THE BASIC AUTONOMY OF METASEQUOIA FEMALE GAMETOPHYTES

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SUMMARY

In France and The Netherlands in *Metasequoia glyptostroboides* (Hu and Cheng) only purely female cones are present budding at the end of February. In the absence of any male gametophytic tissue a normal and complete female gametophyte develops. The developments from free nuclear gametophytes to the mature egg cells are presented at light and electron microscopy level. The functional influence of the male gametophyte as a prerequisite for female gametophyte development is questioned and discussed for the different developmental stages as observed in *Metasequoia* and for other Gymnosperms. In case of *Metasequoia*, it is suggested that the female gametophyte is basically autonomous.

1. INTRODUCTION

In Gymnosperms pollen are shed precociously during the slow and complex development of the female (TISON 1911) gametophyte. Male and female gametophytes coexist over a long period within the ovule as compared to the angiosperms. The question arises about the interactions between these two organisms over this long period of coexistence. Numerous results of histocytological studies on sexual reproduction in Gymnosperms (SINGH 1978) show a relative chronological development of the female and male gametophytes. In some peculiar cases, e.a. in *Sequoia* (LOOBY & DOYLE 1937) the pollen tubes reaching the sides of the female gametophyte still in the free-nuclear stage, are thought to guide the successive differentiation of archegonia. This supports the hypothesis (LOOBY & DOYLE 1937) that the pollen tube is the inductive agent for archegonial differentiation.

In vitro cultures of male gametophytes of *Juniperus communis* (DUHOUX & CHESNOY 1978) show indeed a lower percentage and asynchronous production of sperm cells, but their structure is comparable with those found in vivo. The addition of amino acids proportional to those found in the ovule did increase significantly male gamete production (DUHOUX & PHAMTHI 1980). These results suggest the female gametophyte to produce substances regulating the male gametophyte development.

The influence of the male gametophyte on the development of the female gametophyte is not well understood today.

Experiments on in vivo culture of female gametophytes of Gymnosperms during oogenesis ended in a deadlock (ROHR 1980), while experiments with ovule

or female cones in bags preventing pollination, show cone abscission or a stop of the ovule development (FAVRE-DUCHARTRE, 1970; HAGMAN & MIKKOLA, 1963; PLYM-FORSHELL, 1974; OWENS et al., 1981a). In *Ginkgo* and *Cephalotaxus* (FAVRE-DUCHARTRE, 1957, 1958), dioecious trees, not-pollinated ovules aborted whereas in members of *Abies, Larix, Pinus, Pseudotsuga, Thuja* and *Tsuga* (see OWENS et al., 1981b) in which pollen dehiscence occurred, some ovules without pollen in the integument or pollen tubes in the nucellar tissue, could develop a mature female gametophyte.

Pollination with heat-killed pollen grains (MCWILLIAM 1959) or application of phytohormones did prevent cone detachment and in the second case (HAGMAN & MIKKOLA 1963) the cones remain on the tree with ovules containing abortive prothalli. On the other hand after some hybridizations the stage of abortive embryos was reached indicating the development of a normal female gametophyte (HAGMAN & MIKKOLA 1963). These experiments indicate that pollination is indispensable for attachment of the female cones and ovule development.

Knowledge concerning the influence of the male gametophyte on the development of the female gametophyte in Gymnosperms is insufficient. Therefore the development of cones, ovules and female gametophytes in the only female flowering *Metasequoia glyptostroboides* was studied and compared with the results already described for normal sexual reproduction (WANG & CHIEN 1964). The results are discussed in relation to the dependency of male and female gametophyte in their development to each other.

2. MATERIAL AND METHODS

Observations were made in 1981–1982 on *Metasequoia glyptostroboides* (Hu and Cheng) in the botanical gardens of the Agricultural University of Wageningen (The Netherlands) and in the Jardin alpin du Muséum d'Histoire Naturelle (Paris, France).

