



## EMBRYOLOGICAL PROCESSES IN OVULES OF *RUDBECKIA LACINIATA* L. (ASTERACEAE) FROM POLAND

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**Abstract.** Embryological researches of an invasive plant *Rudbeckia laciniata* focused on the process of megasporogenesis, female gametophyte formation, and fertilization. A disturbed first meiotic division results in the formation of restitution nucleus. In some megaspore mother cells micronuclei or vacuoles were observed near the restitution nucleus. Different course of megasporogenesis led to the development of unreduced embryo sacs with various organization which is reflected in the embryo formation and capacity of seed germination. Our research confirmed the occurrence of hemigamy in the Polish representatives of *R. laciniata*. In spite of a reduced germination capacity of *R. laciniata* seeds, their production allows a long-distance dispersal and expansion of the species.

**Key words:** *Rudbeckia laciniata*, Asteraceae, megasporogenesis, female gametophyte, hemigamy

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### Introduction

In the Polish flora, the *Rudbeckia* genus is represented by two species: *R. hirta* L. and *R. laciniata* L. Both species are kenophytes which arrived from North America and they are agriophytes permanently established in natural or seminatural communities (ZAJĄC *et al.* 1998). *R. laciniata* is a very common species in Poland, occurring especially in the southern part of the country (ZAJĄC & ZAJĄC 2001). At present, it is considered an invasive species, competing with native species and displacing them, which is a potential threat to biodiversity (TOKARSKA-GUZYK 2005). Therefore a detailed knowledge of reproductive biology is essential for an effective protection of the native ecosystem. The roles that reproductive mode plays in the invasion of alien species are largely unknown despite being essential to the understanding of the invasion mechanisms (DONG *et al.* 2006). Invasive plants often have the capacity of rapid vegetative propagation. However, it is also significant to determine the importance of sexual and/or apomictic reproduction in the successful invasion.

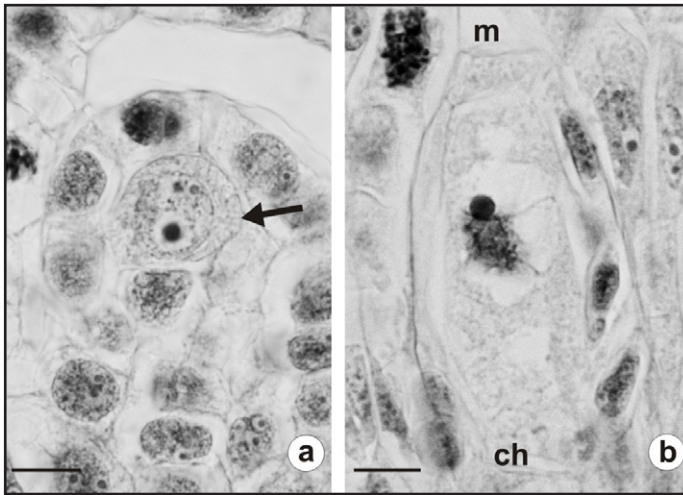
Although *R. laciniata* is an invasive plant rapidly spreading in Europe, the mode of

reproduction of the Polish representatives of this species was not analyzed. Previous embryological investigations within *Rudbeckia* genus showed differentiation of developmental pathways of female gametophyte (BATTAGLIA 1945, 1955, 1981; KOŚCIŃSKA 1980; KOŚCIŃSKA-PAJĄK 1985; MUSIAŁ *et al.* 2012). Moreover, studies of fertilization in *Rudbeckia* species revealed a relatively common occurrence of hemigamy (BATTAGLIA 1945; SOLNTZEVA 1968, 1974; KOŚCIŃSKA 1980).

The aim of the present studies was to examine the course of megasporogenesis, female gametophyte formation as well as analysis of fertilization in the Polish representatives of *R. laciniata*.

### Material and methods

Inflorescences of *R. laciniata* were sampled from randomly selected plants within natural population in Balice near Cracow (50°4'40"N, 19°47'5"E). Whole capitula at various developmental stages were fixed in FAA for at least 24 h and stored in 70% ethanol. Individual flowers, ovaries or ovules were then isolated and dehydrated in ethanol series. A part of the samples was embedded in paraffin, cut



**Fig. 1.** Longitudinal section of young *Rudbeckia laciniata* ovules: **a** – ovule primordium with archesporial cell (arrow); **b** – early prophase in megaspore mother cell. Abbreviations: **ch** – chalazal pole; **m** – micropylar pole. Scale bars: 10  $\mu\text{m}$ .

to 8-10  $\mu\text{m}$  thick sections, and stained with Heidenhain's hematoxylin with alcian blue. Sections were examined using a Nikon 400 Eclipse microscope.

