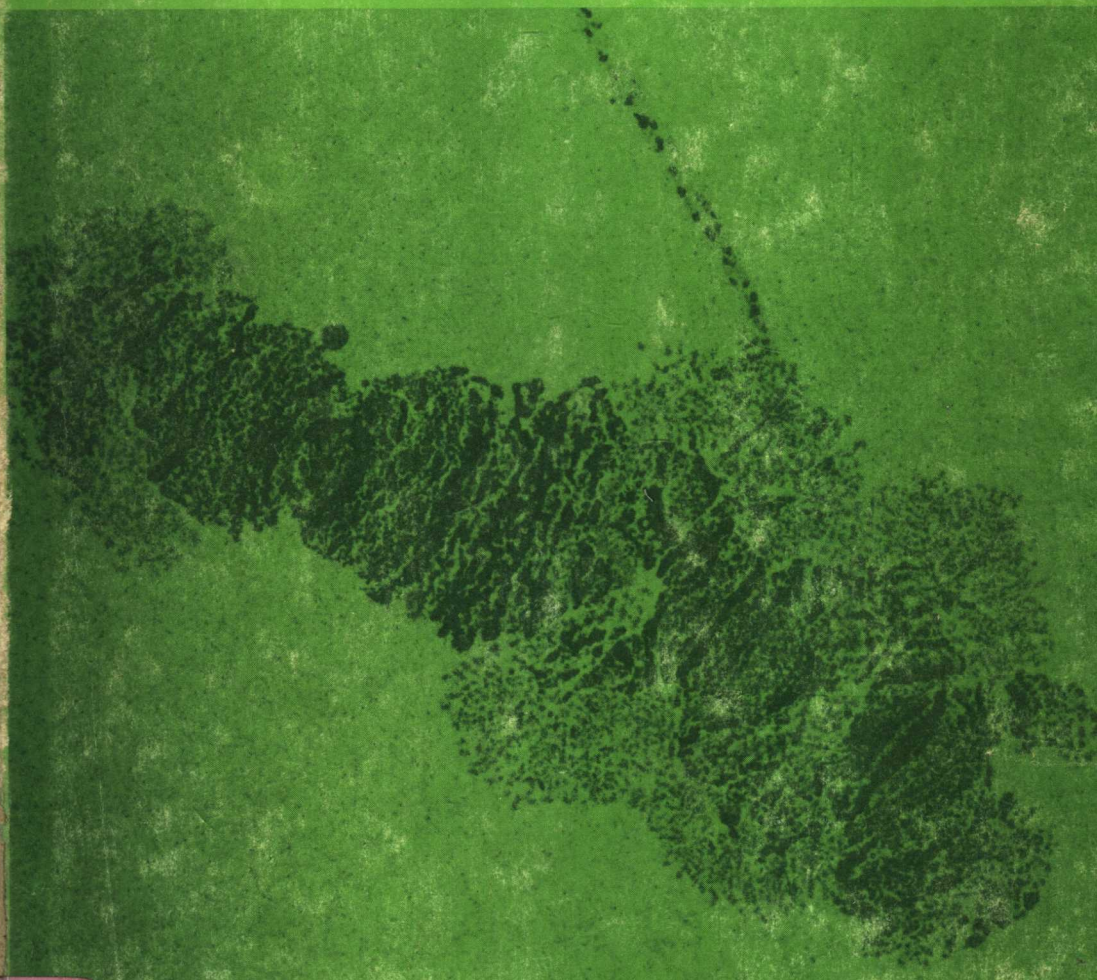
Developmental and Cell Biology 6

Nuclear Cytology in Relation to Development

F. D'Amato



NUCLEAR CYTOLOGY IN RELATION TO DEVELOPMENT

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Preface

When the advisory editors of the Developmental and Cell Biology series of the Cambridge University Press asked me to write a monograph on 'Nuclear Cytology in Relation to Development', I accepted their proposal with great interest. It seemed to me that such a monograph could offer an excellent opportunity to discuss comparatively many aspects of development and growth in plants and animals, trying to properly emphasize peculiar features in plants which do not commonly find due appreciation in discussion of the long-standing problems of development.

When writing the book, I have made all efforts to achieve a balanced treatment of animal and plant systems. Whether my endeavour has been successful, at least partially, can only be judged by the reader. I am aware of the vast fields I have left unexplored (among these, important aspects of abnormal and pathological development) and of the many papers which I have not cited. I apologize to all who feel that their contribution should have been included. They may, however, contemplate that the length assigned to the text demanded many omissions. I have tried to compensate for them, where possible, by giving references to review articles. The citation of literature ends about December 1975 (papers dating 1976–7 were known to me as preprints).

