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PERIODIZATION IN THE DEVELOPMENT OF FLOWERING PLANT REPRODUCTIVE STRUCTURES: CRITICAL PERIODS

TATYANA B. BATYGINA* AND VALENTINA E. VASILYEVA

Department of Embryology and Reproductive Biology, Komarov Botanical Institute, Prof. Popov Str. 2, 197376 St. Petersburg, Russia

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The theory of critical periods in plant ontogenesis has been elaborated from studies of integral morphogenetic processes on different levels. The periodization of the development of various reproductive structures (anther, microspore, pollen grain, ovule, megagametophyte, egg cell, zygote and embryo) has been worked out from data on morphogenesis using systemic and complex morphophysiological approaches. Critical phases, stages and periods have been revealed, for example the stage of autonomy in different flowering plants, by means of culture in vitro. The concepts of "critical period" and "critical mass" in relation to embryonal structure periodization are discussed here. Also addressed are the question of allometry and the significance of morphogenetic fields and rhythms of cell division for revealing critical periods and the management of ontogenesis. Examination of the genesis and structure of anthers and ovules in various flowering plant species has permitted us to discover general regularities in their development and the occurrence of three common critical periods: premeiotic, meiotic and postmeiotic. Embryo development in angiosperms is characterized by two common phases (proembryonal/blastomerization and embryonal/organogenesis) and five critical periods (zygote and proembryo, globular, heart-shaped, torpedo-shaped, and mature embryo). The combination of common and specific critical periods and stages determines the taxon-specific morphogenesis of reproductive structures and contributes to the plasticity and tolerance of the reproductive systems of different species of flowering plants, and of ontogenesis as a whole.

Key words: Critical period, critical mass, morphogenetic fields, reproductive structures, switching over development program.

INTRODUCTION

Elaborating the theory of critical periods, stages and phases during ontogenesis is an important problem in biology. The information gained through investigation of the integral events in the morphogenesis of reproductive organs in flowering plants and the changing pattern of their spatial organization offers the possibility of controlling individual development (Stockard, 1921; Svetlov, 1960; Tokin, 1987). We have suggested the periodization of the development of the basic reproductive structures – anther, ovule, egg cell, zygote and embryo – and put forward a strategy for studying reproductive structure: (1) investigation of the dynamics of anther, ovule and seed structures and their interrelation with the surrounding tissues; (2) comparison of the kinetics of morphogenetic and physiological biochemical processes; (3) comparison of the regularities of anther, ovule and embryo differentiation in situ, in vivo and in vitro; and (4) choice of model plant species, contrasted by their embryological features, ecotype and ecology, and etiology of germination in relation to the plant's position in the phylogenetic system of angiosperms (Batygina et al., 1992).

*e-mail: batygina@TB1390.spb.edu

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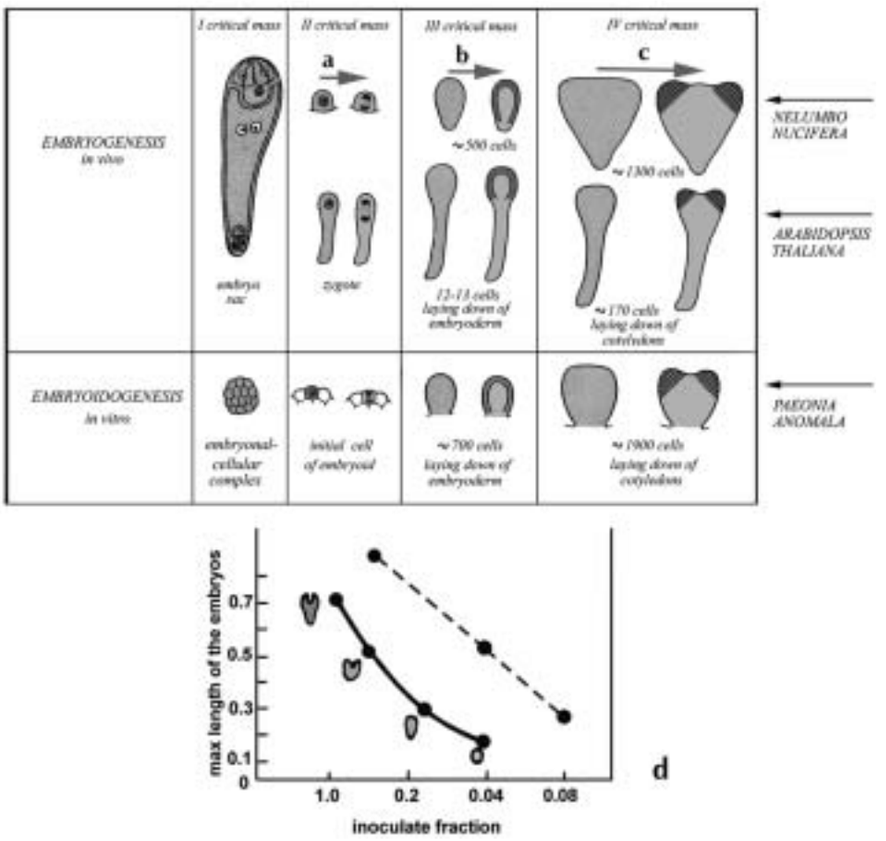

