Floral anatomy and micromorphology of *Hyacinthoides italic* (L.) Rothm. A case of complete stachyospory in Asparagaceae

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Abstract

Floral vasculature and gynoecium architecture were studied in *Hyacinthoides italic* (L.) Rothm. Each locule shelters two ovules inserted basally and surprisingly supplied by axis bundles only, while lateral bundles of the carpels branch out into strands along the epidermis of septal nectaries. We brought then to the fore an unexpected trade-off between the supply of upper ovules in *H. non-scripta* and that of secretory tissues in the studied species. Moreover, a new pattern of septal nectary is described, with outer and inner cavities.

Keywords: *Hyacinthoides italic*, Hyacinthaceae, Asparagales, carpel, floral anatomy, trade-off, vertical zonality of gynoecium

Introduction

The genus *Hyacinthoides* Heist. ex Fabr., previously nested in Hyacinthaceae (Kubitzki et al. 1998), is now placed in Asparagaceae (APG 2009; APG et al. 2016) and its phylogeny was recently revised (Grundmann et al. 2010). It comprises 11 species, growing around the western Mediterranean basin and Atlantic European regions as far as the British Isles and Netherlands to the north. Among them, *H. italic* (L.) Rothm. is well distinguished by its distribution area circumscribed to the maritime Alps of France and Italy. This species was previously named *Scilla italic* L. and *Endymion italic* (L.) Chouard. It is a perennial bulbous plant, blossoming from March to May in shady places (Coste & Flahault 1937), and often introduced for ornamental purposes.
The floral anatomy, especially gynoecial vasculature, was studied for near species by Van Tieghem (1875). Gatin provided additional data about pedicel and receptacle anatomy of Liliaceæ s.l., examined briefly *H. italica* (under *Scilla italica*) and emphasized the occurrence of “glandes septales” i.e. septal nectaries between the carpels (Gatin 1920). Septal nectaries are the result of a postgenital and partial fusion of contiguous carpels (Van Tieghem 1875).

**Material and methods**

A complete inflorescence of *H. italica* was collected at May 16th 2013 in the Botanical Garden at the Muséum national d’Histoire naturelle in Paris (48°50’36.521″ N, 2°21’36.839″ E) and fixed by FAA (90% ethanol 70%, 5% formalin, 5% acetic acid) for 48 h, then preserved in a mixture of water, ethanol and glycerol (equal volumes). Inflorescence structure, as well as those of six anthetical flowers (and gynoecium in two of them) were studied with a stereoscopic microscope (Fig. 1). Four flowers were dehydrated through a t-butyl series and embedded in paraffin (melting point: 58–60°C, Gerlach 1984). Serial transverse and longitudinal sections were cut at a thickness of 15 µm by a rotary microtome Leitz 1512 (Germany), then stained by Astrablue [Chroma® 1B 163] 0.5% aq. and Ziehl’s fuchsine [RAL® 320490-1000] 10% aq. All slides were mounted in Eukitt [O. Kindler GmbH® E0214]. Slides are kept in the plant histological collection of the Muséum national d’Histoire naturelle, Paris, under the range references Zalko 1–4.

Floral vasculature was reconstituted by drawings of the serial sections using a camera lucida, and then by superimposing tracing papers on them.

Succinct observations of *H. italic* flowers visitors were made in the Botanical Garden of Paris on March the 28th and the 1st of April 2017 in order to understand the workings of the different highlighted structures.

