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Translocation of the generative cell towards the centre of the pollen grain due to changes in the vacuolar system in *Ranunculus repens* L.

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Abstract

On the basis of observations performed under the light microscope on the development of the male gametophyte of *Ranunculus repens* L., the hypothesis is advanced that the translocation of the generative cell from a position adjacent to the wall towards the centre of the pollen grain, associated with change in the shape of this cell, is the result of vacuolization of the generative and vegetative oells and the interaction of these two vacuole systems.

INTRODUCTION

The developing pollen grain of angiosperms is the male gametophyte with two cells, the generative and the vegetative one, formed as the result of a differential mitosis in the microspore, and surrounded by a common sporoderm (pollen grain wall). The generative cell which at first is lenticular and lies applied to the wall, is translocated in the course of further development deeper into the grain and eventually is completely surrounded by the vegetative cell cytoplasm.

This fact is commonly known. However, in spite of the great number of studies on pollen grain development the mechanism of the generative cell translocation has not been yet elucidated.

Studies on the translocation and change of shape of the generative cell are scarse. In the fundamental embryological texts (Maheshwari, 1950; Steffen, 1963) there is neither a description or explanation of this process. Vazart (1958) quotes the opinion of Strasburger (1908) and Hakansson (1924), attributing to the generative cell the ability of autonomic movement, and Kostryukova (1947, 1948) considers the translocation of the generative cell as a trend towards that pollen grain part which is rich in nutrient substances.

The author's own observations on the development of the male gametophyte of *Ranunculus repens* led to the hypothesis that the generative cell translocation in the pollen grain is due to the vacuolization and change of configuration of the vacuolar system of the generative and vegetative cells.

MATERIAL AND METHODS

The anthers were fixed in the modified Navashin fixative CrAF 0.5-1-20 and CrAF 0.4-0.5-20 (Teleżyńska and Teleżyński 1973) during 48 h or 2 weeks, processed and embedded in paraffin, and sectioned at 5 μm. Sections were stained by the Feulgen-fast green method or PAS-reaction, observations and microphotographs were made under a Zeiss-Lumipan light microscope.

OBSERVATIONS AND RESULTS

The young *Ranunculus repens* microspores when released from the tetrads are spherical. The nucleus is centrally located and in the cytoplasm there are small vacuoles. Soon, a central vacuole develops which displaces the nucleus towards the pollen grain wall. In the period preceding the microspore mitosis, the central vacuole fall apart into several smaller ones of various sizes, usually symmetrically located, which keep the nucleus close to the wall. In this position, mitosis and cytokinesis occur (Plate I, photos 1—5).

The two cell of the male gametophyte formed as the result of differential mitosis and cytokinesis, a generative and a vegetative one, differ in their size and shape, in structure and size of the nuclei and in the degree of vacuolization of the cytoplasm.

The newly formed generative cell is lenticular and lies close to the wall, that is a part of its surface adheres to the pollen grain wall (Plate I, photo 6). At first, it is deprived of vacuoles, then undergoes gradual vacuolization. Four not large, rather regularly distributed vacuoles arise in the generative cell (Plate II, photos 1 and 2) which further join in pairs to form two vacuoles symmetrically placed in respect to the generative nucleus (Plate II, photos 3 and 4). These vacuoles shift towards the centre of the generative cell into the cytoplasm area between the nucleus and the pollen grain wall and form one vacuole (Plate II, photo 5). Under its pressure the whole generative cell protrudes into the pollen grain. The generative cell surface gradually becomes detached from the pollen grain wall and its lenticular shape changes to club-like (Plate II, photos 5 and 6). The changes of the vacuolar system in the vegetative cell occurring at this time contribute to the changes in shape and position of the generative cell. The vacuoles of vegetative cell are rather large at the end of mitosis (Plate II, photo 1), but they fall into a number of smaller ones, and new tiny vacuoles are arising at the base of the generative cell (Plate II, photos 5, 6). The pressure exerted by the system of the vegetative cell vacuoles contacting with one another contributes to immerse the generative cell in the cytoplasm of the vegetative one. Finally the generative cell is completely surrounded by the cytoplasm of the latter. This process is illustrated in Fig. 1.

