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A Developmental Analysis of the Inflorescence and the Flower of *Lotus corniculatus* (*Fabaceae-Loteae*)

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With 4 figures

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Summary: The ontogeny of the inflorescence and flower of *Lotus corniculatus* was studied by scanning electron microscopy (SEM). On the inflorescence the flowers are initiated in a zig-zag-pattern from one side to the other. Developmental differences disappear during ontogeny, and the mature flowers of a single inflorescence open almost synchronously. The mode of organ formation varies from the acropetal initiation, because the organs of the sepal and the petal whorl overlap at the time of initiation, and the carpel is formed before the first stamen becomes visible. The organ initiation within the flower varies in all whorls from the unidirectional mode, which is regarded to be the rule in *Fabaceae*. In the sepal whorl the adaxial sepals are formed successively, which is interpreted as a remnant of an originally helical initiation. In the petal whorl the two lateral petals and the adaxial petal are formed simultaneously, which is seen as a tendency towards whorled organ formation. The episepalous stamens arise simultaneously, and in the epipetalous stamen whorl the two abaxial stamens and the adaxial stamen are formed first, followed by the two lateral stamens. The latter case is a special form of stamen initiation, which has never been found in *Fabaceae* before. The presented analysis shows striking differences to preliminary studies in *Fabaceae*. Further studies have to broaden the data set, on the basis of which a detailed phylogenetic analysis will be possible.

Kurzfassung: Eine entwicklungsgeschichtliche Analyse des Blütenstandes und der Blüte von *Lotus corniculatus* (*Fabaceae-Loteae*). – Die Entwicklung des Blütenstandes und der Blüte von *Lotus corniculatus* wurde mittels Rasterelektronenmikroskopie (REM) untersucht. Innerhalb des Blütenstandes werden die Blüten einem Zick-Zack-Muster folgend von einer Seite zur anderen angelegt. Unterschiede der Primordiengröße verschwinden im Laufe der Entwicklung, und die Blüten eines Blütenstandes öffnen sich beinahe synchron. Die Art der Organanlage unterscheidet sich von der acropetalen Organanlage, da die Anlage der Sepalen und Petalen überlappt und das Gynoeceum vor dem ersten Staubblatt sichtbar wird. Innerhalb der Wirtel unterscheidet sich die Organanlage in allen Organkreisen vom unidirektionalen Modus, der als Norm innerhalb der *Fabaceae* angesehen wird. Im Kelch erscheinen die beiden adaxialen Sepalen sukzessiv, was als Rest einer ursprünglich schraubigen Anlage gedeutet wird. In der Krone erscheinen die beiden lateralen und das adaxiale Petalum simultan, was als Tendenz zu wirteliger Organanlage interpretiert wird. Die episepalen Staubblätter erscheinen simultan. Im epipetalen Staubblattkreis werden die beiden abaxialen und das adaxial Stamen simultan sichtbar, unmittelbar gefolgt von den beiden lateralen Stamen. Dies ist eine Sonderform der Organanlage, die bislang bei keinem Vertreter der *Fabaceae* nachgewiesen wurde. Die präsentierte Analyse zeigt deutliche Unterschiede zu bislang untersuchten *Fabaceae*. Weitere Untersuchungen müssen den Datensatz erweitern, auf dessen Basis eine detaillierte phylogenetische Analyse möglich werden wird.

1. Introduction

Lotus corniculatus L. (Birdsfoot Trefoil) is a perennial herb, native to Europe and western Asia. Furthermore it is widely naturalised throughout the temperate regions of South and North America, Asia Minor, North Africa, Australia and New Zealand (Garcia de los Santos & al. 2001). Birdsfoot Trefoil is an increasingly popular forage crop in both hay and pasture systems, and the species has been dealt with in different

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studies: Garcia-Diaz & Steiner 2000 focus on different aspects concerning seed production, Beuselinck & McGraw 2000 describe a floral mutant which shows a vestigial corolla, Steiner & Garcia de los Santos 2001 demonstrate the adaptive ecology of *L. corniculatus*, and the condensed tannin concentration in different lineages of the plant has been determined by Wen & al. 2003. Floral ecology and the pump mechanism of the flowers are treated in detail by Müller 1873: 217–222, and do not have to be commented in this paper.