Professor John G. Torrey of Harvard University read all of the manuscript and made many minor corrections and some useful suggestions for which I am very grateful to him. I am also grateful to Mrs M. Velia Bellani and Mr P. L. Leoni of our Institute of Genetics at the University in Pisa, for typing the manuscript, and to Mr Leoni for preparing the drawings for the original figures.

Pisa
June 1976

F. D'AMATO

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General

An essential feature of living organisms is reproduction, the ability to produce other individuals of the same species. Although the continuous flow of generations in any species of animals or plants presumably did not escape man's observation for centuries, the material basis for the transition from one generation to the next remained obscure until about a hundred years ago. It was in the second half of the last century that Hertwig and Strasburger, working on sea urchins and plants respectively, showed that the key event in fertilization is the fusion of the nucleus of the male gamete with the nucleus of the female gamete. Further observations led, within a few decades, to the demonstration that:

(i) during cell division, the nucleus produces by mitosis, or karyokinesis, two daughter nuclei each of which is identical in its chromosome complement to the other and to the original nucleus;

(ii) the chromosome number of gametes (n)* is half the chromosome number of the zygotes and of the somatic cells derived from it (2n);

(iii) the production of gametes – and, in many plants, of cells with n chromosome number (spores) which give rise to gamete bearing individuals (gametophytes) – is the result of meiosis, consisting of two successive nuclear divisions which lead to reduction of the chromosome number from 2n to n.

The above observations have laid the foundations for a correct interpretation of life cycles in sexually reproducing eukaryotes, i.e. organisms, both unicellular and multicellular, which have typical nuclei with a nuclear envelope and chromosomes, as well as nuclear divisions in the form of mitosis and meiosis. In eukaryotes, fertilization involves the fusion of the two gametes to form a zygote containing two complete genomes. These zygotes are called holozygotes in contrast with the merozygotes (Wollman, Jacob & Hayes, 1956) in bacteria (protokaryotes), in which only a part of the genome of a donor cell is transferred to a recipient cell which contributes both its cytoplasm and its entire genome.

* In diploids, n corresponds to the basic chromosome number (x): $2n = 2x$. In polyploids, the gametic or sporic (n) and the zygotic or somatic (2n) chromosome numbers are further specified by adding the level of ploidy. Thus, e.g. $2n = 4x = 24$ designates a tetraploid individual or species having 6 as its basic chromosome number; in case of a regular meiosis, the resulting cells (gametes or spores) have $n = 2x = 12$ chromosomes.

2 *Life cycles*

In most plants and in some groups of animals, besides sexual reproduction, different methods of asexual (= agamic) reproduction may operate: these are often much more efficient than sexual reproduction in the dispersal of the species. During asexual reproduction, new individuals are formed by mitosis from single cells (agamogony), groups of cells or even organs (vegetative reproduction or propagation) which are detached from the individual. The population of individuals derived asexually from a common ancestor is said to form a clone. Clones are either haploid or diploid depending on the chromosome complement of the ancestor.

In analyzing the life cycle of sexually reproducing species it is essential to ascertain the relative positions and the extent of the temporal separation of the two events which alternate in the cycle: fusion of the gametes (amphimixis) and reduction in chromosome number (meiosis). In the following pages, some patterns of life cycles will be discussed. For more extensive treatments of life cycles, including terminological questions and genetic and evolutionary aspects, reference is made to Martens (1954 *a*, *b*), Wetmore, De Maggio & Morel (1963), Alston (1967), Resende (1967), Raper & Flexer (1970) and Feldmann (1972).

Diplontic organisms

In all metazoans and in most protozoans development normally starts from a zygote, which undergoes a series of mitoses to produce a diploid organism. At maturity, this organism reproduces sexually by forming haploid gametes from diploid cells by means of meiosis. Since meiosis forms gametes, it is called gametogenic or, less properly, gametic. Some authors use the term terminal meiosis to emphasize that it occurs at the end of a life cycle, which is wholly occupied by diploid individuals, the haploid state being represented by single cells: the gametes (Fig. 1.1). In describing diplontic as well as haplontic life cycles (see below), several authors use the term 'phase' to denote the haploid and the diploid state. This would mean that in a diplontic life cycle, although no alternation of generations occurs, there is an alternation of two nuclear phases, one haploid and the other diploid. As pointed out by Martens (1954*a*) and subscribed to by others (e.g. Feldmann, 1972), the concept of phase should imply a process of development by mitosis, the minimum requirement for the establishment of a phase being one mitosis. Martens' distinction between nuclear state (the state of single cells as gametes, spores or zygotes) and nuclear phase (a generation, reduced as it may be, which originates by mitosis from either a zygote or a spore: diplophase and haplophase respectively) acquires special significance when the evolution of the haplo-diplontic life cycle in phanerogams, the seed plants (see below), is considered.