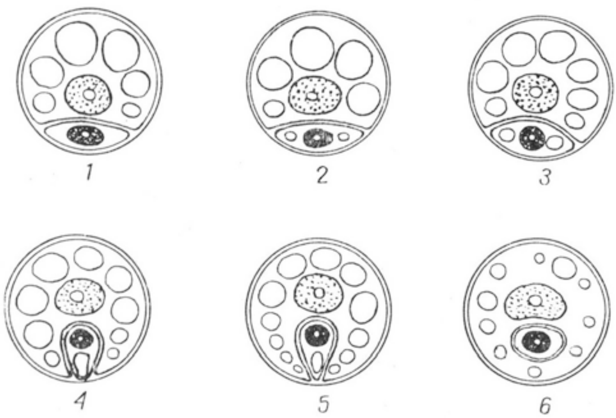


Fig. 1. Translocation of the generative cell towards the centre of the pollen grain as the result of interaction of vacuole systems of two male gametophyte cells.

After shifting into the vegetative cytoplasm, the generative cell lies in the immediate vicinity of the vegetative nucleus in the centre of the pollen grain (Plate III, photos 1, 2, 3). The small vacuoles of the generative cell, visible at this time, rapidly disappear and during maturation of the pollen grain vacuoles are not observed.

The developmental changes observed during maturation of pollen grain of *Ranunculus repens* include changes in the shape of the generative cell and in the shape and structure of its nucleus as well as changes in the shape and structure of the vegetative nucleus. The growth of the grain as a whole is due mainly to an increment in the cytoplasm of the vegetative cell correlated with its devacuolization. The generative cell, at first ellipsoid (Plate III, photos 1, 2), gradually elongates and finally acquires a fusiform shape (Plate IV, photo 2). The generative nucleus is at first spherical, then ellipsoidal, compact in structure, and with a visible nucleolus. In the ripening pollen grain it becomes fusiform and its structure resembles that of an early prophase nucleus (Plate IV, photo 3).