**Results**

**Anthetical floral morphology**

Each individual of *H. italic* builds a single blue-purple racemose inflorescence, bearing ca. 20 actinomorphic trimerous flowers, erect on long ascending pedicels, and axillate by two unequal bracts (Fig. 1 C). Each flower comprises six tepals in two whorls, six stamens and three carpels (Fig. 1 A, B, E, G–J). Tepals are fused together at their bases and stamen filaments are adnate to them along a short base (Fig. 1 A, B). Anthers are introrse. Ovary is externally very papillate – except for the short basal narrowed smooth region, which may be named gynophore (Figs 1 D, F, 2 E–I and 4 H, I), – while it exhibits six depressions i.e. prints of the stamens (Fig. 1 E). Ovary has three locules (Fig. 3 G), each with two anatropous ovules inserted at its base (Figs 1 D–F, 3 T, U and 4 H, I). Carpels are wholly fused in the lower part of the ovary (where ovules occur), and partially above (Figs 1 F, 3 T, U and 4 H). In cross section, narrow clefts are observed between the carpels, corresponding to outer opening of septal nectaries (Figs 1 E, 3 A and 4 C, E, F).

**Flower vascular anatomy**

In the pedicel, the stele is ordered in 6 main bundles in two alternate triangles, whose branching wholly provide above the perianth and androecium. Small intermediate bundles, variable in number, are visible too (Fig. 2 A). Tepals (t) and stamens (e) are
Figure 1. Morphological study of *Hyacinthoides italica* reproductive structures: A, B – anthetical flower (top and side views); C – top of the inflorescence; D – longitudinal-tangential section of gynoecium; E – transverse-median section of gynoecium at its basis; F – longitudinal-median section of gynoecium; G–J – floral buds just before anthesis, which were used for seriate paraffin sections.
Figure 2. Transverse ascending sections of the flower (A–J) and gynoecium (K–Q) of *Hyacinthoides italic*a, from pedicel to ovule insertion level: e – stamen; lc – lateral carpel bundle; mc – median carpel bundle; n – septal nectary (inner or outer); slc – synlateral carpel bundle; t – tepal. Dotted line delimits a central zone with small clear cells (E–J). Broken line is carpel epidermis at the center (P–Q). Arrows show structures quoted in the texte. Xylem in black, phloem left in white, all vasculature dashed when confused, secretory zones dotted (D–Q).
Figure 3. Transverse ascending sections of the gynoecium of *Hyacinthoides italica*, from the ovule insertion level to stigma (A–R) and longitudinal sections of ovary (S–U): same abbreviations as on Fig. 2.
supplied by one bundle each, their traces being fused in a common bundle. The two organs break up from the gynoecium together (Fig. 2 F–H), then become distinct (Fig. 2 I). Gynoecium is supplied by ca. 12 unequal bundles (Fig. 2 G). Three bundles are wider and well distinct at the gynophore level (Fig. 2 G, H, arrows), each of them branch off above in a median carpel bundle and two mediolateral ones (Fig. 2 I, J, mc, mlc), all of them becoming peripheral inside the ovary wall (Fig. 2 K), just below the locules. At the center of gynoecium (i.e. placenta) remain three synaxial bundles (Fig. 2 L, arrows) and three synlateral carpel bundles (Fig. 2 M, slc). Each synaxial bundle divides in two axial ones (a), which get closer to the ovules (Figs 2 O–Q, arrows, and 3 A), and at least supply them (Figs 3 B and 4 B, arrows). So the two ovules of a same carpel are fed by a single axial bundle, while each carpel synlateral bundle (slc) divides in two lateral ones (Fig. 2 M, N, lc), whose branches do not irrigate any ovule, but the inner part of the septal nectaries obviously outlined by the epidermises of the adjacent carpels (Figs 2 P and 3 C). Lateral carpel bundles branch out into five pairs of secondary strands which enter progressively the carpel walls, the first pair diverges rather low (Fig. 2 M, arrows), four lateral bundles are seen above (Fig. 3 E, arrows), while the first diverging bundles on each side fuse again (Fig. 3 G, H, arrows). All this vascular network closely surrounds the septal nectaries (Figs 2 P and 3 A–J). Carpel epidermises, which are more or less confused at the center of ovary (Figs 2 P, Q and 3 A–D), are more distinct at the level of the ovule apices (Fig. 3 E–H), and wholly separate above (Fig. 3 I). So all carpels open in a single ovary locule (Figs 3 I–P and 4 H–I), trilobate in cross section and gradually decreasing toward the stylar zone (Fig. 3 I–P). At the top of the ovary, the last three secondary lateral carpel bundles wholly fused (Fig. 3 L, M, U), on each side of the three clefts between the carpels and then fade above (Fig. 3 M, N, arrows). As usual, only median carpel bundles occur in the style (Fig. 3 N–Q), fading at the level where stylar canal is filled by secretory cells.

