New Aspects in Floral Development of Papilionoideae: Initiated but Suppressed Bracteoles and Variable Initiation of Sepals

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INTRODUCTION

Papilionoideae are, with approx. 12 000 species, the largest subfamily of Leguminosae. They are widely distributed from rainforests to the edges of dry and cold deserts, and they play an important role in human nutrition as well as in soil fertilization. The increase of molecular data and the resulting new insights into legume systematics make the search for new morphological characters and a careful reinvestigation of already stated characters necessary. Bracteoles are small, reduced leaves borne close to the base of lateral branches. Although they seem unimportant in older buds, they have an ecological function in protecting the sepal primordia. Furthermore, a morphogenetic function in mediating the onset of sepal initiation is suspected in the literature. The occurrence of bracteoles varies within Papilionoideae, and their distribution is used in legume systematics. But this is open to criticism, because there is a tendency to use ‘absent’ for ‘caducous’. Thus attention here was paid to the initiation of bracteoles as well as to the sequence of sepal initiation.

The occurrence of bracteoles varies within Papilionoideae (cf. Tucker, 1987), and the character ‘bracteoles present versus absent’ is used frequently in cladistic analyses of the subfamily (e.g. Crisp and Weston, 1987, 1995; Lavin, 1987, 1995; van Wyk and Schutte, 1989; Sousa and Rudd, 1993; Breteler, 1995; Schrire, 1995; Tucker and Douglas, 1994; Barker et al., 2000; van der Bank et al. 2002). Tucker (1987) states that the veriﬁcation of the occurrence of bracteoles could be useful, because there is a tendency to use ‘absent’ for ‘caducous’, which could lead to erroneous conclusions. For the decision as to whether or not bracteoles are initiated, a careful investigation of the floral primordium is necessary.

In contrast to the variable occurrence of bracteoles, in papilionoids the initiation of sepals is said to be almost uniformly unidirectional from the abaxial to the adaxial side of the flower (Tucker, 1984, 1987, 2003). Exceptions are only rarely reported (e.g. Tucker and Stirton, 1991; Klitgaard, 1999). Considering the above-cited morphogenetic function of bracteoles, additional attention was paid to the sequence of sepal initiation.

The aim of this study is to present initiated but early suppressed bracteoles as a ‘new’ character in Papilionoideae, and to show a broad variability in sepal initiation. This variability can be derived from the helical pattern of the occurrence of bracteoles could be useful, because there is a tendency to use ‘absent’ for ‘caducous’, which could lead to erroneous conclusions. For the decision as to whether or not bracteoles are initiated, a careful investigation of the floral primordium is necessary.

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Caesalpinioideae. A possible morphogenetical function of bracteoles as well as systematic aspects of the presented characters are discussed.

MATERIALS AND METHODS

Material
SEM micrographs are shown for Baptisia australis R. Br.: Prenner 249, cult. Austria, Hortus Botanicus Graecensis (HBG); Dorycnium germanicum Rouy: Prenner 288, cult. HBG; Ebenus cretica L.: Prenner 440, Greece, Crete, Psiloritis; Laburnum alpinum J. Presl.: Prenner 408, cult. HBG; Lathyrus latifolius L.: Prenner 272, cult. HBG; Kennedia nigricans Lindl.: Prenner 491, cult. HBG; Petteria ramentacea (Sieber) Presl: Prenner 183, cult. HBG; Thermopsis lanceolata R. Br.: Prenner 242, cult. HBG. Voucher specimens have been deposited in the herbarium of the Institute of Botany, Karl-Franzens-University Graz (GZU), and liquid-preserved collections are held by the author.

Scanning electron microscopy (SEM)
For SEM, young inflorescences and flower buds of different sizes and ages were collected, immediately fixed in FAA (5 parts formalin : 5 parts 100 % acetic acid : 90 parts 70 % ethyl alcohol) and stored in 70 % ethyl alcohol. Floral parts were dissected in alcohol under a Zeiss stereomicroscope. The specimens were dehydrated in formalin-methylacetate (FDA) for at least 24 h and critical-point dried with liquid CO₂ in a Polaron 7010 CPD. The dried specimens were mounted with nail polish on aluminium stubs, on which dissection was completed. The buds were coated with gold in an Agar sputter-coater. SEM studies were done with a Philips XL 30 ESEM at 20 kV at the Institute of Plant Physiology, Karl-Franzens-University Graz. The micrographs were saved as TIF files and labelling was done with Adobe Photoshop 6.0.

