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FLORAL VASCULAR PATTERNS OF THE DOUBLE-FLOWERED AND WILD-TYPE MORPHS OF *NIGELLA DAMASCENA* L. (RANUNCULACEAE)

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Abstract. The perianth of the double-flowered morph of *Nigella damascena* L. consists of spirally inserted petaloid sepals and sepal-like organs, similar in shape and colour to the petaloid sepals of the wild-type flower. It is devoid of petals. We compare the vascularization of each organ category of the double flower with that of the wild-type. We show that the vascular patterns of the sepal-like organs and of the petals are identical, and found an inverse relationship between the number of bracts and the number of sepals in the double-flowered morph. These two surprising findings will influence the future evo-devo studies on this plant model.

Key words: *Nigella damascena*, double flower, flower anatomy, merism, nectary, perianth

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Introduction

Two floral morphs can be found in *Nigella damascena* L. The wild-type morph has a bipartite perianth with five petaloid sepals and five to ten nectariferous petals, whereas the double-flowered morph has a perianth with many petaloid sepal-like organs, exhibiting more stamen characteristics as they are positioned closer from the androecium (Gonçalves *et al.* 2013). No major differences in the organization (number and arrangement) of the fertile organs have been noticed between the two morphs. The double-flowered morph has been known since the premises of the 17th century (Clusius 1601), and has been reported and described in many publications since then (e.g. Linnaeus 1753; Hoffmann 1875; Blaringhem 1910). The genetic control of this floral dimorphism has been first investigated by Toxopéus (1927), who showed using segregation studies that a single bi-allelic locus was involved, with the petalous form (homozygous *PP* and heterozygous *Pp* genotypes) dominant over the apetalous one (homozygous *pp* genotype). Recent studies showed that the dimorphism was controlled by the *NdAP3-3* locus, a B-function gene (Gonçalves *et al.* 2013; Zhang *et al.* 2013). Gonçalves *et al.* (2013) showed that, in addition to its role in petal identity specification, this gene plays a role in meristem patterning.

At the adult stage, sepal-like organs and sepals are morphologically similar. Moreover, previous developmental studies highlighted the fact that inner sepal-like primordia and outer stamen primordia are morphologically undistinguishable (Payer 1857; Jabbour *et al.* 2009; Zhao *et al.* 2011; Gonçalves *et al.* 2013; Jabbour *et al.* in press). In this context, the objective of this study is to describe for the first time the vascularization of the numerous petaloid sepal-like organs found between the sepals and the stamens in the double flower, to examine whether it is more similar to that of sepals, petals, or stamens. To do so, we compare the anatomy of the floral organs in anthetic flowers of the wild-type and the double-flowered morphs of *N. damascena*.

Material and methods

Plant material

Seeds from progenies homozygous for the *P* or the *p* allele were sown. Plants were grown in a growth chamber under long day period (18h

day/6h night) at 25°C during the day, 16°C during the night and 60% relative humidity. Flowers were fixed in FAA (90% ethanol 70%, 5% formalin, 5% acetic acid), and then stored in a mixture of water, ethanol and glycerol (equal volumes).

Anatomical observations

Two flowers from each morph 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 10 µm by rotary microtome Leitz 1512 (Germany), then stained with Astrablue 0.5% aq. and Ziehl's Fuchsine 10 % and mounted in Eukitt. Floral vasculature was reconstructed by drawing the serial sections using a camera lucida. Moreover, six and four flowers were dissected to check organ arrangement and number in the double flower and the wild-type, respectively.

Results

***Comparison of the floral anatomy of the wild-type and double-flowered* Nigella damascena**

Ascending series of cross sections through anthetic flowers of both morphs (Fig. 1 A-N) allowed us to reconstruct the vascularization of every floral organ and to locate precisely the point where the traces get divided into the vascular bundles that are observable in the different organs (Fig. 2 A, B; Fig. 3 A, B).

The bracts of both morphs have three vascular bundles originating from the same point at the base of the floral receptacle (Figs. 2 A, 3 A). Sepals in the [P] morph have five vascular bundles, originating from three independent traces, the two lateral ones being divided into two just before entering the sepal. In the double-flowered morph, five vascular bundles still characterize the sepals, but the median trace fuses relatively deeply with one of the two lateral ones, resulting in two independent traces at the level of the receptacle. In this morph, the number of bracts seems correlated with the number of sepals, the first being lower when the second is higher. Petals (Fig. 2 B) and sepal-like organs (Fig. 3 B) have three vascular bundles originating from the same trace. Stamens have a single vascular bundle.