The tribal delimitation of *Loteae* and *Coronilleae* is discussed in Allan & Porter 2000, and Arambarri 2000 gives an overview of its infrageneric division. Allan & Porter 2000 note that *Lotus* is by far the taxonomically most complex of all the genera within *Loteae*, which could be one reason for the different statements on the number of species. It varies from 100 (Polhill 1981, Diez & Ferguson 1994) to 188 (Arambarri 2000).

Concerning the floral development, Tucker 1987a quotes an unidirectional organ formation in *Lotus tetragonolobus* L., but shows only an older flower bud, in which all organs are formed. Hence a complete ontogenetic series of a member of *Loteae* is still lacking. Because of this, and because of the heterogeneity and complexity of the genus, the aim of this study is to present a complete ontogenetic series of a member of *Loteae*, and to search for "new" developmental characters, which possibly could be useful in a better understanding of evolution within the tribe.

2. Material and Methods

Floral buds of different size of *Lotus corniculatus* were collected on 24th of June, 2001 on the roadside of Schubertstraße 21 (Graz, Austria). The specimens were immediately fixed in FAA (5 parts formalin : 5 parts acetic acid : 90 parts 70% ethanol) and stored in 70% ethanol. Floral parts were dissected under a Leica MZ6 stereomicroscope in alcohol of the same concentration. For scanning electron microscopy (SEM) the specimen were dehydrated in formalindimethylacetal (FDA) for at least 24 hours, and afterwards critical-point dried with liquid CO_2 in a Polaron 7010 CPD. The dried specimens were mounted on aluminium stubs with nail polish. The buds were coated with gold in an Agar sputter coater. SEM studies and micrographs were taken in a Philips XL 30 ESEM at 20 kV at the Institute of Plant Physiology, Karl-Franzens-University Graz. Photographs of flowers show the buds with the abaxial subtending bract at the bottom of the image. In total 73 micrographs were taken and analysed.

3. Results

3.1 Organography

The umbellate inflorescence is (1)3–6(8) flowered, and at its base there are three small hypsophylls. On the mature flower neither floral bracts nor bracteoles are visible. The yellow flowers are 0.8–1.3 cm long and pedicellate for about 1 mm. At the floral base an about 0.5 mm long hypanthium is formed. The calyx is synsepalous and 5 mm long. It consists of a 2.5 mm long tube, and lanceolate teeth, which are as long as the tube. The standard has a broad 5 mm long claw. Its blade is 1 cm in diameter and curved upwards. The blades of the wing petals are 10 mm long with a claw of 3 mm. The claws of the keel petals are as long as that of the wings, and their blade is 7 mm long. The blades of the keel petals are curved upwards and are connate along their margins. Only the apex remains free. In the androecium nine stamens are connate to an adaxial open sheath. The adaxial epipetalous stamen remains free, and to the left and right of it two nectar windows are formed.

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3.2 Flower Initiation on the Inflorescence

On the **inflorescence** axis the flowers are initiated in short succession from one side to the other (Fig. 1a–c). Instead of **bracts** or **bracteoles** only hairs along the margins of the roundish primordia are visible (Fig. 1a–c). Organ initiation in the individual flowers of one inflorescence sets in sequential (Fig. 1b–c). But the developmental differences disappear during ontogenesis (Fig. 1d), and at anthesis the flowers of a single inflorescence open almost synchronously.

3.3 Organ Initiation

The sequence of organ initiation is quite fast and varies from the acropetal mode. The organ formation in the sepal and the petal whorl overlap in the time of initiation (Fig. 2a), and the carpel arises before the first stamen becomes visible (Fig. 2c).

The initiation of the **sepals** starts with the abaxial sepal, which is followed by the lateral sepals (Fig. 2a). Shortly later the two adaxial sepals arise in short succession (Figs. 2a–b).

The formation of the **petals** overlaps with the sepals and starts at a floral diameter of about 160 µm. The two abaxial keel petals are formed first (Fig. 2a–b). Afterwards the remaining three petals arise simultaneously (Fig. 2c).

Synchronous with the last initiated petal the **carpel** becomes visible as a shallow bulge in the flower's centre (Fig. 2c).

