Participation of Male Cytoplasm During Gamete Fusion in an Angiosperm, *Plumbago zeylanica*

Abstract. *The embryo sac of* Plumbago, *lacking synergids, reveals an alternative course for pollen tube growth in angiosperms and provides ultrastructural evidence for transmission of sperm cytoplasm into the zygote and endosperm. Study of such evolutionarily reduced female gametophytes may aid in elucidating the structural basis for genetic transmission of plastids and mitochondria in flowering plants.*

Plumbago zeylanica possesses one of the most highly reduced female gametophytes among angiosperms. The female gametophyte, or embryo sac, of this plant consists of the egg, the central cell, and three accessory cells (one at the chalazal end and two lateral cells). Synergids, which are assumed to receive the pollen tube in all flowering plants outside of the tribe Plumbagineae, are absent in embryo sacs of *Ceratostigma* (*1*), *Dyerophytum* (*2*), *Plumbagella* (*3*), and *Plumbago* (*1*, *4*-*6*). However, several structural modifications of the egg of *Plumbago* suggest that the egg has assumed some of the physiological functions of synergids (*6*). These modifications include the development of extensive wall ingrowths (the so-called filiform apparatus) at the micropylar end of the embryo sac and greater apparent activity in mitochondria and dictyosomes. These adaptations are consonant with the function of the egg as a transfer cell (*6*, *7*).

I have discovered two aspects of the reproductive anatomy and fertilization of *P. zeylanica* that are of embryological interest, namely (i) the path of the pollen tube in the embryo sac of this synergidless angiosperm and (ii) the fate of the male cytoplasm upon gametic fusion. Despite the brief time between syngamy and fertilization in this plant, I was able to reconstruct events related to gamete fusion that occur before fertilization.

A number of gynoecial modifications make *P. zeylanica* suitable for such a study. Among these are the uniovulate ovary, closed transmitting tissue, obturator, and upwardly directed (circintropous) ovule (*1*, *4*, *5*). This may represent the most highly controlled system possible for the study of fertilization in vivo, since only the most rapidly growing pollen tube is successful, and this maximal growth rate can be reliably predicted. Under these controlled conditions, timed collections of hand-pollinated flowers were made and the ovaries were processed in accordance with published procedures (*6*).

The pollen tube enters the ovule through the micropyle and penetrates the embryo sac by displacing several nucellar cells, which then degenerate. As the pollen tube reaches the ovule, the two male gametes and tube nucleus approach the growing tip. The pollen tube enters the filiform apparatus at the base of the egg, penetrates the egg wall, and discharges the male gametes and tube nucleus between the egg and central cell (Fig. 1). (I did not observe the direct penetration of any cell during pollen tube growth or gamete discharge.) The plasma membranes of the egg and central cell remain intact and appear to exclude the electron-dense discharged pollen cytoplasm.

The tube nucleus is closely associated with the two sperm cells, which travel in tandem within the pollen tube (*8*). Apparently this linear array is maintained at discharge. Near the time of gamete fusion, the discharged tube nucleus is 15 to 25 μm from the pollen tube aperture. Sperm cells may fuse with the egg or central cell as close as 3 μm to the aper-

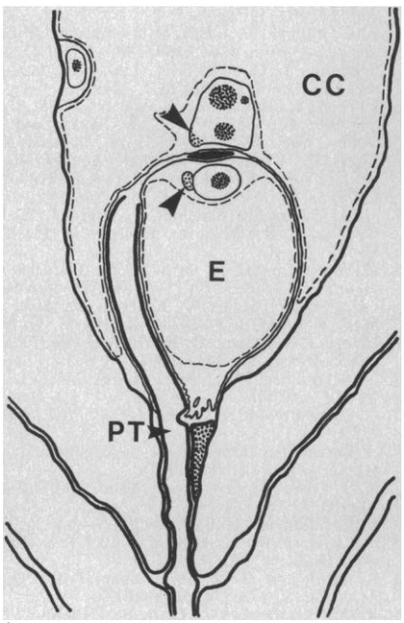

Fig. 1 (left). Diagram of a fertilized embryo sac of *P. zeylanica*. Arrowheads indicate the two sperm nuclei in different stages of fusion. The tube nucleus remains between the egg and the central cell (often near the summit of the egg). A typical lateral cell is shown in the upper left corner. Abbreviations: *CC*, central cell; *E*, egg; *PT*, pollen tube.

