International Journal of Plant Developmental Biology ©2010 Global Science Books

Development and Evolution of the Unique Floral Organ Arrangement of *Lacandonia schismatica*

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ABSTRACT

Lacandonia schismatica (Triuridaceae) is the only known angiosperm species with flowers composed of central stamens surrounded by carpels. If the reproductive axes of this species are interpreted as heterotopic flowers, crucial questions on the evolution of morphological novelties arise, such as: a) is this phenotype fixed or whether intermediate floral variants within *L. schismatica* populations exist, and b) what is the nature and number of molecular alterations involved in such a morphological saltation. Furthermore, the temporal progression of floral organ formation in this taxon is unaltered with respect to the great majority of angiosperms (perianth, then stamens and finally carpels). This suggests that the regulatory mechanisms underlying the spatial and temporal morphogenetic patterns of flower development can be altered independently of each other. Through developmental genetic studies, the underlying molecular components involved in the unique position of sexual organs in *L. schismatica* have started to be unravelled. However, studies on floral meristem identity genes, including B-function genes and their regulators (LFY, UFO and SEP) will be important to address the molecular basis of any regulatory alterations. In this contribution we summarize the developmental, systematic and structural data that nurture the on going debate concerning the nature of the Triurid reproductive structures, considered either true flowers (euanthia) or compressed inflorescences (pseudanthia). Finally, we discuss the theoretical approaches that are helping us to understand developmental constraints of the ABC gene regulatory network, and how such theoretical analyses could help explain the arrangement of *L. schismatica* flowers.

Keywords: ABC model, developmental genetics, homeosis, reproductive structures, Triuridaceae

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INTRODUCTION

The field of Evolutionary Developmental Biology or *Evo-Devo* has profited from the data and conceptual inputs from a diverse set of scholars and fields of study. The morphology-centered school of the 18th and 19th centuries was fundamental in describing plant sexual organs, which would prove fundamental for taxonomic and comparative morphology studies (Smyth 2005). In the 20th century, biologists such as C.H. Waddington and D'Arcy Thompson

Received: 9 July, 2009. Accepted: 25 May, 2010.

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among others, made important propositions regarding the role of interactions among genetic and non genetic elements during development, as well as the need to explicitly acknowledge the role of physical and mechanical constraints affecting organismal form. More recently, *Evo-Devo* has benefited from the growing field of developmental genetics, which has utilized empirical data attained through the study of homeotic transformations in model systems, to propose developmental models such as the ABC model of plant floral organ formation (Bowman *et al.* 1989, 1991; Coen and Meyerowitz 1991; Smyth 2005). Such models have been instrumental to assess the role of homeotic genes in the development of the flower structure in both model and non-model plant species (Soltis *et al.* 2007).

Experimental data attained from *Arabidopsis thaliana* has also enabled the development of computational models that address the role of Gene Regulatory Networks (GRN) in the emergence of evolutionarily stable genetic expression patterns associated with organ development (Espinosa-Soto *et al.* 2004; Álvarez-Buylla *et al.* 2008). These contemporary experimental and theoretical approaches have benefited particularly from ideas put forward by Waddington (1957) who introduced the notion of epigenetic landscapes as a metaphor to explain the dynamic nature of development. In such a metaphor, the landscape topology is a dynamic system defined by complex interactions among genetic and non-genetic factors, and basins correspond to stable developmental pathways. In the epigenetic landscape, once a basin has been reached, the developmental process will be buffered against external or internal interventions, thus being *canalized* (Slack 2002). This and other concepts put forward by embryologists and developmental biologists, have been important in shaping the way we think about development (see review by Slack 2002). However, the *Modern Synthesis* did not consider developmental processes explicitly (Huxley 1942) thus failed to provide a useful theoretical framework for understanding phenotypic evolution from an ontogenetic perspective. Developmental constraints, epigenesis, and the dynamics and structure of gene regulatory networks should be explicitly addressed and ideally, comprehended, in order to contribute to the understanding of the evolution of organismal form and function and to map genetic variation into phenotypic variation.

In contrast to the *Modern Synthesis*, *Evo-Devo* has incorporated data and insights from comparative embryology, developmental biology, developmental genetics and theoretical biology, to understand the evolution of organismal form. *Evo-Devo* has also attempted to fulfill what has been called the most ambitious promise of the discipline: "[a] full conceptual continuity between evolutionary and developmental mechanisms and explanations" or, "[a] conceptual continuity from ontogeny through phylogeny" (Bolker 2008).

