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Cytoembryological aspects of reduced seed setting in *Ranunculus ficaria* L. subsp. *bulbifer* (Marsden-Jones) Lawalrée

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Abstract

Highly disturbed micro- and macro-sporogenesis in *R. ficaria* L. subsp. *bulbifer* gives rise to cytologically and genetically unbalanced reproductive cells. In plants from different populations, pollen degenerates in various percentages. The majority of apparently normal 7-nucleate ES's undergo abortion. Fertilization occurs sporadically only in ca 7% of ovules. Numerous young achenes undergo degeneration resulting from unviable genotypic combinations. Only 1% of seeds contain multicellular embryos, endosperm tissue and are capable of germination.

Ranunculus ficaria L. is karyologically differentiated (Pogan, Wcisło, 1975). The tetraploid taxon subsp. *bulbifer* (Marsden-Jones) Lawalrée (2n = 32) is known to be highly seed-sterile; it reproduces mainly vegetatively by axillary bulbils.

Previous studies aiming at elucidation the seed sterility of this taxon are only fragmentary. It was supposed that this phenomenon may be connected with the formation of bulbils (Kindler, 1914; Marsden-Jones, 1935; Metcalfe, 1939), with self-sterility (Marsden-Jones, 1935), with low viability of pollen unable to germinate on the stigma (Kindler, 1914; Neves, 1942; Perje, 1952). The disorganization of mature embryo-sacs and of nucellus has been also taken into consideration as a primary cause of restricted fructification (Kindler, 1914; Metcalfe, 1939; Perje, 1952).

In the present work, cytoembryological studies of the reproductive cycle of *R. ficaria* subsp. *bulbifer* have been performed; they were supplemented by experimental studies and by observations of plants in natural habitats and in culture.

Microsporogenesis is highly disturbed. Various chromosome associations (quadrivalents, trivalents, bivalents and univalents) occurred with various frequency in particular metaphase plates. Univalents were scattered along the metaphase spindle or eliminated into cytoplasm. Uneven distribution of chromosomes to the poles in the I anaphase, lagging

chromosomes and anaphase bridges persisting to the I telophase were observed.

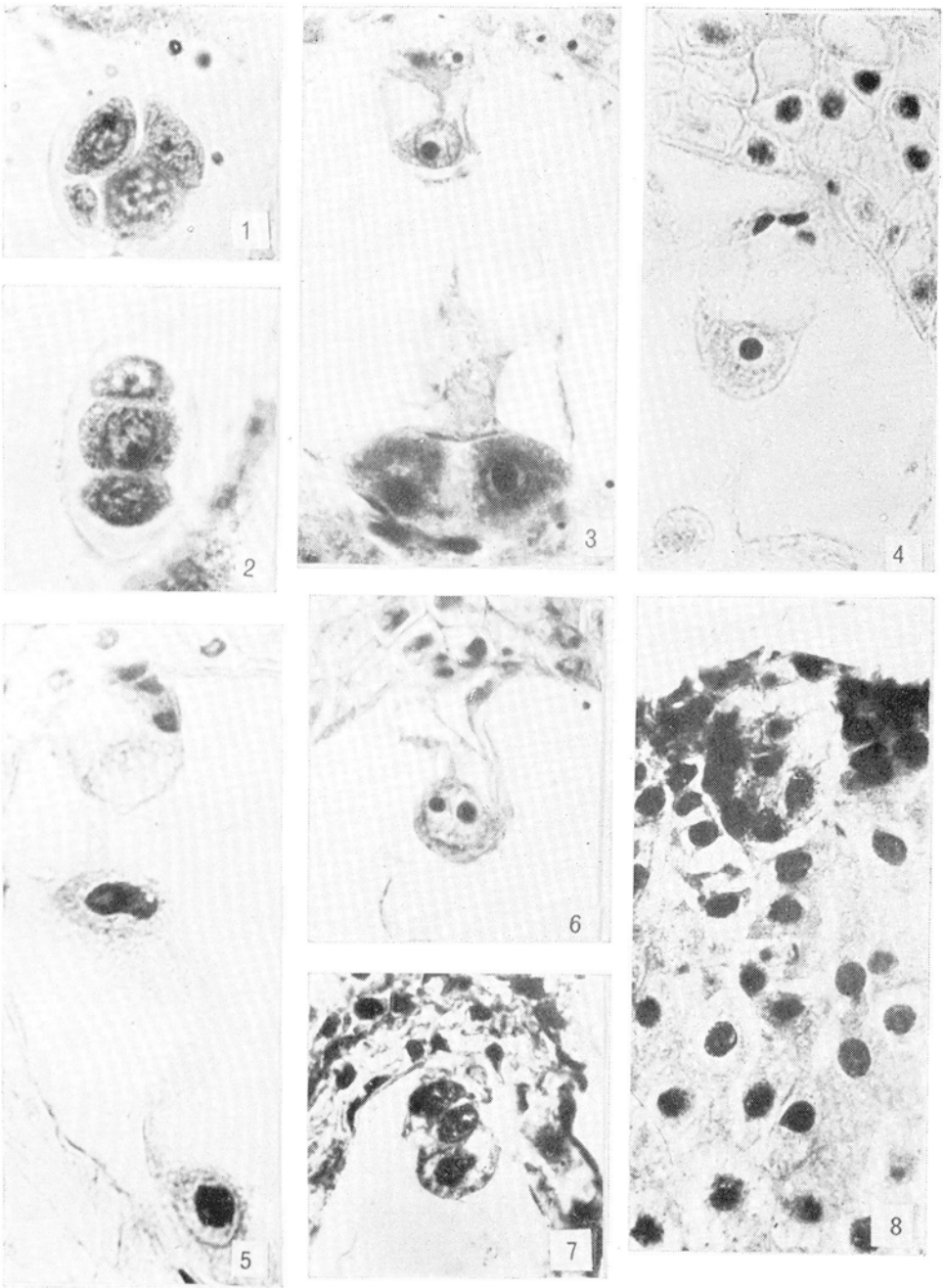
Chromosome counts in the corresponding anaphase groups (eg. 17 + 18, 19 + 20, 19 + 21) showed that most probably some univalents are able to divide already in the I meiotic division. Unequal distribution of chromosomes, their frequent elimination were the most striking disturbances of the II meiotic division. Tetrads represent the prevailing type of sporads in subsp. *bulbifer;* they contain as a rule microspores of various sizes, differing also in the size of their nuclei (Fig. 1). Other types of sporads: monads, triads (Fig. 2), pentands and hexads were formed only sporadically. In some microspores micronuclei were visible.

