Although chemical analysis played a secondary role at that time, in the later period it has, of course, come to occupy the centre of the stage, and analysis is most frequently in terms of molecules or at least sub-cellular particles. But even though binocular microscopes, hair loops and glass needles have given way to ultracentrifuges, counters and fraction collectors, and the level of analysis pushed down from the cellular to the molecular, the basic problem remains: what causal processes bring about the changes which we can see or otherwise detect as an egg develops into an adult? The new methods of study have still to be applied to embryos if they are to provide answers to embryological problems.

These volumes survey various types of embryos or other developing systems in an attempt to bring out the types of developmental problems for which they seem to offer specially favourable approaches. Biological research, even in its most abstract phases, has usually depended critically on the exploitation of a few particularly appropriate experimental materials; witness the roles played, in various aspects of genetics, by *Drosophila*, *Neurospora*, *Chlamydomonas* and *E. coli*. The older tradition of embryology explored many developmental systems, and went far to determine their particular merits and demerits for the types of experimentation then possible, and to define the essential problems arising from the facts which could be observed. The volumes discuss how the old problems can be restated in terms of the newer insights, what progress has been made towards their solution, and the opportunities the different experimental materials offer to the more recent analytical tools.

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1 | Oogenesis¹

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I. INTRODUCTION

Today we are rightly proud of and enthusiastic over the great advances that have been made in molecular biology. We know the chemical characteristics of the genetic code and we feel confident that the basics of information transcription and translation are understood. This just enthusiasm for modern molecular approaches bears with it unfortunately a sense of frustration for the complexities of the actual living cell. We do not know how the genetic material is actually structured with proteins, phosphoproteins, phospholipids, RNA, etc. to form the complex organelle, the eukaryotic chromosome. We do not, in fact, have clear ideas about the structure in molecular terms of any of the organelles of higher organisms.

Probably the most complex cell is the oocyte. It is not only the largest cell type, but it also has the potential to develop into the larval or nymph stages,

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for example in insects, without exterior influence except gaseous and sometimes fluid exchange. We have only the bare outlines of some of the key facts in this amazing potential. The egg has completed meiosis and thus has a haploid set of genes. It contains sufficient nitrogen and energy reserves to form all of the cells of the embryo. During the initial hours of embryonic life ribosomes and probably mitochondria and other cellular organelles are already present in sufficient numbers so that multiplication of nuclei is the first chief event. But many experiments in both amphibians and echinoderms have made it clear that informational RNA is also present, and recently Lockshin (1966) and Hansen-Delkeskamp (1969) have shown that insects probably have the same reserve. Moreover, experimental embryology has abundantly indicated the presence of organization in the egg which is necessary for normal development (Krause and Sanders, 1962; Counce, 1972). Studies on female sterility factors (e.g. Beatty, 1949; King, 1970; Wright, 1970) have indicated the role of some genes in the process of oogenesis and it is assumed that the cytoplasmic information present in the egg and necessary for the total development of the embryo is also produced by nuclear genes. Thus it is evident that the process of oogenesis is the central preparatory event for embryogenesis.

Many aspects of the total story on oogenesis will not be treated here, although some of them will be covered in other chapters. For example, the process of egg production is dependent upon various hormonal controls, but the various hormonal relationships existing in insects are treated separately (Doane, 1972). There have been many female sterility genes studied especially in *Drosophila*, but since many of these have been studied principally because of the changed developmental potentialities of the egg, they will be considered in other chapters (Wright, 1970; Counce, 1972). Those that have clear effects on the process of oogenesis have been reviewed recently (King, 1964, 1970) and will be introduced here to illuminate certain areas.