Vascular architecture of the gynoecium of *H. italica* is sketched on Fig. 5 A.

**Flower micromorphology and gynoecium structure**

Secretory tissues are recognized basally, between the tepals (Fig. 2 D–I), between stamens and gynoecium (Fig. 2 E–H) and between tepals and stamens (Fig. 2 G, H). All these secretory regions stained deeply with fuchsine (Fig. 4 A). Outer clefts of the ovary wall, related to septal nectaries (n), are distinguished just below the level of the locules floor (Fig. 2 I) and their covering epidermis is secretory shortly above (Figs 2 J, 3 S and 6, NSE). A septal nectary is wholly visible in tangential section (Figs 3 T, 4 H and 5, NSI). Just above the ovules insertion, carpel epidermises are well distinguished in the central placentary region (Figs 2 P and 4 D, arrow). Outer and inner parts of the septal nectaries join at the top of the ovary (Figs 3 K–M and 4 F, H, arrow) in a
wide secretory cavity (Fig. 3 U, n). Septal nectaries extend all along the style as outer clefts, but devoid of any secretory activity (Fig. 3 N–Q).

As complete sections ranges were obtained, it is easy to characterize and measure the different morphological zones after the extent and degree of fusion between the carpel epidermises, septal nectaries resulting from their separation combined to a secretory activity.

Five zones are recognized here: a short ovarian base (or gynophore, BO), below the floor of locules; a synascidiate zone (ZS), from the floor of locules to that of the inner part of septal nectaries (NSI); a hemisynascidiate zone (ZHA) until carpel epidermises are distinct at the center of the placenta; a second hemisymplicate zone (ZHP) from the separation of these epidermises to the junction of the external and inner parts of nectaries (NSE and NSI); at least an asymplicate zone (ZA) comprising style and stigma. Relative proportions are: BO = 6.5 %, ZS = 8 %, ZHA = 14.5 %, ZHP = 12 % and ZA = 59 %, which are to be compared with those in H. non-scripta: BO = 6 %, ZS = 11 %, ZHP = 15 %, ZA = 68 % (Deroin 2014).

**Flower visitors**

Basal secretory regions – between stamens and the gynoecium (Figs 2 E–H and 4 A) – appear, after field observations, to be exploited mainly by Apidae (*Apis mellifera* L., 1758) (Fig. 7). On the other hand, upper parts of the septal nectaries are exploited by Syrphidae.

**Discussion**

Our results strengthen the receptacle vascular architecture of *H. italica*, previously described by Gatin (1920), as well as the occurrence of minute peripheral

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**Figure 5.** Comparative vascular diagrams for: **A** – *Hyacinthoides italica* and **B** – *H. non-scripta* (after Deroin 2014):

- **a** – ovular bundle from the floral axis;
- **mc** – median carpel bundle;
- **mlc** – mediolateral carpel bundle;
- **n** – septal nectary (inner part);
- **slc** – synlateral carpel bundle. All **dotted bundles** are considered homologous.
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Figure 6. Vertical zonality of *Hyacinthoides italica* gynoecium. BO – ovary base; NSE – external part of septal nectary; NSI – inner part of septal nectary; ZA – asymplicate zone; ZHA – hemisynascidiate zone (from the distinction of carpel epidermises at the center of the placenta to their separation) carpel epidermises are shown in dots; ZHP – hemisymplicate zone (from separation of central epidermises to fusion of outer and inner parts of septal nectary); ZS – synascidiate zone (from the lowest locule level to ovule insertion).

bundles in the pedicel whose meaning remains yet unclear.