Some animals actually show alternation of two or more morphological types due to the occurrence, in addition to sexual reproduction, of asexual propagation processes including pedogenesis (asexual propagation of immature diploid ani-

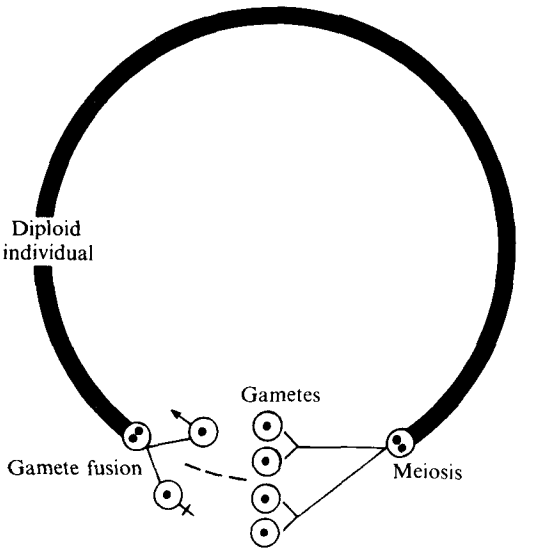

Fig. 1.1. Diagram of a diplontic life cycle. The diploid phase is represented by a thick line and the outline of a nucleus with two nucleoli; the haploid state, comprising single cells (gametes), is represented by the outline of a nucleus with one nucleolus.

mals), viviparity, parthenogenesis, predominance or exclusiveness of the female sex. Other factors contributing to the complexity of life histories in some animals are migration to different hosts and metamorphosis. As an example, consider the complex life history of the liver flukes (trematodes). The adult sheep fluke (*Fasciola hepatica*) inhabiting the liver releases fertilized eggs which are excreted by the host and develop into a miracidium, a ciliated free-swimming larva. The miracidium bores into a certain type of snail where it forms a cyst (sporocyst) from which many larvae of a second type, the rediae, are produced. A third type of larva, the cercariae, develops from the rediae within the tissues of the snail: the cercariae leave the snail and swim actively to reach vegetation on which they encyst. If consumed by a sheep, the cyst resumes development into an hermaphroditic adult fluke. The sequence of pedogenetic developments, which precedes the attainment of the adult state in the sheep liver fluke and in other trematodes, appears most striking if it is considered that the three different larval types and the adult animal all have the same genetic endowment, namely that of the original zygote.

Another example of the ability of the same set of genes to express alternative forms of the organism in the course of a life cycle is offered by the complete metamorphosis in holometabolous insects. The larva, which originates from the egg, may differ completely from the adult; it contains, in addition to its larval structures, a number of primordia, the imaginal disks, which give rise during

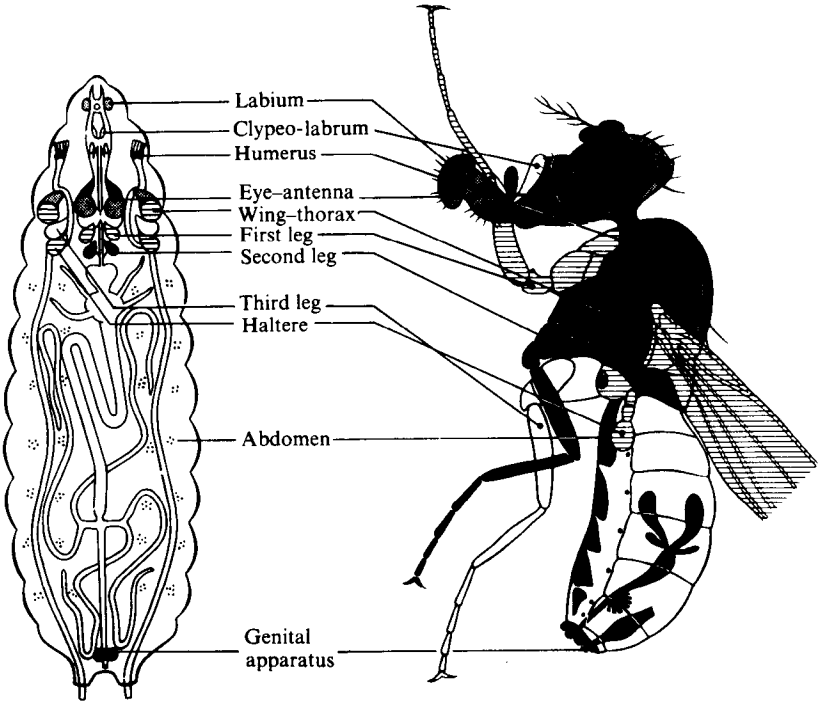

Fig. 1.2. Schematic representation of the larval organization and the location of different imaginal disks in Diptera. Disks and their corresponding adult derivatives are connected by lines and given the same hatching or shading (after Nöthiger, 1972).

metamorphosis to specific structures of the adult. When the larva enters the pupal stage, most of the larval tissues are destroyed by histolysis and the adult is practically formed anew from the imaginal disks, whose cells undergo dramatic differentiative changes. In the intensively studied *Drosophila*, most, if not all, imaginal disks first appear as invaginations or thickenings of the epidermis in the later embryonic stages; moreover, the imaginal disks responsible for the formation in the adult (imago) of the head, the thorax and its appendages, the integument of the abdomen and several internal organs (gut, salivary glands, gonads) have been identified (Nöthiger, 1972) (Fig. 1.2). Since the imaginal disks have provided outstanding material for studies on developmental processes, we will refer to them on other occasions.

