OPINION PAPER

Spatial orientation of gynoecium in legumes and beyond: Commentary to the paper of Wang et al. (2021)

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A B S T R A C T

The third largest angiosperm family, Leguminosae, exhibits a relatively wide range of variation in morphology of gynoecium. Some of gynoecial patterns found in this taxon are of special interest, as they resemble ones previously described in the earliest angiosperms. The different orientations of carpels in legumes appear easily switchable through changes in flower symmetry and floral meristem sizes. Regardless of orientation of a single carpel with respect to the inflorescence axis, the placenta-bearing suture invariably remains adaxial as related to the floral axis. This conclusion relaxes the existing controversies between the supposed megasporophyll-derived nature of the carpel and observed diversity of placentation in known Mesozoic angiosperms.

Keywords: angiosperms, inflorescence, carpel, monosymmetry, placenta, polysymmetry

Р Е З Ю М Е

Синюшин А.А. Пространственная ориентация гинецея у Бобовых и не только: комментарий к статье Wang et al. (2021). У представителей третьего по величине семейства покрытосеменных, Бобовых (Leguminosae), отмечается значительное разнообразие в структуре гинецея. Некоторые варианты его строения особенно интересны, поскольку сходны с типами, описанными у древнейших покрытосеменных. Вероятно, различные варианты ориентации плодолистиков у Бобовых сравнительно легко переходят друг в друга при изменениях симметрии цветка и размеров флоральной меристемы. Независимо от ориентации единственного плодолистика относительно оси соцветия, несущий семязачатки шов неизменно остается адаксиальным по отношению к оси цветка. Это заключение снимает противоречия между представлениями об эволюции плодолистика из мегаспорофилла и наблюдаемым разнообразием гинецеев у мезозойских покрытосеменных.

Ключевые слова: покрытосеменные, соцветие, плодолистик, зигоморфия, плацента, актиноморфия

The spatial arrangement of floral parts is one of the main sources of variation in flowering plants. Understanding mechanisms which govern this patterning is essential to explain both natural diversity of angiosperms and the evolutionary origin of flower. In their recent paper, Wang et al. (2021) touched upon the problem of gynoecium architecture in the third largest angiosperm family, Leguminosae. The authors of cited paper wished more botanists to join their enquiring of this question. This topic really requires different approaches for solution.

The diversity of gynoecia in Leguminosae deserves a brief overview. Despite an outstanding variation of flowers and fruits, all Leguminosae possess apocarpous gynoecia, typically unicarpellate, which is a synapomorphy of this family within Fabales (Bello et al. 2012). A single carpel is usually placed with its ventral suture towards the adaxial side of flower or slightly turned to the right or to the left of the median plane (Prenner 2004). This holds true for the most of Leguminosae, especially ones with monosymmetric flowers. Such morphology of carpel with clearly discernible dorsal and ventral sides is convenient for investigation of factors

defining its ontogeny and orientation and further approximation of the obtained data to the most of angiosperms.

In polysymmetric flowers (e.g., *Acacia*, *Gleditsia*), orientation of a single carpel is highly variable (Gómez-Acevedo et al. 2007). In particular individuals of *Gleditsia*, most of carpels develop with lateral or even abaxial, i.e. inverted cleft position (Tucker 1991).

The multicarpellate gynoecia evolved through different mechanisms and independently in different leguminous lineages (Paulino et al. 2014, Sinjushin 2021 and references cited therein). There are two basic plans of polymerous gynoecia, viz. with two clefts turned to the adaxial side (*Swartzia* p.p.) or with all carpels more or less facing a center of receptacle (*Acacia* p.p., *Inga*, *Thermopsis* p.p.). The '*Acacia* type' of Wang et al. (2021), i.e. with all carpels' clefts turned 'outwards' of floral center, was never recorded in Leguminosae. Wang et al. (2021) point at the gynoecium of *Acacia celastrifolia* as an example of such type (*figure 4f, h* in Paulino et al. 2014). However, it can be clearly seen from *figure 4e* from the same paper, as well as from images in the paper (Prenner 2011), that carpels are facing their dorsal,

not ventral, sides outwards. The '*Acacia* type' and '*Inga* type' of (Wang et al. 2021) are actually the same.