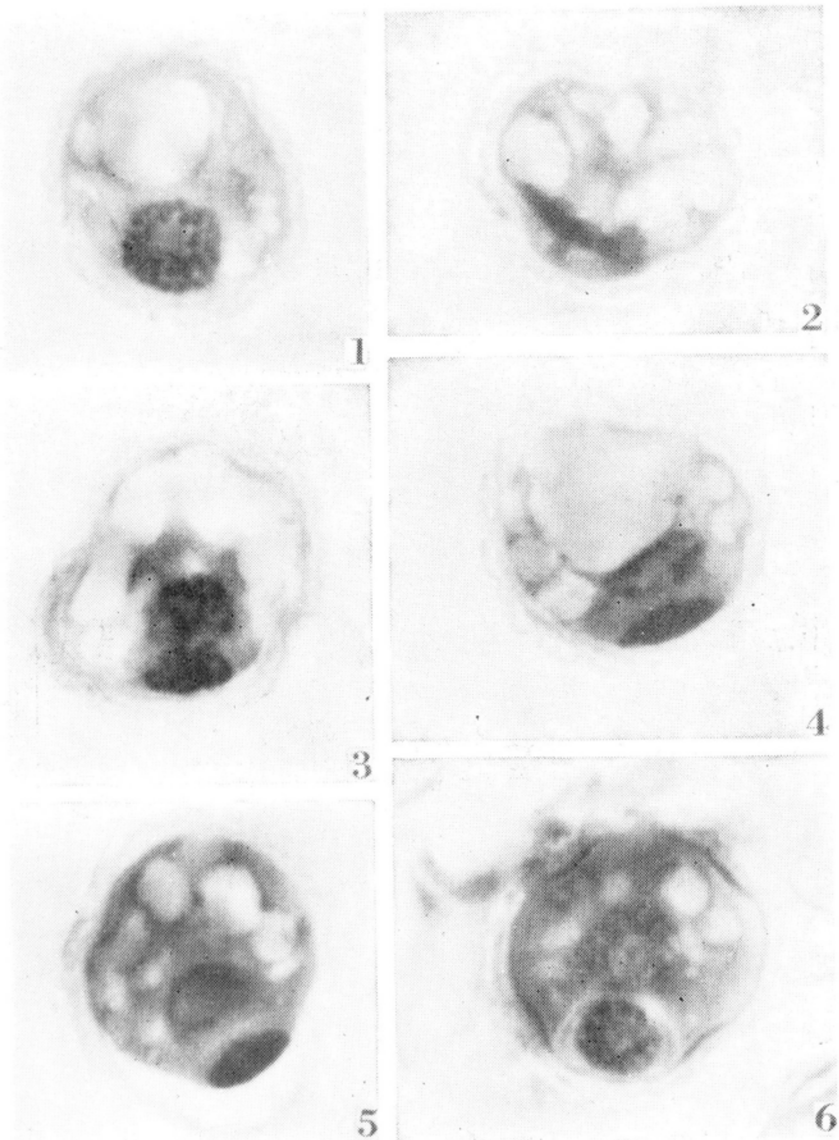
The vegetative cell nucleus has from the beginning a much looser structure than the generative one and gives a much less intensive Feulgen reaction. It contains a large nucleolus. The spherical shape of the vegetative nucleus, after translocation of the generative cell to its neighbourhood, changes to bonnet-like and its surface increases considerably owing to the formation of characteristic lobes (Plate III, photo 4). At shedding, mature pollen grains of *R. repens*, are two celled. The border between the generative and vegetative cell cytoplasms is clearly outlined during the entire development of the pollen grain (e.g. Plate III, photos 1, 2, 3), but in the mature pollen they are less distinct. The vegetative nucleus with a highly developed surface and irregular shape surrounds horseshoe-like the fusiform generative cell (Plate IV, photos 1, 2). The vegetative cell cytoplasm is filled with starch grains, (Plate IV, photo 4).

CONCLUSIONS

Observations carried out on the development of pollen grains in *Ranunculus repens* allow the conclusion that the translocation of the generative cell from the parietal position towards the centre of the pollen grain, associated with a change in cell shape, is the result of vacuolization and of the interaction of the vacuole systems of both the generative and the vegetative cells.

This hypothesis is in agreement with the new data on the physico-chemical character of the border between the generative and vegetative cells. It is known that the surface of the lenticular generative cell is adjacent, in parts, to the pollen grain wall, and to the protoplast of the vegetative cell. As early as 1908, Strasburger claimed that, after cytokinesis in the microspore, each of the two cells possesses its own plasma membrane, and that between them there is a wall capable of swelling which dissolves later. Also Vazart (1958, 1969) mentions the stiffness of the wall separating the lenticular generative cell, and its later plastic character. Electron-microscope examination performed by numerous authors (Chardard, 1958; Larson, 1963; B. Vazart, 1969; J. Vazart 1970 and others) on various angiosperm species confirmed the suggestion that the limiting border between the generative and vegetative cell consists of the two plasma membranes of these cells and of a homogenous, non cellulosic substance contained between them. The chemical character of the substance forming the stiff wall between the lenticular generative cell and the vegetative one was until recently controversial. Some authors even considered it to be made of cellulose and pectin (Maruyama, 1965; Dexheimer, 1965). Górska-Brylass (1967) was the first to identify this substance as callose by the fluorescence method with the use of aniline blue. She believed that this callose not