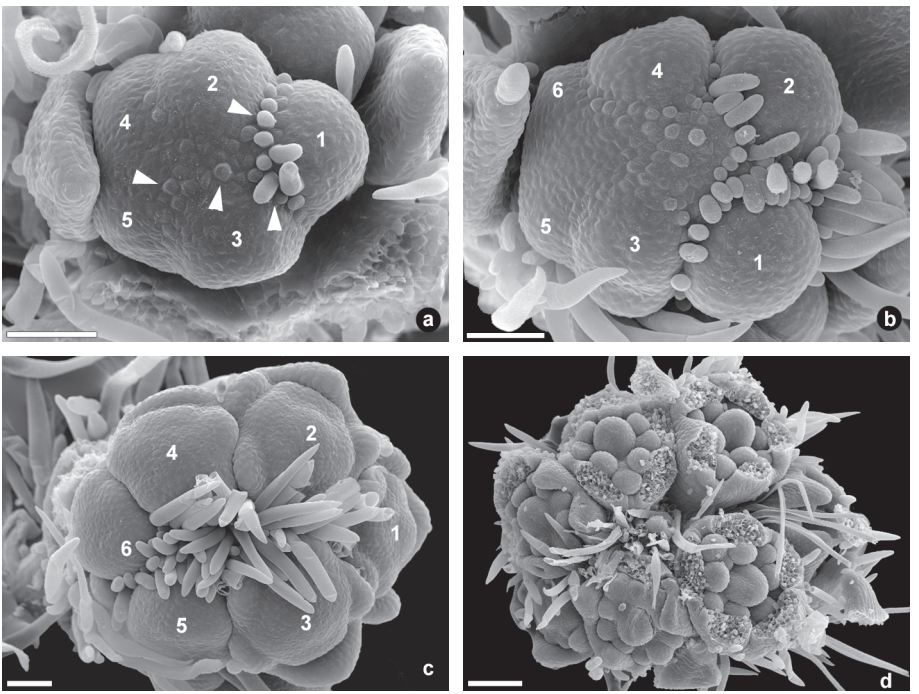

Fig. 1: *Lotus corniculatus*, ontogeny of the inflorescence: **a.** Five flowered inflorescence. The flowers are numbered in the sequence of their initiation (1–5). Note the developing hairs at the margins of the floral primordia (some are marked with arrowheads). **b.** Six flowered inflorescence. The primordia are free of organs. **c.** Six flowered inflorescence. The organ initiation sets in sequential in the order of the initiation of the floral primordia. **d.** Young inflorescence in which the individual flowers already show organs. The developmental differences between the flowers have decreased. (Scale bar in a–c = 50 µm, d = 100 µm)

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The stamens of the **outer androecial whorl** are formed simultaneously, after all petal primordia and the carpel are visible (Fig. 2d). The floral diameter at this ontogenetic stage is about 250×210 µm.

The initiation of the **inner androecial whorl** starts after a developmental pause in which the primordia of the episepalous stamens and the carpel enlarge markedly (Fig. 2e). This makes the observation of the precise sequence of organ initiation in this whorl somewhat difficult. The initiation starts with the two abaxial and the adaxial stamen (Fig. 2f). The floral diameter at this stage is about 295×250 µm. The two lateral stamens are initiated

