LIFE HISTORY IN *DROSERA* L. (DROSERACEAE) EMPHASIZING THE NATURAL HYBRID *DROSERA X OBOVATA* MERT. AND KOCH

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ABSTRACT

A study about the sporogenesis and gametogenesis of the hybrid *Drosera x obovata* and its parental species (*D. rotundifolia* and *D. anglica*) was carried on. *D. x obovata*, even if sterile, produces pollen tetrads and a male gametophyte structurally the same as the parental species' one. However the pollen grains appear poor of protoplasmic papillae, which indicates low vitality. The investigations on the ovules allowed to follow the megasporogenesis and the megagametogenesis till the development of the embryos in the parental plants whereas in *D. x obovata* it has never been possible to observe an embryo.

KEY WORDS

Drosera, pollen, ovule, sporogenesis, gametogenesis, hybrid sterility.

INTRODUCTION

The genus *Drosera* L. (sundews), which includes over 150 species of insectivorous plants, counts in Italy (and in Europe) 3 species and a natural hybrid. They are rare, show restricted ranges, and are closely linked to wet and oligotrophic habitats with specific edaphic-climatic characteristics. These sites of high naturalistic value, own an intrinsic critical state and have to be considered very vulnerable if the rising temperature trend and the rainfall reduction will continue at the present rate. Sundews populations almost disappeared from the plains because of the eutrophication. Also in montane habitats they are at great risk because of the climatic changes and the anthropic activities.

In Pian di Gembro bog (SO, Italy, fig. 1), 1350 m a.s.l., three of the four European *Drosera* cohabit in vigorous populations: *D. rotundifolia* L. (2n=20), *D. anglica* Huds. (2n=40) and their natural hybrid *D. x obovata* Mert. and Koch (2n=30) (Fig. 2) (Andreis and Rodondi, 1982).

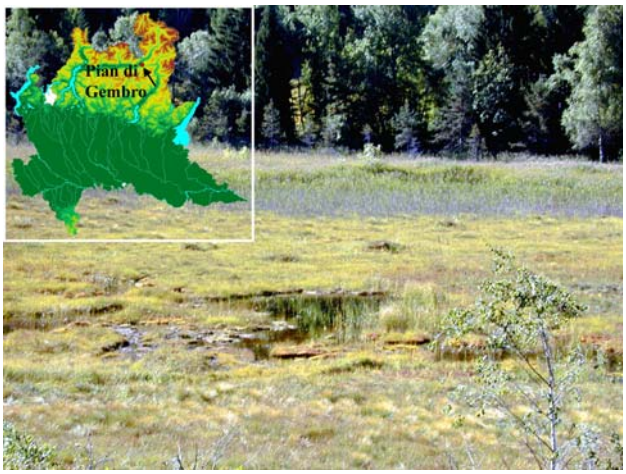


Figure 1. Pian di Gembro bog and its geographycal location.

Figure 2. *Drosera x obovata* in the wild.

D. x obovata is known to be sterile, presents a particular meiosis (Rosenberg, 1904; Kondo and Segawa, 1988) and a vigorous vegetative reproduction. Previous studies demonstrated that *D. x obovata* releases its pollen in tetrads with echinate ornamentations, morphologically similar to the parental species' one (Rodondi et al., 2004). *Drosera* 's ovules are bitegmic, anatropous with an embryo sack in the micropylar region of the nucellus and the dust-like seeds are dispersed prevalently by wind (Boesewinkel, 1989; Murza and Davis, 2003). Since there are few data about sporogenesis and gametogenesis of the european sundews an investigation of these processes has been undertaken with particular attention to the sterility of *D. x obovata* goal of this study.

MATERIALS AND METHODS

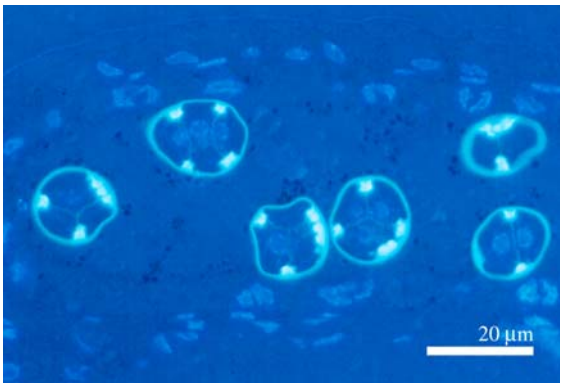
The racemes of the three sundews, collected in the Natural Reserve of Pian di Gembro in July 2006, were fixed in loco in formalin-acid-alcohol (FAA) (Krishnamurthy, 1988). For LM observations the samples were dehydrated through a graded ethanol series, embedded in methacrylate resin (Technovit 7100; Heraeus Kulzer, Werhrheim, Germany), stained with toluidine blue, DAPI to detect DNA (Vergne et al., 1987) and aniline blue to detect callose (O'Brien, 1981). Observations were made with a LEICA DM-RD epifluorescent microscope equipped with a Ploëmopack 1 System and a mercury lamp (OSRAM HBO 100 W) with an excitation filter 340–380 μm, a dichroic beam-splitting mirror RKP 400, and a barrier filter at 420 μm.