Depending on the investigated species 60 to 180 pictures were taken and analysed. Complete ontogenetic series of three species are shown in Prenner (2003a, b, 2004). Ontogenetic series of the other species will be published elsewhere.

Interpretation of the SEM micrographs
For an accurate interpretation of the SEM micrographs, great attention was paid to ensure that separated flowers and floral primordia were investigated in exact frontal view, in which all organs are clearly visible. Furthermore, an attempt was made to find as many different developmental stages as possible. Under optimal conditions, only stages in which the organ just becomes visible were analysed. If such exact developmental stages were lacking, the size of the organ was used to interpret the time of organ initiation. But it should be emphasized that this could easily lead to erroneous interpretations, since the growth rate can vary notably. Therefore, such data were treated with caution. Analysis of tilted specimens and/or side views of specimens were avoided whenever possible, in order to assure an accurate analysis.

Terminology and systematic treatment
The terminology used refers to Tucker (1987). ‘Adaxial’ means the upper side of the flower, which is closest to the inflorescence axis, and ‘abaxial’ means the lower side of the flower, which is closest to the subtending bract. The tribal classification of the mentioned taxa follows Polhill (1994).

RESULTS

Bracteoles initiated but suppressed
Bracteoles are initiated to the left and right of the floral primordium and are clearly discernable before the first floral organ becomes visible (Fig. 1A). The protuberances remain small during initiation of the first sepals (Fig. 1B), and they disappear completely in the course of floral development (Fig. 1C). Hence they are no longer visible at anthesis. This character was observed in the Thermopsisae Baptisia australis (Fig. 1A–C) and Thermopsis lanceolata (Fig. 1D–F), in Kennedia nigricans (Fig. 1G–I; Phaseoleae–Kennediinae), and in Petteria ramentacea (Fig. 1J–L; Genistae). In Ebenus cretica (Hedysareae) bracteoles are initiated before the floral apex becomes visible, and they are discernable to the left and right of the floral bract (Fig. 2A). These small protuberances disappear early and are no longer visible when the first sepals are formed (Fig. 2B, C).

Bracteoles initiated and fully developed
This character was found in 16 species, and as an example Laburnum alpinum is shown (Fig. 2D–F). The bracteoles are initiated to the left and right of the floral primordium (Fig. 2D), and they enlarge parallel to the initiation of the sepals (Fig. 2E, F). Thus, they have to be removed for the analysis of later developmental stages.

Bracteoles entirely suppressed
No bracteoles were found in ten species, which is illustrated for Lathyrus latifolius (Fig. 2G–I) and Dorycnium germanicum (Fig. 2J–L). In both taxa the abaxial sepals are the first organs initiated on the floral primordium (see below).

Sepal initiation
Six different patterns of sepal initiation were found (Fig. 3), which are shown in the following sections by means of selected examples.

Sepal initiation modified unidirectional. In Baptisia australis the initiation starts in a unidirectional manner in that the abaxial sepal is formed first (Fig. 1A), and the two lateral sepals arise simultaneously (Fig. 1B). The modification is found in the two adaxial sepals, which are formed successively (Fig. 1C). Modified unidirectionality is also found in Genista sagittalis, Lotus corniculatus, Sophora davidii and S. flavescens (Fig. 3D).
Sepal initiation whorled. In *Thermopsis lanceolata* and *Petteria ramentacea* all sepals are formed in a simultaneous whorl (Fig. 1F, L). Whorled sepal initiation is also found in *Daviesia cordata* (Fig. 3H).

Sepal initiation unidirectional. In *Kennedia nigricans* sepal formation is unidirectional from the abaxial to the adaxial side. The abaxial sepal is initiated first (Fig. 1G), followed by the two lateral sepals, and by the two adaxial...
sepals (Fig. 1H, I). Unidirectional sepal initiation was found in nine of the studied taxa (Fig. 3E).