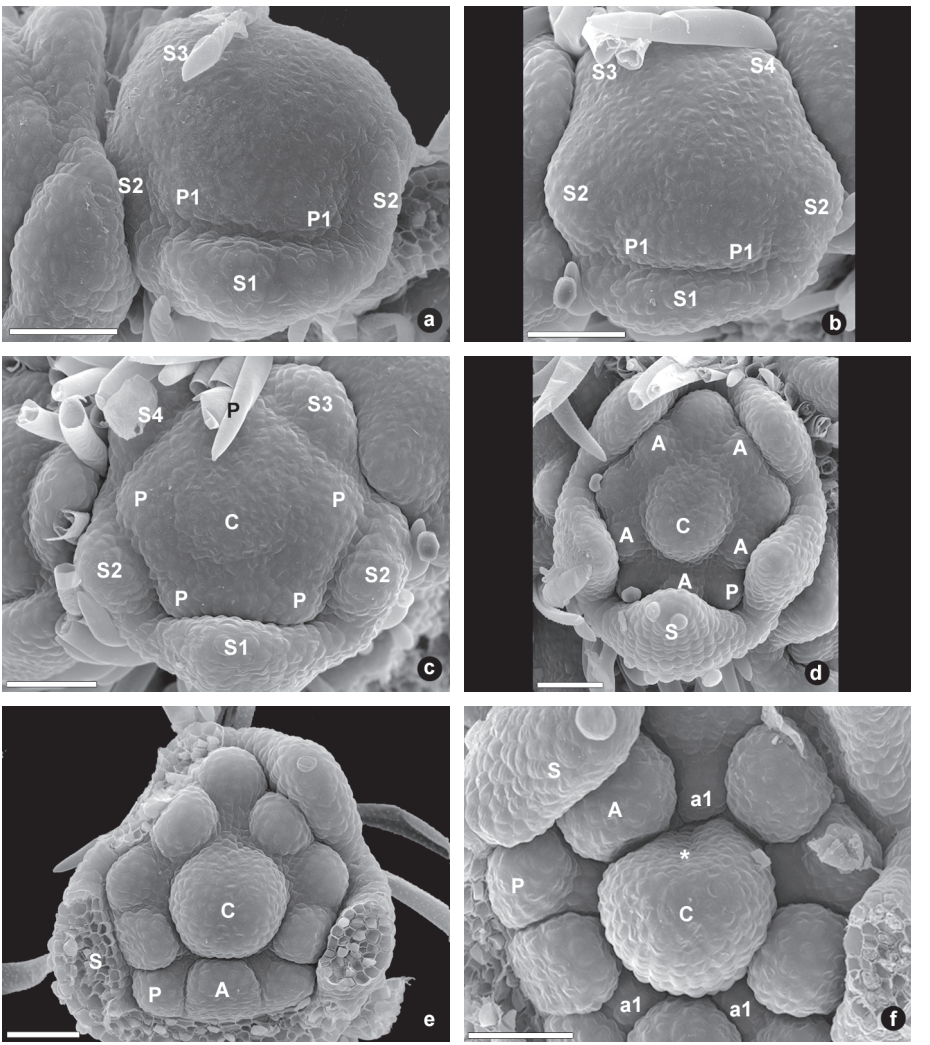

Fig. 2: *Lotus corniculatus*, early floral ontogeny: **a.** The initiation of sepals starts with the abaxial sepal (S1), followed by the lateral sepal pair (S2) and one adaxial sepal (S3). Two abaxial keel petals (P1) are initiated before the last adaxial sepal is formed. **b.** The last adaxial sepal (S4) on the right side is formed. **c.** All petals and the carpel are initiated. Sepals enlarge. **d.** Simultaneous formation of the episepalous stamens (A) and enlargement of sepals. The carpel shows an adaxial flattening. **e.** Developmental pause in which the carpel enlarges markedly (four sepals removed). **f.** Initiation of the first three epipetalous stamens (a1), two in abaxial and one in adaxial position. The beginning formation of the carpellary cleft becomes visible (asterisk). (Scale bar = 50 µm)

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later, which can only be observed after removing the episepalous stamens (Figs. 3a–b). At a floral diameter of about 290–330 µm all floral organs are visible. They are initiated alternating with the former whorls, and the young floral bud is zygomorphic (Fig. 3a).

3.4 Organ Enlargement and Differentiation

The growth of the **sepals** is moderate, so that the initiation of the petals and the outer stamen whorl is observable without additional preparations (Figs. 2c–d). In contrast to this, the observation of the epipetalous stamens makes additional preparations necessary, in which parts of the calyx and the episepalous stamen whorl have to be removed (Figs. 2f, 3a). Late in floral ontogeny the sepals fuse to an about 2.5 mm long tube, and lanceolate teeth of the same length remain free.

Due to the growing processes within the **petal** whorl, the difference of the time of initiation soon disappears, and the petal primordia become almost equal in size (Figs. 2c–e). The further growth of the young petals goes along slowly, and the episepalous stamens and the carpel soon overtop them. Late in floral ontogeny the abaxial keel petals fuse apically and only on the tip a small slit remains open, through which pollen can emerge.