Different developmental stages of the female gametophyte were observed after fixation and staining with glutardialdehyde-OsO$_4$ and embedded in ultra-low viscosity medium (Ladd).

Light microscopic observations were performed on phase contrast semi-thin sections (2–3 μm). Electron microscopic observations were made on double post-stained ultra-thin sections (Ur-acetate, leadcitrate). Polysaccharides were traced by PATAg (THIERY 1967). In vitro cultures of mature female gametophytes were made (GREEN & PHILLIPS 1975).

3. RESULTS

3.1. The female gametophyte and the oogenesis

In France and The Netherlands only female cones of *Metasequoia* bud at the end of February. Microsporangia are never developed. The ovules secrete their pollination drop as do different species of Gymnosperms. None of the trees

Plate I. Light micrographs of not-pollinated ovules with female gametophyte development in *Metasequoia glyptostroboides.*

Fig. 1. The free nuclear female gametophyte (FG) with the large vacuole (V) and prothallial nuclei (PN). The nucellus (N) does not show any pollen tube. × 170.

Fig. 2. Very young apical archegonial initials (AI) placed side by side and surrounded by the prothallial cells (PC). The nucellar tissue (N) free from pollen tubes. × 170.

Fig. 3. The neck-cell initials (NCI) can be recognized and the central cells (CC) with large vacuoles are now surrounded by a definite jacket layer (JL). × 170.

Fig. 4. Mature egg cell (EC) with a ventral nucleus (VN) and egg cell nucleus (ECN) and already secreting neck-cells (NC). The degeneration of the egg cell (A) is announced by very small vacuolizations (arrow). × 450.

examined developed a single male cone, so local self-pollination is excluded. Cross- or interspecies pollination could be excluded since *in toto* sectioning of ovules did not show any pollen grain present in the ovules. All cones with their ovules were unpollinated.

Cellularization of the free-nuclear gametophyte (*fig. 1*) occurs during the first weeks of July and the archegonial initials are easyly identified (*fig. 2*). Oogenesis is completed within four weeks. Up to ten adjacent archegonial initials are placed in an apical complex in the cellular gametophyte (*fig. 2*). The archegonial initials are elongated and supplied by a large vacuole assuring cellular growth. The divisions of the archegonial initials (taking place in Mid-July) give rise to the initiation of a still vacuolated central cell and a neck-cell initial (*fig. 3*). The neck-cell initial will further divide to form one tier of 4–6 neck-cells (*figs. 3, 6, 9*).

The central cell nucleus divides and an important and rapid synthesis of cytoplasm takes place wh˙ ;h ends at the mature egg cell stage at the end of July. The egg cell contains a large voluminous nucleus situated in the middle of the cell and a ventral canal nucleus pressed to the plasmalemma of the egg cell just underneath the neck-cells. Cell wall synthesis to separate a ventral cell fails (*fig. 4*). The central vacuole, although always important, decreases in volume during this period (*figs, 4, 5, 6*). The cytoplasm of the egg cell contains numerous small cytoplasmic inclusions, bordered by planes of smooth endoplasmic reticulum; a vacuolated zone is thus created (*fig. 7*). The mitochondria are accumulated together or fused one to another (*fig. 7*). The leucoplasts are assembled and they all are enclosed by smooth endoplasmic reticulum (*fig. 8*).

In the nucleus of the egg cell numerous larger ring-shaped nucleoli or fine micronucleolar bodies are dispersed in the nucleoplasm (*fig. 6*). The development of the ovules within one cone is asynchronous but within one ovule the development of the different archegonia is nearly synchronous. In at least 10% of the ovules additional archegonial complexes are found on the lateral sides of the female gametophyte. These complexes are identical in development with the apical complex (*fig. 5*).

The cells of the jacket layer with dense cytoplasm surround the central cells and the egg cells. The other prothallial cells are poorly provided with cytoplasm, they do contain a large vacuole (*figs, 5, 6*).