Fig. 1. Critical mass formation during morphogenesis in situ and in vitro. (**a**) Mature zygote transition to division, (**b**) Transition from globular stage of embryo development to embryoderm formation, (**c**) Transition to heart-shaped stage of embryo development (after Batygina, 1984), (**d**) Influence of the density of the population on embryoid differentiation and their viability in cell culture of carrot (after Halperin, 1967, modified).

CRITICAL CELLULAR MASS AND MORPHOGENETIC FIELDS IN THE REPRODUCTIVE SYSTEM

The totipotency of the plant cell defines the continuation of morphogenesis throughout ontogenesis. The whole of ontogenesis could be determined as a particular function of meristem growth to a certain critical mass (Fig. 1). We understand critical cellular mass as the minimum number of cells or the mass of a single cell required for morphogenesis of any structure, or in other words "not more than is absolutely needed" in accord with the embryogenetic law of economy (Johansen, 1950). For example, at a definite critical stage of embryo development, differentiation of protoderm, conditioned by the appearance of embryo critical mass, takes place simultaneously with the beginning of exponential growth (Batygina, 1984a; Vallade, 1989). In carrot cell suspension, the correlation between the degree of differentiation of embryoids, their survival, and the degree of inoculate dilution (Halperin, 1967), that is, the critical mass of cells, was established (Fig. 1).

For plant reproductive structures, the origin of all cells and their relationship can be revealed at early stages. At later developmental stages the role of each single cell in the system decreases. However, intercellular cooperation significantly increases and morphogenetic fields are formed (Batygina, 2000). From the standpoint of modern ideas and facts (Gilbert et al., 1996), the concept of "morphogenetic field" acquires a new significance when one examines the periodization of reproductive structures. We refer to the morphogenetic field as a definite morphogenetic zone in which the pattern of cell division and the direction of growth is strictly determined, and in which these cells occupy positions corre-

sponding to their roles in this system (in agreement with the law of disposal, Souèges, 1937). Each species is characterized by a definite number of critical masses and morphogenetic fields. Differentiation of the reproductive structures and the entire organism may be accompanied by the formation of several critical masses, which determine the "threshold of factors" required for establishing any morphogenetic pathway (embryogenesis, embryoidogenesis, organogenesis and histogenesis) both in situ and in vitro.

CRITICAL PERIODS, CRITICAL STAGES IN REPRODUCTIVE STRUCTURE DEVELOPMENT

Earlier investigators examined the "critical period", the "critical stage" ("transitional moment") as the stage of ontogenesis in which the structures display the greatest sensitivity to unfavorable environmental factors. We interpret the critical period more loosely as including a number of stages. Our basis for distinguishing critical moments consists in the morphogenetic and morpho-physiological correlations that cause allometries of the reproductive structures at different developmental stages and their architecture. In investigating the critical periods and stages, we observed the specific and nonspecific reactions of the plant to one or another source of influence. Comparative analysis of data on the genesis of anthers and ovules in various flowering plant species permitted us to outline the periodization of their development and discover common (premeiotic, meiotic, postmeiotic) and specific critical periods and stages.

CRITICAL PERIODS AND STAGES IN ANTHER DEVELOPMENT

Anther development in cereals offers an illustration of the critical periods (premeiotic, meiotic, postmeiotic) and critical stages within each period, as presented in detail in Figure 2. Numerous works are devoted to investigation of meiosis in plants and animals, but certain mechanisms of this process have not been studied sufficiently. Several types of meiosis are known. For most flowering plants, "spore meiosis" is characteristic. In this case, meiosis is part of the general process of sporogenesis and is accompanied by interchange of phases (diplophase – haplophase), or "generations" (sporophyte – gametophyte). Meiosis is a multistage process in ontogenesis, a transitive critical period between sporophytic and gametophytic generations. In the classic understanding, meiosis is completed with the formation of micro- and megaspore tetrads. However, some embryological data (on the development of embryo sacs of different types) suggest that the meiotic period can finish later, namely after disintegration and specialization; that is, it draws in the whole process of micro- and megasporogenesis. Microspores represent the initial cells of gametophyte generation. The formation of the gametophyte proper provisionally begins in the postmeiotic period with microspore germination – its division. The occurrence of enormous abnormalities during micro- and megaspore development confirms our suggestion. It should be emphasized that the division of the developmental process into premeiotic, meiotic and postmeiotic periods is rather a conditional one, as meiosis itself is a highly plastic process and cannot coincide precisely with the stages of micro- and megasporogenesis; moreover, meiosis I is sometimes absent (in the case of diplospory).

