

On some specificities of seed formation in *Salvia nemorosa* (Lamiaceae)

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Abstract. The embryological processes leading to seed formation in *S. nemorosa* are comprehensively studied for the first time. A number of embryological specificities have been established, typical of the malesterile plants. They show that the investigated species manifests a tendency for transition from hermaphrodite to the evolutionally more advanced unisexual flowers in *Magnoliophyta*.

Key words: development of the female gametophyte, embryo, endosperm, *Lamiaceae*, macrosporogenesis, *Salvia*, seed

Introduction

Salvia nemorosa L. is an essential-oil plant widely distributed on grassy and shrub terrains in the foremountains, up to 2000 m a.s.l., all over Bulgaria. It yields some 0.01–0.04 % of essential oil during blossoming, with pleasant aroma resembling that of *S. sclarea* L.

In the Bulgarian folk medicine *S. nemorosa* is used chiefly to treat stomach ache, diarrhoea, haemorrhages, furuncles, etc.

We have found no data on the embryology of *S. nemorosa* in the world literature. Along with other medicinal plants, it has been object of comparative anatomical research of the spermoderm (Ryding 1995; Wojcechowska 1966). Furthermore, male sterility was also established in *S. nemorosa* (Linnert 1955; Mohan & Kans 1990), and in the Bulgarian representatives of the species only a tendency for transition to functionally female flowers (Daskalova 1999). All this calls for a more thorough study of the processes in the female generative sphere of the flower, in order to trace out how much it has been influenced by the evolved sterilisation processes.

Material and methods

The studied material (flower buds, flowers in different stages of development and seeds) was collected in the period 1993–1994 from two natural habitats of *S. nemorosa*: the first near Bistritsa village (Vitosha region) and the second near Novo Selo village, Veliko Turnovo district (the Forebalkan). The material was fixed with Navashin's mixture and treated according to the classical paraffin methods. The staining was made with Heidenhain's hematoxylin. Eight to twelve μm thick sections were cut with Minot rotation microtome and the observations were made with Amplival light microscope. The microphotos were made with MF-matik.

Results and discussion

Similarly to all representatives of family *Lamiaceae* (Junell 1934; Kamelina & Dzevaltovsky 1987; Ryding 1995), the ovary in *S. nemorosa* is in upper position, 4-locular. In each loculus, an anatropous, tenuinucellate

unitegmic ovule forms and develops (Plate I, Fig. 1). Already in the comparatively young ovule, only one archesporial cell forms subepidermally, contrary to *S. pratensis* L. (Daskalova 2002), *S. officinalis* L. and *S. sclarea* (Kamelina & Dzevaltovsky 1987), in which occasionally two archesporium cells form. A multicellular female archesporium consisting of eight cells that simultaneously set into meiosis was found out in *Stachys trinervis* (Kamelina & Dzevaltovsky 1987).

The archesporium cell differs distinctly from the remaining nucellar cells by its larger size and characteristic orthogonal to polygonal shape. It does not divide further and directly transforms into a macrospore mother cell (MMC).

The type of the ovules and the only one-celled archesporium formed in each, without cover cells, testify to a high level of development of the female generative sphere of the flower, judging by these embryological characteristics. The same was found out in other investigated representatives of genus *Salvia* (Daskalova 1997) too, as well as in the evolutionally most advanced taxa of family *Lamiaceae* (Dzevaltovsky 1979; Poddubnaya-Arnoldi 1982; Kamelina & Dzevaltovsky 1987).

Macrosporogenesis in MMC runs normally, resulting in a linear macrospore tetrad. The chalazal cell becomes the embryo sac mother cell (Plate I, Fig. 2). The remaining macrospores degenerate comparatively slowly in acropetal succession, occasionally lingering until the one-celled embryo sac stage (Plate I, Fig. 2). Development of the four macrospores in the tetrad was also observed in *S. officinalis*, as well as formation of a greater number of tetrads in the ovule, subsequently with a greater number of embryo sacs in *Stachys trinervis* (Kamelina & Dzevaltovsky 1987).

