ONTOGENY AND STRUCTURE OF ARCHEGONIUM IN *CERATOPTERIS THALICTROIDES* (L.) BRONGN.[1]

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Abstract: A mature archegonium of *Ceratopteris thalictroides* (L.) Brongn. consists of a short neck with three to five (typically four) tiers of cells curved backward away from the notch, an enlarged venter surrounded by a layer of sterite jacket cells and an axial row of a binucleate neck canal cell, a ventral canal cell and an egg. The archegonium is derived from the archegonial inital together with its surrounding prothallial cells rather than just from a single initial as commonly thought. Furthermore it was observed that the archegonial initial also gives rise to the prothallial tissue which is not related to archegonium.

INTRODUCTION

The gametophytes of pteridophytes are easily observed under the microscope without sectioning the material because they are small and simple in structure, consisting of only one to several layers of cells in thickness. Therefore numerous observations have been made on them, especially on their morphology. On the contrary, only few works provide information regarding the ontogeny of the sex organs of the ferns (Nishida and Sakuma, 1961). The present work is concerned with the ontogeny and structure of the archegonium in *Ceratopteris thalictroides* which is an aquatic or semi-aquatic leptosporangiate fern belonging to the family Parkeriaceae.

MATERIALS AND METHODS

The prothallia of *Ceratopteris thalictroides.* (L.) Brongn. from Taiwan were grown from spores in $\frac{1}{2}$ Hoagland's solution in Petri dishes under continuous white light (Chiang and Chiang, 1967), and fixed in Craf III (Sass, 1958). After dehydration with a tertiary butanol series, the material was embedded in paraffin, cut at 8 μ in series and stained with safranin and fast green (Johansen, 1940).

OBSERVATIONS AND DISCUSSION

Ontogeny.

The main portion of the archegonium of *Ceratopteris thalictroides* arises from a

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single cell, known as an archegonial initial, in the gametophyte (Figs. 1A, 1B). It occurs usually close to the apical notch of the prothallium in a region which is one cell in thickness. At its early stage, this initial is difficult to be distinguished from the other prothallial cells. Later, however, it grows slightly larger than its neighbors and becomes wedge-shaped in longitudinal section. The nucleus of this cell is also larger. At the start, the archegonial initial divides periclinally into a primary cover cell and an inner cell. This is followed by a similar division of the inner cell, forming a central cell and a basal cell (Figs. 1B, 1C, 2B, 2C). The central cell lies between the primary cover cell and the basal cell, making the characteristic tier of three cells (Figs. 1C, 2C). Next ot follow is the formation of the four quadrately arranged neck initials which are produced by two anticlinal divisions occurring in the primary cover cell. By several anticlinal divisions these neck initials give rise to a neck composed of typically four rows of three to four tiers of cells (Figs. 1C–1G, 2C–2G, 3). In the strongly curved neck, the adaxial side has three tiers of cells whereas the abaxial side is five cells in height (Figs. 1G, 2G). As the neck is developing the central cell divides periclinally into a primary canal cell and a primary ventral cell (Figs. 1E. 2E). As seen in longitudinal section, the primary canal cell is lenticular in shape whereas the primary ventral cell is tabular in shape. While the neck is in the two-tier stage, the the primary ventral cell divides periclinally into the ventral canal cell and the egg cell (Figs. 1F, 2F). As the neck increases its length by one or more divisions and also by cell enlargement, the primary canal cell elongates vertically and pushes out into the neck (Figs. 1F, 2F). Its nucleus divides into two, but this is not followed by cell wall formation, resulting in a binucleate neck canal cell (Fig. 4A). At maturity, the neck canal cell and the ventral canal cell shrink and gradually dissolve or disappear to become a duct or the neck canal which is the pathway of the spermatozoids (Figs. 1G, 2G, 4A, 4D). The basal cell gives rise to a part of the sterile jacket layer surrounding the venter and also to become a part of the ordinary prothallial tissue (Figs. 2E, 2F, 2G, 3).

Venter.