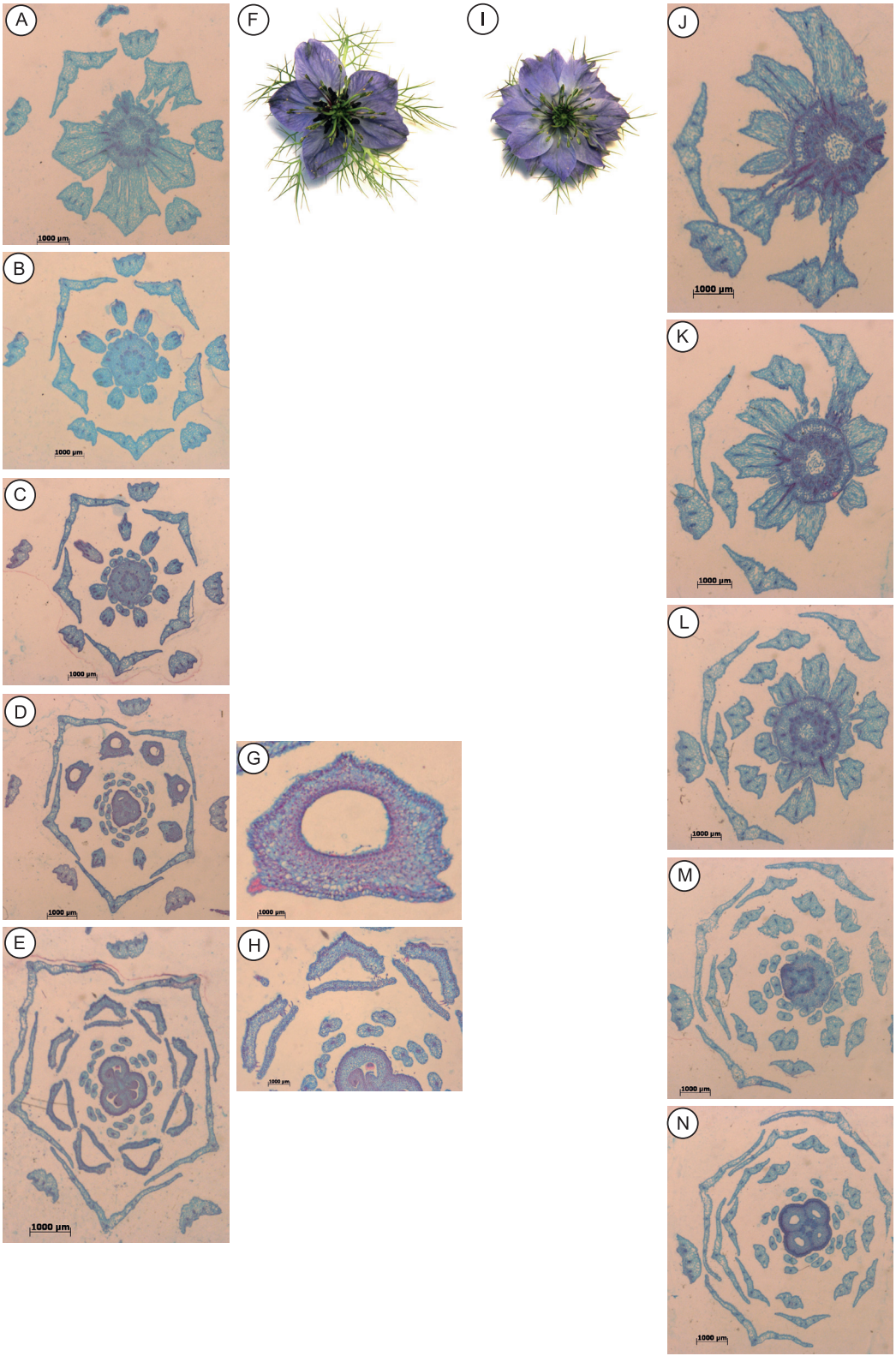
Nectariferous cells with outsized nuclei are visible on Fig. 1 G. They are distributed all around the cavity at the base of the petal pouches. Fig. 1 H shows trichomes inserted on the margins of both lips of the petals, and growing in the pouch.

***Comparison of the floral organization of the wild-type and double-flowered* Nigella damascena**

Floral organs of both morphs initiate on a spiral. Wild-type flowers generally consist of five bracts, five sepals, five to ten petals, c. 25 stamens, and three to five carpels. In the wild-type flower we investigated (Fig. 1 A-H), the perianth consists of five sepals in a quincuncial aestivation, and eight nectariferous petals. Twenty stamens are organized into eight parastichies. The gynoecium is trimerous.