Development of the female gametophyte follows the *Polygonum*-type, the only type identified so far within the entire family *Lamiaceae* (Davis 1966, Kamelina & Dzevaltovsky 1987) and in most of the investigated species of genus *Salvia* (Carlson & Stuart 1936), particularly in those referred to the so-called *S. mellifera*-type.

After the end of nuclear division in the one-celled embryo sac, the formed central vacuole pushes the two resulting nuclei out towards the poles of the sac. The subsequent mitotic division and polarisation of the nuclei in the 2–4 celled embryo sacs respectively run normally.

In the 8-nuclear embryo sac, first differentiate the elements structuring the egg apparatus, which in their

morphology do not differ from those described for other investigated species of genus *Salvia* (Dzevaltovsky 1979; Daskalova 1997).

In the initial development stages of the embryo sac the egg cell is almost equal in size to the two synergids. In most of the observed embryo sacs it does not occupy a central position, but is slightly laterally shifted, most often overlaying one of the two synergids. The egg cell is pyriform, with a big nucleus and distinct vacuole. Most often it maintains direct contact with the two polar nuclei, respectively at a later stage of development of the embryo sac with the secondary nucleus, which after fertilization moves to the chalazal end of the embryo sac.

Similarly to the egg cell, the synergids are often also pyriform (Plate II, Fig. 3), but smaller in size. They are strongly narrowed towards the micropyle and cling haustorially to it. Synergids with hook-shaped outgrowths and a well-shaped filiform apparatus identified in other investigated species of family *Lamiaceae* (Dzevaltovsky 1979) were not found in *S. nemorosa*.

Pollination and subsequent fertilization usually lead to faster degeneration of one of the two synergids, later on followed by the other too.

In the mature embryo sacs there are three antipodes, distinctly pyriform and comparatively large in size (Plate II, Figs 1, 2). At a later stage of their development, their lower apical part rounds up and strongly vacuolises (Plate II, Fig. 2), while their basal part strongly lengthens and most often haustorially clings to the chalazal part of the embryo sac (Plate I, Fig. 3; Plate II, Figs 1, 3). In the process of our investigations we have established several patterns of arrangement, but most often two antipodes were positioned side by side, while the third one lay under them. Contrary to other authors that have studied some species of family *Lamiaceae* (Dzevaltovsky 1979), we have found that the antipodes of *S. nemorosa* are not ephemeral, but often linger until the outset of endospermogenesis that starts in the chalazal part of the embryo sac.

Development of the female gametophyte in *S. nemorosa* has been accompanied by a more distinct lengthening of the chalazal part of the embryo sac and a stronger widening of the micropylar part (Plate I, Fig. 3). Consequently, the chalazal end of the embryo sac cavity shifts laterally and is placed under an angle to its micropylar end (Plate I, Fig. 3), contrary to the initial, almost ovate shape of the embryo sac (Plate II, Fig. 1). The two parts of the embryo sac are linked by a short narrow passage.

