



# DEVELOPMENTAL SWITCH DURING EMBRYO SAC FORMATION FROM A BISPORIC MODE TO THE TETRASPORIC FRITILLARIA TYPE IN *HYACINTHOIDES VINCENTINA* (HOFFMANNSEGG & LINK) ROTHM. (HYACINTHACEAE)

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Developmental transitions between the major types of embryo sac formation, in which development starts according to one type and continues according to another type, have been reported seldom in the embryological literature. Here we describe in a sample of the rare taxon *Hyacinthoides vincentina* (a species of the Hyacinthaceae related to *H. italica*, sometimes considered conspecific with *H. mauritanica*) a bisporic start of embryo sac development and its tetrasporic continuation according to the Fritillaria type. The decisive events are the postmeiotic dissolution of the cell wall, which had been formed after the first meiotic division, the assumption of a 1+3 position of the spore nuclei in the germinating coenomegaspore, and the formation of a triploid chalazal spindle during the first embryo sac mitosis (Carano-Bambacioni phenomenon). The endosperm is pentaploid. While the bisporic *Hyacinthoides* type is considered characteristic for the genus *Hyacinthoides*, the Fritillaria type has not been found in the family hitherto. These observations add one further and particular example to the remarkable diversity of embryo sac types in the family Hyacinthaceae, in which the Polygonum, Allium, and Drusa-I types have been described, in addition to the *Hyacinthoides* type.

**Key words:** *Hyacinthoides*, embryo sac, *Hyacinthoides* type, Fritillaria type, developmental plasticity.

## INTRODUCTION

*Hyacinthoides* Medicus is a Mediterranean genus of the monocotyledonous family Hyacinthaceae, order Asparagales sensu Dahlgren et al. (1985). Among the later synonyms of *Hyacinthoides*, *Endymion* Dumortier and *Agraphis* Link have been more widely used. Baker (1873) included the genus in *Scilla* L. Two subgenera (Speta, 1987, 1998) are distinguished: *Hyacinthoides* with *H. hispanica* (Miller) Rothm. and *H. non-scripta* (L.) Chouard ex Rothm.; and *Somera* (Salisb.) Speta, including all other presently acknowledged species, that is, *H. aristidis* (Coss.) Rothm., *H. italica* (L.) Rothm., *H. kabylica* (Chab.) Rothm., *H. lingulata* (Poir.) Rothm., *H. mauritanica* (Schousboe) Speta, *H. paivae* S. Ortiz & Rodr.-Oubina, *H. reverchonii* (Degen & Hervier) Speta and *H. vincentina* (Hoffmannsegg. & Link) Rothm. Ortiz and Rodríguez-Oubiña (1996) treat the subgenera as sections. Heywood (1980) clarified the priority of the name *Hyacinthoides*.

The genus lends its name to the bisporic *Hyacinthoides* type of embryo sac development (Battaglia,

1958, as *Endymion* type). This type differs from the structurally similar *Allium* type in the competence of the micropylar dyad cell rather than the chalazal one, and in the prolonged survival and even some limited development of the incompetent dyad cell, called the "antigone" (Battaglia, 1958). In the mature state, both types are structurally indistinguishable from a *Polygonum*-type embryo sac. The *Hyacinthoides* type seems to be restricted in occurrence to this genus, with the exception of *Smilacina amplexicaulis* Nutt. ex Baker, a species of the *Smilacaceae* (Björnstad, 1970).

Vesque (1879) already gave a basically correct depiction, though an invalid interpretation, of embryo sac development in *H. non-scripta* (as *Agraphis nutans* Link) (Battaglia, 1958). Treub and Mellink (1880) are considered to be the first to correctly describe the type in *H. hispanica* (as *A. patula*), followed by Guignard (1882) in *H. non-scripta* (as *A. nutans*) and *H. hispanica* (as *A. campanulata*). Maheshwari (1937, 1948) recalled that Strasburger (1879) had already basically described this mode of embryo sac development in *Allium fistulosum* L. (but note that here the chalazal dyad cell

is the competent one). He suggested the name *Allium* type instead of *Scilla* type, the latter term having been in use because Baker (1873) had included *Hyacinthoides* in *Scilla*. Battaglia (1958) improved the terminology, correctly suggesting removal of the *Scilla* type and differentiation of the *Allium* type from the *Hyacinthoides* type (then termed *Endymion* type). Later studies on embryo sac development showed that the genus *Scilla* in the current wide circumscription is heterogeneous: *Polygonum*, *Allium*, and *Drusa* types have been found, and even within narrow taxonomic groups, such as in the *Scilla bifolia* L. alliance or the *S. siberica* Haw. in Andr. alliance (genus *Othocallis* Salisb.), more than one type occur (Svoma, 1981; Svoma and Greilhuber, 1988, 1989).