Plate I

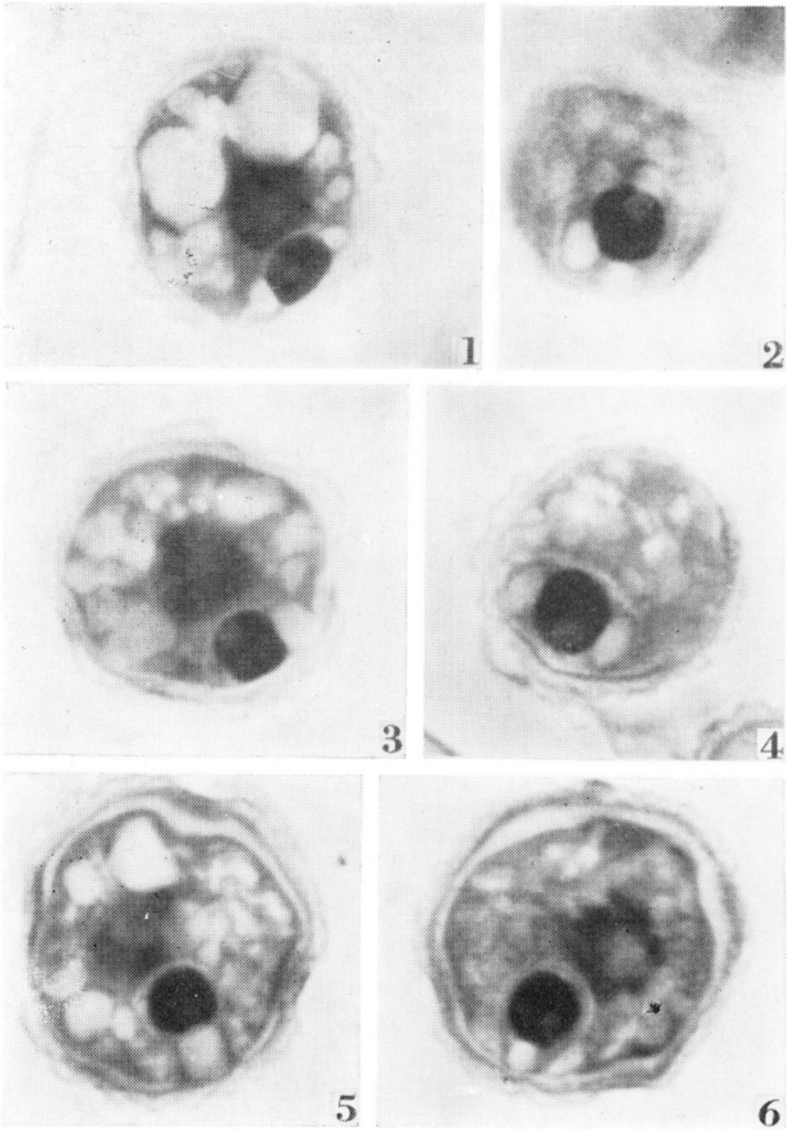


Photos 1—5. Mitosis and cytokinesis in the microspore

Photo 6. Two-celled pollen grain with lenticular generative cell adjacent to pollen grain wall

Feulgen reaction with fast green counter-staining, × 2000

Plate II



Photos 1 and 2. First stage of vacuolization of the lenticular generative cell. Small and large vacuoles in vegetative cell.
Sections through generative cell in two planes:
Fig. 1 — two vacuoles visible, fig. 2 — all four vacuoles visible

Photos 3 and 4. Two vacuoles in generative cell; numerous vacuoles in vegatative cell. Sections through generative cell in two planes: fig. 3 — one vacuole visible, fig. 4 — two vacuoles visible

Photos 5 and 6. Generative cell in the course of translocation towards the centre of the pollen grain. (One vacuole visible).
In vegetative cell small vacuoles are present at the base of the generative cell.

only separates the lenticular generative cell from the vegetative one, but also surroundes it completely when it shifts into the pollen grain.

The investigations on cytokinesis in the microspore and on the chemical character of the wall between the generative and the vegetative cell performed on *Convallaria maialis* by the aniline blue fluorescence method (Charzyńska and Pannenko, 1972, in preparation) demonstrated that callose appears within the cell plate in telophase at the initial stage of cytokinesis. The callose wall separating the lenticular generative cell from the vegetative one is a stiff wall. It was definitively established that callose dissolution in *Convallaria maialis* occurs before the translocation of the generative cell and the change of its shape. The space between the two plasma membranes is later filled with a substance of carbohydrate character, but different from callose, which seems to be liquid (in agreement with Jensen's 1968).