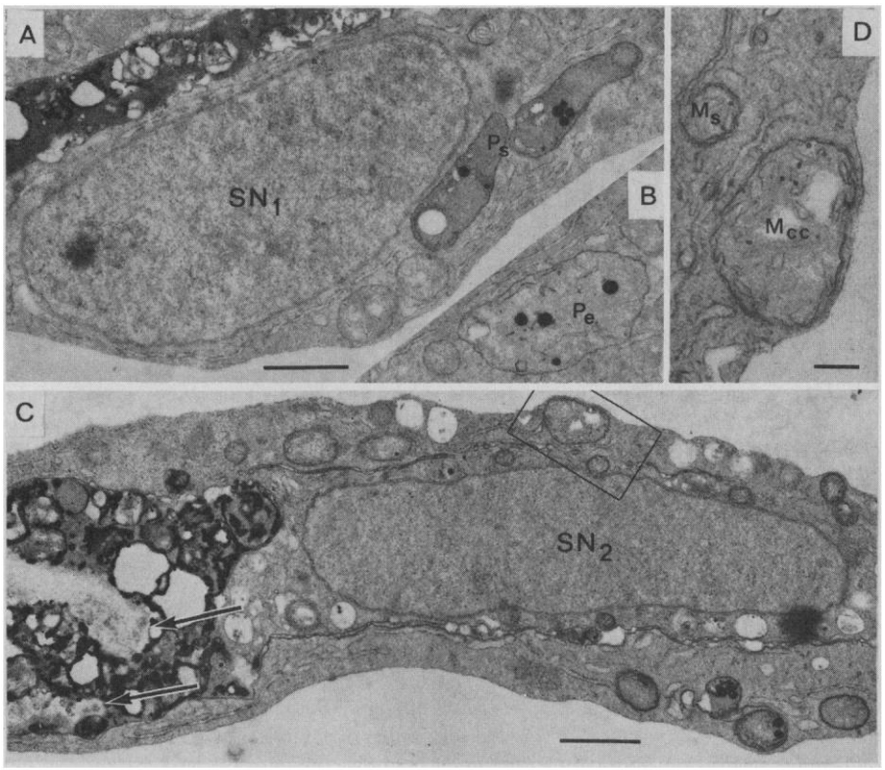

Fig. 2 (right). Embryo sac of *P. zeylanica* shortly after gamete fusion. (A) Sperm nucleus (SN_1) and two sperm plastids (P_s) in the egg cytoplasm (scale bar, 1 μm). (B) Typical egg plastid (P_e) at the same magnification as (A). (C) Second sperm nucleus (SN_2) shortly after fusion with the central cell. The arrows indicate both halves of the ruptured pollen tube wall (scale bar, 1 μm). (D) Enlargement from (C) showing sperm mitochondrion (M_s) at the periphery of the sperm nucleus and a nearby central cell mitochondrion (M_{cc}) (scale bar, 0.2 μm).

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ture (Fig. 2C). Eventually, one sperm nucleus fuses with that of the egg and the other fuses with the central cell nucleus (Fig. 1), confirming Dahlgren's report (*4*) of double fertilization without the involvement of synergids.

Male cytoplasmic organelles can be identified in both the egg and central cell immediately after entry of the sperm nuclei. Sperm plastids are distinguished by their smaller size (0.5 to 0.7 μm wide), elongate shape (up to 2.2 μm long in section), inflated internal lamellae, and relatively electron-dense stromata (*9*). Such plastids are seen in Fig. 2A at the periphery of the sperm nucleus that entered the egg. Egg plastids are larger (about 1.2 by 2.0 μm), circular to elliptical in section, less electron-dense (Fig. 2B), and occasionally contain starch grains. Mitochondria of male origin can be distinguished from those of the female gametophyte by their size and cellular location (Fig. 2, C and D). Sperm mitochondria next to the sperm nucleus are usually circular in section and 0.2 to 0.3 μm wide. Mitochondria of the female gametophyte are elliptical and 0.5 to 0.8 μm wide. The sperm organelles appear normal in their ultrastructure and presumably are capable of replication under the right conditions. Meyer and Stubbe (*10*) reported the occurrence of ultrastructurally identifiable sperm plastids in zygotes of *Oenothera erythrosepala*. Chlorophyll mutations have been used to identify sperm and egg plastids in this and other species of *Oenothera*, and they are present throughout the life of the plant (*11*).