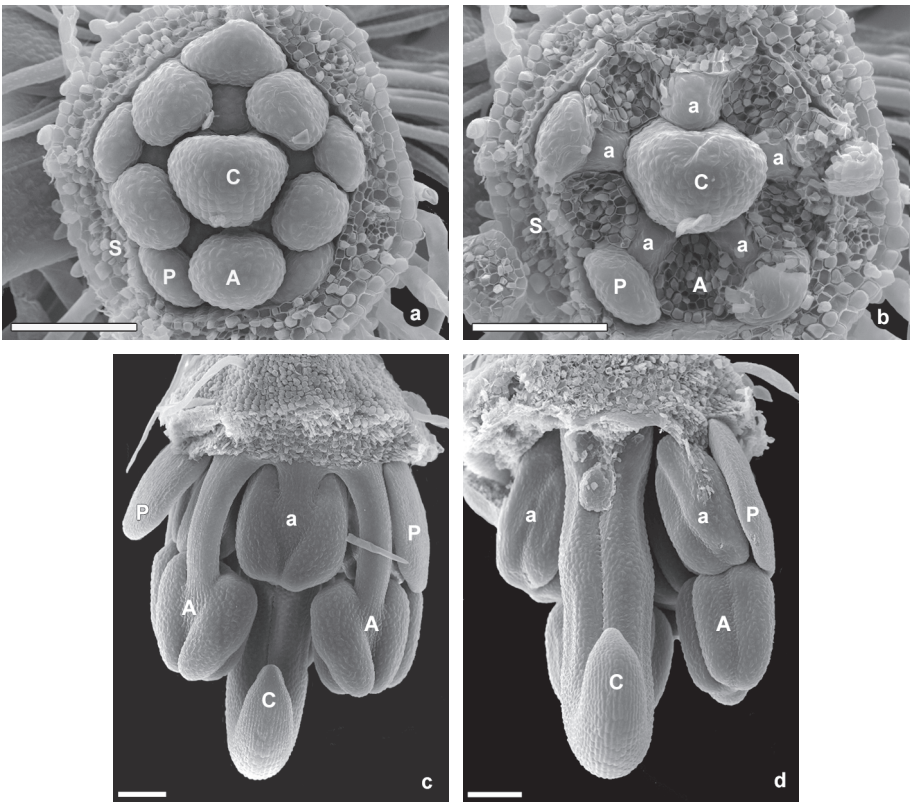

Fig. 3: *Lotus corniculatus*, organ enlargement: **a.** The episepalous stamens and the young carpel are enlarged and mask the epipetalous stamen whorl (sepals removed). **b.** Same as a., episepalous stamens, laterally right and adaxial petal removed. All epipetalous stamens are initiated, but they are still small and undifferentiated. **c.** View of the adaxial side of a young floral bud, showing the episepalous stamens already with distinct filaments, while the epipetalous stamens are still small (sepals and standard removed). **d.** Same as c., stamens partially removed. The young carpel (C) shows the adaxial suture and bends at the apex towards the adaxial side. (Scale bar = 100 µm)

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Because of their simultaneous initiation, the **episepalous stamen primordia** are more or less equal in size from the beginning (Figs. 2d–e). In the **epipetalous stamen whorl** the difference in size, which emerges due to stepwise initiation, disappears soon