**Sepal initiation bidirectional.** In *Ebenus cretica* sepal initiation is bidirectional, with the two lateral sepals formed first (Fig. 2B). The abaxial sepal is initiated next and the two adaxial sepals arise at the end (Fig. 2C). The same mode of sepal formation is found in *Crotalaria pallida*. In *Galega officinalis* this mode is modified in that the two adaxial sepals are formed successively.

**Tendencies towards whorled sepal initiation.** In *Laburnum alpinum* calyx initiation starts with the abaxial sepal (Fig. 2E), and the remaining four sepals arise...
simultaneously (Fig. 2F). This is seen as a tendency towards whorled initiation, which is also found in Lespedeza thunbergii and Teline nervosa (Fig. 3F). Another tendency towards whorled sepal initiation can be found in Lathyrus latifolius, in which the abaxial and the two lateral sepals are formed simultaneously (Fig. 2H), while the two adaxial sepals arise later (Figs 2I, 3G).

Sepal initiation sequential. In Dorycnium germanicum all five sepals are formed sequentially, beginning with the abaxial sepal, which is followed either by the lateral left (Fig. 2J) or by the lateral right sepal (not shown). The second lateral sepal arises next (Fig. 2K), and the two adaxial sepals are formed finally in succession (Fig. 2L). Further examples of successive sepal initiation have been found in Dussia discolor, Machaerium arboreum, Lotus berthelotii, Indigofera gerardiana and Styphnolobium japonicum (Fig. 3C).

**DISCUSSION**

Bracteoles initiated but suppressed

In the present study initiated but early suppressed bracteoles are shown as a ‘new’ character in Papilionoideae. Early suppressed bracteoles were found in Petteria ramentacea (Genistaeae), Ebenus cretica (Hedysareae), Kennedia nigricans (Phaseoleae–Kennediinae), Baptisia australis (Thermopsideae) and Thermopsis lanceolata (Thermopsideae).

Petteria ramentacea (Genistaeae). The observations presented here on bracteoles in Petteria ramentacea are in contrast to those by Polhill (1976), Bisby (1981), and Tucker (1987). Bracteoles are not present in mature flowers. However, bracteoles are initiated, stop growing early, and are no longer visible at maturity. Thus the occurrence of bracteoles can be confirmed in a modified sense, van Wyk and Schutte (1989) state for Melolobium, Polhillia and Argyrolobium brevicalyx that bracteoles are ‘+/± absent’, which should be checked by use of SEM. Considering that bracteoles are ‘+/± absent’, Argyrolobium brevicalyx and Schutte (1989) state for Melolobium bracteoles can be confirmed in a modified sense. van Wyk and Schutte (1989) say for minute bracteoles (Hutchinson, 1964; Tucker, 1987), while they are lacking in the remaining genera. In contrast, in the present study it was found that in B. australis and T. lanceolata two bracteoles are initiated at the floral primordium stage, but these are suppressed early in floral development. Doyle et al. (2000) mention uncertainties in the classification of Thermopsideae (see also Käss and Wink, 1995; Crisp et al., 2000). Thus a clarification of the presence or absence of bracteoles in the remaining genera Ammopiptanthus, Anagyris and Piptanthus is desirable.

Bracteoles developed and bracteoles absent

The findings of developed, alternatively absent bracteoles largely confirm indications from the literature (e.g. Hutchinson, 1964; Polhill, 1976, 1981a, c, 1982; Baudet, 1978; Bisby, 1981; Lackey, 1981; Kupicha, 1981; Tucker, 1987; Schrire, 1995; Crisp and Weston, 1987, 1995; Crisp, 1995). Nonetheless some outstanding results should be mentioned, as detailed below.

Cicereae. Hutchinson (1964) mentions the absence of bracteoles in Cicer, and Tucker (1987) cites Cicereae as having bracteoles which are converted into spines. However, according to the observations reported here, bracteoles are clearly visible on the mature flower of *C. arietinum*. Spines, which occur in some species of *Cicer*, are seen as the sterile outgrowth of the reduced inflorescence rather than as converted bracteoles (cf. Wydler, 1860).