To resolve the problem of possible interrelations between different morphologies of gynoecia in Leguminosae (and other angiosperms with a variable gynoecium merism), two principal questions need to be addressed.

First, what is the exact position of a single carpel on floral axis, i.e. is it terminal or lateral? Geometrically, a carpel is a distal-most and a central part in an unicarpellate leguminous flower. It has been long debated if it is a true terminal or lateral derivative of floral meristem (FM). This topic is reviewed by Derstine & Tucker (1991), who concluded that the carpel is a terminal structure on a floral axis.

In my opinion, a single leguminous carpel is a lateral derivative. In some legumes, a floral apex can be visually recognized (Newman 1936, criticized by Derstine & Tucker 1991; see also *figure 3j, m* in Sinjushin 2018). Derstine & Tucker (1991) mentioned the axiom that a carpel (a leaf homologue having abaxial and adaxial surfaces) could not be terminal without providing a substantial rebuttal against it. Flowers of caesalpinioids *Gleditsia* and *Gymnocladus*, although possessing polysymmetric perianth and androecium, cannot be called fully polysymmetric because of their unicarpellate gynoecia. Both genera produce truly terminal flowers in their racemose inflorescences. The existence of a terminal unicarpellate flower seems a serious counterevidence against the true terminal position of a single carpel in such flower (although its ontogeny and morphology deserve a special revision).

The exact position of a single carpel remains a problem which requires a cross-examination from different perspectives, such as classical morphology, developmental genetics or teratology (often neglected source). Whether a floral apex persists in the leguminous flower (and is overtopped by developing carpel) or fully converts into a carpel, this apex provides certain morphogenetic signals during a definitive period, thus assigning the abaxial-adaxial structure of all floral elements including a conduplicate carpel.

Second, what are the sources of positional information in an unicarpellate flower? The position of each structure with respect to other parts of developing organism is a key determinant of this structure's fate, the phenomenon known as positional information (Wolpert 1989). The following sources of this information can be hypothesized for an unicarpellate gynoecium since its inception or even earlier, at the stage of its patterning.

(1) A gradient of signal molecules, such as phytohormones or transcription factors. Except for the true terminal flower, each lateral FM inevitably initiates and differentiates between the inflorescence apex and the flower-subtending phyllome, such as bract (sometimes cryptic). The most logically parsimonious agent of this polarity is an auxin, which is synthesized by meristems (inflorescence apex, FM or primordia of floral organs) and then subject to a polar transport (Müller et al. 2017, Cucinotta et al. 2021). Each lateral FM therefore occurs between two sites of auxin biosynthesis (inflorescence apex and bract primordium) and synthesizes auxin itself.

Additional source of abaxial-adaxial polarity in FMs is preferentially adaxial expression of TCP genes, which were repeatedly recruited for control of floral monosymmetry in different angiosperm lineages (Preston & Hileman 2009). Both these factors may contribute to the orientation of gynoecium, not only uni- but also multicarpellate. Although a role of TCP transcription factors is mostly discussed with respect to the perianth (mono)symmetry, it is not a trivial question why carpels are oriented as they are and not otherwise. For example, two carpels are in a lateral (not median) position in disymmetric flowers of *Arabidopsis*. The extra complexity if that the flowers in many angiosperm families have a different merism in gynoecium and other floral whorls, so the position of carpels cannot be readily derived from the location of other floral parts.

(2) Space availability and/or physical pressure from adjacent organs, i.e. spatial constraints. Besides chemical cross-talk, some structures of flower and associated phyllomes may interact directly, which probably has a significant morphogenetic potential. Several genera of Caryophyllaceae have a common floral formula K5 C5 A5+5 G5 but differ in the orientation of carpels (antesepalous or antepetalous). These types were distinguished by Wei & Ronse De Craene (2020) by unequal space availability for emerging stamens and carpels. A shape and size of available FM are probably a key factor of a single carpel's orientation in Proteaceae (Douglas & Tucker 1996).

The most probable is that a superposition of all forces (mechanical/hormonal inhibition from a subtending bract + TCP-mediated regulation of the adaxial floral domain) predisposes a preferential initiation of a carpel with respect to the floral axis, i.e. from what side of floral apex the uppermost primordium (future carpel) will emerge. The floral apex itself provides a positional information for differentiation of this primordium, and its cleft will be oriented towards a floral apex.