The present analysis adds, in *Hyacinthoides vincentina*, to the variety of embryo sac types in *Hyacinthaceae* and, as new to the genus *Hyacinthoides*, a unique variation of the tetrasporic *Fritillaria* type. In our material, development starts in the bisporic mode, followed by wall dissolution and a bipolar tetrasporic course with the Carano-Bambacioni phenomenon. This means that in the first embryo sac mitosis there is formation of a chalazal triploid spindle after assumption of the 1+3 position of the spore nuclei, the result being triploid nuclei in the chalazal part of the embryo sac (Carano, 1925; Bambacioni, 1928). A short report was given as a poster abstract by Ebert (1987).

#### MATERIALS AND METHODS

Several plants of undocumented natural origin, which upon identification represent *H. vincentina* (Hoffmannsegg & Link) Rothm., were cultivated originally as *H. italica* (L.) Rothm. in the Botanical Garden of the University of Vienna (HBV). The taxonomic identification as *H. vincentina* was made possible by morphological and chromosomal comparison with identified plants collected on 29 September 1987 by Christiane König, Vienna, from the *locus classicus* of *H. vincentina* in SW Portugal, Cabo de S. Vicente (compare: Coutinho, 1913), the karyotypes of which closely resemble the very characteristic C-banded karyotype found in the investigated individuals (Ebert, unpubl. data). A quantified C-band idiogram of the present material was given by König and Ebert (1997). This karyotype ( $2n = 16$ ) differs greatly in its C-band patterns from the heterochromatin-poor karyotype of *H. italica* from gardens and from the *locus classicus* in Italy (Ebert, unpubl. data). Herbarium specimens of the present material are deposited in the Vienna University Herbarium (WU).

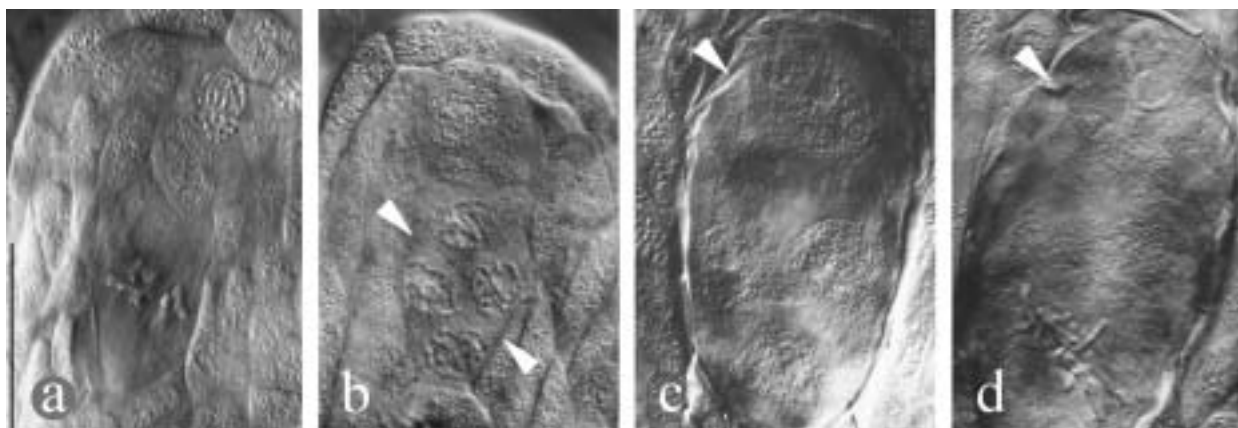
Flower buds of various stages of development were fixed in FPA50 overnight, transferred to 70% ethanol, and stored at  $-20^{\circ}\text{C}$  (FPA50: 36% formaldehyde, propionic acid, 50% ethanol, 5:5:90). Ovules were dissected, the outer integument removed if necessary, and transferred to a drop of Herr's clearing fluid (Herr, 1971) on a glass slide. Cover slips were mounted, supported to prevent damage to the ovules. Preparations were investigated under differential interference contrast (DIC) optics. Camera lucida drawings were made with a Zeiss Standard RA microscope using the Zeiss drawing device.