The premeiotic period in anther development includes three critical stages: (1) differentiation of certain cells of the anther primordium to archesporium; (2) division of archesporial cells into sporogenous tissue and parietal layer; and (3) determination of a centripetal pattern of anther wall development.

The meiotic period is characterized by the transition from diplophase to haplophase and includes two critical stages: (1) initiation of meiosis in the microspore mother cells, formation of callose, formation of microspore tetrads; and (2) development of the microspore.*

The postmeiotic period (formation of the gametophyte proper) includes three critical stages: (1) microspore "germination" and unequal microspore division, yielding the generative and vegetative cells, together making up the pollen grain; (2) displacement of the generative cell and vegetative cell nucleus in the pollen grain; and (3) gametogenesis. The postmeiotic period is associated with maturation and developmental disintegration of some tissues of the anther wall.

The meiotic and postmeiotic periods are considered to be the critical ones in anther development in respect to moisture supply and adequately high air temperature. Deficits of microelements, in particular boron, result in the breakdown of nucleic acid exchange, which in turn leads to the disruption of meiosis and the formation of abnormal pollen. A correlation between such genetic features as cyto-

*Included in postmeiotic period by most authors [ed.]

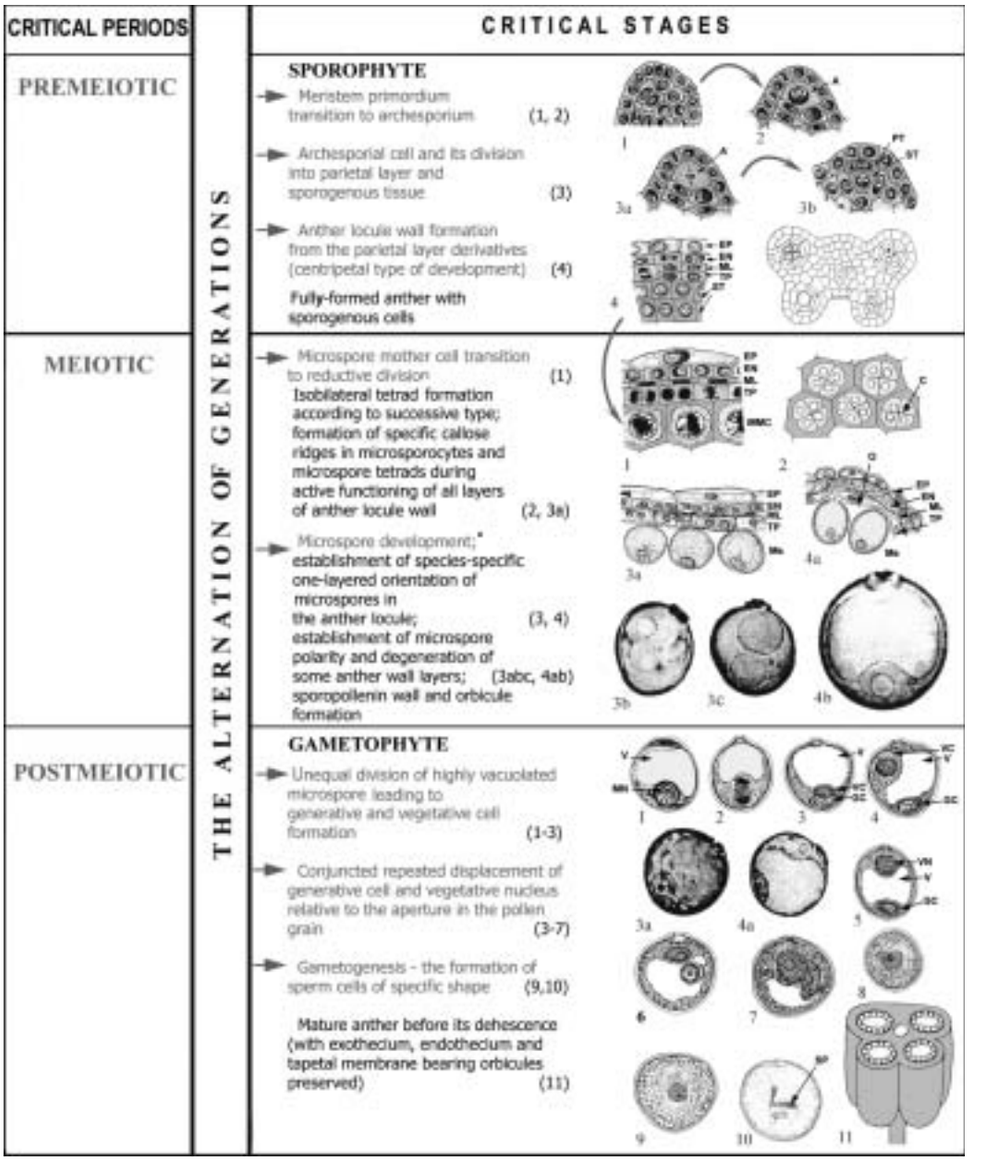

Fig. 2. Anther development in grasses. A – archesporium; C – callose; EP – epidermis; EN – endothecium; GC – generative cell; ML – middle layer; MMC – microspore mother cell; MN – microspore nucleus; Ms – microspore; O – orbicule; PT – parietal tissue; ST – sporogenous tissue; SP – sperm cell, TP – tapetum; V – vacuole; VC – vegetative cell; VN – vegetative nucleus. *Classified as postmeiotic period by most authors [ed.].

plasmic male sterility (CMS) and disturbed anther wall development, occurring mainly in the postmeiotic period, has been demonstrated.