Ovules are supplied straight by the floral stele (Figs 2 O, P and 4 B, arrows), in other words they have no vascular connection with lateral bundles of the carpels. They are homologous to the basal ovule pairs in *H. non-scripta* (Deroin 2014), and demonstrate here a surprising total stachyospory in Asparagaceae. Using a vascular criterion appears here to us quite relevant for
defining the right ovule nature (cauline or carpellary): ovular bundles originate from the stele just below the boundary between synascidiate and symplicate zone, where funicles are inserted (Fig. 6). So, inside the Angiosperms, the distinction phyllospory/stachyospory, suggested yet during the last century (Lam 1950), is somewhat valuable, at least for the gynoecium, and these both conditions being in no way exclusive, as we observed in *H. non-scripta* (Deroin 2014). The most recent paleobotanical researches (Wang 2010) suggested strongly the putative angiosperm ancestors should be likely stachyosporous, with carpels only sheltering cauline ovules. Transfer of some or all ovules to the carpel margins should be secondary, perhaps improving feeding (especially during the fruit set) and allowing a better link with stigmatic and stylar regions (during the pollination and fertilization processes).

Furthermore, a comparison of the gynoecial vascular diagrams of *H. italica* and *H. non-scripta* (Fig. 5) makes obvious lateral bundles play different roles, despite their homology. In *H. non-scripta*, the upper 3–4 ovules pairs are supplied by secondary lateral branches from the lateral carpel bundles, which in *H. italica* irrigate the septal nectaries by two changes: 1) they deviate to external layers of the ovary wall; 2) they build a vascular network along and below the nectarial epidermises.

In such a context it appears yet uneasy to ascertain the cauline ovules are the ancestral condition in *Hyacinthoides*, or they

Figure 7. Bees (*Apis mellifera*) prospecting on flowers of *Hyacinthoides italica* grown in Jardin des Plantes, MNHN Paris, 1st April 2017. Scales: 1 cm.
are a reversion from a classical placentation with carpellary ovules only.

Septal nectaries of *H. italic* were not described by Daumann (1970), who worked within Monocotyledons clade. Moreover, it is noticeable this kind of nectary was never observed before. Indeed, internal septal nectaries were reported in *Scilla* L. (a genus close to *Hyacinthoides*) as well as external ones (in e.g., *Sabal* Adans., Arecaceae family), but not the both together. Conferring to the above mentioned specific conformation in *H. italic* we suggest the recognition of a new pattern of nectary.

These nectaries are more secretory (Fig. 6) in comparison with those of *H. non-scripta* (Deroin 2014). In fact, homologous lateral carpel bundles seem to play an important role in the secretions within the gynoecium (Fig. 5). We can infer the existence of a trade-off (Garland 2014) between the vasculature of the nectaries versus the supplementary ovules pairs between *H. italic* and *H. non-scripta*. According to this hypothesis, the two functions could not be provided concurrently.

Brief *in vivo* observations showed that two distinct regions of *H. italic* flowers are prospected by insects: one between the stamens and the gynoecium and the other at the septal nectary level. The first zone is visited by *Apis mellifera* (Fig. 7) and corresponds to the gynophore, which is smooth, unlike the papillosse gynoecium (Fig. 1 D–F). Therefore, an accumulation of exudates in this region could create a reserve for potential visitors. Furthermore, Syrphidae seem to feed on external parts of the nectaries. Thus we can wonder if a second type of trade-off could exist, namely a resources reallocation between the different secretory zones in both species.