Traditionally, the sterile jacket layer surrounding the venter (cavity containing the egg) in the archegonium of ferns is referred to as a part of the archegonium although this layer is known to be composed of ordinary gametophytic cells not derived from the archegonial initial (Cronquist, 1961). The archegonium is the female sex organ in bryophytes, pteridophytes and gymnosperms and is thought to have arisen from a single superficial cell of the gametophyte, the archegonial initial (Stokey, 1961). In *C. thalictroides*, it was found that the first cell of the jacket layer surrounding the venter is produced by a periclinal division occurring in the basal cell which is derived from the archegonial initial (Fig. 2F). Another cell produced by this divison becomes an ordinary prothallial cell which does not

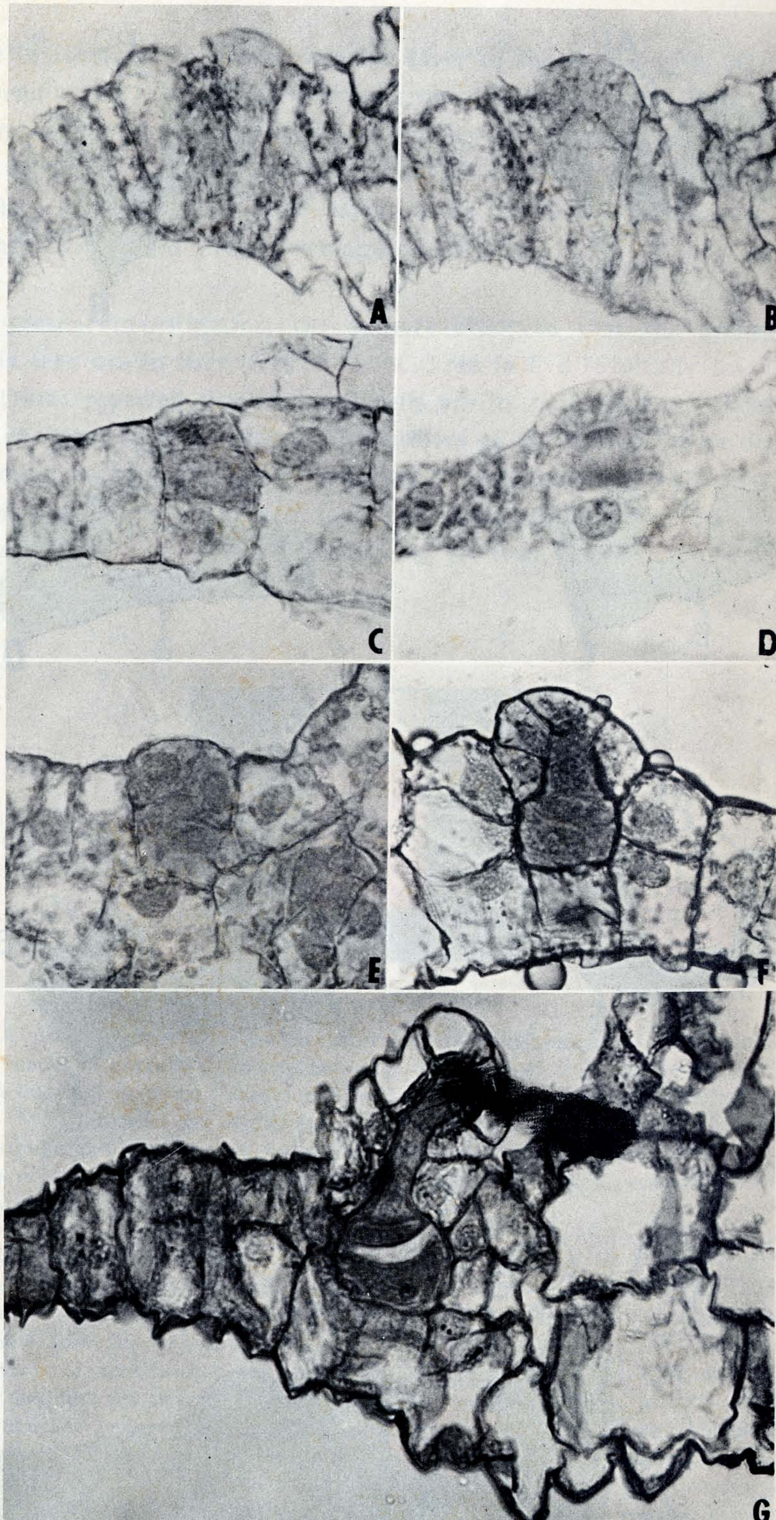


Fig. 1. Microphotographs showing successive stages in the development of an archegonium. ×430. For details see Fig. 2.