Morphogenesis and critical periods in tissue culture

There are two morphogenesis pathways to obtain haploids as presented in Figure 3. The critical stage in anther development for obtaining haploids is species-specific. For cereals in vivo the highly vacuolated microspore is that critical stage, during which the developmental program is transferred to the gametophytic pathway.

By changing the conditions of anther cultivation, it is possible to obtain a great number of regenerants from a single microspore, by deriving secondary embryoids from the epidermal cells of callus tissue pro-

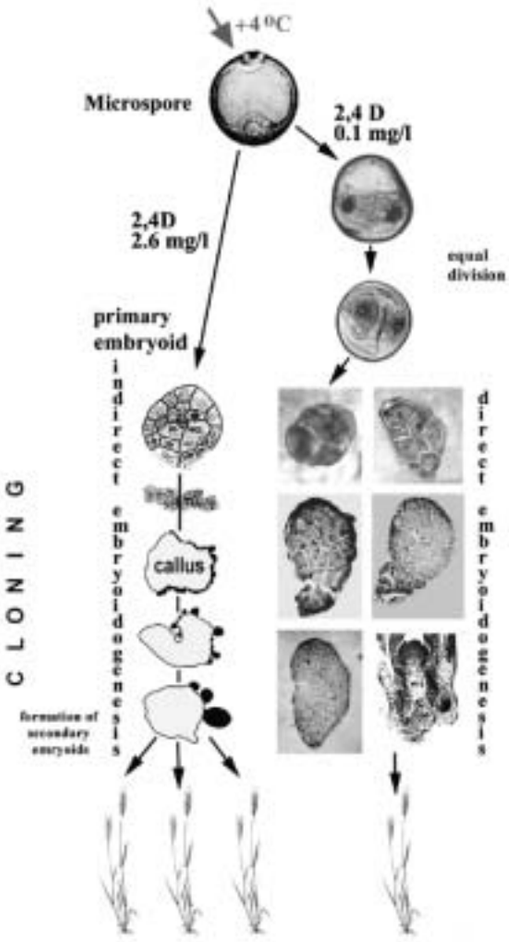

Fig. 3. Haploids obtained from highly vacuolated cereal microspore – the critical stage of anther development.

duced from the primary embryoid. Such cloning produces a large quantity of haploid plants.

CRITICAL PERIODS AND STAGES IN OVULE DEVELOPMENT

The premeiotic period in ovule development includes two critical stages: (1) formation of the ovule primordium and (2) initiation and switching over of the developmental program, differentiation of certain cells (archesporium) and structures of the nucellus, and the establishment of the three-dimensional organization of ovule structures. This period of ovule development is the critical one, because it determines the mode of reproduction (sexual or asexual) and the morphogenetic pathway (embryo-, embryoidogenesis). The character of this period as it proceeds, when the genetic program for apospory, diplospory, and the "dormant meristem" can be switched over, later determines the diversity of reserves (parthenogenesis, nucellar and integumentary embryoidogeny, etc.) and consequently the heterogeneity of seeds (Batygina, 1984b, 1999).

The meiotic period is characterized by certain events connected with meiosis and the transition from diplophase (sporophyte) to haplophase (gametophyte). It includes two critical stages: (1) the beginning of meiosis in the megaspore mother cell, establishment of polarity, and the formation of callose; and (2) the formation of megaspore tetrads. However, this stage is clearly separated only in monosporic megagametophytes.