For detailed karyological investigations, previously cleared ovules of interest were rinsed in 96% ethanol and subjected to the Feulgen procedure, basically following Greilhuber and Ebert (1994). Embryo sacs were dissected in 45% acetic acid on a glass slide, gently flattened under filter paper, and photographed.\*

#### RESULTS

The ovules of *Hyacinthoides vincentina* (Hoffmannsegg & Link) Rothm. are bitegmic, crassinucellate, initially with two to four layers of parietal cells which are resorbed later during embryo sac expansion, and anatropous. The first meiotic division is accompanied by complete cell division, while the second is not (Figs. 1a,b, 2a,b). The completeness of the cell division after telophase I is well seen under DIC optics thanks to the thin cell wall that separates the dyad cells (Fig. 1b). Exceptions such as coenomegaspores with four nuclei in linear arrangement were not found. This course of meiosis conforms to the schedule of that bisporic mode of embryo sac development which hitherto has been found almost exclusively in the genus *Hyacinthoides*: the *Hyacinthoides* type. In the present material, however, further development takes a different course than known hitherto. The wall between the dyad cells is dissolved and a common cytoplasm is formed (Figs. 1c, 2c). The remnants of the original transversal wall emergent from the embryo sac wall can still be seen occasionally in later stages (Fig. 1c,d). Herewith the bisporic mode of development shifts over to the tetrasporic mode. The four nuclei assume the 1+3 position, with the single micropylar haploid nucleus being separated by a vacuole from the chalazal group (Figs. 1c, 2c, 3a). During the first embryo sac mitosis, the three chalazal nuclei form a common triploid spindle as is characteristic for *Fritillaria*-type embryo sac development (the Carano-Bambacioni phenomenon; Figs. 1d,

\*Because of the untimely death of the first author after a long sickness, the present work could be prepared for publication only now, without the participation of the main contributor. Due to the circumstances, the materials belonging to this work were only partially recovered, that is, the poster materials (Ebert, 1987) and photographic negatives, so that details on the number of observed stages cannot be given. Although the observations, and especially those on the course of meiosis, were reproduced in successive years, one should be aware that for any developmental stage the number of observations necessarily must have been low.



**Fig. 1.** *Hyacinthoides vincentina*, sporogenesis and early embryo sac development. (a) Nucellus with megaspore mother cell in metaphase I, (b) Postmeiotic dyad, oblique wall between cells (arrowheads), (c) Coenomegaspore with nuclei in 1+3 position, (d) Embryo sac in first mitosis, with haploid micropylar and triploid chalazal metaphase. Arrowheads indicate cell wall residues of the first meiotic division in (c) and (d). Cleared whole-mount preparations under DIC optics. Bar = 50  $\mu$ m.

2d, 3b). A secondarily four-nucleate embryo sac is formed, in which the two chalazal triploid nuclei are conspicuous by their size (Figs. 2d,e, 3c). The nuclei are arranged mostly along the embryo sac axis or oblique, but a transversal position has also been found. The more chalazal of the triploid nuclei or one of these nuclei, when transversally arranged, lags somewhat behind in the nuclear cycle; this is conspicuous during the prophase of the second mitosis (Fig. 3c). The second mitosis results in an embryo sac with four micropylar haploid and four triploid chalazal nuclei (Figs. 2f, 3d,e). The mutual orientation of the micropylar spindles is perpendicular and clearly indicates the destiny of the division products, that is, the two most micropylar synergids in the apex of the embryo sac, and below the egg cell and the haploid micropylar polar nucleus. An early stage of cell wall formation with phragmoplasts is shown in Figure 3e. In a later stage, the somewhat larger size of the egg nucleus and micropylar polar nucleus compared with the synergid nuclei is obvious (Fig. 3f). In the chalazal antipodal group, a tendency towards pycnosis is observed in the most chalazal sister nuclei (Fig. 3f).