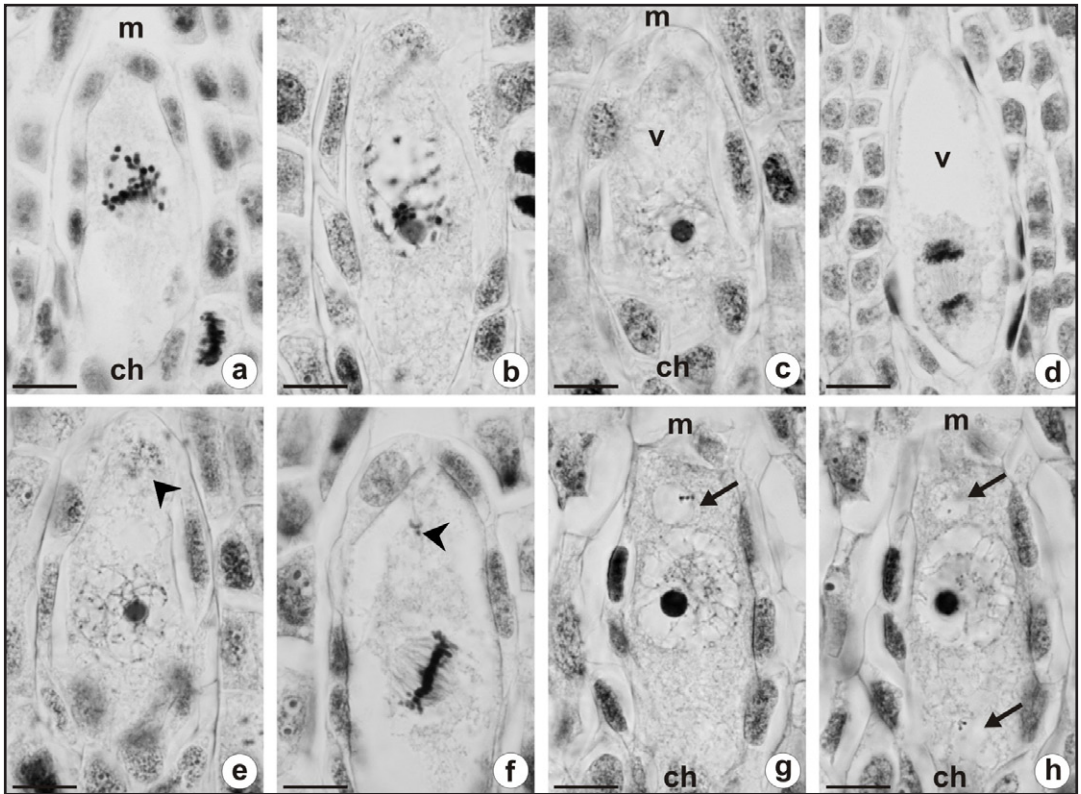
### Results and discussion

The ovule of *R. laciniata* is anatropous, tenuinucellate and unitegmic as in other members of Asteraceae. In young ovules of *R. laciniata*, a single hypodermal archesporial cell differentiates (Fig. 1 a) and functions directly as a megaspore mother cell (MMC) which becomes distinctly elongated in the micropylar-chalazal axis before meiosis (Fig. 1 b). In the analysed material, three types of megasporogenesis were found, which led to the formation of unreduced female gametophytes with different structure. In all of the analysed ovules the first meiotic division was strongly disturbed, chromosomes were scattered along the spindle during the metaphase (Fig. 2 a). Most frequently, one centrally situated restitution nucleus was formed after the irregular first meiotic division (Fig. 2 b). The second meiotic division was not followed by cytokinesis and gave rise to a binucleate coenomegaspore. Then, two successive mitosis lead to an unreduced eight-nucleate embryo sac (Fig. 3 a). This developmental pathway follows the diplospory of *Ixeris* type which was also described in the representatives of *R. laciniata* investigated by

BATTAGLIA (1945). This mode of female meiosis also occurs in *R. speciosa* Schrad., *R. triloba* L. as well as in *Erigeron annuus* (L.) Pers. (for review see JOHRI *et al.* 1992).