At time of maturation of the archegonia, there is an intensive amylogenesis observed in the prothallial cells just near the archegonial complexes. This amylogenesis is noticed by an axial band of amylose in the female gametophyte (*fig. 18*).

The absence of fertilization is marked by a selective lysis of the mature egg cells and approximately three weeks later the other parts of the female gametophyte (*figs. 4, 18*) will degenerate.

In vitro culture of the mature female gametophytes results in a delay of degeneration of another two weeks, during which no further developments occur. A parthenogenetic development is not observed, neither in vivo nor in vitro.
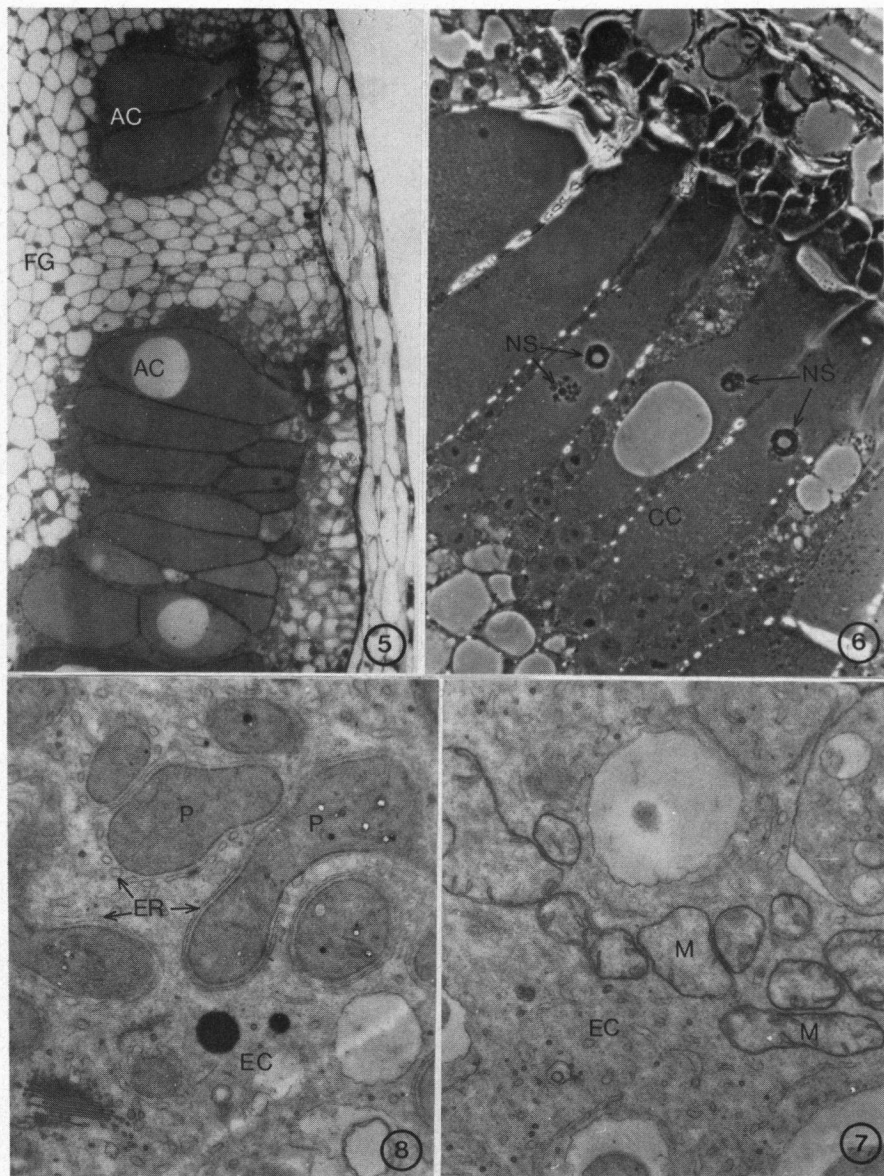
Plate II. Light (5–6) and electron (7–8) micrographs of mature egg cells of not pollinated ovules in *Metasequoia glyptostroboides.*

Fig. 5. Additional archegonial complex (AC) placed at the lateral sides of the female gametophyte (FG). × 170.