In contrast, in the double flower of *N. damascena*, and according to the type of vascularization, we identify only three sepals and 17 sepal-like organs with a vascularization similar to that of the petals of the wild-type. The sepal-like organs are inserted along the eight parastichies of the 17 stamens. The gynoecium is tetramerous. Based on observations of the visible vascularization made on six additional flowers from the double-flowered morph (Fig. 4), we noticed that the flowers had three sepals when the number of bracts ranged from one to three, two sepals when there were four bracts, and a single sepal when five bracts were present.

Fig. 1. Ascending series of cross-sections through the anthetic flower of *Nigella damascena* wild-type (**F, A-E, G, H**) ▸ and double-flowered (**I, J-N**). **A-E** – Five bracts surround five sepals, which cover eight nectariferous petals. The eight parastichies of stamens and the trimerous gynoecium are clearly shown in **D** and **E**; **G, H** – Magnifications of the petals from the pictures **D** and **E**, respectively; **J-N** – A single bract subtends the flower, and three sepals (clearly visible in **L**) surround the sepal-like organs (with three vascular bundles) and the stamens (with a single vascular bundle). The gynoecium is tetramerous (**N**).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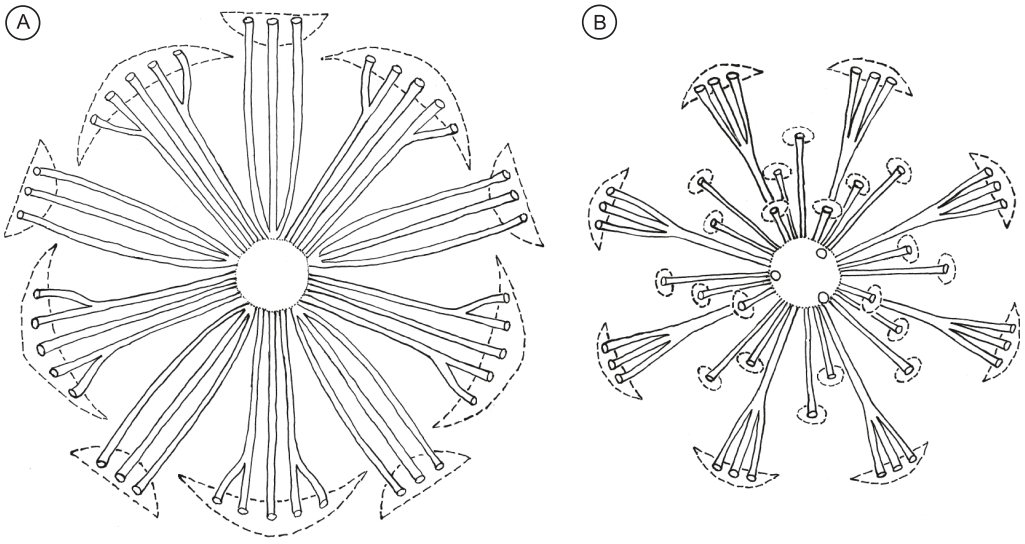

Fig. 2. Reconstruction of the flower vascularization of *Nigella damascena* (wild-type). **A** – Bracts (three vascular bundles) and sepals (five vascular bundles). **B** – Petals (three vascular bundles) and stamens (a single vascular bundle). The position of the carpel median bundles is shown.