In some ovules, after irregular first meiotic division, a large vacuole arises in the micropylar region of MMC thus moving the restitution nucleus to the chalazal pole of the megasporocyte (Fig. 2 c) where the second meiotic division (Fig. 2 d) takes place, giving rise to a binucleate unreduced megaspore. In the course of further development, only one mitosis occurs leading to the formation of a four-nucleate embryo sac. In these cases, there is no egg apparatus at the micropylar pole of the embryo sac and the female gametophyte consists of three antipodal cells and a central cell containing only one nucleus (Fig. 3 b). Such organization of the female gametophyte, which is devoid of the egg apparatus, was described in other species within *Rudbeckia* genus (BATTAGLIA 1946).

Quite often, two nuclei unequal in size were formed as a result of the first disturbed meiotic division (Fig. 2 e). Both nuclei undergo second meiotic division (Fig. 2 f) resulting in four nucleate coenomegaspore. Mitotic division of this coenomegaspore leads to the formation of an eight-nucleate embryo sac in which four micronuclei at the micropylar pole and four significantly larger nuclei at the chalazal pole were visible. After the formation of the cells in the embryo sac, a miniature egg apparatus,



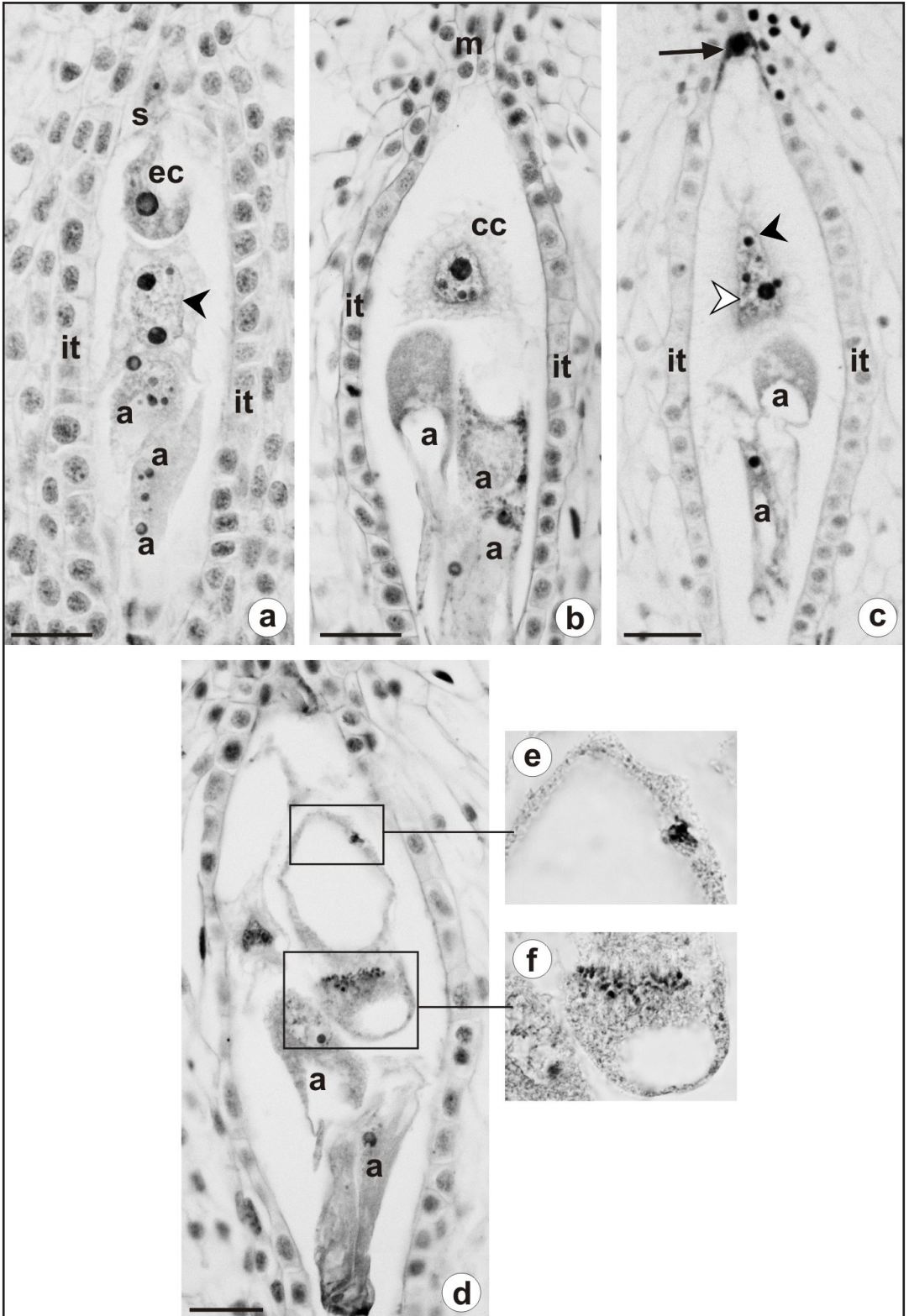
**Fig. 2.** Megasporogenesis in *Rudbeckia laciniata*: **a** – disturbed metaphase I in megaspore mother cell; **b** – centrally situated restitution nucleus formed after irregular first meiotic division; **c** – megaspore mother cell with vacuolated micropylar pole and restitution nucleus visible in the chalazal part; **d** – telophase II at chalazal pole of megaspore mother cell; **e** – two nuclei unequal in size formed as a result of the first disturbed meiotic division, arrowhead points to micronucleus; **f** – metaphase II in megaspore mother cell, arrowhead shows dividing micronucleus; **g, h** – two successive sections of megaspore mother cell with micronuclei (arrows) visible above and below restitution nucleus. Abbreviations: **ch** – chalazal pole; **m** – micropylar pole; **v** – vacuole. Scale bars: 10  $\mu$ m.