Fig. 6. Nucleolar structures (NS) observed in the central cells (CC) just before final central cell division. × 450.

Fig. 7. Irregular shaped and accumulated mitochondria (M) in the egg cell (EC). × 14,000.

Fig. 8. In the maturing egg cell (EC) all the plastids (P) are surrounded by a plane of the smooth endoplasmic reticulum (ER). × 14,000.
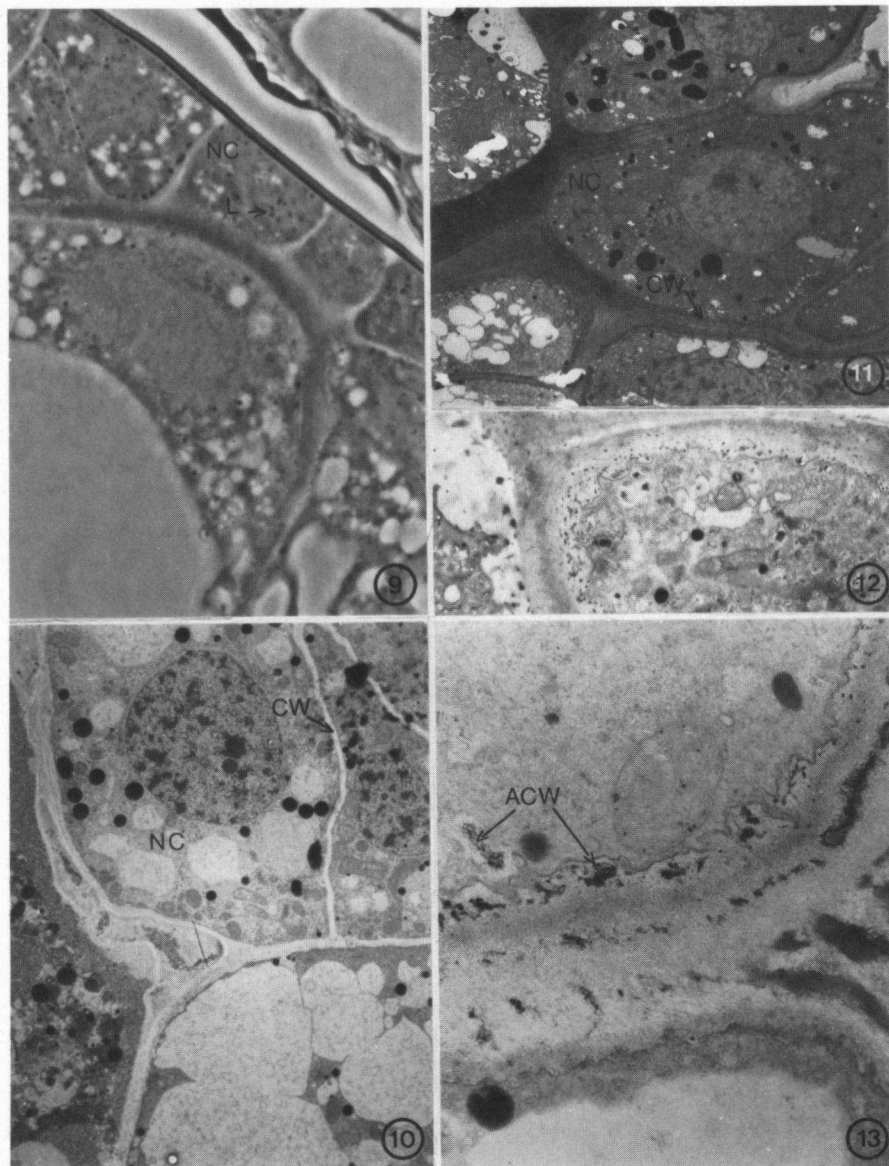
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Plate III. Light (9) and electron (10–13) micrographs of the early (9–10) and late (10–13) development of neck-cells in not-pollinated ovules of *Metasequoia*.