The analysis of the gynoecium structure reveals that the three carpels are irregularly fused. Indeed, *H. italic* is neither syncarpous (carpels are incompletely fused, due to the septal nectaries), nor apocarpous (carpels are not entirely isolated from each other). This peculiar gynoecial pattern was described by Leinfellner (1950), through the frame of vertical zonality, a concept which has recently been examined and widely developed (e.g., Novikoff & Odintsova 2008; Dyka 2013; Odintsova et al. 2013; Fishchuk & Odintsova 2014). According to that, the gynoecium is subdivided in different regions, depending on the type and the degree of carpellary epidermis fusion. In *H. italic*, four zones are noticeable (from the bottom to the top): 1) synascidiate (ZS); 2) hemisynascidiate (ZHA) 3) hemisymplicate (ZHP) and 4) asymplicate (ZA). This description could match with the hemisyncarpous gynoecium characterized in Leinfellner (1950), if no synascidiate zone was reported and if a symplicate zone was found in *H. italic*. Nevertheless, the structure of the gynoecium seems closely similar to the hemisyncarpous type, thus we choose to consider it as a hemisyncarpous gynoecium sensu lato. This conclusion was also established for *Dracaena fragrans* (L.) Ker Gawl., *Sansevieria parva* N.E. Brown and *S. trifasciata* Prain in Odintsova et al. (2013) and for *Aechmea fulgens* var. *discolor* Morr. and *Pseudananas sagenarius* (Arruda) Camago whose gynoecium vertical architecture is exactly the same as *H. italic* (Novikoff & Odintsova 2008). Indeed, those three species have an identical structural type of hemisyncarpous gynoecium s.l.: type A, that corresponds to the consecutive zones cited above (Novikoff & Odintsova 2008).

However cauline ovules appear to be linked to the synascidiate zone (ZS), where carpel margins are wholly fused, while carpellary ovules – if any – are inserted in
the hemisymplicate zone (ZHP), where carpels are partially fused. It is noticeable all these structural features are tightly linked to the septal nectaries outline (related to pollination biology), also foreshadowing the fruit dehiscence (related to dispersal), as previously seen in *H. non-scripta* (Fig. 6 in Deroin 2014). Thus gynoecial anatomy (especially specialized tissues and vascular architecture) is to be interpreted in a flower-fruit continuum.

**Conclusions**

As emphasized by several recent studies (Novikoff 2008; Novikoff & Odintsova 2008; Novikoff & Kazemirska 2012; Dyka 2013; Odintsova 2013; Odintsova et al. 2013; Fishchuk & Odintsova 2014; Deroin 2014) structural features of Monocot flower are far from being fully understood, and much work remains to do before all of them are recognized, described and their relationships drawn up or at least sketched. The anatomy of the anthetical flower is to be considered as evolving from developmental processes, functioning for pollination as well as fertilization, and foreshadowing the fruit set stage including dispersal events. In this scope vertical zonality concept reveals as a highly significant morphogenetical frame, allowing to put seemingly static features (e.g., vasculature and secretory tissues) in a dynamical context. A complete stachyospory appears to occur in *H. italica*, strengthening the partial stachyospory previously recognized at the level of basal ovules in *H. non-scripta*. A revision of placentary vasculature in and beyond Asparagaceæ is urgent and should result in a better morphological definition of the angiospermous carpel, including the likely – and until now controversial – role of an axial contribution to the emergence of syncarpy. In some way, the stachyospory/phyllospory transition (supported by vasculature) should be paralleled by the apocarpy/syncarpy one (supported by vertical zonality), the both explaining the complex gynoecial pattern of Asparagaceæ. It is noteworthy anatomy could support in some cases this occurrence of angiospermous stachyospory, often suggested by morphogenetical studies (see e.g., Payer 1857 and Moeliono 1970).

In this morphological frame, the functional role of the nectariferous structures in the *H. italica* flower cannot be asserted with too few observations. However, we can postulate the evolutive advantage that could endow the flower with the possession of different secretory areas, permitting the attraction of different types of pollinators.

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**References**