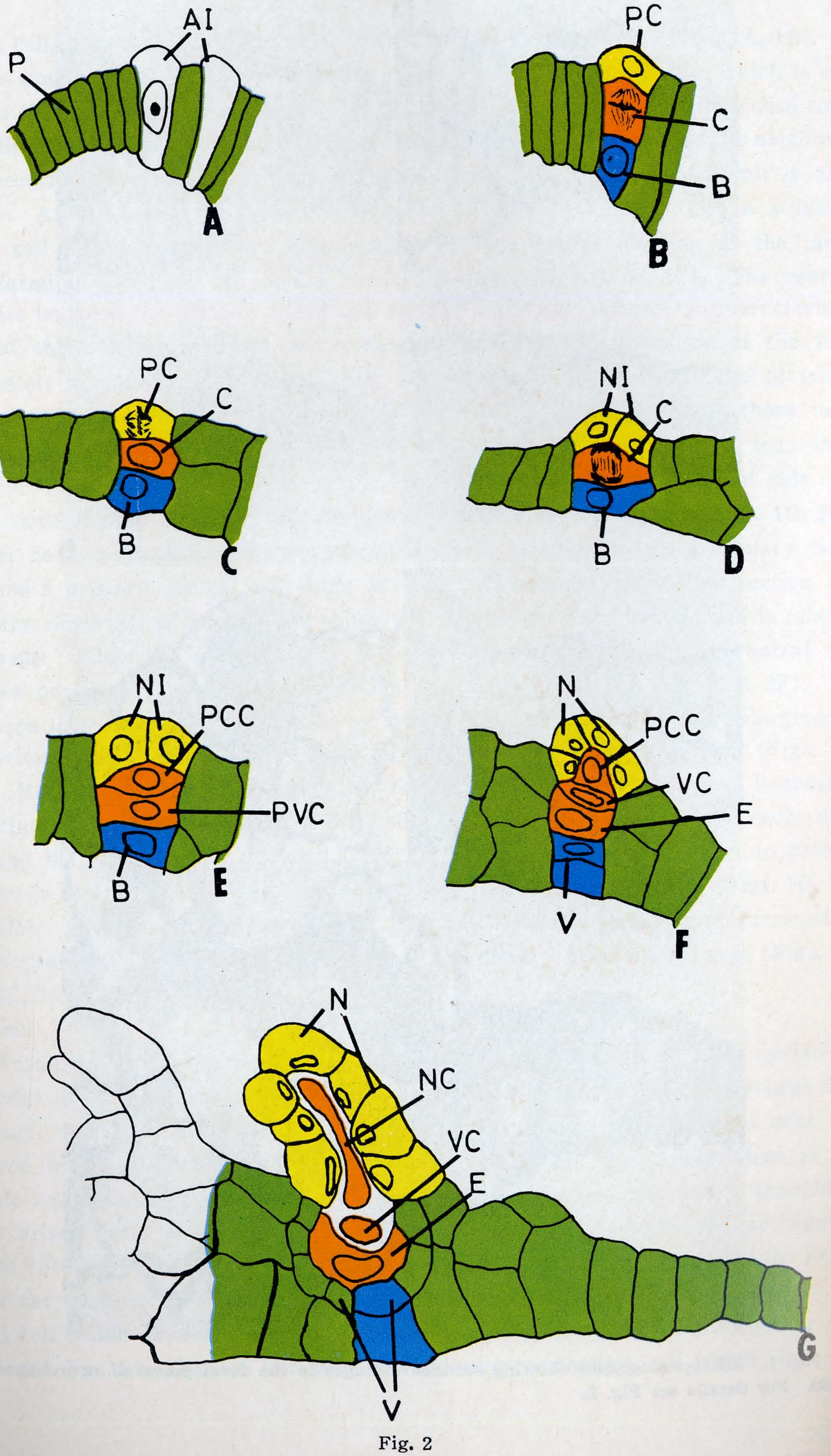


Fig. 2

participate in forming a continuous jacket layer of the venter (Fig. 3G). All other cells surrounding the venter are derived from the prothallium (Figs. 2G, 3). The jacket layer surrounding the venter, is a bladder-like layer consisting of relatively small, flat cells, in which the egg cell and the ventral canal cell are developed. This nutritive and protective jacket layer of the venter is meristematic. It grows as the embryo grows and always remains uniseriate.

Neck.

In the present investigation, the authors observed that the archegonial neck cells of this fern are in four tiers of about three to five (typically four) cells each. Mahabale (1948) reported that the neck cells are in four tiers of about five cells each in *C. thalictroides* from India. The authors of the present paper imagine that the archegonial neck contributes by isolating the egg farther from the outside world. The species with longer necks are therefore suggested to be more protected from sudden changes of environment, pathogens, or from harmful agents. Since the

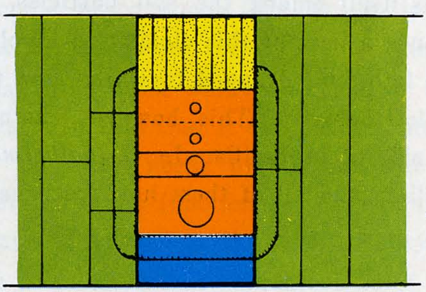


Fig. 3. A diagrammatic illustration showing the development of archegonium. Circles indicate the nuclei of potential gametes and egg. The largest circle is the nucleus of the egg cell. Broken line indicates the undeveloped wall of the neck canal cell. Yellow cells consist of the neck. (⁄⁄⁄⁄) indicates the sterile jacket cell surrounding the venter. The cells indicated by yellow, orange and blue are derived from archegonial initial, whereas those cells indicated by green are prothallial cells. Note that both archegonal inital and prothallial cells contribute to the formation of venter.