Because of the importance of understanding oogenesis for any investigation of development of the early embryo, it is not surprising that the area had been reviewed frequently. Raven (1961) has surveyed the whole field of oogenesis and discussed it in the context of a process of storage of developmental information for the embryo. Oogenesis in insects is considered in some detail in Engelmann's (1970) treatise on the physiology of reproduction in insects while Bonhag (1958) has thoroughly reviewed the cytological data in insects obtained with the light microscope, and Telfer (1965) has analyzed comprehensively the data on vitellogenesis including the ultrastructural data that had accumulated to that date. Recently, Bier (1967) has discussed the process of insect oogenesis from the viewpoint of the variety of methods used by insects to produce eggs with their characteristically low DNA/cytoplasmic

ratios. In a more general review, Nørrelung (1968) has surveyed the ultrastructure of oogenesis in which brief mention of certain features of insect oogenesis have been included. And finally, King (1970) has reviewed oogenesis in *Drosophila melanogaster*, emphasizing genetic studies. The central focus of this chapter is a comparative analysis of the process of oogenesis in the different orders of insects, depending primarily on autoradiographic and electron microscopic data. Insofar as necessary, histochemical and cytological studies with the light microscope will be used as they have added to our basic understanding of the events described with the aid of the electron microscope, or where these results have gone beyond those previously reviewed.

The point of view taken in this chapter is that oogenesis is the necessary first step for development of the animal embryo since only in this unique cell does the nucleus exhibit its totipotent properties required for supporting normal embryogenesis. Hence the process of oogenesis can be considered to be a series of biological functions whereby the egg cell is endowed both with the nutritional prerequisites for normal development and with the necessary informational content so that in interactions with the zygote nucleus proper differentiation occurs.

The cytological mechanisms involved in each of these steps appear to be sufficiently discrete to allow independent treatment. However, within the class Insecta there are very distinct modes of solving the biological problem of oogenesis, and consequently careful distinctions will be made in the ways in which different orders of insects proceed in oogenesis.

II. TYPES OF OÖGENESIS IN INSECTS

The developmental process leading to the production of a mature egg varies greatly from one group of organisms to another. Within the class Insecta there are two major types of oogenesis, the distinction being based primarily on the cellular mechanisms used for the synthesis of the reserves of RNA-containing organelles, although trophic tissues contribute more than just RNA to the egg. The first type, the panoistic ovary, resembles the common form of oogenesis found throughout most of the animal kingdom in which the oocyte nucleus is the source of all of the RNA contained within the mature egg. The second type, the meroistic ovary, is characterized by the presence of a cluster of cells, the nurse cells or trophocytes, which have their origin from germinal tissue but do not form part of the mature egg. They produce all or nearly all of the RNA of both the growing oocyte and the mature egg. There are many variations of this type of ovary, but for the most part meroistic ovaries fall into two further classes: the telotrophic ovary, in which the trophocytes are located at the anterior-most portion of the ovary and are

connected to the growing oocytes by cytoplasmic connections called nutritive cords or tubes through which RNA and other substances move to the growing oocyte; and the polytrophic ovary, in which a cluster of nurse cells are derived from the germ line and form a part of the growing egg chamber. These nurse cells produce all or nearly all of the RNA of the egg and ultimately most of the ooplasm through fusion of the nurse cell cytoplasm with the egg. Only their nuclei are excluded from the mature egg. This mode of egg formation appears especially adapted to the mechanism of rapid and continuous egg production found in dipterans, hymenopterans, lepidopterans, etc. The source of much of the protein yolk in all of these types of oogenesis has been clearly shown to be the hemolymph and ultimately the fat body (Telfer, 1965; Pan *et al.*, 1969).

First, I will give a brief review of the structure of these major classes of ovaries, their histological differentiation from embryonic and larval tissue, and then proceed to analyze in detail the process of oogenesis in each. Because of the many differences in the histology of the ovarioles in each type, it is difficult to find consistent terminology in the literature. However, for consistency in the subsequent description, I will use the following divisions of the insect ovary. The ovary is usually composed of linear arrays of growing oocytes called ovarioles. The ovariole can be divided into five regions: Region 1, the terminal filament; Region 2, the germarium; Region 3, the previtellogenic stages of the vitellarium; Region 4, the vitellogenic stages; and Region 5, the mature egg. In more detailed studies, further subdivisions of specific regions into zones or stages have been made to facilitate description, and these subdivisions will be indicated in their appropriate place.