Figs. 9–10. Young neck-cells (NC) with rather thin cell walls (CW) (0.5 μm) and globular lipids (L). × 1700, × 2800, respectively.

Fig. 11. Thickening of the cell walls (CW) of the neck cells (NC) at the time the egg cells will mature; after PATAg staining. × 1,900.

Fig. 12. Additional "cell wall" (ACW) material is excreted by means of large vesicles containing PATAg positive substances. × 1,900.

Fig. 13. Detail of onset of PATAg positive material excretion (ACW) by means of vesicles. × 19,000.
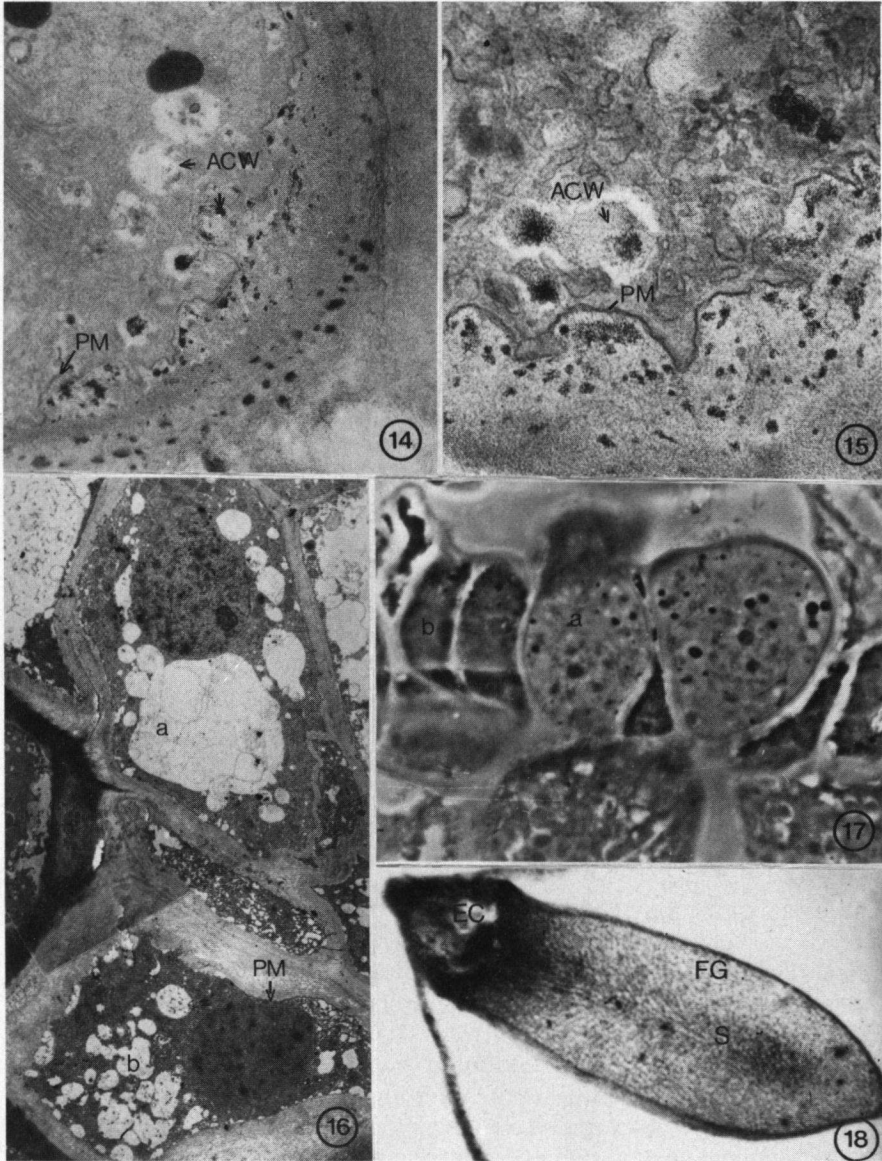
Plate IV. The neck-cells at the time that oogenesis is completed in not-pollinated ovules of *Metase-quoia.*

Figs. 14–15. Successive stages during secretion (ACW) of PATAg positive material, × 19,000, × 25,000, respectively.