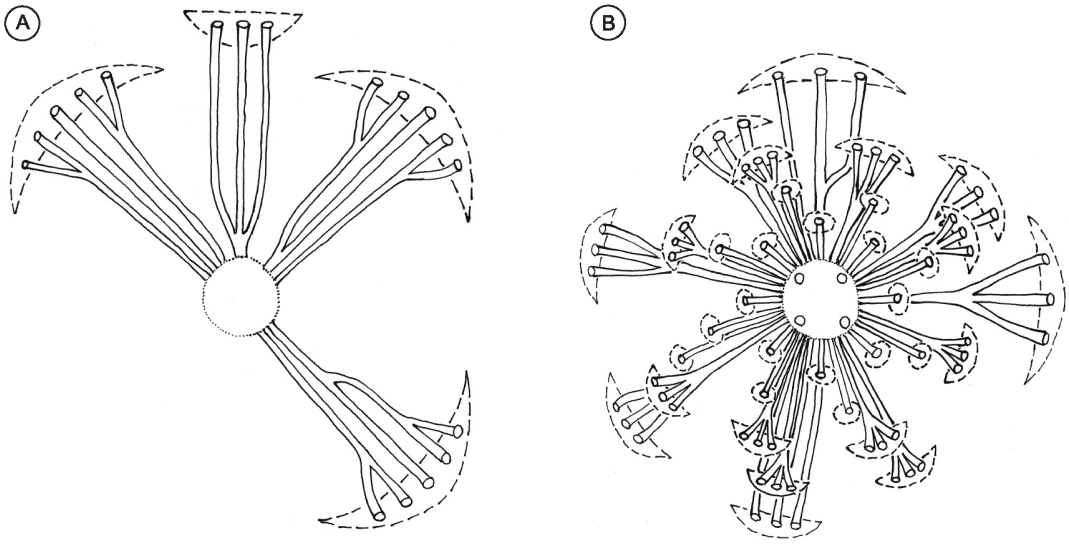

Fig. 3. Reconstruction of the flower vascularization of the double-flowered *Nigella damascena*. **A** – Bract (three vascular bundles) and sepals (five vascular bundles). **B** – Sepal-like organs (three vascular bundles) and stamens (a single vascular bundle). The position of the carpel median bundles is shown.

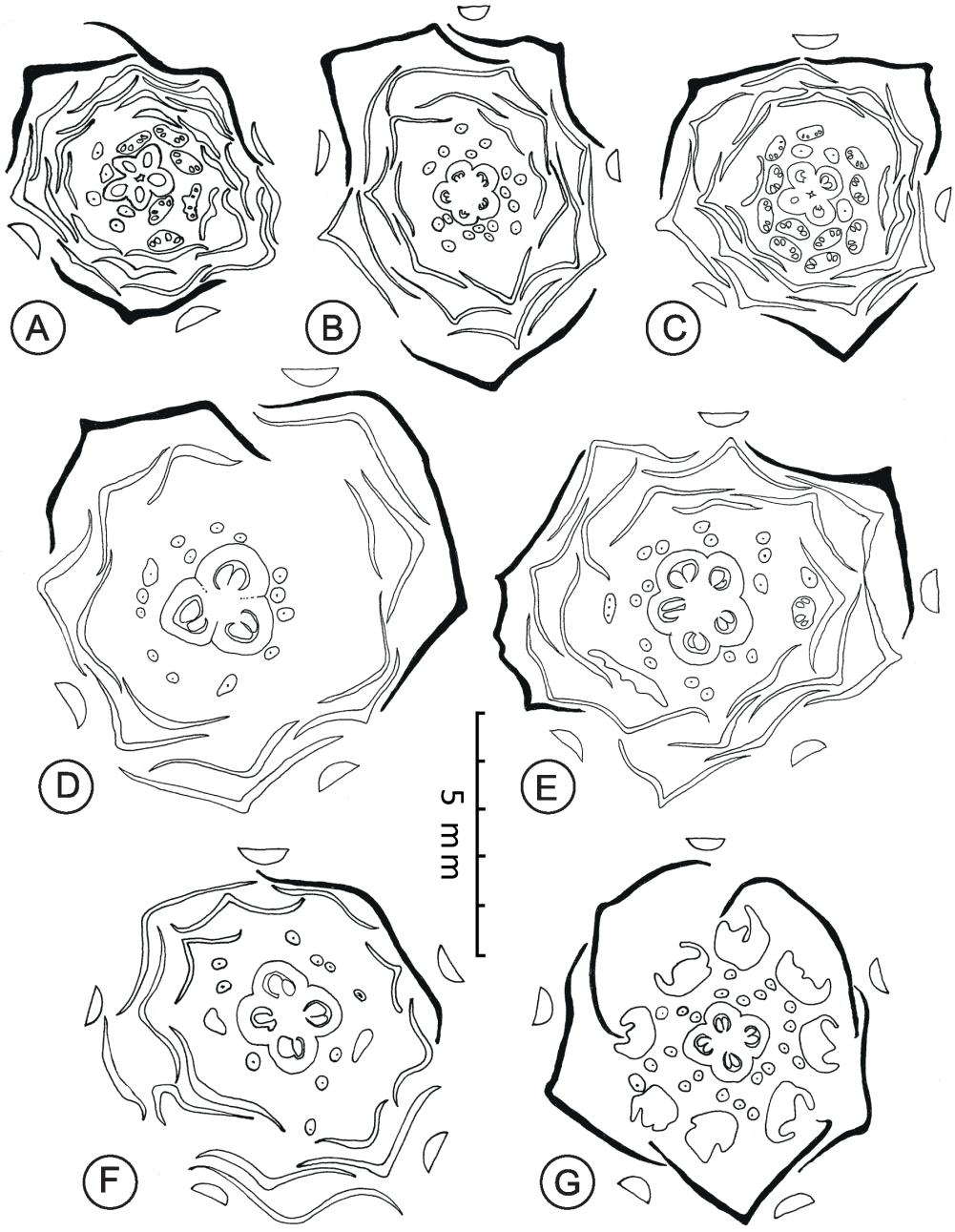

Fig. 4. Hand cross-sections of some double flowers of *Nigella damascena* (**A**-**F**), arranged in increasing bract numbers, sepals in black; **G** – wild-type flower for comparison.