In different populations of subsp. *bulbifer* the viability of pollen varied from 57 to 97%. Chromosome counts in the first and second pollen mitoses showed that viable pollen grains were cytologically and genetically unbalanced; the chromosome number amounted to n = 8, 9, 10, 11, 12, 15 and 16.

Similar disturbances like in PMC's could also be observed in EMC's. The first meiotic division led to the formation of a dyad. In some of them the micronuclei could be discerned. Further development may proceed according to two patterns: in some ovules the embryo sac develops according to *Polygonum* type. In some others, the micropylar cell of the dyad degenerates and the chalazal one divides, giving rise to two macrospores; the chalazal macrospore develops into the 8-nucleate embryo-sac. In spite of the highly disturbed macrosporogenesis, normal embryo sacs (Fig. 3) developed as a rule in flowers just after anthesis; it may be supposed, however, that like pollen grains, also egg cells should be cytologically and genetically unbalanced.

In a great majority of ovules, the process of seed formation becomes inhibited; eight- or seven-nucleate embryo-sacs undergo degeneration; this process is followed by degeneration of the nucellus. The results of experimental pollination showed that the development of seeds in subsp. *bulbifer* is highly retarded as compared with that in the fertile diploid subsp. *calthifolius* (Pogan, Wcisło, in press). In the latter, already 24 hrs after pollination, early stages of seed development could be observed in the majority of ovules; after 120 hrs, in all of them, endosperm accompanied by the egg cell or zygote could be identified. In subsp. *bulbifer* this developmental stage could be identified only in a few ovules since 192 hrs after pollination. By contrast, in the majority of ovules, degeneration of embryo sacs was already highly advanced.

Fertilization was not observed in *R. ficaria* subsp. *bulbifer* by any of the previous authors. In the present work, sperm cells in the embryo sacs (Figs 4, 5, 6) as well as fertilization were observed. The endosperm,



Figs 1-8. *Ranunculus ficaria* subsp. *bulbifer* (2n = 32)
1) Tetrad of microspores — note the differences in size of spores. 2) Triad of microspores. 3) 7-nucleate ES. 4) Egg cell and sperm cells. 5) Nuclear endosperm; pollen tube in the micropylar region of ES's. 6) Zygote; note two nucleoli of different size. 7) Young embryo. 8) Multicellular embryo and endosperm tissue

nuclear in its origin (Fig. 5), precedes in its development the divisions in the zygote cell.

Enlarged, apparently well developed achenes were formed only in 7% of the pistils. In some of them, however, only remnants of the degenerated embryo sacs could be still recognized. In some others, young seeds of various developmental stages were degenerating. Only in a few achenes, the process of development proceeded further (Fig. 7) and could be accomplished giving rise to seed with endosperm tissue and a multicellular embryo (Fig. 8). These achenes represented about 1% of pistils.

The present results show that the reduced seed setting of *R. ficaria* subsp. *bulbifer* is primarily due to the cytologically and genetically unbalanced character of the reproductive cells. The retarded growth of pollen tubes might be responsible for the lack of fertilization in the majority of ovules. The pollen tubes either do not enter the embryo sac or they may enter it too late, at the time when the egg cell is already unable to undergo fertilization. In consequence, the majority of embryo sacs degenerate. But even in fertilized embryo sacs, degeneration of the zygote or of achenes in various developmental stages can occur in connection with unviable genotypic combinations.

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