Fig. 16. Retraction of the plasmalemma (PM) as a result of secretion and subsequent degeneration of the neck-cell cytoplasm (b). × 2,200.

Fig. 17. Light micrograph with the asynchronous stages of development of the neck-cells; a. relatively young; b. older and d. generating neck-cell. × 1,700.

Fig. 18. Light micrograph of starch (S) accumulation axially in the mature female gametophyte (FG). The egg cells (EC) are completely degenerated. × 40.

3.2. Development of the neck-cell complex

In the young central cell stage the neck-cells are characterized by their dispersed and large quantity of globular lipids (*figs. 9, 10*). This lipid is transported towards the cell walls, suggesting a secretion mechanism.

The first step in development of the neck-cells coincides with the growth of the central cell. During the first phase the wall thickness measures 0.5 μm. The cytoplasm is rich in small vacuoles, lipid droplets, mitochondria and leucoplasts and the nucleus represents the structures as a low differentiated parenchymous cell (*fig. 10*).

At the time of central cell cytoplasmic synthesis and during egg cell maturation a rapid structural change occurs in the neck-cells. The vacuoles are reduced in volume, the cytoplasm gets denser and is enriched with ribosomes. Characteristic for the last step in development is the considerable structural specific thickening of the cell wall (*fig. 11*). Together with cell wall thickening a new cell wall material heavyly stained by PATAg is found just between plasmalemma and the existing cell wall (*fig. 12*). This mass of polysaccharides is produced by a large activity of the golgi apparatus inside the cytoplasm (*fig. 14*). The dictyosomes issue large quantities of polysaccharides containing vesicles. These fuse, giving rise to large vesicles that are excreted into the periplasmic cavity (*figs. 13, 14, 15*). This phase of secretion results in a progressive retraction of the plasmalemma (*fig. 16*).

At time of the mature archegonia, secretion of polysaccharides still occurs in the already degenerating cytoplasm of the neck-cells. The ribosomes are no longer detectable and a successive disorganization of plastids and mitochondria is observed (*figs. 16, 17*). The desintegration of the neck-cells is asynchronous, disordered and intact neck-cells are found side by side (*fig. 17*).

4. DISCUSSION

The female gametophyte of *Metasequoia* has been studied in the scope of sexual reproduction only by light microscopy by WANG & CHIEN (1964). In their material male and female gametophytes coexist over three months within the ovules. Despite some time scale differences due to the climate our observations agree in all details with the descriptions of WANG & CHIEN (1964) for the female gametophyte development; that is, duration of oogenesis, structure and position of archegonial complexes, sequence of events in oogenesis, structure and function of the neck-cell complex and percentage of additional lateral complexes. Our results demonstrate a normal and complete female gametophyte development in total absence of any male gametophytic tissue on the tree, on the cones and in the ovules. The male gametophytic tissue thus cannot exercise any influence on the female gametophyte and the female gametophyte must be considered as basically autonomous.

In members of *Abies, Larix, Pinus, Pseudotsuga, Thuja* and *Tsuga* (see OWENS et al. 1981b) mature female gametophytes were observed in the absence of pollen in the integument or pollen tubes in the nucellar tissue. All species did show

a normal sexual reproduction in that all trees investigated developed male strobi-li, shed their pollen, did receive pollen in cones, but not all ovules did get pollen. The influence of the male gametophyte on the female one is possible at any stage of its development. In these species it is very difficult to prove basic auton-omy of the female gametophyte because the male gametophyte tissue has to be ruled out even on the level of initiation of its development. On exception has been found in the Himalayas, where an only female flowering *Abies pindrow* developed a para-normal oogenesis (DOGRA 1966). In dioecious *Ginkgo* and *Ce-phalotaxus* the absence of pollen inevitably leads to abortion of all ovules at an early stage of development (FAVRE-DUCHARTRE 1957, 1958) indicating the female gametophyte to be dependent on the presence of the male gametophyte.