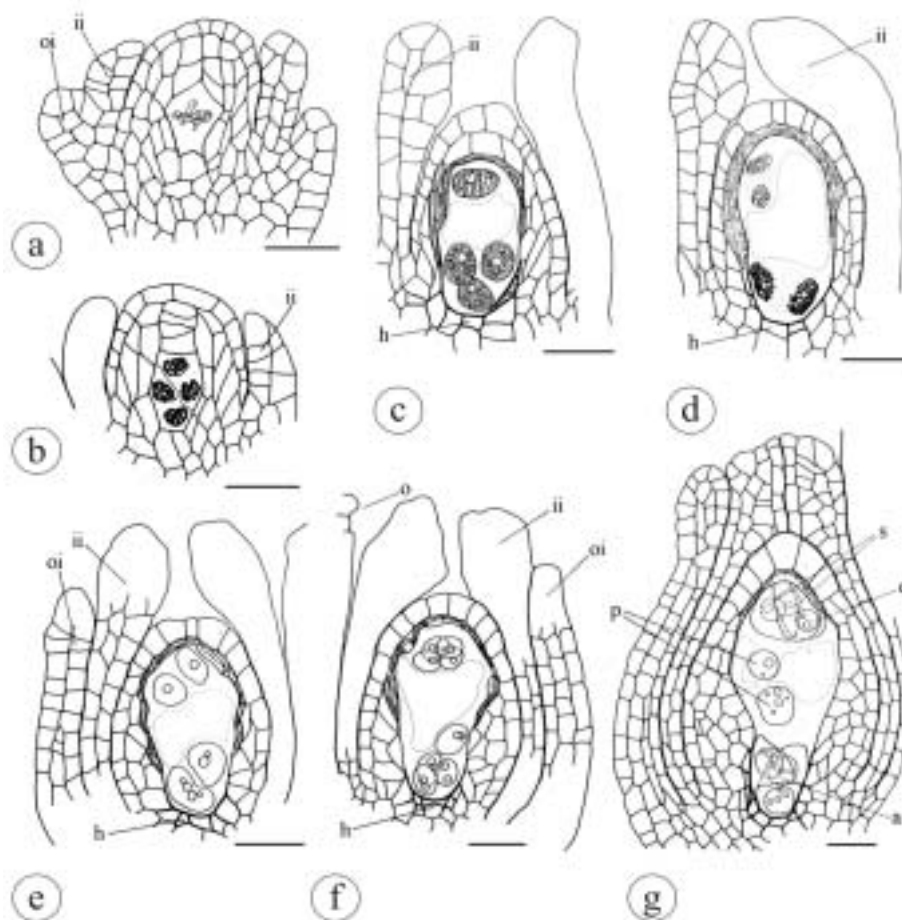
In the mature embryo sac, the polar nuclei are found close to each other more in the middle or more chalazal part of the central cell (Fig. 2g). Thus, the endosperm is expected to be pentaploid, and this was confirmed by DNA measurements (Ebert, 1987).

## DISCUSSION

Developmental shifts from one embryo sac type to another one have not often been reported in the literature. An example from the dicotyledons is the composite *Leontodon hispidus* L., in which Bergman (1935)

found, among normal plants with Polygonum-type development, one plant in which about half of the ovules formed tetrasporic embryo sacs by dissolving the cell walls between the megaspores. As regards Hyacinthaceae, in a tetraploid accession of *Prospero autumnale* (L.) Speta (as *Scilla autumnalis* L.), a plant taxon which normally has the Polygonum type, Battaglia (1959) found two plants with often 16 and rarely 14, 12 or 10 nuclei in the mature embryo sac. In meiosis, one, two or three cross-walls were formed, which were later dissolved. In these plants the further embryo sac development was irregular. Battaglia (1959) considered these plants to be embryological mutants. In some mature embryo sacs of *Scilla sibirica* (as *S. sibirica* Satô), Bhandari and Mal (1989) described "trabecula," which resemble the wall residuals as found here in *H. vincentina*. This species normally has Allium-type development (Svoma, 1981). Significantly, these deviating embryo sacs were abnormal in having seven or eight antipodal nuclei, while three – which could occur here in the same ovary – were normal. Although not interpreted that way by the authors, postmeiotic partial wall dissolution could have led to Drusa-type development. Possibly the higher temperature in India was responsible for these deviations from the normal course of development.