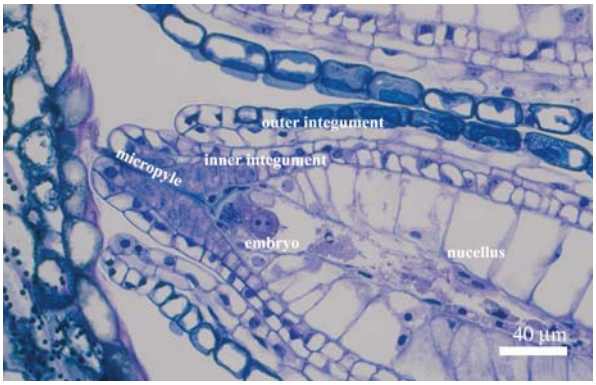
For SEM observations the pollen grains were acetolyzed according to Erdtman (1960) or fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, post-fixed in 1% osmium tetroxide, dehydrated in an acetone series, criticalpoint dried with CO_2, mounted on aluminium stubs, gold coated, and observed under a LEO 1430 scanning electron microscope.

The exicata are housed in the herbarium of the Department of Biology, University of Milano: Herbarium Universitatis Mediolanensis (MI).

RESULTS AND DISCUSSION

In Pian di Gembro bog *D. x obovata* is present with a population as vigorous as the parental species' one. Previous investigations on pollen micromorphology (Rodondi et al. 2004) and new data confirmed that the sterile hybrid *D. x obovata* realeases its pollen in tetrads which means that all the steps of microsporogenesis goes on regularly (Fig. 3). In particular figure 4 shows the envelopes of callose around the microspores at the end of the meiosis. The investigations on *D. rotundifolia* and *D. anglica* allowed to follow the micro-megasporogenesis and the development of the gametophytes and the embryos in the micropylar portion of the nucellus (Fig.5).





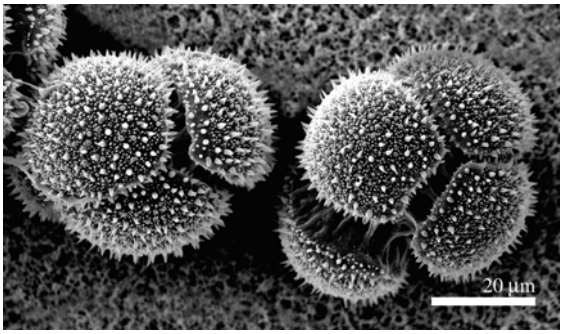


Figure 3. Pollen tetrads of *D. x obovata* (SEM micrograph).

Figure 4. Microsporogenesis in *D. x obovata*: callose stained with aniline blue (LM micrograph).

Figure 5. First stage of embryo in *D. anglica* stained with toluidine blue (LM micrograph).

In *D. x obovata* also the gametogenesis seems to proceed regularly: in figure 6 are clearly visible the vegetative and one of the generative cell inside a pollen grain. However the pollen of this plant appears often poor of protoplasmic papillae, which indicates low vitality.

Also the embryo sacs have been frequently observed but it has never been possible to observe a developed embryo (Fig. 7). Usually the embryo sac degenerates soon.

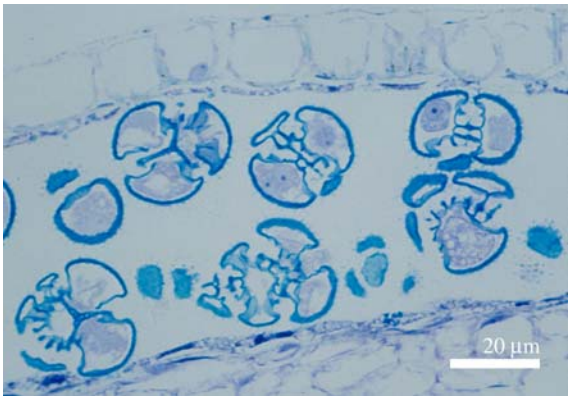


Figure 6. Microgametogenesis in *D. x obovata* stained with toluidine blue (LM micrograph).

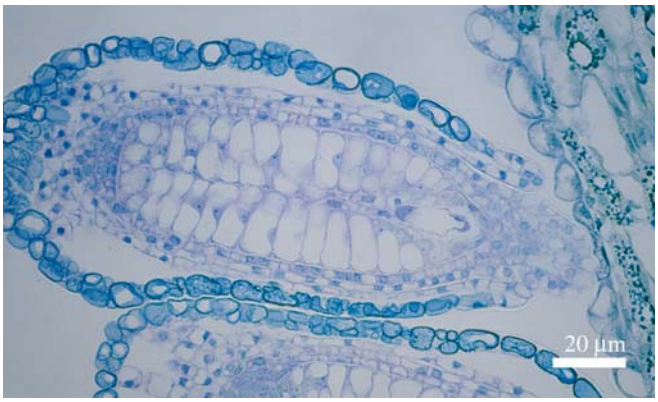


Figure 7. Embryo sac in *D. x obovata* stained with toluidine blue (LM micrograph).

CONCLUSIONS

Experiments in crossing and further investigations will test vitality and germination of the gametophytes of the hybrid. Even the interactions between the three populations in their natural habitat will be investigated with a new project.

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