Probably the development of purely female cones only is not restricted to The Netherlands and France as in the whole of Western Europe only non-viable seed production is found (BELDER & WIJNANDS 1979). Recently in the USA male strobili were found, resulting in the production of viable seeds (KUSER 1982). It is questioned whether the basic autonomy of the female gametophyte will be lost when male strobili will appear on a female tree and pollination occurs.

The ultrastructural organization and cytoplasmic characteristics of the egg cells of numerous representatives of Gymnosperms are well known today (CHES-NOY 1971, 1973, 1977; GIANORDOLI 1978; SINGH 1978): We observed in *Metase-quoia* structures as described for many egg cells of Gymnosperms, e.g. storage of membrane bound proteins, accumulation and connection of mitochondria (CHESNOY 1971; GIANORDOLI 1978).

The cytoplasmic development of the neck-cells and the specific cell wall devel-opment in non-pollinated *Metasequoia* ovules is comparable to that in other Gymnosperms investigated on this peculiar point (CHESNOY 1971; GIANORDOLI 1978). The polysaccharide secretion of the neck-cells in *Metasequoia* is compara-ble to the observations made in *Biota orientalis* (CHESNOY 1971, 1977), *Sciadopy-tis verticellata* and *Cephalotaxus drupacea* (GIANORDOLI 1978). In the last men-tioned two species an ultrastructural and cytochemical investigation showed that the polysaccharide secretion, preceding the fertilization, was exclusively com-posed of pectins that assure the dissociation of the neck-cells at time of penetra-tion of the pollen tube (CHESNOY 1971, 1977; GIANORDOLI 1978). This prepara-tion of the neck-cells just before fertilization is thus autonomic and not regulated by pollen tube arrival in *Metasequoia*.

The selective autolysis of the female egg cell cytoplasm in non-pollinated *Metase-quoia* corroborates the hypothesis (GIANORDOLI 1978) stated for Gymnosperms after cyto-enzymological studies. The enzymes necessary for the lysis of the egg cell cytoplasm are synthesized by the egg cell itself and are not carried by the pollen tube. The experiments on *Juniperus communis* pollen cultured in vitro are not only indicative for basic autonomy of the female gametophyte, but also for a regulating mechanism on male gametophyte development (DUHOUX & CHESNOY 1978; DUHOUX & PHAMTHI 1980).

In Sequoia (LOOBY & DOYLE 1937) the pollen tube is considered to be inductive of the development and positioning of archegonia. In the neighbourhood of

the sperm cells the archegonia are more advanced in their development and the number of archegonial complexes corresponds to the number of pollen tubes penetrating laterally between nucellar tissue and female gametophyte. In *Metasequoia* lateral complexes occur and not all archegonia and neck-cells are synchronous in development. This could mean that also for *Sequoia* the archegonia are guiding the pollen tubes during growth and the positioning of the sperm cells as well.

In *Pinus* unpollinated ovules abort (SARVAS 1962). Differences in enzym activity (KORUMTAK 1976) or gibberelins (KAMIENSKA & PHARIS 1975) and auxins (SWEET & LEWIS 1969) were found. However, general agreement does still exist about pollen and pollen tube influence on ovule development in *Pinus* (MCWILLIAM 1959 PLYM-FORSHELL 1974; SARVAS 1962). A comparative determination of growth substances from pollinated and non-pollinated ovules could provide new evidence about the coexistence and development of male and female gametophytes in Gymnosperms.

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