If we now turn our attention to the plant kingdom, we see that the diplontic life cycle is rare. It is found in some diatoms, in some brown algae (Fucales) and in species of lower fungi. In some species, the diploid individuals are monoecious, because they produce male and female gametes on different portions of their body

(thallus); in other species, male and female individuals occur (dioecism). Besides sexual reproduction, asexual propagation by means of propagules, either unicellular (conidia) or multicellular, is very common.

Haplontic organisms

In relatively few species of Protozoa and in species of green and red algae and of fungi, development starts from a single haploid cell, a spore, which results from meiosis in a zygote. In general, from one zygote four spores (tetrad) are formed; in most species of ascomycetes, however, the zygote undergoes a third mitosis after two meiotic divisions so that eight haploid nuclei and spores are produced. In exceptional cases, more than eight spores are formed, due to the occurrence of two or more mitoses after the meiotic process. In unicellular species, each spore produces a sometimes very large progeny of identical individuals (colony, clone); within the colony, the fusion of two cells behaving as gametes (conjugation) forms a diploid zygote which undergoes meiosis (meiocyte or gonotokont). In multicellular organisms, each spore forms one individual which reproduces sexually; here again, monoecy or dioecy may occur. Since meiosis forms spores, it is called sporogenic; some authors speak of zygotic meiosis, others of initial meiosis, because it takes place at the beginning of a life cycle which is wholly

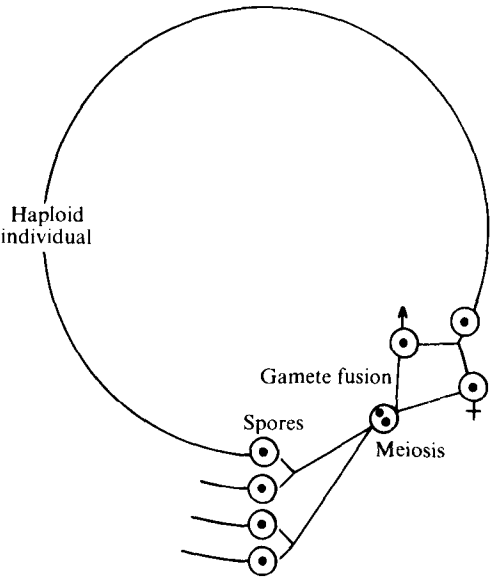

Fig. 1.3. Diagram of a haplontic life cycle. The haploid phase is represented by a thin line and the outline of a nucleus with one nucleolus the diploid state, comprising single cells (zygotes), is represented by the outline of a nucleus with two nucleoli.

occupied by haploid individuals (haplontic cycle), the diploid state being represented by single cells, the zygotes (Fig. 1.3). In haplontic organisms, asexual reproduction may occur by means of single vegetative cells, the conidia, which by mitosis form new haploid individuals identical to those which originated from spores. Conidia and spores, then, share a common mode of development (Haeckel's monogony as opposed to amphigony or development by fusion of gametes); they are, however, quite distinct in origin, the former being vegetative cells and the latter products of meiosis. In view of the significance of this distinction (Battaglia, 1955*a*), the indiscriminate use of the term spore in some text books, and even in scientific papers, is much to be regretted, because it may render the description of some life cycles incomprehensible to a non-specialist. This is the more so when the term spore is also applied to diploid conidia which are released from asexually reproducing diploid individuals both in diplontic and diplo-haplontic life cycles.

In a few species of green algae (e.g. *Acrosiphonia spinescens*), the zygote undergoes extensive growth in the absence of mitosis and has a much longer life-span than the haploid individuals which develop from the spores.

Diplo-haplontic organisms

The diplo-haplontic life cycle is by far the most widespread life cycle among plants. It is characterized by an intermediate meiosis and consequently by an alternation of generations, one haploid and one diploid. The diploid zygote produces by mitosis a diploid individual, a diplophyte, also called a sporophyte because it forms spores by meiosis. Spores undergo mitotic divisions and develop into haploid individuals (haplophytes) which eventually contain the gametes (gametophytes) (Fig. 1.4). Since gametophytes are haploid as their gametes are, they are correctly interpreted as gametiferous (gamete bearing) individuals, in opposition to the gametogenic individuals such as the diplonts, which form gametes by meiosis (D'Amato, 1971).