Plate I

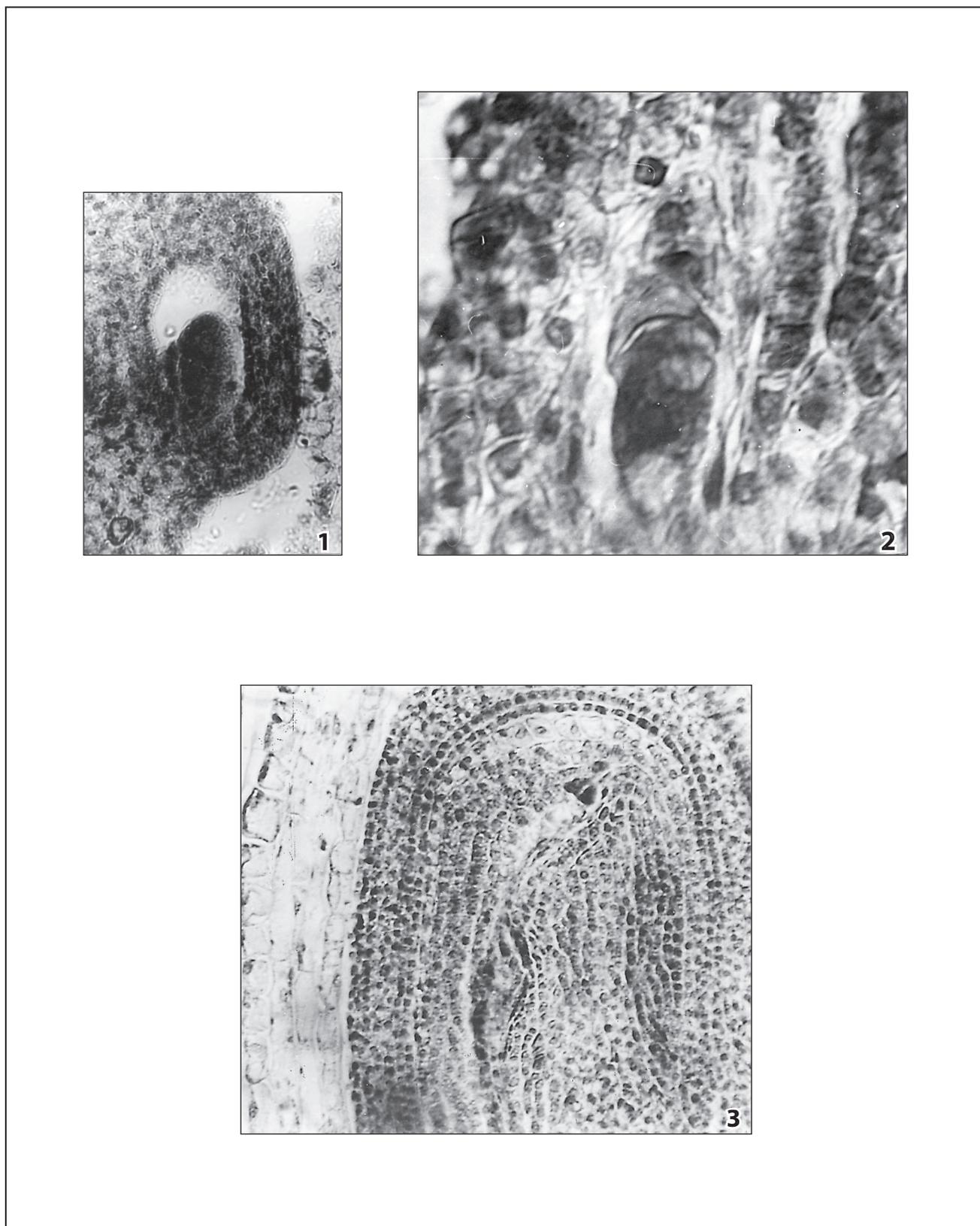


Fig. 1-3. Female gametophyte in *Salvia nemorosa*.

1, tenuinucellate unitemic ovule ($\times 160$); 2, one-celled embryo sac with degenerating macrospore above ($\times 500$); 3, mature embryo sac with two antipodes ($\times 250$).