The postmeiotic period begins with the formation of the gametophyte and includes four stages: (1) the coenocytic phase of the megagametophyte; (2) cellularization and gametogenesis; (3) the fully cellularized megagametophyte not yet ready for fertilization; and (4) the mature embryo sac with complete specialization of its elements, ready for fertilization. Such structures as the hypostase, operculum and others can be observed in various species in different critical periods.

Critical periods in egg cell and zygote development

In the periodization of egg cell and zygote development, three common critical periods (egg apparatus organization, fertilization and zygote formation, transition of zygote to proembryo) were distinguished. Additionally, seven general stages in egg cell and zygote formation could be distinguished (Fig. 4). Pollination, pollen tube growth in the pistil and its entry of the megagametophyte undoubtedly are critical moments and possibly signals for reorganization of the egg apparatus and other female gametophyte elements.

In the "egg cell → zygote" system there are periodically recurring processes such as meristematization, differentiation, specialization, dedifferentiation and again meristematization.

The moment the sperm enters the egg cell is a common critical stage for most flowering plants (egg cell → zygote). During the uniting of female and male gametes, differentiation begins in the initial zygote. It continues at the stages of resting zygote and zygote in interphase. The cytoplasm, cell wall and nucleus undergo several changes which on the one hand result in the formation of the taxon-specific appearance of the mature zygote, and on the other

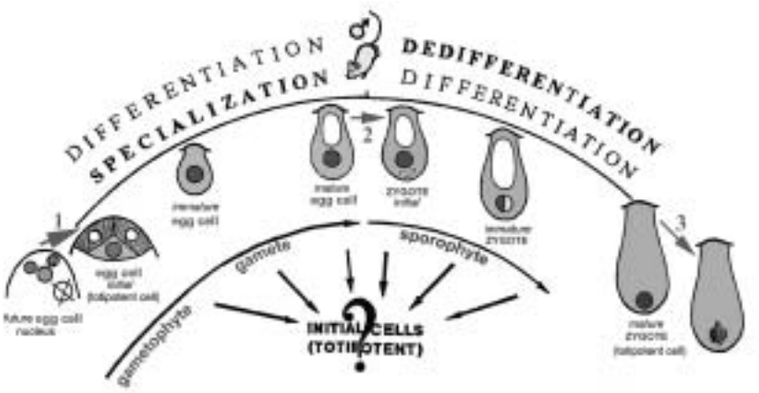

Fig. 4. Critical periods and stages of egg cell and zygote development as a source of embryos in situ and in vitro. 1 – transition from coenocyte stage of embryo sac to determination of egg apparatus cells; 2 – transition from mature egg cell to zygote initial; 3 – transition of mature zygote to proliferation (after Batygina and Vasilyeva, 2001, modified).

hand restore it to the meristematic state and enable cell proliferation.

"Egg cell → zygote" development may be compared with the initials of somatic embryos where at a certain stage of their development they are also characterized by the meristematic state (Fig. 4). Direct determination of the stage of egg cell, zygote and somatic cell isolation during experimental investigations is required for control of the next development processes.

Critical periods and stages in embryo development

In the last decade, geneticists have begun to pay more attention to the theory of critical periods in embryo development. Thus, in *Zea mays* the critical periods in early embryogenesis have been identified, characterized by the breakdown of the mechanism for the establishment of basal-apical polarity and symmetry (Sheridan and Clark, 1993). Studies of the influence of auxin inhibitors on polar transport in the embryo of *Brassica juncea* deserve special attention (Chaudhury et al, 1993; Liu et al, 1993). The globular stage of embryogenesis is one of the stages most sensitive to the effects of different environmental factors. Different auxins expressed in culture of embryos at the late globular stage caused embryos with fused cotyledons to form. This influence did not operate in heart-shaped embryos because of the changed auxin and cytokinin levels in the embryo.

In embryo evolution the tendency toward common developmental regularities has been clearly observed, manifested in five common and specific critical periods and stages, and the formation of embryo critical masses:

(1) the proembryonal phase (blastomerization), with a common critical period, that is, division of the zygote and further development of the proembryo. Establishment of the polarity axis, symmetry and the beginning of three-dimensional embryo organization, accumulation of critical cellular mass, promoted protoderm differentiation and the formation of the hypophysis and epiphysis are the main features of this period;

(2) the embryonal phase (organogenesis), with a common critical period, that is, the formation of a globular embryo (early, middle, later stages) This stage is transitional to organogenesis;

(3) the heart-shaped embryo (early, middle, later stages), with the appearance of morphogenetic zones (fields) and the beginning of the formation of cotyledons, the shoot and main root apexes. This period is characterized by stepped determination of the globular embryo;

(4) the torpedo-shaped embryo, with development of the cotyledons, roots, vascular system and shoot apex;

(5) the mature embryo, the complicated period in seed development (transition to dormancy).