A. The Panoistic Ovary

The panoistic ovary consists of several ovarioles, the number of which varies among species; for example, there are eight in *Periplaneta americana* and 40 to 50 in *Tachycines asynamorus* (von Kraft, 1960b). Each ovariole (Fig. 1) consists of a linear array of oocytes in different stages of development. At the anterior end of the ovariole (region 1) is the terminal filament, composed of flattened cells, surrounded by basement lamina and an ovarian sheath. These two latter structures are found in every ovarian type and surround the entire ovariole. The second region¹ is the germarium and can be

¹ The most detailed studies of the histology of the panoistic ovary have been made by Bonhag (1959) and Anderson (1964) and they divide the ovariole into six zones. I have included their zones two and three within the germarium in order to obtain some consistency in terminology among the three types of insects ovarioles. In the two types of merioistic ovarioles, the germarium includes all the oogonia, oocytes in meiotic prophase, and the early stages of oocyte growth prior to the formation of the layer of follicle cells surrounding individual oocytes. By including zones two and three of Bonhag within region 2, the terminology for all of the ovarioles can be made consistent. Furthermore, the germarium demands further subdivision in each type, so that the clarity of Bonhag's description is not lost.

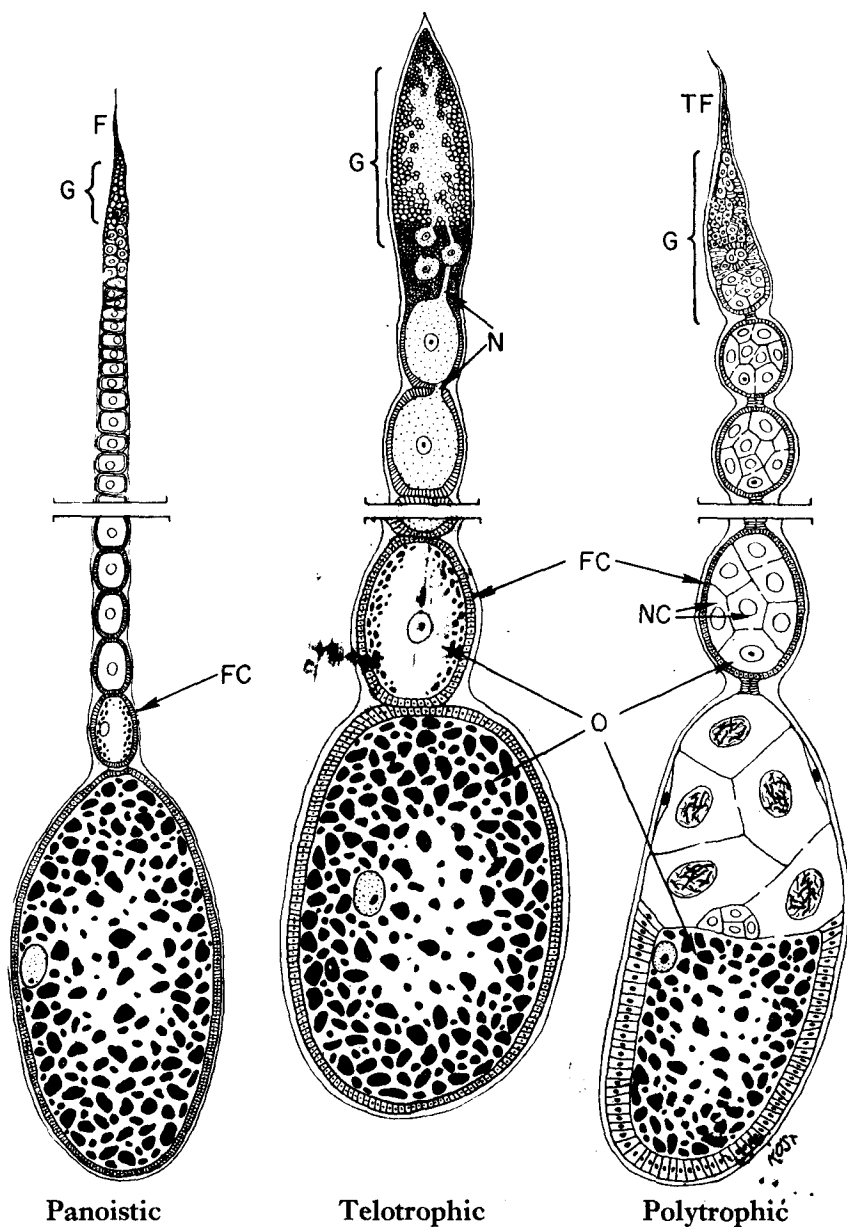


FIG. 1. Diagrams of panoistic, telotrophic and polytrrophic ovarioles. The upper portion of the figure shows the germarium and the early growth stages while the lower portion has the pre-vitellogenic and vitellogenic stages. No attempt has been made to draw the different ovarioles to scale. The upper portion of the figure is enlarged to a greater extent than the lower in order to make details of germarial structure clear. Labels: FC, follicle cell; G, germarium; N, nutritive cord; NC, nurse cell; O, oocyte; and TF, terminal filament. (Drawings made by Mrs. Kathleen T. O'Sullivan.)

subdivided into the anterior and posterior portions or zones. The anterior portion of the germarium is composed of oogonia adjacent to the terminal filament and separated from it by the transverse septum. The oogonia are followed by oocytes in the early stages of meiosis. In the posterior portion, the meiotic chromosomes pass from a compact diplotene to a diffuse stage, both the nucleus and the cytoplasm become very basophilic and rich in RNA, and multiple nucleoli appear. At this time the individual oocytes are still incompletely surrounded