Fig. 2. Camera lucida drawings showing successive stages in the development of an archegonium. ×340. A, occurrence of archegonial initial in an uniseriate prothallium; B, a characteristic tier of three cells, note that the central cell is dividing periclinally; C, a tier of three cells, note that the primary cover cell is dividing anticlinary; D, formation of neck initial, note that the neck initials are formed while the central cell is dividing; E, formation of primary canal cell and primary ventral cell; F, formation of egg, ventral canal cell, the first ventral cell and neck cells; G, a nearly mature archegonium, note the shrinkage of neck canal cell and ventral canal cell. AI, archegonial initial; B, basal cell; C, central cell; E, egg cell; N, neck; NC, neck canal cell; NI, neck initial; P, prothallium; PC, primary cover cell; PCC, primary canal cell; PVC, primary ventral cell; V, venter; VC, ventral canal cell. Four different colors are used in these figures in order to make it easier to trace the lineage of cells.

species with shorter necks and those with longer necks survive under the same environmental condition, it is obvious that those with shorter species are more adaptable to the environmentent than those with longer necks. It is thought therefore that those with longer necks are primitive, whereas those with shorter are derived. It is known that the archegonium with relatively long, straight neck occurs in the primitive ferns, whereas those with shorter, strongly curved necks are found in the derived ferns (Foster and Gifford, 1959). One of the most primitive familes of ferns of sporophytic characters is the Gleicheniaceae, which has long archegonial necks ordinarily with 7 to 14 cells in a tier (Stokey, 1950). The present authors think that that the species with a shorter curved neck is more favorable for fertilization. It needs only a film of water on the surface of the prothallium for fertilization. The species with a straight long neck, on the other hand, needs more water for fertilization.

