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Structural aspects of ovule and seed development and nonrandom abortion in *Melilotus officinalis* (Fabaceae)

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Summary. Only one ovule matures into a seed in *Melilotus officinalis*. Although eight ovules form within an ovary, only the basal ovule develops into a mature seed, whereas the other ovules degenerate. The investigation of ovule and seed structure at different developmental stages and a comparison of quantitative characters of differently fated ovules within an ovary were undertaken by light, phase contrast, and fluorescence microscopy. In this species, campylotropous ovules develop simultaneously on marginal placentae in an apocarpous unilocular gynoecium. Megasporo- and megagametogenesis proceed normally and are completed in bud. The maturation of the Polygonum type embryo sac takes place after the flower opens. Shortly before fertilization, synergids show signs of degeneration in all ovules. At this stage, neither the structure nor the sizes of ovules within one ovary differ significantly. In spite of this, only the basal ovule develops into a seed. Rarely, one of the upper-situated ovules or the basal and another ovule mature into seeds. Seed enlargement is insignificant until the stage when globular embryo and nuclear endosperm are formed. At the seed-filling stage, other ovules have collapsed and the seed gradually comes to occupy the total volume of the pod. The fruit-to-seed length ratio decreases considerably during seed ripening. At fertilization, ovary length is four times greater than ovule length. In the mature state, the fruit and seed lengths are approximately equal. Seed size and weight diminish with an increase in seed number within a pod, although pod size remains constant. It is assumed that nonrandom abortion of young seeds in *M. officinalis* is under maternal control and is not related to structural abnormalities in ovule development or with limitation in pollen. We suppose that evolution of this species may have proceeded in the direction of a decrease in seed number and an increase in its sizes, which may play an important role in seed dispersal and seedling establishment.

Keywords: Fabaceae; Fertilization; *Melilotus officinalis*; Seed abortion; Seed development; Seed size.

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Introduction

In common with many other legumes, *Melilotus officinalis* matures only one seed per flower (Lachashvili 1981). Although eight ovules form in an apocarpous, unilocular gynoecium, the number of maturing ovules is reduced after fertilization to one (rarely to two), by abortion (Wiens 1984, Wiens et al. 1987). The abortion of developing seeds in different species may occur randomly or nonrandomly with respect to ovule position within an ovary (Horovitz et al. 1976, Hossaert and Valero 1988). A study (Schouten 1998) of the effect of ovule position on seed formation in different populations of *M. officinalis* has shown that seed set occurs predominantly in the basal ovule position, is very low in the second ovule position from the base, and is extremely rare in the upper ovule positions. It is not known, however, which structural or functional characters determine nonrandom seed set in this species. Earlier investigations on embryology in *M. officinalis* have not been concerned with the process of seed abortion (Cooper 1933, Rembert 1969, Jha and Pandey 1989). In some plant species, structural studies were performed and comparisons were made between successfully maturing embryos and those that aborted, emphasizing the different roles of maternal, offspring and endosperm tissues (Sangduen et al. 1983, Sage and Webster 1987). Phenotypic analyses of female-gametophyte mutants in *Arabidopsis thaliana* and maize suggest that low seed set in these plants depends on the activities of many genes expressed in the female gametophyte and sporophyte (Moore et al. 1997, Drews et al. 1998, Grossniklaus and Schneitz 1998). However, remarkably little is known about the actual

differences at the cytological level in the developmental process of individual ovules of different fates before and during fertilization and early abortion (Cooper et al. 1937, Cooper and Brink 1940, Bubar 1958, Briggs et al. 1987). The nonrandom character of seed maturation with respect to ovule position within an ovary of *M. officinalis* makes it possible to use structural and developmental differences between differently fated ovules to examine the importance of various types of phenotypic variability to the success of seed formation. For this purpose we performed structural and quantitative analyses of the reproductive structures of *M. officinalis* before and after fertilization and during seed maturation.