These findings represent aberrations, which seem to occur now and then in isolation in the angiosperm system at different frequencies. However, in certain species of Convallariaceae *sensu* Dahlgren et al. (1985), wall dissolution during megasporogenesis is a regular phenomenon. In a synopsis of older findings, Björnstad (1970) found ephemeral walls in species of *Polygonatum* Mill., *Smilacina* Desf., *Maianthemum* F. H. Wigg. and *Convallaria* L.; *Polygonatum verticillatum* (L.) All. and *P. multiflorum* (L.) All. show the Polygonum type,

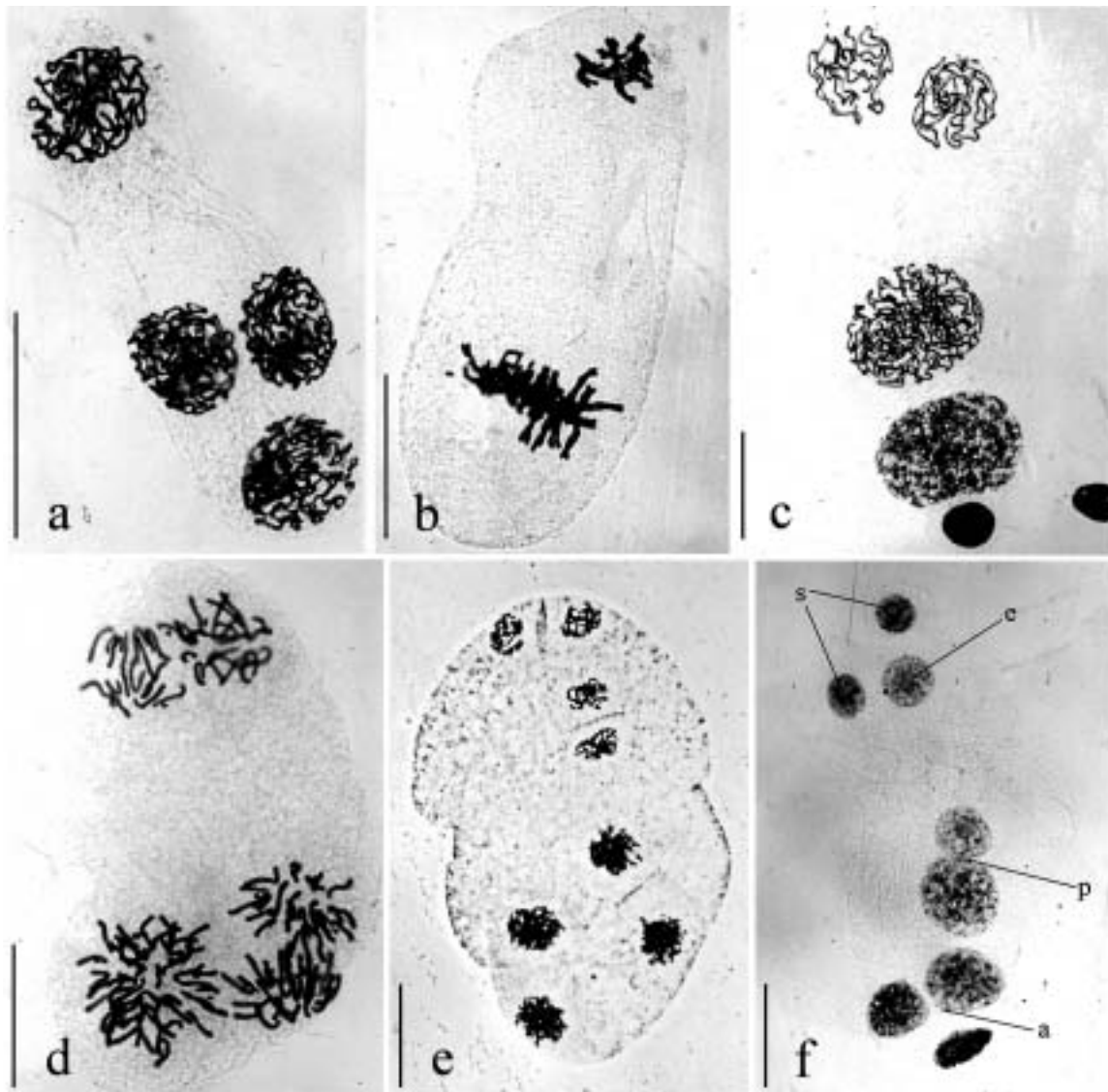


**Fig. 2.** *Hyacinthoides vincentina*, ovules, megasporogenesis and Fritillaria-type embryo sac development. (a) Metaphase I, (b) Telophase II, (c) Coenomegaspore, 1+3 position of nuclei, (d) Telophase of first embryo sac mitosis, secondary 4-nucleate stage following the Carano-Bambacioni phenomenon; (e) Secondary 4-nucleate stage, interphase, (f) Interphase following second embryo sac mitosis, (g) Organized embryo sac. ii – inner integument; oi – outer integument; o – obturator; a – antipodals; e – egg cell; s – synergids; p – polar nuclei. Camera lucida drawings of cleared whole-mount preparations under DIC optics. Bar = 50  $\mu$ m.

the latter with bi- and tetrasporic deviations. *P. odoratum* (Mill.) Druce, however, starts monosporic and continues with the Allium type, as does *Convallaria majalis* L. *Smilacina amplexicaulis* Nutt. ex Baker starts monosporic but continues with the Hyacinthoides type, in that the upper dyad cell develops. *Smilacina stellata* (L.) Desf. starts monosporic and continues with the Drusa type after all walls are dissolved. The same occurs in *Maianthemum bifolium*, as already found by Stenar (1934). In these plants, the walls destined to be dissolved are noticeably thinner than those not so destined (Björnstad, 1970).

The present report in *H. vincentina* adds a so-far unique example of a developmental switch from the bisporic mode of megasporogenesis (the premise of the Hyacinthoides type) to the Fritillaria type of embryo sac development, which as a consequence of its structure conditions a pentaploid endosperm. This is the

case here also (Ebert, 1987). Despite the limitation of the material, the observed sequence of unequivocal stages, from meiosis up to a pentaploid endosperm, suggests a stabilized program rather than an aberrant or pathological process. A different question, however, concerns the genetic uniformity of the natural population in regard to embryo sac development. One could hypothesize polymorphism for two types, Hyacinthoides