The question remaining for consideration is how the similarity of embryonic organization in modern plants can be explained, whether from parallel evolution or from their origin within a community (Teryokhin, 1996).

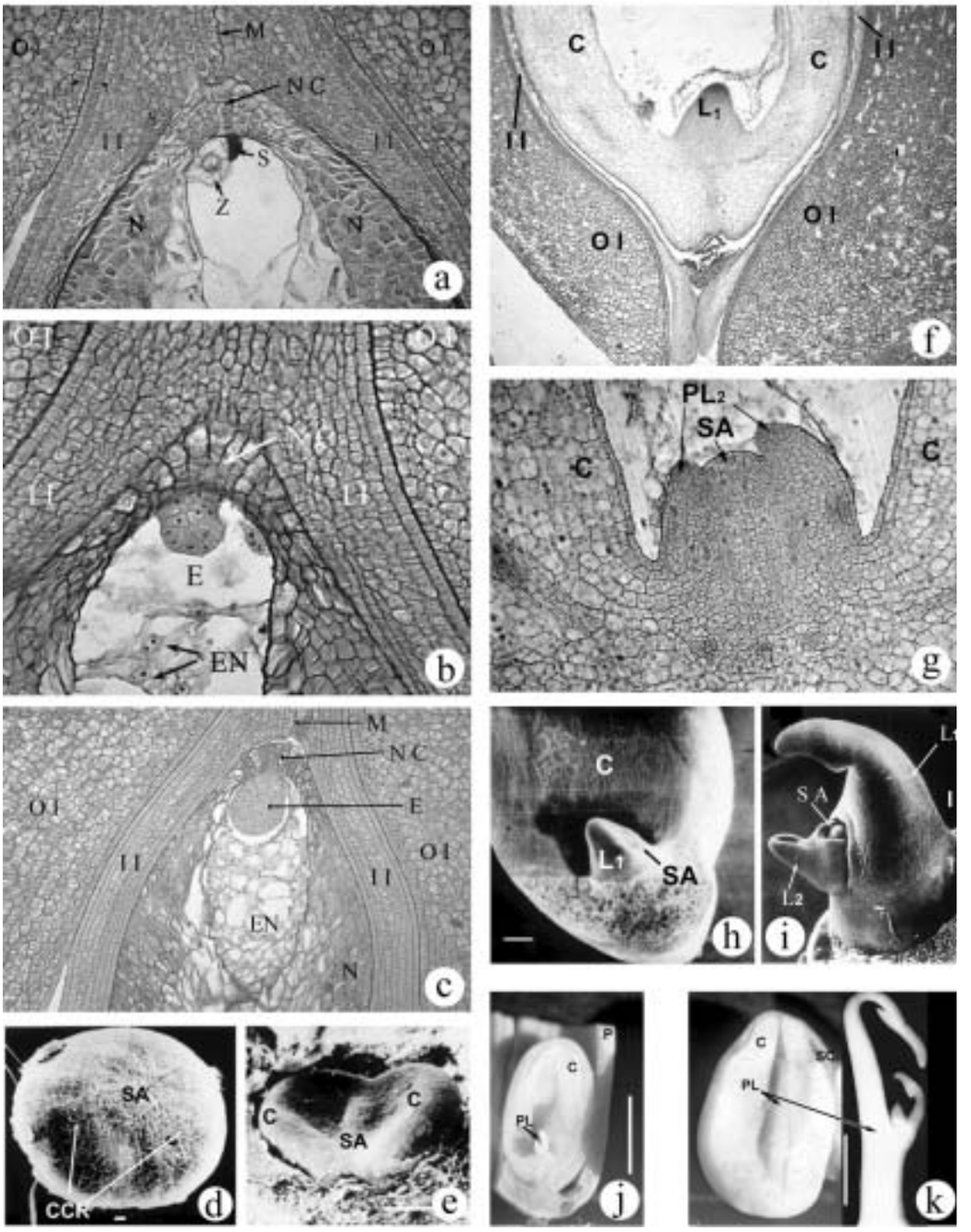

Fig. 5. The critical periods and stages of embryo development in *Nelumbo nucufera* L.: (**a,b**) First critical period – zygote (a) and proembryo (b), (**c**) Second critical period – globular embryo, (**d,e**) Third critical period – heart-shaped embryo: critical stages – formation of common cotyledon ridge (d), laying down of cotyledons and shoot apex (e), (**f–j**) Fourth critical period – torpedo-shaped embryo: critical stages – laying down of first leaf primordium in plumule, root apex and conductive system (f,h), laying down of second leaf primordium (g), formation of blade of first leaf (i,j), (**k**) Fifth critical period – mature embryo. C – cotyledon; CCR – common cotyledon ridge; E – embryo; EN – endosperm; II – inner integument; L_1 – first leaf primordium; M – micropyle; N – nucellus; NC – nucellar cap; OI – outer integument; P – pericarp; PL – plumule; PL_2 – primordium of second leaf; S – synergid; SA – shoot apex; SC – seed coat; Z – zygote; a–c and f, g – LM, d,e,h,i – SEM, j,k – longitudinal section of fruit (photo).

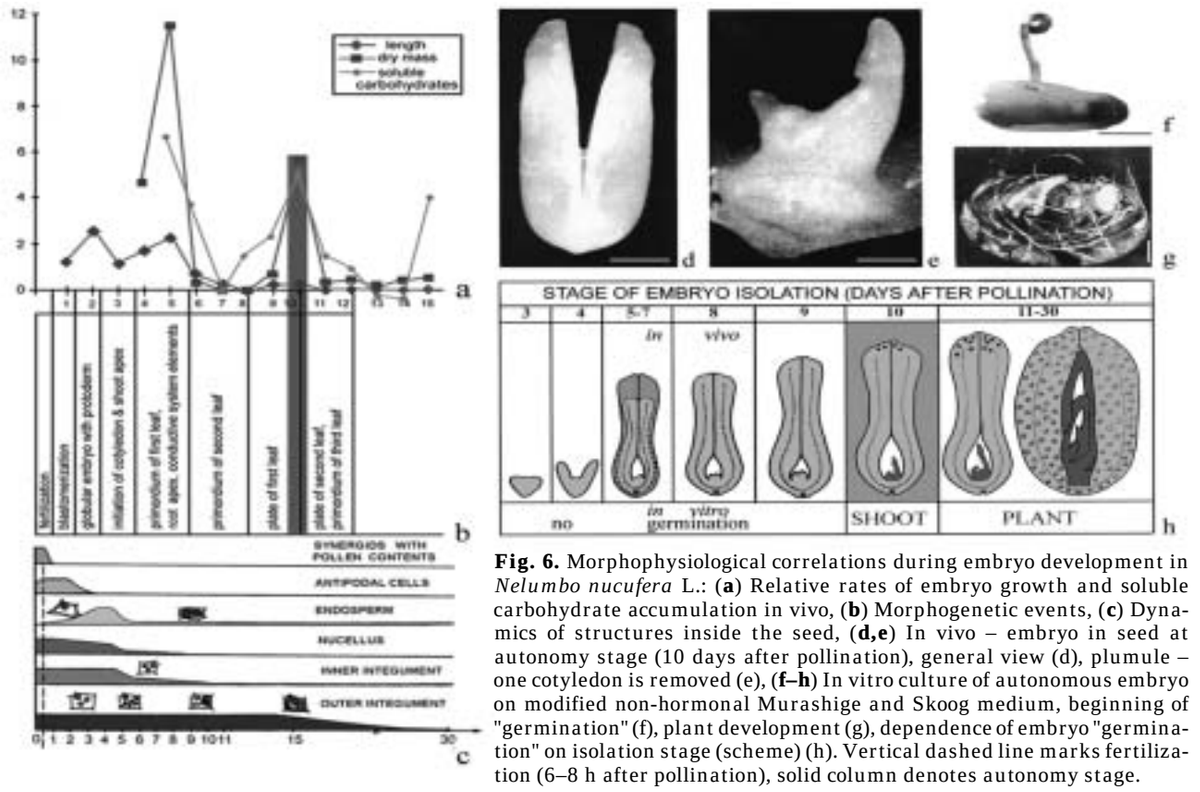

Fig. 6. Morphophysiological correlations during embryo development in *Nelumbo nucufera* L.: (**a**) Relative rates of embryo growth and soluble carbohydrate accumulation in vivo, (**b**) Morphogenetic events, (**c**) Dynamics of structures inside the seed, (**d,e**) In vivo – embryo in seed at autonomy stage (10 days after pollination), general view (d), plumule – one cotyledon is removed (e), (**f–h**) In vitro culture of autonomous embryo on modified non-hormonal Murashige and Skoog medium, beginning of "germination" (f), plant development (g), dependence of embryo "germination" on isolation stage (scheme) (h). Vertical dashed line marks fertilization (6–8 h after pollination), solid column denotes autonomy stage.