Most ferns have archegonial necks bent backward directed away from the notch. Coulter (1959) thinks that this is probably the inevitable result of obvious mechanical forces. He believes that the neck of a young female sex organ protrudes straight down and imbeds itself among the soil particles. Since the gametophyte is still elongating, and since a part of this elongation involves the cells around this particular sex organ, the latter is "dragged forward", and the imbedded tip of its still elongating neck is "left behind". This, however, can not explain the archegonia that grow on the dorsal side of prothallia. In *C. thalictroides*, archegonia occur on both sides of the prothallium and all of them have recurved necks which are bent backward. (Fig. 4B).

Neck canal cell.

It can be seen from Fig. 3 that the growth of the neck cells is faster than that of any of the other cells in the formation of archegonium. The neck canal cell is drawn out rapidly by this fast growth of the jacket neck cells. This mechanical rapid stretching of the neck canal cell in a lengthwise direction may be unfavorable for its wall formation, resulting in a coenocytic cell. In the present study, two nuclei are found occurring in the neck canal cell of *C. thalictroides* from Taiwan. In the neck canal cell of the same species from its natural habitat in India, Mahabale (1948) observd three nuclei. Nishida and Sakuma (1961), on the other hand, reported four unclei from Japanese species. Obviously the number of nuclei in the neck canal cell is quite variable. Nishida and Sakuma (1961) state that the aberrant archegonia with multinucleate neck canal cell or with two neck canal cells, or sometimes with two ventral canal cells, are not rare but common in so-called primitive ferns, except Ophioglossales. In the present work, the authors observed that the ventral canal cell is formed before the neck canal cell. Nishida and Sakuma (1961) showed that in the primitive ferns the ventral canal cell is completed before the neck canal cell. Although the number of nuclei in the neck canal cell is variable, the number of the neck canal cell in the archegonium is constant. The archegonium of *C. thalictroides* only has of a single neck canal cell in its neck.

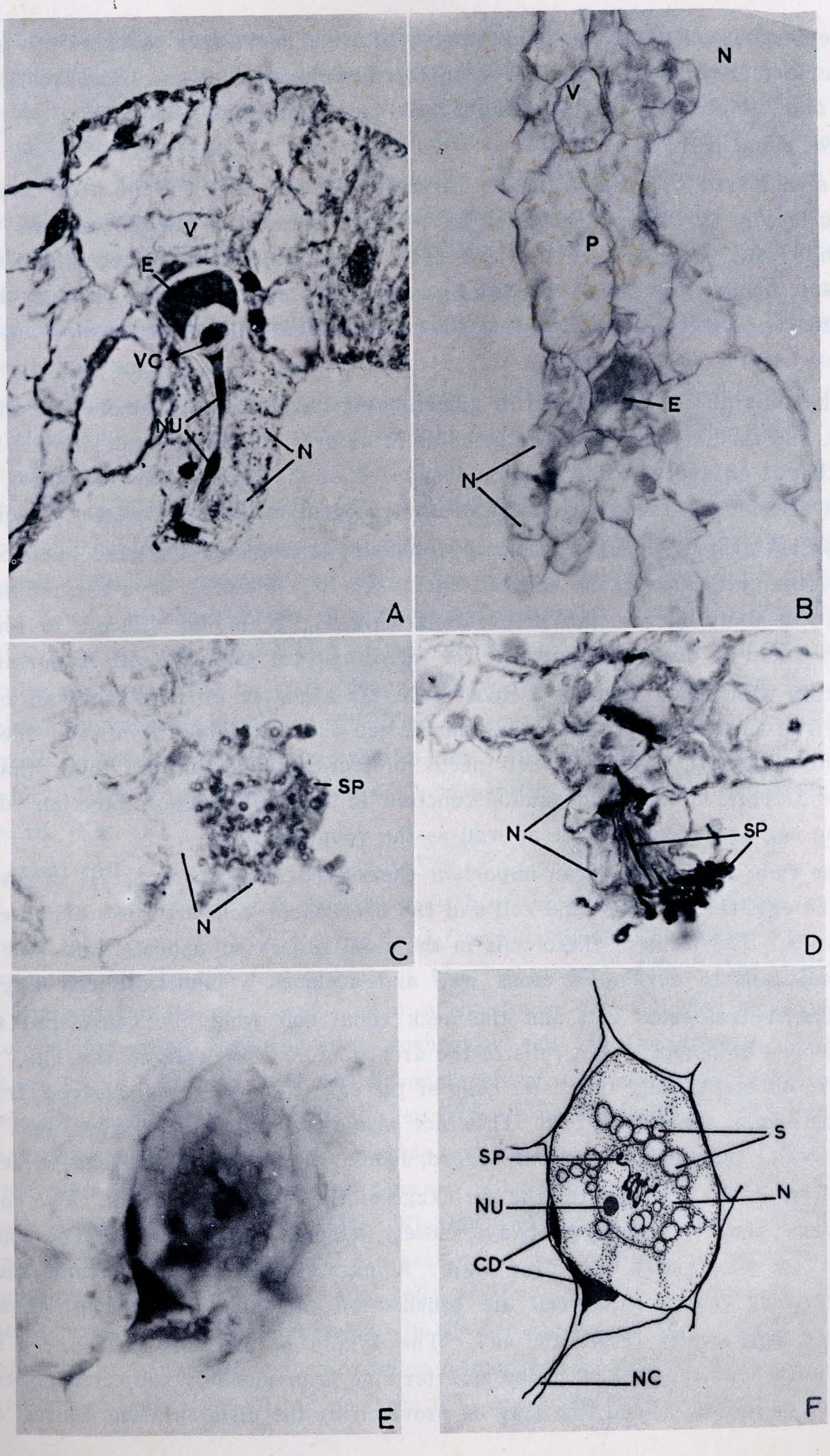
Ventral canal cell.