When the callose wall becomes liquefied, the pushing off interaction of the vacuoles in both cells may easily cause a change in the shape of the generative cell and its translocation into the cytoplasm of the vegetative cell.

The *in vivo* observations carried at present on the development of pollen grains of various angiosperms will make it possible to establish whether the above proposed translocation mechanism of the generative cell is common in the angiosperms.

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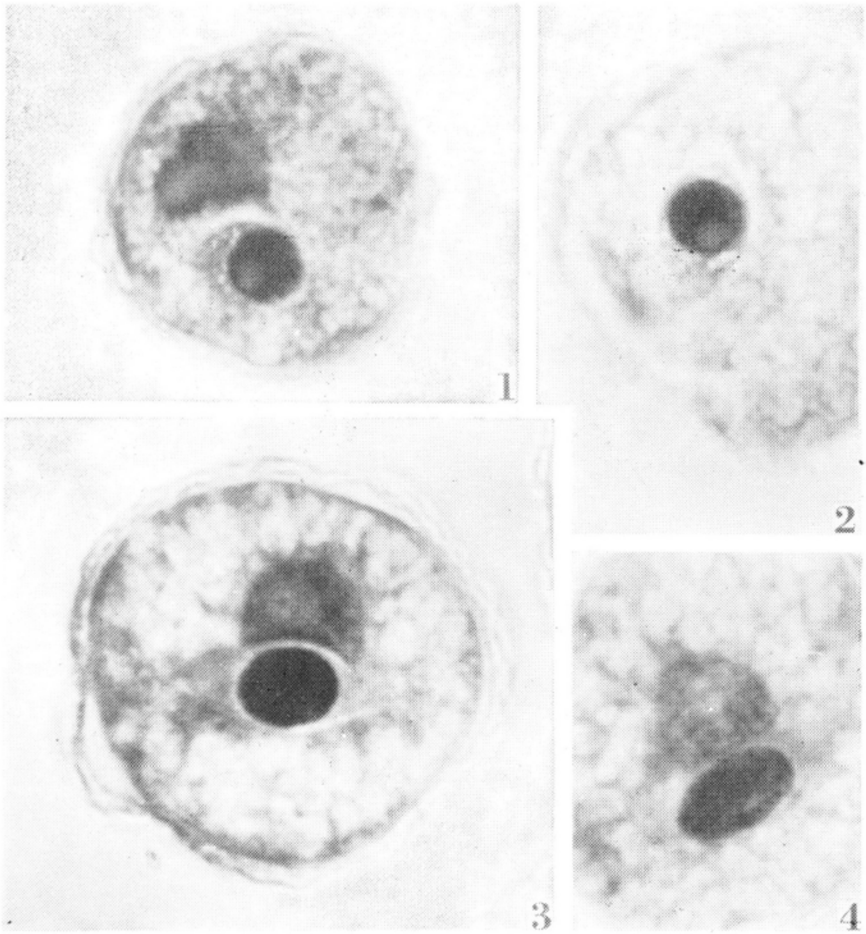
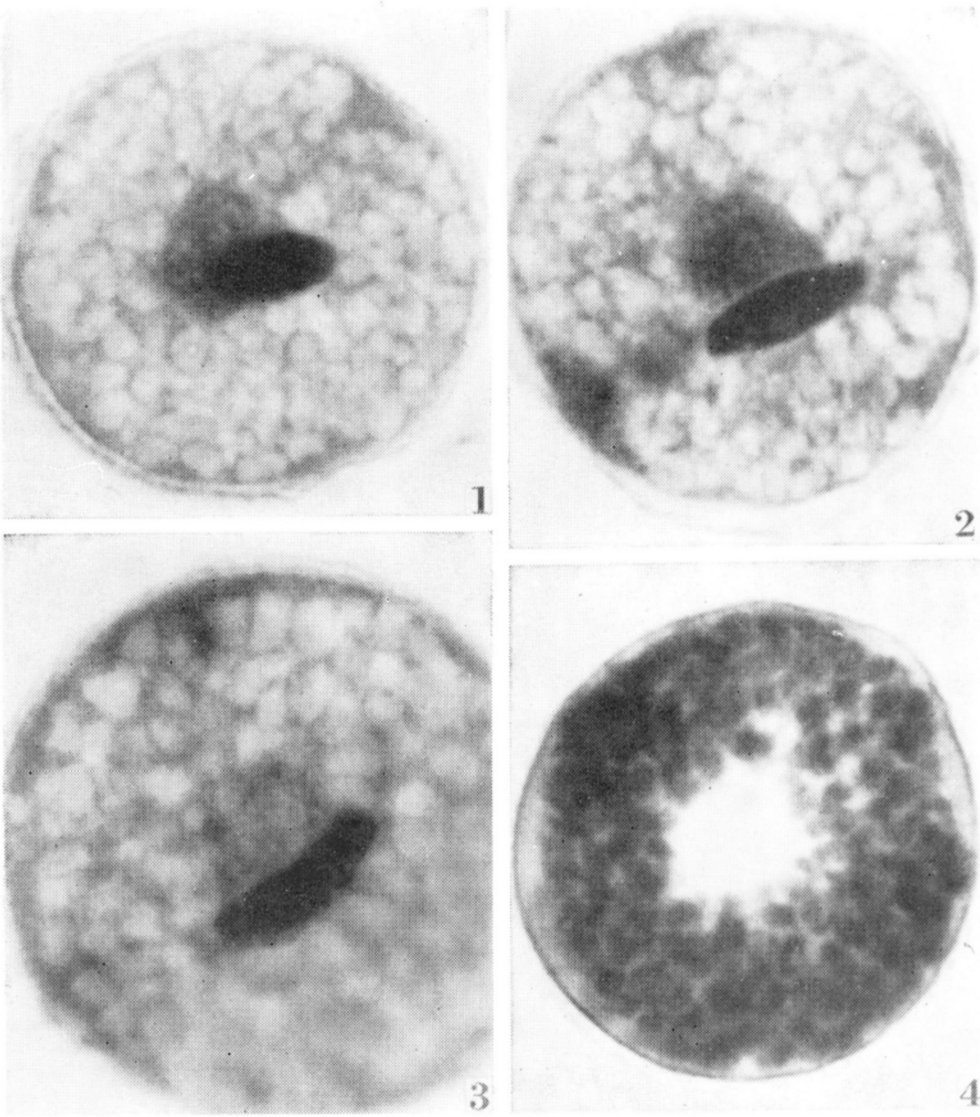
Plate III