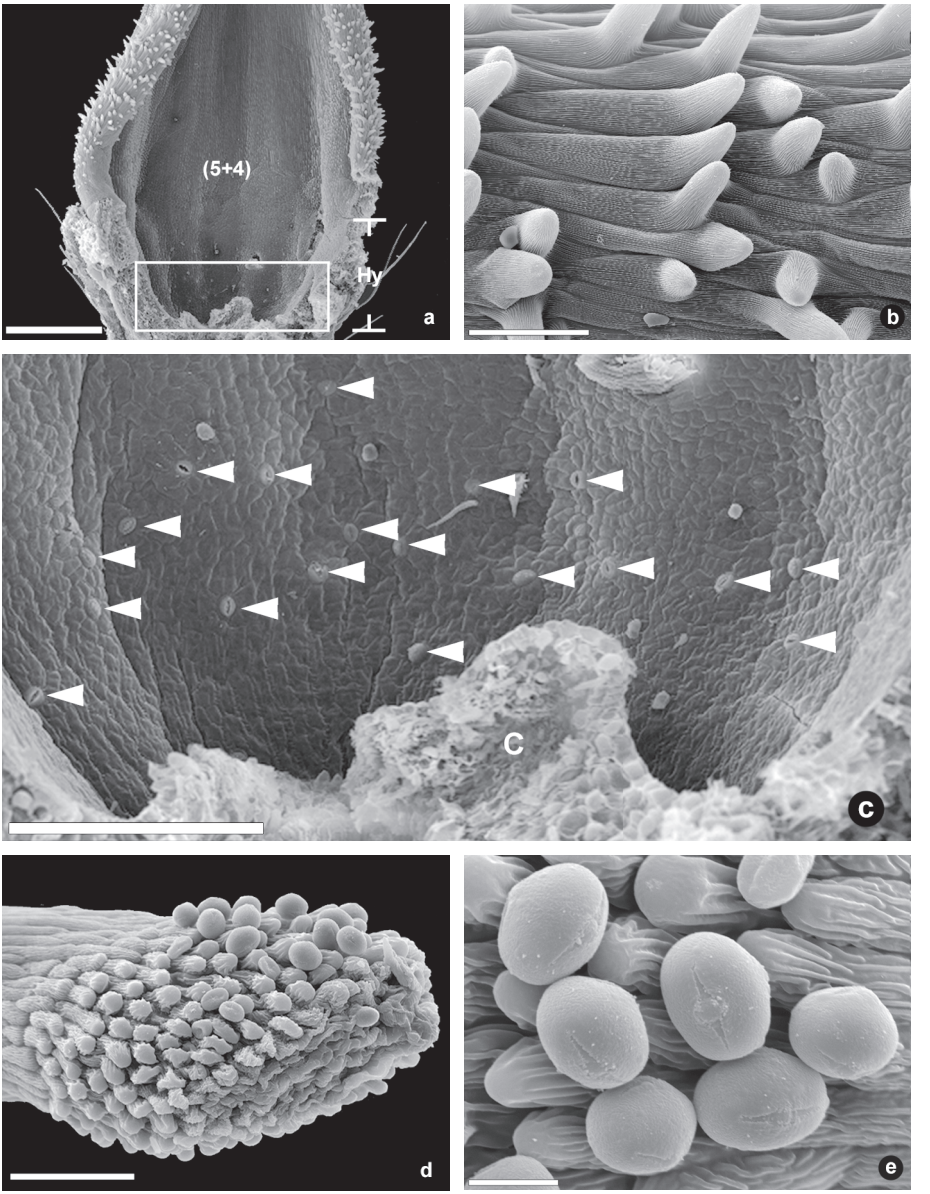

Fig. 4: *Lotus corniculatus*, characters of the anthetic flower: **a.** The filament sheath (5+4) with the adjoining hypanthium (Hy), seen from the adaxial side. The rectangle marks the area where nectar stomata are located on the base of the hypanthium. The adaxial stamen and the carpel have been removed (detail given in c.). **b.** Surface of the rim of the filament sheath with tooth like outgrowths at the upper end of the longitudinally elongated epidermis cells. **c.** Detail of a. Floral nectary on the inside of the hypanthium. Nectar stomata are marked with arrowheads. **d.** Papillate stigma with adhering pollen grains, frontal view. **e.** Tricolporate pollen grains on the surface of the stigma. (Scale bar in a = 500 µm, b and d = 50 µm, c = 200 µm, e = 10 µm)

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(Figs. 3b). Late in the floral development the filaments fuse in diadelphic manner, nine forming an adaxially open sheath, and the adaxial epipetalous stamen remains free. The filament sheath bends outwards at the base of the flower, and two nectar widows are formed, through which nectar is accessible. The surface of the rim of the filament sheath is peculiar in that, that the longitudinally elongated epidermis cells show tooth like outgrowth at their upper end (Fig. 4b).

Soon after its initiation, the **carpel** primordium shows an adaxial depression, which is a result of increased abaxial and decreased adaxial growth. The formation of the adaxial cleft starts parallel with the initiation of the episepalous stamens (Fig. 2f). At a bud length of about 0.7 mm, the apex of the gynoecium begins to bend in adaxial direction (Figs. 3c–d).

The **nectary** is located on the abaxial side of the hypanthium, where the nectar stomata are located (Figs. 4a, c).