As compared with the variablity of the number of nuclei in the neck canal cell, the nucleus in the ventral canal cell as well as in the egg is always constant. They are unincleate. The number of these cells in an individual archegonium is also constant; one archegonium consists of one ventral canal cell and one egg in the venter.

Egg.

The cells of the prothallium or gametophyte have a haploid number or chromosomes, and theoretically they are gametes in nature. They however, have a thick wall that is unfavorable for gametic fusion. It seems to the authors that a series of cell divisions in the development of an archegonium is the process by which a thin walled gamete or egg with dense cytoplasm is formed. The gradual concentration of the cytoplasm in the egg cell during the development of archegonium can be seen in many microscopic preparations (Fig. 1). It is not difficult to imagine that it would be of great advantage for fertilization if the egg cell is surrounded by a thin wall rather than by a thick wall. As a matter of fact the wall of the egg cell of *C. thalictroidcs* is so thin that it can only be stained 'faintly in the preparation (Figs. 1G, 4A). Such cell needs protection. The authors think that the neck and venter of the archegonium function as the wall in the protection of this delicate egg and of the zygote as well as the young embryo.

The thin wall nature is an important characteristic of the egg cell or gamete. Like the egg, the ventral canal cell and the neck canal cell are also delimited by thin walls. The walls of these cells in the axial row is so delicate that the neck canal cell fails to develop a cross wall and becomes a binucleate cell (Fig 4A). Thus the ventral canal cell and the neck canal cell would be considered to be undeveloped or potential egg cells in the archegonium. As well as the egg, these cells are ontogenetically the sister cells of the egg, *i. e.*, they are derived from a common origin, the central cell. This fact also may support the view that they are potential gametes (Foster and Gifford, 1959). A crescent-shaped egg is left in the bottom of the venter after the shrinkage of the ventral canal cell. The convex spherical surface of the egg always closely adheres to the venter. The concave side of the egg has a thin free wall. A mass of excess spermatozoids and the disintegrating ventral canal cell are crushed on this side of the egg when the fertilized egg swells (Figs. 4E, 4F). The zygote is therefore surrounded by a non-uniform environment, *i. e.*, the anterior side is presumably subjected under the influence of nucleic acid which may be provided by the disintegrating ventral canal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cell and excess spermatozoids, whereas the posterior side of the egg is free from the influence of nucleic acid. This unequal environment is presumably one of the important factors affecting the differentiation of the embryo. M. Niu (1956) suggested the possible influence of nucleic acid on the differentiation of an animal embryo.