and Fritillaria type, on a simple genetic basis. The bi-tetrasporic shift after meiosis then could indicate a heterozygous constitution, while the homozygous individuals would have either Hyacinthoides- or Fritillaria-type embryo sacs. However, such polymorphism would have consequences for the endosperm ploidy level, with possible negative consequences for fertility. Deeper insights could be gained only by a population study, which is desirable in any case.



**Fig. 3.** *Hyacinthoides vincentina*, Fritillaria-type embryo sac development. (a) Prophase of first embryo sac mitosis, 1+3 position of nuclei, (b) First embryo sac mitosis with Carano-Bambacioni phenomenon, (c) Secondary 4-nucleate stage, prophase, with the most chalazal nucleus in a retarded state (pycnotic nuclei at bottom are from the nucellus), (d) Anaphase of second mitosis, (e) Telophase of second mitosis, phragmoplast formation, (f) Organized embryo sac, showing egg apparatus, two polar nuclei of unequal size and chromatin content, and three antipodal nuclei, the two more chalazal sister nuclei being more strongly pycnotic than the sister nucleus of the triploid polar nucleus. e – egg cell; s – synergids; p – polar nuclei; a – antipodals. Feulgen staining after FPA50 fixation. (a–d, f) – bright field optics, (e) – phase contrast. Bar = 50  $\mu$ m.

The Fritillaria type is characteristic of genera in the Liliaceae *s.str.*, but elsewhere is of only sporadic occurrence. It seems that in the Hyacinthaceae the embryo sac types are less rigidly determined than their stability in many major taxonomic groups would suggest. Possibly the delicateness of the cell walls separating the megaspores or coenomegaspores is in *H. vincentina* a prerequisite for secondary spore fusions. In *H. vincentina*, the position of the nuclei in the dyad cells after wall dissolution leads by itself to a 1+3

position in the coenospore. The longer persistence of the micropylar dyad cell in the *Hyacinthoides* type may play a role in positioning the central vacuole, which upon spore germination separates the micropylar spore nucleus from the three chalazal ones in the fusion coenomegaspore. However, the precise Carano-Bambacioni phenomenon observed, rather than the expected more or less regular separate mitotic spindles in the chalazal part of the embryo sac, as characteristic for the Drusa type, is remarkable. We have to note that

nothing is known about the embryo sac types and endosperm ploidy status in the remaining seven species of *Hyacinthoides* subg. *Somera*.

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