The presence of extranuclear sperm organelles in the cytoplasm of the egg and central cell supports the view that syngamy in angiosperms is initiated by cellular fusion (*12*). Therefore, both male organelles and nuclei enter the egg and central cell and may contribute to the development of the embryo and endosperm.

The genetic impact of male cytoplasmic inheritance during development on the mature plant depends on whether male organelles remain viable, reproduce, and transmit genetic information within the embryo. The region of the egg where gamete fusion occurs is destined to become part of the suspensor, but it seems unlikely that sperm organelles remain fixed and thus unincorporated in the embryo. Evidence suggests that angiosperms with plastids in the generative cell display biparental patterns of plastome mutation inheritance (*13*). However, full understanding of the impact of male cytoplasmic inheritance during later development in *P. zeylanica* must await research on the inheritance of mitochondrial or chloroplast genes in this plant.

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9. Gametophytic organelles vary somewhat from ovule to ovule, a condition presumably favored by a biparental cytoplasmic inheritance mechanism. Therefore, organelle measurements given in this paragraph were restricted to the embryo sac illustrated in Fig. 2. Only organelle sections with clearly distinguishable membranes (that is, near-median sections) were measured.
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Cytostructural Localization of a Tumor-Associated Antigen

Abstract. *Tumor cell membrane glycoproteins may be involved in the induction of tumor immunity or in the escape of tumors from immunologic defense mechanisms. Forty-four benign and malignant breast lesions were examined for the presence of a carbohydrate precursor antigen (T antigen) of the human blood group system MN. T antigen was demonstrated by means of an immunohistochemical technique to detect tissue binding of peanut agglutinin, a plant lectin, with affinity for T antigen. Malignant breast lesions showed a pattern of T antigen expression different from that of benign breast tissues. A possible role for T antigen in the modulation of the immune response to breast carcinoma is suggested.*

Springer and colleagues have serologically identified an antigen that is present on malignant but not benign breast tissues (*1*). This antigen, termed T antigen, elicits delayed hypersensitivity reactions in patients with breast cancer but not in healthy controls (*2*). In additional studies, antibody present in normal human serums was used with an immunohistological technique to demonstrate that a similar or identical antigen may be detected on malignant but not benign breast epithelium (*3*). We have now further clarified the nature and specificity of this antigen and its relation to neoplastic transformation.

An immunoperoxidase technique was developed to detect binding of a plant lectin with affinity for T antigen to histological sections of breast tissue. This lectin is derived from peanuts (*Arachis hypogaea*) and is termed peanut agglutinin (PNA). The difference in the pattern of expression of T antigen between benign and malignant breast epithelium suggests that it may have a possible role in the induction of the immune response to neoplasia.

Tissues from breast carcinomas and benign breast lesions were selected, according to their recorded pathological diagnosis, from the surgical pathology files

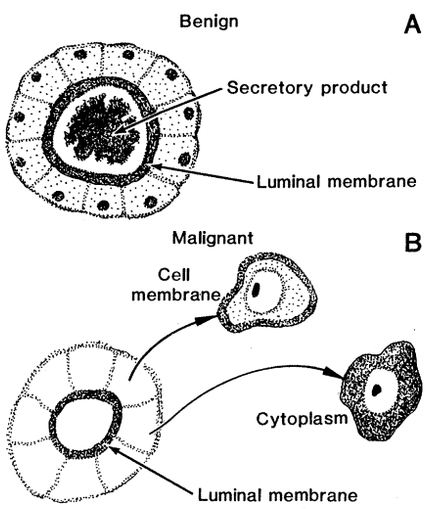

Fig. 1. Pattern of T antigen expression (dark stippled areas) in (A) benign breast tissues and (B) malignant breast tissues. (A) Peanut agglutinin binding occurs along the luminal cytoplasmic membrane with or without binding to intraductal secretory products. (B) Peanut agglutinin binding may occur along the luminal cytoplasmic membrane in the well-differentiated malignancies. Unique to neoplastic cells is the presence of T antigen in a diffuse pattern in the cytoplasm or along the peripheral cell membrane.

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