Sophoreae. Bracteoles were found in Dussia discolor and Styphnolobium japonicum, while in Cladrastis sinensis, Sophora davidii and *S. flavescens* no bracteoles were observable. These findings agree with Sousa and Rudd (1993), who mention bracteolate flowers for Styphnolobium and ebracteolate flowers for *Sophora* s.s. The absence of bracteoles in *C. sinensis*, and their presence in *D. discolor*, confirms Hutchinson (1964).

Amorpheae. Statements on the occurrence of bracteoles within this tribe are somewhat inconsistent (e.g. Hutchinson, 1964; Tucker, 1987). No bracteoles were found in Amorpha fruticosa.
Systematic treatment of initiated but suppressed bracteoles

While in Mimosoideae bracteoles are lacking, they are frequently found in the polyphyletic ‘Caesalpinioideae’, in which bracteoles are absent only in more derived lineages (Herendeen et al., 2003). Considering the ancestors of Papilionoideae within progenitors of Caesalpinioideae (e.g. Doyle et al., 2000; Bruneau et al., 2001; Wojciechowski, 2003), the occurrence of bracteoles can be seen as a plesiomorphic character in Papilionoideae. Consequently, the character ‘initiated but suppressed’ is seen as a step towards fully absent bracteoles. Initiated but suppressed bracteoles are found in Thermopsidoeae, Genisteae, Hedysareae and Phaseoleae. Hence the character is scattered almost throughout the papilionoid phylogeny, which is a hint for convergent reduction of bracteoles. Nonetheless, at lower taxonomic levels the occurrence of initiated but suppressed bracteoles could be a useful character, and the study of ‘bracteolate’ taxa could bring new insights to legume evolution.

Sepal initiation variable

Considering that unidirectional organ formation is said to be the rule in Papilionoideae (e.g. Tucker, 1984, 1987, 1989, 2003; Endress, 1994; Erbar and Leins, 1997), the variability in sepal initiation is unexpected. Besides the unidirectional pattern sensu Tucker (1984), the following were found in this present study: modified unidirectionality, tendencies towards whorled and whorled initiation, bidirectional, and sequential formation of all sepals. Considering that in Caesalpinioideae helical sepal initiation is common (cf. Tucker, 1989, 2003; Endress, 1994 in Delonix regia; G. Prenner, unpubl. res.), and that Papilionoideae are nested within this subfamily (cf. Doyle et al., 2000), a model has been generated to deduce the variability of sepal initiation from the helical pattern of caesalpinioideae. Evidence for helical sepal initiation has already been found in Psoralea pinnata (Tucker and Stirton, 1991) and Dalbergia brasiliensis (Klitgaard, 1999).

Both helices to the right and to the left occur. For the interpretation of the different developmental patterns, a helix to the left is chosen as a basis (Fig. 3A). The first modification of the helix is that the adaxial right sepal, which would arise after the abaxial sepal, is delayed in the time of its initiation. Continuing the helix, despite of this sepal, the lateral left sepal is formed next. According to the ongoing helical sequence, the second lateral sepal now appears, which is followed by the adaxial left sepal. Finally the delayed sepal arises, resulting in a sequential organ formation from the abaxial to the adaxial side of the flower (Fig. 3B). This pattern is found in Dorycnium germanicum, Dussia discolor, Machaerium arboreum and Lotus berthelotii × maculatus (Fig. 3C). Indigofera gerardiana and Styphnolobium japonicum deviate from this pattern in that the adaxial sepals are initiated in reversed sequence.

In Caesalpinioideae the lateral sepals are initiated in very short succession. The plastochron between these organs decreases (= harmonizes) until they arise simultaneously. As a last reminder of the originally helical initiation, the adaxial sepals are initiated in succession (Tucker et al., 1985). The same pattern was found in Genista sagittalis, Lotus corniculatus (Prenner, 2003a), Sophora davidii, Sophora flavescens and Baptisia australis (Figs 1C, 3D).