The diplo-haplontic life cycle shows great variation in different taxonomic groups in relation to specific adaptations, phylogenetic position of the taxa etc. A developmentally interesting type of the diplo-haplontic life cycle is that found in some species of algae belonging to the genera *Ulva*, *Cladophora*, *Zanardinia*, *Dictyota*, *Rhodochorton*, which show an alternation between morphologically indistinguishable (homomorphic) and sometimes equally sized (equivalent) gametophytes and sporophytes. In dioecious species (*Dictyota dichotoma*), the life cycle comprises three identical individuals: a sporophyte, a male gametophyte and a female gametophyte, whose distinction is only possible on cytological grounds. As shown by a now classical tetrad analysis (Schreiber, 1935), of the four spores which are produced meiotically in each sporangium in *Dictyota*, two give rise to female gametophytes and two to male. In many other species of algae (see list in Feldmann, 1972), there is an alternation of morphologically different (hetero-

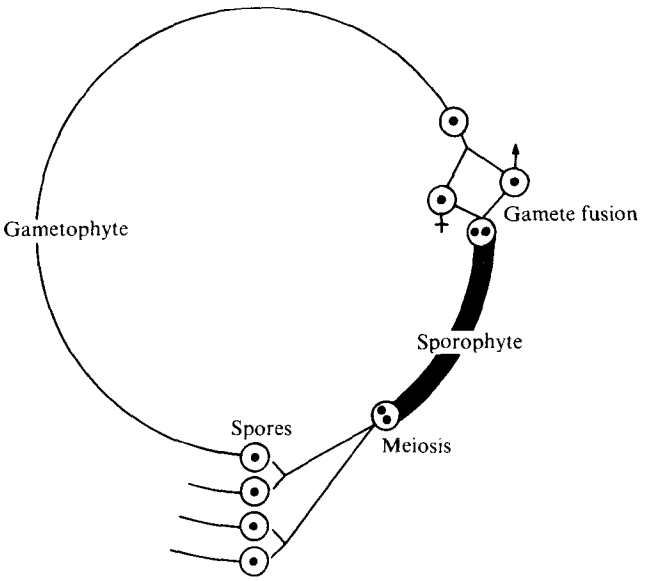

Fig. 1.4. Diagram of a haplo-diplontic life cycle with predominance of the haplophase (e.g. mosses). The haploid phase is represented by a thin line and the outline of a nucleus with one nucleolus; the diploid phase is represented by a thick line and the outline of a nucleus with two nucleoli.

morphic) generations, generally with one generation dominating the other. A more complex life cycle which occurs in red algae (e.g. *Polysiphonia*) comprises one gametophyte and two separate diplophytes. The zygote develops inside the carpogonium (female organ) of the gametophyte to form a diplophyte (carposporophyte) which lives as a parasite of the gametophyte; this diplophyte produces diploid conidia ('carpospores') which form an independent diplophyte (tetrasporophyte) reproducing by typical haploid spores (four spores in each sporangium). Gametophytes and tetrasporophytes are practically indistinguishable: however, carposporophytes and tetrasporophytes, both diploid, are completely different in structure.

Among higher plants, i.e. the Embryophyta comprising the Bryophyta (mosses and liverworts), the Pteridophyta (ferns, lycopods, horsetails, etc.) and the Phanerogamae (seed plants), the diplo-haploid life cycle shows a progressive decrease in the extent and size of the haplophase in the series from the mosses to the seed plants.

In the bryophytes, the leafy plant represents the haplophyte, which originates from a spore and reproduces sexually by means of egg cells (one in each archegonium) and motile sperm cells contained in great numbers in each antheridium. After fertlization, the zygote grows *in situ* into a diploid embryo and afterwards

a non-leafy sporophyte in whose sporangia spores are produced by meiosis. Spores are morphologically alike; in dioecious species, they give rise to male and female gametophytes in a 1:1 ratio.

In the pteridophytes, the trend towards a progressive morphological and physiological dominance of the diplophase over the haplophase, which reaches its end-point in the angiosperms, is very clear. The diplophyte (e.g. the fern or the horsetail plant) produces sporangia, generally grouped in sori, on either vegetative or modified leaves (sporophylls): in each sporangium, the spore mother cells undergo meiosis to form tetrads of spores. In the isosporous species (e.g. isosporous ferns, horsetails), the spores give rise to minute free-living monoecious gametophytes (prothalli). From the fertilized egg a diploid embryo is formed, which further develops into the leafy plant (sporophyte). In the heterosporous species (e.g. heterosporous ferns, *Selaginella*, *Isoëtes*), the sporophyte produces sporangia of two different types (megasporangia and microsporangia) borne on different sporophylls (mega- and microsporophylls). From the two types of spore produced (megaspores and microspores) female and male gametophytes (mega- and microgametophyte respectively) develop: these gametophytes are even smaller than the gametophytes of isosporous pteridophytes. Indeed, they generally occupy the cavity of the original spore, whose wall was broken during development of the gametophyte.