Two-celled pollen grain after translocation of generative cell.

Photos 1 and 2. Ellipsoidal generative cell with spherical nucleus.
Photo 3. Slightly fusiform generative cell with spherical nucleus
Photo 4. Vegetative cell nucleus with characteristic lobular protuberances, generative nucleus ellipsoidal.
Feulgen reaction with fast green counter-staining $\times$ 2000

Plate IV



Photos 1 and 3. Sections through maturing pollen grain with fusiform generative cell, in two planes.
Photo 3. Mature pollen grain, characteristic structure of generative cell nucleus.
Photos 1—3. Feulgen reaction with fast green counter-staining.
Photo 4. PAS reaction. Starch grains stained $\times$ 2000

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Przemieszczenie się komórki generatywnej wgłąb ziarna pyłku pod wpływem zmian systemu wakuolarnego u Ranunculus repens L.

Streszczenie

Na podstawie obserwacji przeprowadzonych w mikroskopie świetlnym nad rozwojem męskiego gametofitu *Ranunculus repens* L. postawiono hipotezę, że przemieszczenie się komórki generatywnej z położenia przyściennego w głąb ziarna pyłku, któremu towarzyszy zmiana kształtu tej komórki, jest wynikiem zwakuolizowania komórki generatywnej i wegetatywnej i współdziałania tych dwóch systemów wakuol.