In the unicarpellate leguminous flowers, this 'polarizing' factor probably has the decisive value. In monosymmetric taxa, the preferential orientation of a single carpel's cleft towards the adaxial side suggests a structural restriction of its initiation on the adaxial side of a floral axis. Moreover, clefts of extra carpels are also turned to the adaxial side rather than to the presumptive floral apex, regardless if these carpels result from prolonged FM proliferation (*Thermopsis turcica*) or are homeotically derived from stamens (some mutants of *Pisum sativum*) (Sinjushin 2021). Both carpels in *Swartzia dipetala* are adaxially oriented with their clefts (Paulino et al. 2014). These facts indicate the exclusive significance of floral monosymmetry for carpel orientation in Leguminosae, as well as in many other families. In all flowers of a monocot genus *Posidonia* (Posidoniaceae), a single carpel is abaxially oriented with its cleft (Remizowa et al. 2012). More examples are discussed by Sokoloff et al. (2017).

Oppositely, all known cases of variable carpel position are restricted to the polysymmetric leguminous flowers (even bract-subtended), as those of *Acacia* and *Gleditsia*. The latter genus is also remarkable with a completely helical succession of all floral organs and unstable position of the first initiated primordium (Tucker 1991). In such flowers, the adaxial inhibition seems less pronounced, permitting diverse placement of a carpel. Similar variation is also

found in some unicarpellate Ranunculaceae, with both poly- (*Actaea*) and monosymmetric (*Consolida*) flowers (Sinjushin, unpublished data).

Of most importance is that carpel clefts in such flowers may be in lateral or abaxial position with respect to the axis of inflorescence – but they are invariably adaxial with respect to the floral axis (Fig. 1). In these terms, shift between 'abaxially' or 'adaxially' oriented carpel is a simple rotation around a floral apex and may occur rapidly, without any rudiments or intermediate stages. The same ease characterizes variations of merism in leguminous flowers, where the polymerous gynoecia result from the longer proliferation of FM and/or its larger sizes (Sinjushin 2021).

The paper of Wang et al. (2021) is seemingly in the context of previous works on the origin of an angiosperm carpel (e.g., Liu & Wang 2018). This discussion is primarily founded on observations of fossil angiosperms with a placentation supposedly divergent from adaxial. However, the decisive ambiguity awaiting resolution is to distinguish first between solitary flowers and many-flowered inflorescences, which is not an easy challenge in the lack of perianth and stamens in many fossils (e.g., Liu & Wang 2018). The fossilized postanthetic inflorescences of *Gleditsia*, *Acacia* or *Actaea* would provide a clear impression of atypical placentation, while the position of ovule-bearing suture in carpels of these plants is in fact adaxial, like in more 'conventional' flowers. Similarly, flowers of the extant genus *Cercidiphyllum* (Cercidiphyllaceae) possess the unusual carpel orientation, but the demarcation between flower and inflorescence is complicated and probably impossible in this case (Yan et al. 2007). The female reproductive units of Cymodoceaceae may also correspond to reduced spikelets thus explaining a dorsal insertion of ovules (see Remizowa et al. 2012 for references).

Regarding the 'flower or inflorescence' controversy, one of the most notable cases is the extinct (and probably one of the oldest known) angiosperm *Archaefructus*. Since the first fossils of *Archaefructus* were discovered, it has been debated if reproductive structures terminating its shoots are solitary flowers or the inflorescences of unisexual flowers (Friis et al. 2003). As this plant was aquatic, it might possess the highly specialized inflorescences of perianthless (or with strongly reduced perianth) unisexual flowers, like those of the extant *Hippuris* (Plantaginaceae), *Myriophyllum* (Haloragaceae) or seagrasses, such as *Posidonia* (Posidoniaceae).
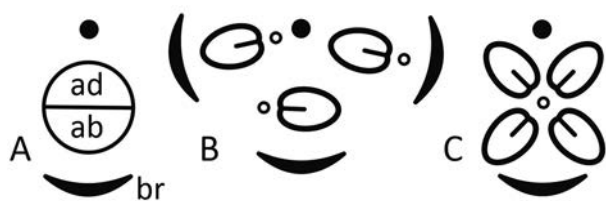
Although the specialized phyllomes (bracts and bracteoles) are the key markers of the mutual position and orientation of flowers within an inflorescence, these phyllomes may be fully reduced (as in *Pisum*, if continue listing examples from the Leguminosae), early deciduous (*Lupinus*) or very minute. That is why these indicators can be either absent in living plant or not preserved in the fossil specimens and hence not informative. However, a paired arrangement of flowers (at least female) in each node observed in *Archaefructus* is not rare among extant angiosperms including legumes (e.g., *Phaseolus*).