Fertilization.

The spermatozoids are coiled in the mature antheridium. They are usually held in large numbers in the slime before the mouth of the archegonium (Fig. 4C). This is also observed in *Onocla* (Shaw 1898). The spermatozoids at the tip of the neck are also coiled, usually having about two turns. In the entrance of the neck canal, the spermatozoid becomes elongate. It again coils after entering the venter. Only one spermatozoid is permitted to enter the cytoplasm of the egg. After the spermatozoid has entered the cytoplasm of the egg, the egg cell becomes turgid and swells, pushing the excess spermatoroids to the base of the neck. At the time of fertilization, the nucleus of the egg is circular and centrally located. As in other ferns (Shaw, 1898, Atkinson, 1943), the fusion of two sexual nuclei occurs, when the egg nucleus is in the resting condition. Before entering the nucleus of the egg, the spermatozoid becomes much elongated though it is still somewhat coiled (Fig. 4F). As can be seen in Figs. 4E, 4F, many spherical bodies of varying sizes are found occurring close to the periphery of the nucleus of the egg cell during fertilization.

LITERATURE CITED

ATKINSON, L. R. 1943. A Preliminary report of fertilization in *Marsilea vestita.* Amer. J. Bot. **30**: 401-404.

CHIANG, Y. L. and S. H. CHIANG. 1967. Influence of shading and Gibberellic acid on growth and sex of the prothallia of *Ceratopteris thalictroides.* Taiwania **13**: 179-193.

COULTER, M. C. 1959. The story of the plant kingdom. The Univ. of Chicago Press, Chicago.

CRONQUIST, A. 1961. Introductory botany. Harper & Row Publishers, New York.

FOSTER, A. S. & E. M. GIFFORD. 1959. Comparative morphology of vascular plants. W. H. Freeman and Co. San Francisco.

JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill Book Co. Inc., New York.

MAHABALÉ, T. S. 1498. Prothallia of *Ceratopteris thalictroides.* Bot. Gaz. **109**: 349-354.

NISHIDA, M. and T. SAKUMA. 1961. Abnormalities in archegonia of the fern prothallia and its bearing to systematics. Journ. Jap. Bot. **36**: 142-115.

Fig. 4. Photographs and an illustration showing the structure of archegonium, the entrance of spermatozoid through the neck canal and fertilization. A, an archegonium with a binucleate neck canal cell, a ventral canal cell and a crescent-shaped egg. ×300; B, a section from a prothallium, showing two archegonia on both upper and lower sides. ×300; C, a photograph showing numerous coiled spermatozoids crowded on the neck. ×300; D, a section through the neck canal, showing an uncoiled spermatozoid in the canal and many spermatozoids crowded at the entrance of the neck canal. ×300; E and F, a photograph and an illustration showing fertilization. ×980. Key to labeling. CD, disintegrating canal cells and crushed excess spermatozoids; E, egg; N, nucleus; NC, neck canal; NU, nucleolus; P, prothallium; S, spherical body; SP, spermatozoid; V, venter; VC, ventral canl cell.

NIU, M. C. 1956. New approaches to the problem of embryonic induction. *In*. An introduction to embryology (Rudnick. D. ed.): Cellular mechanisms in differentiation and growth. Princeton University Press, Princeton.

SASS, J. E., 1958. Botanical microtechnique. Iowa State College Press, Ames, Iowa.

SHAW, W. R. 1898. The fertilization of Onoclea. Annals Botany **12**: 261-285.

STOKEY, A. G. 1950. The gametophyte of the Gleicheniaceae. Bull. Torry Bot Club **77**: 322-339.

STOKEY, A. G. 1961. Archegonium. In the encyclopedia of the biological sciences (Gray, P. ed.): 56-56. Reinhold Publishing Corporation, New York.