The floral formulas of both morphs, according to morphology (✳ = actinomorphic flower, B = bract, K = calyx (number of sepals), C = corolla (number of petals; superscript = petaloid), A = androecium (number of stamens), G = gynoecium (number of carpels), $\underline{G}$ = superior ovary, "+" indicates when several whorls of the same organ can be distinguished, see RONSE DE CRAENE *et al.* 2014) are the following:

Wild-type flower examined:

✳ B5 K^c5 C8 A20 $\underline{G}$3

Double flower examined:

✳ B1 K^c20 A17 $\underline{G}$4

Thanks to the anatomical observations, the 20 petaloid sepals and sepal-like organs could be broken up into three petaloid sepals, a single petaloid sepal-like organ with a vascularization showing features of both sepals and petals, and 16 petaloid sepal-like organs with a vascularization identical to that of the petals of the wild-type. The floral formulas, according to anatomy (the number of vascular bundles is specified with subscripts, and the level of the subdivision of the initial trace(s) is noted) could then be written as follows:

Wild-type flower examined:

✳ $B_3$5 $K^c_{3\Rightarrow5}$5 $C_{1\Rightarrow3}$8 $A_1$20 $\underline{G}$3

Double flower examined:

✳ $B_3$1 $K^c_{3\Rightarrow5}$1+$K^c_{2\Rightarrow5}$2+$K^c_{2\Rightarrow3}$1+$K^c_{1\Rightarrow3}$16 $A_1$17 $\underline{G}$4

Discussion

The vascular patterns found in flowers of *N. damascena* wild-type and double-flowered are consistent with the typical patterns found in Ranunculaceae. For instance, SMITH (1928, *Ranunculus* and *Caltha*), BROULAND (1935, *Nigella diversifolia* Franch.) and NOVIKOFF & JABBOUR (2014, Delphinieae) observed five vascular bundles originating from three traces, three vascular bundles originating from a single trace, and a single undivided trace in the sepals, petals, and stamens, respectively.

The double-flowered morph of *N. damascena* lacks petals, but possesses petaloid sepal-like organs located between the calyx and the androecium. Even though our observations should be confirmed on a larger sample, it is worth noting that the vascularization of the sepal-like organs looks more similar to that of the wild-type petals than to that of the sepals. Hence, even if the sepal-like organs and especially the most external ones look morphologically similar to sepals, they are anatomically (this study) and genetically (GONÇALVES *et al.* 2013, JABBOUR *et al.* in press) distinct. A similar observation was made by SMITH (1928), when describing the vascularization of the supernumerary sepal-like organs in a double flower of *Caltha palustris*. These organs did not have five vascular bundles as in the true sepals, but three, originating from a single trace.

In this article, a possible negative correlation between bract number and sepal number in the double-flowered *N. damascena* is reported. Previous studies did not notice this, and considered that the calyx was composed of five sepals (GONÇALVES *et al.* 2013). In their defence, as a reduced number of sepals was not expected in the double-flowered morph, it is understandable that the authors may have thought that some external sepal-like organs (or primordia, when looking at meristems) were sepals.

The organization of the androecium in eight parastichies was already described by PAYER (1857) and ZHAO *et al.* (2011), among others. In the two flowers we observed, the number of carpels was different (three for the wild-type, four for the double flowered morph). Gynoecium merism is variable in *N. damascena* wild-type (PAYER 1857; BAILLON 1868) as in the double-flowered morph, ranging from two to five carpels (Fig. 5).

Conclusions

We described the vascular anatomy of the wild-type and of the double flower of *N. damascena*. Our study revealed that 1) the vascularization of the petals (wild-type flower) and of the sepal-like organs (double-flowered) is identical, and 2) in the double-flowered morph, the numbers of developing bracts and sepals seem inversely correlated. If confirmed on a larger sample of flowers in the two morphs, this

Fig. 5. Diversity of gynoecium merism in the double-flowered *Nigella damascena*.

relationship could bring novel perspectives for evo-devo studies of structural boundaries in the floral meristem of *N. damascena*.

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