Among phanerogams (seed plants), only two orders of gymnosperms, the Cycadales and the Ginkgoales, have ciliated motile sperm cells; all others (higher gymnosperms and angiosperms) have nonmotile sperm cells or sperm nuclei. The evolutionary change which made the fertilization process independent of a liquid milieu was the germination of the megaspores and the development of the megagametophyte *in situ*; that is, on the maternal plant (sporophyte), which, by nourishing the megagametophyte, allows fertilization and the development of an embryo within a storage and propagation organ, the seed. In gymnosperms and angiosperms, the homologue of the megasporangium of heterosporous pteridophytes is the ovule, made of vegetative tissue (nucellus) containing one or more megasporogenous cells and surrounded by one or two integuments which leave a free passage, the micropyle. The ovules are borne on the carpels, the homologues of the megasporophylls, either at their axil (naked ovules: gymnosperms) or within a cavity (pistil) made of one or more carpels (angiosperms). The homologue of the microsporophylls are the stamens in which the anther contains the microsporogenous tissue. Pistils and/or stamens, sometimes accompanied by other types of transformed leaves (e.g. calix, corolla) form the flower, the typical reproductive organ of phanerogams.

In gymnosperms, the megaspore mother cells (2n) in the ovules produce by meiosis a tetrad of megaspores (n), one of which forms a female gametophyte consisting of a haploid tissue in which one or more archegonia each with an egg cell are differentiated. Tetrads of microspores (n) are produced meiotically from microspore mother cells in the anther. The nucleus of the microspore divides to

form, within the spore wall, the mature pollen grain; that is, the male gametophyte consisting of some vegetative cells and two male gametes. The pollen grains are shed from the anther and, transported by wind or some other agent, may fall on the micropyle of an ovule. Here they germinate by producing a pollen tube which elongates to transport the male gametes into the proximity of the archegonia. After fertilization, the embryo (the new sporophyte) becomes embedded in a nutritive tissue (the vegetative portion of the female gametophyte) and is protected by the seed coat derived from the ovular integument.

In angiosperms, the pollen grains germinate on the stigma of the pistil and the pollen tubes grow in the stylar tissue to reach the female gametophyte (embryo sac) in the ovule. For a review on fertilization in higher plants, reference is made to Linskens (1969). In the ovule, the nucellus bears one or, less frequently, two or more megaspore mother cells, which undergo meiosis. Following meiosis, a variety of patterns in the development of the female gametophyte can be observed. Since an extensive literature has accumulated on the nuclear cytology of the female gametophyte in angiosperms (Maheshwari, 1950; Battaglia, 1951*a*; Johri, 1963) it will be considered in some detail.

Depending upon the number of megaspore nuclei which participate in the formation of the female gametophyte, three types are distinguished:

(i) monosporic, when one of the four megaspores divides to form the gametophyte;

(ii) bisporic, when the second meiotic division is not followed by cell wall formation. Of the two dyad cells (coenocytes with two haploid nuclei each) thus formed, one develops into a gametophyte;

(iii) tetrasporic, when both meiotic divisions are not followed by cell wall formation and the four nuclei are involved in the development of the gametophyte.

The most common types of embryo sac are shown schematically in Figs. 1.5 and 1.6, which are reproduced from Battaglia (1951*a*). In Battaglia's interpretation, the reduction of the female gametophyte from gymnosperms to angiosperms is characterized by the disappearance of the archegonium and a strong reduction of the somatogenic phase; that is, the number of mitotic divisions which follow sporogenesis (tetrad formation). In angiosperm embryo sacs, the maximum number of somatogenic divisions is three (Normal or *Polygonum* type, monosporic) and the minimum is one as in the tetrasporic types *Plumbagella*, *Adoxa* and *Plumbago*. In the development of the *Polygonum* type, which is found in the majority of angiosperms, one of the four megaspores of the tetrad enlarges (vacuolization) and then undergoes the first somatogenic division to form a binucleate cell in which the two haploid nuclei are brought to occupy the cell poles (polarization). Following the second and third somatogenic divisions, a coenocyte with eight haploid nuclei is produced which undergoes cellularization (wall formation). At maturity, when the egg cell has obtained its typical shape and size (oomorphogenesis), the female gametophyte consists of an egg cell, two synergids,