The suggested interpretation of the inflorescence of *Archaefructus* (Fig. 2) is only one among several possible, as a phyllotaxis, a number of flowers per second-order axis or a merism of male flowers may be a matter of debates and easily switchable judging by extant angiosperms. However, the hypothetical model provides a structural explanation of the 'abaxial' seed insertion reported by Wang & Zheng (2012). In a very similar case of the strongly reduced flowers of aquatic *Posidonia*, a single carpel is oriented with its ovule-bearing cleft to the abaxial side (with respect to the inflorescence axis), which is interpreted as a result of reduction of the tricarpellate gynoecium typical for most monocots (Remizowa et al. 2012).

If flowers are borne in few-flowered partial inflorescences which are, in their turn, produced oppositely, as stated by Wang & Zheng (2012), the whole flower-bearing system of *Archaefructus* can be classified as a compound raceme or thyrse, as in many living angiosperms. The condensation of such thyrse to a single verticil would give a clear impression of a single terminal multicarpellate 'flower' (probably the case of *Varifructus*: Liu et al. 2020), as in extant *Monarda* (Lamiaceae), but with an 'atypical' position of carpels.

When there are no possibilities of experiment and detailed ontogenetic and molecular analyses (exactly the case of paleobotany), the key principle to interpret the overpast phenomena is uniformitarianism (Scott 1963). It assumes that, until proven otherwise, nature is uniform in such a way that the regularities observed in present can be approximated to both the past and the future. As seen from
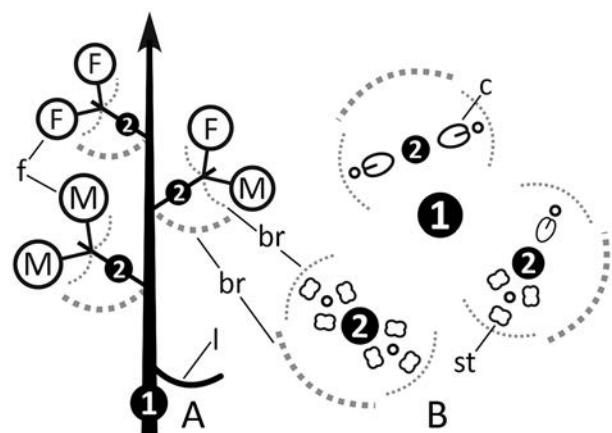

Figure 1 Orientation of flower (A) and carpels (B, C) with respect to the floral (white dot) and inflorescence (black dot) apices in unicarpellate (B) and multicarpellate (C) leguminous flowers. In B, all three carpels are oriented variously with respect to the inflorescence apex but placed with their adaxial sides to the floral apices. Perianth and androecium are not depicted. ab = abaxial domain, ad = adaxial domain, br = bract

Figure 2 Scheme (A) and diagram (B) of reproductive unit of *Archaefructus* assuming its inflorescence nature. Black circles with numbers = axes of the respective (1st or 2nd) orders, white dot = floral apex, br = bract, c = carpel, f = flower (M = male, F = female), l = leaf, st = stamen

several examples listed above, we have a clear understanding which gynoecial morphologies are 'typical' and 'atypical'. In all known cases, the mechanisms can be suggested how the latter ones could be derived from the former ones. Both 'typical' and 'atypical' variants are found among striking floral diversity of the Leguminosae. The ongoing work on the phylogeny of legumes enables to classify different morphologies into ancestral and derived (Sinjushin 2021). That is why even within a single family one can find the interpretation of 'atypical' variants found among fossil angiosperms, (yet) without involving hypotheses of 'unconventional' origin of the angiosperm carpel.

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