by follicle cells. In *Acheta domesticus*, where no further oogonial divisions occur and all the oocytes are in meiotic prophase, this region has been called the end chamber rather than the germarium (Netzel, 1968). Oocytes are considered to be in region 3 when the cytoplasmic volume has increased until the oocyte extends across the whole width of the ovariole and has been surrounded by a complete follicular epithelium. The growth in this region is mostly in cytoplasmic components and the nuclear morphology remains similar to that in the posterior portion of region 2. Region 4 is characterized by vitellogenesis. Initially the yolk appears along the follicular border but it soon fills the ooplasm. The large yolky oocytes have a decreased basophilia in the ooplasm, presumably due to a dilution effect as the yolky elements are produced (Bonhag, 1959). The germinal vesicle becomes located along the mid-dorsal or concave surface of the oocyte throughout the yolk deposition stages and this location may be responsible for the production of the bilateral symmetry of the egg (Netzel, 1968). The final region is filled with mature eggs. The ovariole is joined to the oviduct through a special structure, the pedicel. The ontogeny of the ovary in *Tachycines* and *Acheta domesticus* has been described by von Kraft (1960a) and by Echard (1962) respectively.

The fine structure of the panoistic ovary has been investigated in a number of organisms: *Periplaneta americana* (Anderson, 1964); *Gryllus bimaculatus* (Favard-Séréno, 1964); *Gryllus capitatus* (Favard-Séréno, 1968, 1969); *Blattia orientalis* (Gresson and Threadgold, 1961, 1962); *Locusta migratoria*, *Blattella germanica*, *Acheta domesticus* (Bier et al., 1967); *Aeschna* (Beams and Kessel, 1969); *Tibellula pulchella* (Kessel and Beams, 1969); *Leucophaea maderae*, (Scheurer, 1969); *Leptinotarsa decemlineata* (de Loof and Lagasse, 1970). The emphasis has been either on vitellogenesis or nucleolar changes especially at the time of vitellogenesis and the results will be presented in the appropriate section. The most complete description has been given by Anderson (1964). Mitochondria, Golgi elements and ribosomes are present in great abundance but there is very little endoplasmic reticulum. Lamellar bodies are randomly distributed in the cytoplasm but their function is not known. Prior to vitellogenesis the oocyte plasma membrane is tightly applied to that of the follicle cells and they frequently interlock, but during vitellogenesis an intercellular space appears and pinocytosis is common. Anderson (1964) and Scheurer