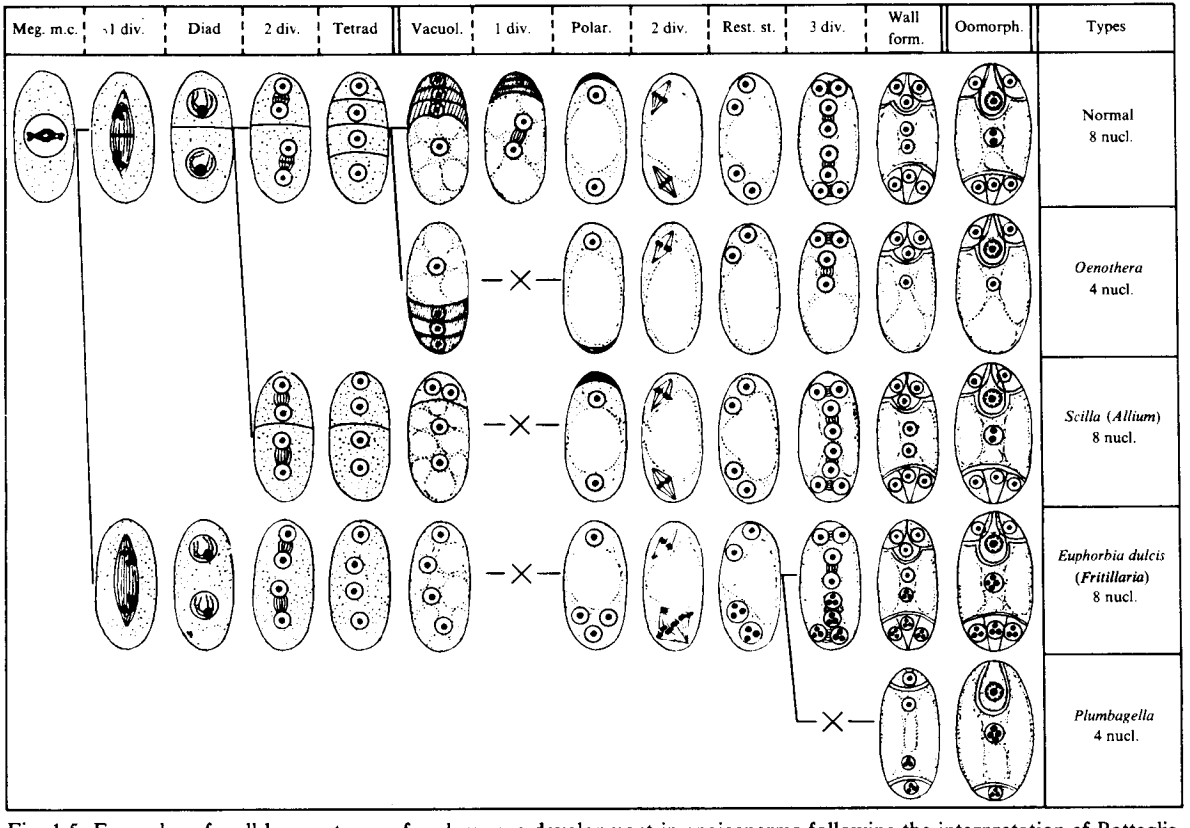

Fig. 1.5. Examples of well-known types of embryo sac development in angiosperms following the interpretation of Battaglia. The ploidy of nuclei is given by the number of nucleoli, from one (haploid) to four (tetraploid). The nucleus of the megaspore mother cell is figured with one bivalent. Vacuol: vacuolization; polar.: polarization; oomorph.: oomorphogenesis (after Battaglia, 1951 *a*).