three antipodal cells, and central cell containing two polar nuclei different in size were observed (Fig. 3 c). Sporadically, micronuclei were visible below and above the restitution nucleus (Fig. 2 g, h). Abnormalities observed in the female gametophyte development and structure affected the viability of seeds. In the studied material, only 36% of mature seeds was capable of germination.

In some embryo sacs, just after fertilization, we observed an independent division of the egg cell and sperm cell nuclei (Fig. 3 d-f). These observations confirmed the occurrence of hemigamy in *R. laciniata*. During this process, also called semigamy, a male nucleus enters the egg cell but does not fuse with the female

one and then both nuclei divide independently giving rise to a mosaic embryo which contains tissue sectors of maternal or paternal origin (BATTAGLIA 1981; JOHRI 1984). Hemigamy was previously recorded in unreduced embryo sacs of other species of *Rudbeckia* (SOLNTZEVA 1974, and references therein) as well as in the meiotic embryo sacs of *R. bicolor* (KOŚCIŃSKA 1980; KOŚCIŃSKA-PAJĄK 1985). However, the recent analysis revealed that in *R. bicolor* hemigamy is not obligatory and most likely depends on the genotype (MUSIAŁ *et al.* 2012). It should be emphasized that sexual reproduction contributes to genotypic diversity, facilitating the adaptation of species to adventive environments (TIÉBRÉ *et al.* 2007). Likewise in





the case of *R. laciniata*, seeds can contribute to the invasiveness of this species, despite of their reduced germination capacity.

### Conclusions

1. The first meiotic division is disturbed in all analysed young ovules of *R. laciniata*.

2. In the studied material, three types of megasporogenesis were found, which led to the formation of unreduced female gametophytes which differ in structure.

3. Our embryological observations confirmed the occurrence of hemigamy in Polish representatives of *R. laciniata*.

4. Disturbances of megasporogenesis and observed abnormalities in the embryo sacs organization affect the viability of seeds.

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◀ **Fig. 3.** Longitudinal section of organized female gametophytes in *Rudbeckia laciniata*: **a** – embryo sac containing typical egg apparatus, central cell with polar nuclei of equal size at the time of fusion (arrowhead), and three antipodal cells; **b** – embryo sac without egg apparatus, in central cell one polar nucleus, three antipodal cells at chalazal pole; **c** – mature embryo sac with a miniature egg apparatus (arrow), central cell containing polar nuclei distinctly differed in size (black and white arrowheads), and antipodal cells; **d** – simultaneous division of the female (large) and male (small) nuclei, framed parts are shown in **e** and **f**. Abbreviations: **a** – antipodal cell; **cc** – central cell; **ec** – egg cell; **it** – integumentary tapetum; **m** – micropylar pole, **s** – synergid cell. Scale bars: 25 μm.