Unidirectional sepal initiation sensu Tucker (1984) is the result of equalization of the plastochrons of the adaxial sepals, and was found in nine species out of five tribes (Fig. 3E).

Assuming that the ongoing process of harmonization of the plastochrons is inherent, the developmental pattern ‘tendencies towards whorled initiation’ can be derived from the unidirectional pattern. Harmonization occurs on either the adaxial or the abaxial side of the flower. Adaxial harmonization is found in Lespedeza thunbergii, Laburnum alpinum and Teline nervosa, in which the lateral and the adaxial sepals are formed simultaneously (Figs 2F, 3F). Abaxial harmonization occurs in Lathyrus latifolius. Here the adaxial sepal and the two lateral sepals arise simultaneously (Figs 2H, 3G; for the complete ontogenetic sequence see Prenner, 2003b).

Due to further harmonization, the simultaneous or whorled pattern results. This is found in Petteria ramentacea, Daviesia cordata (Prenner, 2004) and Thermopsis lanceolata (Figs 1F, L, 3H).

The sequence presented here (sequential → modified unidirectional → unidirectional → tendencies towards whorled → whorled) should be seen as a hypothetical pathway, which needs to be tested on the basis of an enlarged developmental data matrix.

Bidirectional sepal initiation and the morphogenetic function of bracteoles

In Crotalaria pallida, Ebenus cretica and Galega officinalis sepal initiation is bidirectional with the two
lateral sepal is initiated first. The abaxial sepal is initiated next, and finally the adaxial sepal arises either simultaneously or in succession. This uncommon pattern corresponds to the results of Breindl (1934) in Vaccinium rollisoni and Limnanthes douglasii, in which the first two sepals have a lateral position if the bracteoles are lacking or small. Referring to the morphogenetic function of bracteoles, Endress (1994, p. 97) states that ‘as the two first organs at the floral axis they mediate the onset of the spiral of the calyx’. In Galega officinalis bracteoles are lacking, in Ebenus cretica the bracteoles are of the type ‘initiated but suppressed’ and in Crotalaria pallida the bracteoles remain minute. In these taxa the loss or reduction of bracteoles could have influenced the order of sepal initiation. However, in contrast to this hypothesis other investigated taxa with reduced or lost bracteoles do not show bidirectional sepal formation. Hence further observations are necessary for a clarification of this uncommon pattern in Papilionoideae.

Systematic interpretation of the diversity of sepal initiation

Analogous to initiated but suppressed bracteoles, the big diversity of sepal initiation does not show a clear line within Papilionoideae. The different modes seem to be scattered throughout the papilionoid phylogeny. Two different modes of sepal initiation (unidirectional and tendency towards whorled initiation) within the genus Teline corroborate this, and are evidence for a lability of these patterns, which is also found in the petal whorl and in the two stamen whorls (G. Prenner, unpubl. res.). While Tucker and Douglas (1994) highlight the discrete position and monophyly of Papilionoideae on the basis of the analysis of previous ontogenetic characters, the present study corroborates Wojciechowski (2003, p. 9), who suggests that ‘papilionoids are only weakly differentiated molecularly from their caesalpinoid sister groups’. In fact, the derivation from the helical pattern of caesalpinoids and the broad variability of sepal initiation link the papilionoid flower more closely with the caesalpinoid flower than it was thought before (e.g. Tucker and Douglas, 1994; Tucker, 2003).

CONCLUSIONS

In the present paper ‘initiated but suppressed’ bracteoles are shown as a ‘new’ character in Papilionoideae. Furthermore, a remarkable variability of the sequence of sepal initiation was found, which can be derived from the helical pattern of caesalpinoids. With this result the widely stated unidirectionality of Papilionoideae is questioned, and a link between the flowers of Papilionoideae and Caesalpinioideae is shown. Bidirectional sepal initiation is possibly a consequence of the morphogenetic function of bracteoles, although bidirectionality is not found in all taxa with reduced bracteoles. Clarification and a detailed phylogenetic analysis of the presented characters based on a broadened data matrix could allow new insights into legume systematics.

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LITERATURE CITED