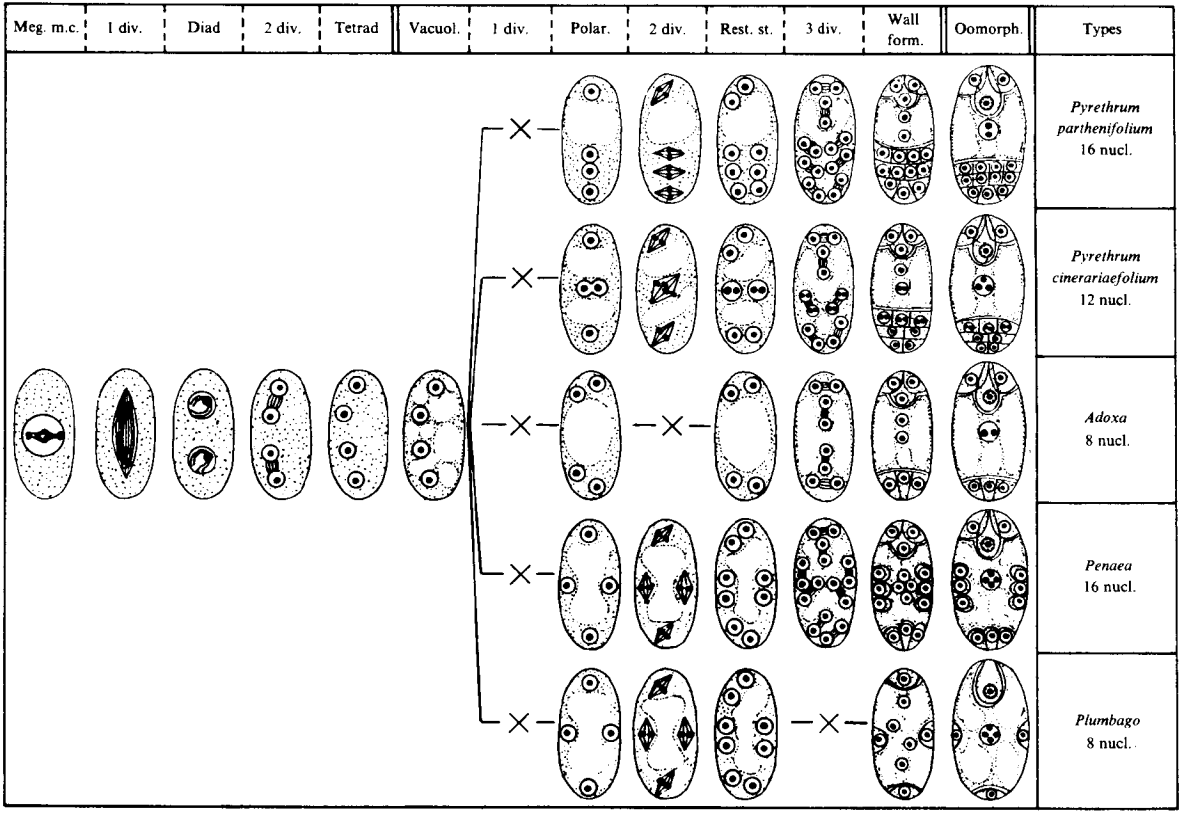

Fig. 1.6. Other examples of well-known types of embryo sac development in angiosperms following the interpretation of Battaglia. For details, see legend to Fig. 1.5 (after Battaglia 1951 *a*).

a central cell (proendospermatic cell of Chiarugi, 1927) with two polar nuclei which fuse to form a diploid secondary nucleus and three antipodals in a chalazal position. In all other embryo sac types shown in Figs. 1.5 and 1.6, the first somatogenic division does not occur; in a few tetrasporic types either the second or the third somatogenic division is lacking. An interesting aspect of the development of some tetrasporic embryo sacs is fusion of interphase nuclei or spindles. In *Euphorbia dulcis*, following a 1+3 polarization of the four megaspore nuclei, the three chalazal nuclei fuse to form a triploid nucleus; in *Fritillaria*, when the three chalazal nuclei divide they fuse their spindles into a bipolar triploid spindle. In both cases, an eight-nucleate embryo sac is formed which contains four haploid micropylar nuclei and four triploid chalazal nuclei. Nuclear fusion is also found in *Pyrethrum cinerariaefolium*, which is characterized by a 1+2+1 polarization. As pointed out by Chiarugi (1950), the fusion of the polar nuclei to produce a diploid or polyploid secondary nucleus is one of the most outstanding features in the construction of the angiosperm embryo sac. The ploidy level of the secondary nucleus is further increased by its fusion with one of the two sperm nuclei during the process of double fertilization which leads to the formation of a diploid zygote and a polyploid proendospermatic cell, which is the initial starting point of the development of a nutritive tissue, the endosperm (see below). According to Chiarugi (1950), the central polyploidization in the angiosperm embryo sac is an adaptation to a dehydration gradient starting from the micropylar and chalazal poles, which may be regarded as the zones of nutrient supply to the gametophyte; by this means, the haploid gametophytic generation is thought to correct its physiological inferiority towards the diploid sporophytic tissues in which it is immersed.

As to the male gametophyte of angiosperms, it originates from a microspore which develops mitotically into a mature pollen grain containing a vegetative cell and two sperm cells and is surrounded by a spore coat (Steffen, 1963). The microspores are produced in tetrads in each of the many microspore mother cells which form the sporogenous tissue in an anther loculus. The first microspore division ends with a typical cell wall which is unpenetrated by plasmodesmata or wider protoplasmic interconnections (Angold, 1968; Heslop-Harrison, 1968): a vegetative and a generative cell are formed. Thereafter, the division of the generative cell produces two separate sperm cells. Pollen grains are shed from the anther in a binucleate or trinucleate state. In the first case, the division of the generative cell to form the two sperm cells occurs in the pollen tube which is growing to reach the egg cell. Binucleate pollen grains characterize roughly 2/3 of angiosperms, namely 170 families; in fifty-four families, trinucleate pollen grains occur. In twenty-four other families, genera with binucleate or trinucleate pollen grains have been recorded, whereas genera in which bi- and trinucleate pollen species have been found are rare (Brewbaker, 1959; Brewbaker & Emery, 1962).

An interesting aspect of the development of the pollen grain, which has been recently clarified concerns the timing of DNA synthesis. In the classical *Trades-*