Critical periods in embryo development of *Nelumbo nucifera*

Comparative, complex investigation of morphogenesis, growth dynamics, differentiation, and accumulation of proteins, nucleic acids and carbohydrates in the embryo, seed coat, pericarp and receptacle development permitted the establishment of five common critical periods in proembryonal (blastomerization) and embryonal (organogenesis) phases in *Nelumbo nucifera*, as presented in Figure 5. *Nelumbo nucifera* offers a model for the gradual establishment of embryo autonomy in the seed in vivo (Vasilyeva et al., 1987; Batygina and Vasilyeva, 1988; Titova and Batygina, 1996).

Autonomy of the embryo

The most important critical stage of embryogenesis is the stage of embryo autonomy. Subsequently the embryo (new sporophyte) begins the transition on the relatively independent (autotrophic) developmental pathway, that is, the gradual autonomization of ontogenesis takes place (Fig. 6). The stage of autonomy can be identified in vitro by the embryo's ability to complete normal embryogenesis and produce a plant in a mineral-sucrose medium without hormones. The autonomy of embryos appears at different stages of development in different plant species (*Nelumbo*, *Capsella*, *Vicia*, *Triticum*, *Oryza*, *Hordeum*, *Arabidopsis*).

The autonomous embryos of all flowering plants are not dependent on exogenous hormones, and possess the ability to germinate.

Peculiarities of periodization in peony embryo development

In peony seeds two types of embryos successively form, differing in origin, namely by sexual and somatic embryogeny. The sexual embryo is created by the sexual process (syngamy) (n+n) and the somatic embryo (2n = 2n) by the formation of an initial embryoid cell from a somatic cell of the protoderm in the sexual embryo. Subsequent stages of sexual and somatic embryo development are presented and described in Figure 7. Unlike in sexual embryo development, the somatic embryo

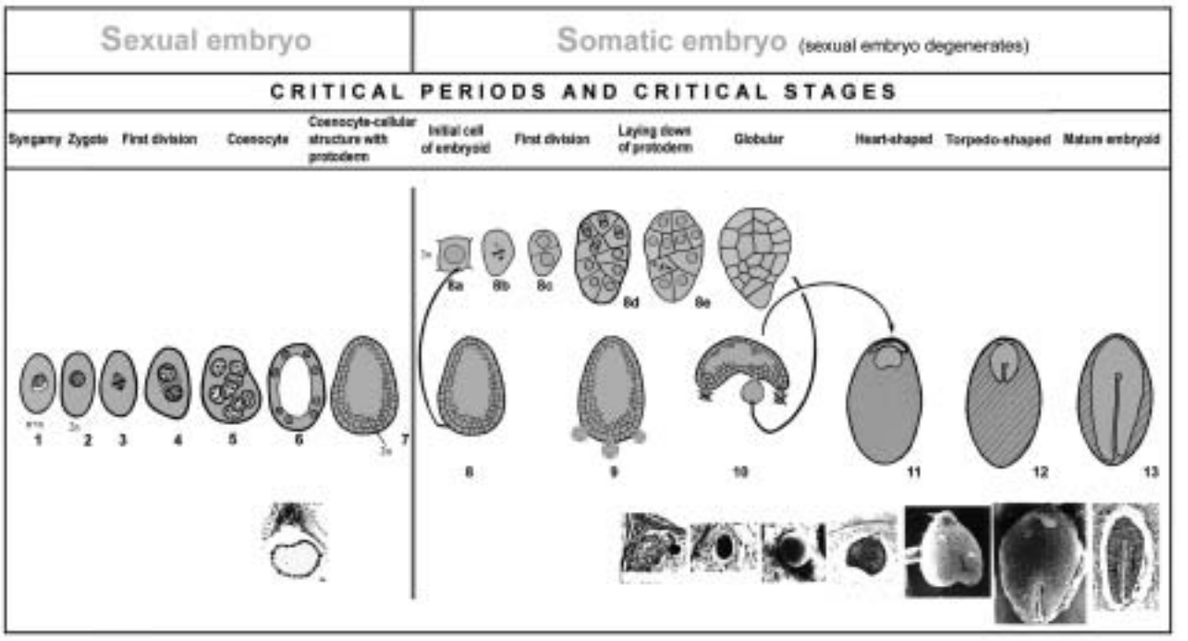

Fig. 7. Switching over the genetic program in *Paeonia* embryo development.

follows the Asterad type, Penaea variation (Johansen, 1950).

The developmental program switches over from a sexual to asexual mode of embryo production at the end of the first common critical period, that is, the formation of the coenocytic-cellular structure with protoderm (proembryo). The tendency to reproduce asexually (vegetatively), with the possibility of cloning (polyembryony), is a peculiarity of the sexual embryo in peony.

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