The **stigma** is oval shaped, papillate and about 150 µm in diameter (Fig. 4d). In fig. 4d a region without papillae is visible in the center of the stigma. Further studies have to show if this is an artefact due to manipulation, or if this region does not contain papillae at all.

The ovally shaped **pollen** grains are 3-zonocolporate, have a smooth tectum and a diameter of about 15×11 µm (Fig. 4e).

4. Discussion

4.1 Ontogeny of the Inflorescence

The umbellate inflorescence of *L. corniculatus* differs clearly from the racemose inflorescence type, which is common in *Fabaceae* (Tucker 1987a, 1987b, personal observations). The zig-zag-pattern of flower formation from one side of the inflorescence towards the other side is remarkable. Similar patterns have been found in the 10–14 flowered globose umbels of *Dorycnium germanicum* Rouy and in the 2–3(4) flowered umbels of *Lotus berthelotii* Masf. × *maculatus* Breitfeld (personal observations). In all these taxa the initiation of floral primordia differs clearly from the helical pattern, which is found in racemose inflorescences (personal observations). This is in contrast to Tucker 1987a: 185, who quotes that spikes and umbels "are not distinguishable in young stages from racemes". For an accurate interpretation of the circumstances in the inflorescence of *Loteae* further studies have to be undertaken.

4.2 Overlapping Organ Initiation Between Whorls

The overlapping of the organ initiation between the whorls is a common phenomenon in *Fabaceae*. Tucker 1989, showed it for *Pisum sativum* L. and quotes 22 taxa out of twelve tribes, in which this phenomenon occurs (see also Prenner 2003). Besides *Lotus tetragonolobus* (quoted in Tucker 1989), the present study is the second proof of overlapping organ initiation in *Loteae*.

4.3 Variations from the Unidirectional Mode of Organ Formation

According to Tucker 1984 and 1987a, the organs in *Fabaceae* arise almost uniformly unidirectional from the abaxial towards the adaxial side of the flower (see also Tucker 2002: 755). Contrary to this, I found a broad variability in the organ initiation of *Lotus corniculatus* and other members of *Fabaceae* (Prenner 2003, personal observations). *L. corniculatus* differs in all floral whorls: The sepal whorl varies least from the unidirectional mode. Here only the two adaxial sepals are formed successively. Tucker & al 1985 found this mode of sepal initiation in *Caesalpinia pulcherrima* (L.) Sw., and interpret it as a remnant of the original helical sepal formation. This goes conform with my own studies,

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which will be published elsewhere. Within the petal whorl, the synchronous formation of the lateral wing petals together with the adaxial standard is remarkable. It is seen as the result of harmonisation of the plastochrons within this whorl. I interpret this as a tendency towards whorled organ formation, which is found in the episepalous stamen whorl. Tendencies towards whorled organ formation have also been found in the sepal whorl and both stamen whorls of *Lahthyrus latifolius* L. (Prenner 2003). Within the epipetalous stamen whorl, the formation of the two abaxial stamens together with the adaxial stamen is a phenomenon, which has not been found in *Fabaceae* until now. Hence the interpretation of this character is difficult, unless more taxa are found, which show the same mode of stamen initiation.

4.4 Characters of the Floral Nectary and the Pollen Grains

The occurrence of nectar stomata on the abaxial side of the hypanthium confirms the observations done by Murrell & al. 1982. An analogous phenomenon has been recently found in *Lathyrus latifolius*, where the nectar stomata are located in a distinct field on the abaxial side of the floral receptacle (Prenner 2003).

According to Diez & Ferguson 1994, the pollen grains of *L. corniculatus* belong to the subtype IIIb of 3-zonocolporate pollen grains in *Loteae*. This subtype shows a psilate-perforate ornamentation and no granules at the colpus margin. Subtype IIIb is the most common pollen type, which comprises of a very wide range of Old World species of *Loteae* (Diez & Ferguson 1994).

4.5 Conclusion

The presented results differ strikingly from preliminary studies on members of *Fabaceae*. *L. corniculatus* differs both in the ontogeny of the inflorescence and in the order of organ initiation within the flower. This study should be a first step towards a better understanding of evolution within *Loteae*. Further studies on more members of this tribe will have to broaden the data set, on the basis of which a detailed phylogenetic analysis will be possible.

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