Plate II

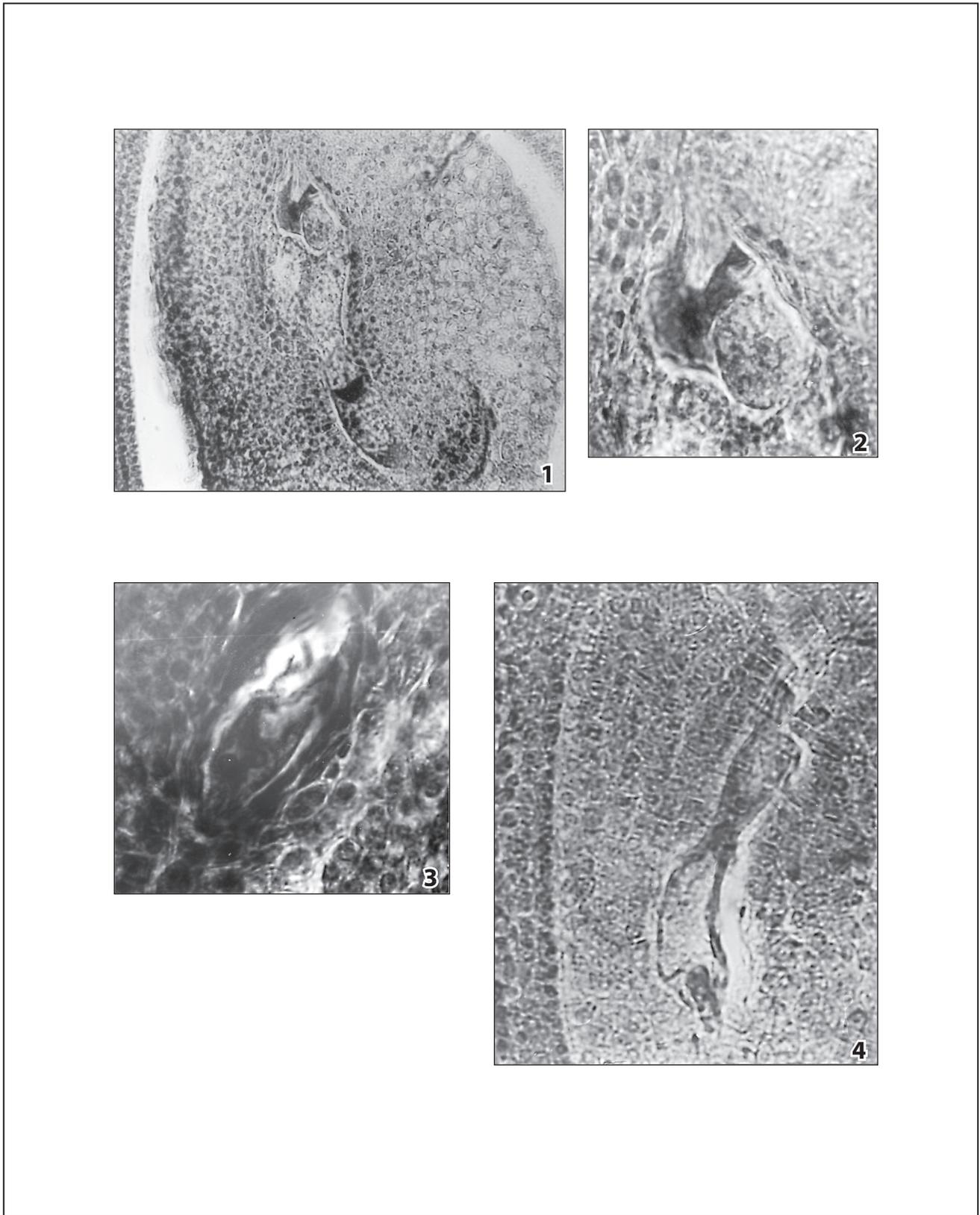


Fig. 1-4. Female gametophyte, embryo- and embryogenesis.

1, the ovate in shape embryo sac ($\times 350$); **2,** antipodes with vacuolising cytoplasm ($\times 600$); **3,** synergids ($\times 300$); **4,** two-celled proembryo and cellular endosperm ($\times 300$).

Differentiation of the integumental tapetum often runs at the stage of mature embryo sac. Most often and similarly to all investigated representatives of *Lamiaceae* (Wunderlich 1967), soon after fertilization it occurs only in the upper and chalazal part (Plate II, Fig. 4), as well as in the passage which connects it with the micropylar part. Thus it obstructs the quick growth of the embryo sac in its chalazal part, contrary to lateral development in the micropylar part, which subsequently determines the shape of the mature embryo sac. According to the reports of some other authors (Wunderlich 1967), against the background of the constant embryological characteristics in the female sphere of the flower in family *Lamiaceae*, this shape is specific for the different investigated species.