Several hypotheses concerning the possible causes and function of flower and seed abortion have been formulated (Stephenson 1981, Queller 1983, Lee 1984, Haig 1986). The most often discussed causal factors of seed abortion are thought to be limitation of resources (Genter et al. 1997) and pollen (Agren 1996), influence of predators (Marshall et al. 1985), pathogens (Jones 1976), or unfavorable abiotic factors (Lee and Bazzaz 1982), and occurrence of sporophytic or gametophytic mutations (Seavy and Carter 1996). Most of these hypotheses generally regarded aborting material as abnormal. However, a different suggestion concerning the fixed rate of random ovule abortion and phenomenon of one-seededness in *Cryptantha flava* has been postulated (Casper and Wiens 1981, Casper 1984). According to this hypothesis, seed abortion is considered as adaptive to increased dispersal of fruits (which are transported by wind) and, consequently, reduced competition among the offspring. This study awoke our interest to investigate the one-seededness in *M. officinalis* in this light. The relation between the size and number of seeds within a fruit and the influence of seed size on germination is another critical aspect to be analyzed in this study.

In this paper we describe several aspects of the embryology and reproductive biology of *M. officinalis* concerned with the process of abortion of ovules and young seeds. Three questions will be addressed in the analysis. What is the structural difference between aborted and successfully matured ovules before and during fertilization? How does seed maturation proceed with respect to abortion of other ovules? Is there any relationship between size and number of seeds within a fruit? Finally, we discuss the adaptive significance of one-seededness in *M. officinalis* and its importance to the seed dispersal and seedling establishment.

Material and methods

Plant material and study sites

Melilotus officinalis (L.) Pall. is an annual or biennial herb, 30–250 cm high. Inflorescence is spikelike raceme, to 10 cm long, bearing 20–30 spirally arranged flowers. The papilionaceous yellow flowers are 4–6 mm long. They stay open for approximately one week and mature asynchronously. The fruit is a simple ovoid legume, 3–5 mm long. Fruits require 7 to 9 weeks to mature, are tardily or not dehiscent. The number of seeds per fruit is usually 1, rarely 2. *Melilotus officinalis* grows in ruderal habitats, both in natural populations or as a weed of cultivation. It is distributed throughout most of the entire northern hemisphere. It was introduced to North America from Eurasia and has naturalized.

The material was collected at different sites both in Oregon, U.S.A. and in Georgia, Caucasus. In Oregon, the material was collected to be used in an embryological study and the determination of pollen tube growth characters within an ovary. In Georgia, ovule and seed growth patterns and pollination and seed set characters were studied. Collection sites in Oregon are located in the areas surrounding the cities of Portland and Clackamas. In Georgia three populations of *M. officinalis* were used for material sampling. Population 1 is located in the surrounding of Tbilisi, on Nutsubidze Plateau at 450–500 m above sea level. Individuals are 30–50 cm high. The reproductive period (i.e., time from appearance of the first flower until maturing of the last pod in the population) is May to October. Population 2 is growing in South Georgia, Javakheti, near Ninotsminda at 1650 m above sea level, on a subalpine meadow. Plants are 30–40 cm high. The reproductive period for this population is from July to October. Population 3 is distributed in and around a cultivated area in Kakheti, near Shilda at 540–550 m above sea level. The site is among wheat fields and along the roadsides. Plants are 80–200 cm high. The reproductive period for this population is from June to October.

Material sampling and fixation

Ovaries and fruits at different stages of development were fixed in FAA_{50} (formalin, glacial acetic acid, 50% ethanol, 5 : 5 : 90). Material was embedded in paraffin, sectioned at 12 µm, and stained with safranin-fast green (Jensen 1962). The sections were examined with a Zeiss light microscope. Pollen tube growth characters within an ovary were observed with fluorescence microscopy by the technique of Martin (1959). Photographs were taken on Kodak T-MAX 400 black-and-white film. To study ovule and seed growth patterns and determine pollination and seed set characters, flowers at different developmental stages were dissected only from the middle part of each inflorescence from different individuals inside a population and fixed in FAA_{50}. Material was stored in 70% ethanol. Whole pistils were dissected out of the flowers, mounted on microscope slides in clearing solution according to Herr (1971) and examined with a PZO 30 phase contrast microscope.

Germination test

The seeds of population 1 were collected during September and October of 1997 and stored in paper bags at room temperature. The germination test was conducted in April of the following year. Seeds were dissected from pods and sorted into two groups: those from single-seeded pods and those from double-seeded pods. Two different treatments were carried out for each group of seeds – a total of four treatments. In the first treatment, seeds were germinated with intact seed coats. In the second, seeds were germinated after seed coats were mechanically destroyed to make them permeable for water. 100 seeds from single-seeded pods or 30 seeds from double-