After the end of double fertilization processes in the embryo sac, division of the primary endosperm nucleus considerably precedes that of the fertilized egg cell. In *S. nemorosa*, similarly to all investigated species in family *Lamiaceae* (Davis 1966), the endosperm is cellular. Its development begins in the chalazal part of the embryo sac, where the fertilized secondary nucleus has moved (Plate II, Fig. 4). Its first division is accompanied by the formation of a transverse wall, with subsequent formation of a primary micropylar and chalazal cell. The latter consequently turns into a two-nucleate chalazal haustorium. The second division takes place by the formation of a transverse wall in the formed micropylar cell. The cell situated towards the micropyle turns into a two-nucleate micropylar haustorium, better developed in the later stages of development, as compared to the chalazal one. The further running of endospermogenesis and the development of the two terminal haustoria have shown that endospermogenesis follows the widest spread *Lamiaceae Prunella*-type (Wunderlich 1967), to which all species of genus *Salvia* have been referred to and especially those of the *S. mellifera*-type.

The quickly developing endosperm gradually disintegrates the remnants of the ovule integument, leaving only its outer epidermis in the mature seed to form the seed coat. Endosperm in mature seed is completely disintegrated by the developing embryo.

After the end of porogamous fertilization, contrary to the central cell, the zygote envelopes into a thick coat and usually considerably increases in size, the vacuole disappears, and it fills in with homogeneous cytoplasm.

Our observations of the embryogenesis in *S. nemorosa* have shown that it follows the *Onograd*-type,

widely spread in family *Lamiaceae* (Johansen 1950). The first division of the zygote starts by formation of a transverse wall, usually after the endosperm has considerably advanced in its development (Plate II, Fig. 4). Thus the formed apical cell also divides transversely, while the formed basal cell does not participate in the further formation of the embryo.

The embryo grows comparatively slowly and already in the globular and torpedo stage acquires a long uniseriate, multicellular suspensor to penetrate deep into the endospermal tissue. The basal cell of the suspensor is strongly elongated towards the micropyle and probably has a trophic function.

The embryo in the mature seed is straight, with well-formed cotyledons, each with an outgrowth tightly enveloping the hypocotyl and the radicle. On the strength of this it could be referred to the evolutionary most advanced *Investing*-type in *Lamiaceae* family (Wunderlich 1967) found in all so-far studied species of genus *Salvia* (Daskalova 1993, 1997).

In the course of macrosporogenesis, macrogametogenesis and development of the female gametophyte, we have observed some very distinct degeneration processes affecting single macrospore tetrads and elements of the embryo sac (most frequently the egg cell and the mature embryo sacs). Consequently, we have observed a rather high percentage of empty and sterile seeds in *S. nemorosa* (about 25%).

The distinct, above-described degeneration processes are probably a consequence of the trend towards transition into functionally female flowers established by us (Daskalova 1999). These, in turn, furnish another proof in support of the existing positive correlation of the processes between the male and female generative sphere of the flowers in malesterile plants (Laroche 1964).

Conclusion

During the detailed study of processes leading to seed formation in *S. nemorosa* conducted by us, some embryological structures have been identified, already known for other investigated representatives of genus *Salvia*. Along with this, the species differed from each other both in single insignificant variations of some embryological characteristics (morphological features of the elements forming the mature embryo sac and the time of their functioning, as well as the shape of the mature embryo sac), and in a number of essen-

tial characteristics of proven evolutionary importance within the framework of *Magnoliophyta* (the type of formation of the female archesporium and the degree of transition to malesterile plants).

The formation of one-celled female archesporium in the ovule of *S. nemorosa*, the *Prunella*-type endospermogenesis, the formation of an *Investing*-type mature embryo, and of functionally female flowers, all evidence the high degree of evolutionary development of this species, both within the framework of the genus and within the entire family *Lamiaceae*.

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