Some novel approximations put forward to address the non-linear nature of the phenotype-genotype mapping, have addressed the possible microevolutionary processes underlying morphological innovations in certain plant lineages, for example, discerning whether such innovations can be explained by continuous variation or by a discrete phenotypic change (Vergara-Silva *et al.* 2003). Other approaches have advocated the inclusion of genetic algorithms into evolutionary models. Such algorithms describe the mechanisms that determine how genetic information is mapped onto phenotypic traits (Espinosa-Soto *et al.* 2004). The molecular basis of developmental processes is being understood with greater detail and comparative genomics has revealed conservation in developmental gene families outside model systems. It is therefore becoming possible to address hypotheses concerning candidate genes involved in the origin of major morphological transitions. In fact, there are already some examples of relatively small changes to key regulators that are associated with major evolutionary innovations or morphological saltations (Shapiro *et al.* 2004; Brakefield 2006; Davidson and Erwin 2006; Jeong *et al.* 2008). Among such genetic events are homeotic alterations involving major changes in body plans in the case of flowers, or limb number in arthropods (Bowman *et al.* 1991; Ford and Gottlieb 1992; Ronshaugen *et al.* 2002). Similar homeotic alterations seem to be involved in the case of the remarkable inside-out flower of the Mexican monocot herb, *Lacandonia schismatica*.

Fig. 1 ***Lacandonia schismatica*** **flower.** Note semi-transparent tepals and three central stamens (dark yellow) surrounded by numerous carpels (photo by Esteban Martínez).

As a contribution to this special issue of the IJPDB, we synthesize here the state of knowledge about the evolution and development of *L. schismatica*, a living example of a natural heterotopic species (see **Fig. 1**).

This review provides an overview of the general boilogy of *L. schismatica*; its taxonomy as well as the phylogenetic position of the Triuridaceae within the order Pandanales. We also summarize morphology-based studies of reproductive axes of Triuridaceae (from adult specimens of different Pandanales representatives, to developmental series of *L. schismatica*) (Rudall 2003; Ambrose *et al.* 2006; Rudall and Bateman 2006) and review data generated from developmental genetic studies based on the ABC model for floral organ determination (Bowman *et al.* 1991; Coen and Meyerowitz 1991).

Developmental genetics has generated a body of data that informs the debate regarding the nature of the reproductive axes of *L. schismatica*, a controversy that started immediately following formal description of this taxon (Martínez and Ramos 1989). Furthermore, we also address other questions that have been raised since the introduction of *L. schismatica* into the botanical literature, such as: Which changes in the underlying gene regulatory networks controlling flower morphogenesis enabled the emergence of this plant's unique floral phenotype? What is the identity of the reproductive axes of this species? What can we learn in terms of the mechanisms underlying floral morphogenesis from studying *L. schismatica* flower development? Can the understanding of the particular modifications present in *L. schismatica* at the functional/genetic levels help expand our view of the mechanisms and processes implicated in plant *Evo-Devo*?

We end this review with future perspectives on the research that remains ahead, which we continue to pursue with this challenging and fascinating taxon.

TAXONOMY OF *LACANDONIA SCHISMATICA* AND RELATED TRIURIDACEAE SPECIES

L. schismatica was assigned to a monotypic genus and a new family: Lacandoniaceae, although its affinity with Triuridaceae was also acknowledged (Martínez and Ramos 1989). Currently, morphological and phylogenetic analyses further support that this species should be considered a member of Triuridaceae (Maas van der Kamer 1995; Vergara-Silva *et al.* 2003; Davis *et al.* 2004; Ambrose *et al.* 2006; Rudall and Bateman 2006; Rudall 2008).

The family Triuridaceae comprises approximately 48 extant species and 11 genera (Maas-van de Kamer and Weustenfeld 1998; Cheek 2003) of mycoheterotrophic, achlorophyllous (white, yellow or crimson/purple) monocot herbs distributed in tropical rain forests of the Paleo and Neotropics (Maas-van de Kamer and Weustenfeld 1998). Members of this family are thought to be perennials (although further biological and ecological observations are needed to corroborate this observation for all species within this group). These species bear rhizomes from where simple stems with reduced leaves arise and give place to a raceme bearing minute flowers (2-10 mm in diameter) during the reproductive season. *L. schismatica* is a hyaline, racemose, bisexual species with central stamens (3 on average) surrounded by numerous (40-80) free carpels (polyapocarpy) and an actinomorphic perianth (Martínez and Ramos 1989).

Triuridaceae is subdivided in three tribes based on stamen morphology, embryo development, and the sexual reproduction system (Maas-van de Kamer and Weustenfeld 1998; Rudall and Bateman 2006; Rudall 2008). The basal tribe Kupeaeae, with monoecious unisexual or dioecious species includes the genera *Kupea* and *Kihansia* and is only distributed in Africa (Cheek 2003; Cheek *et al.* 2003). The tribe Sciaphileae comprises monoecious, dioecious and bisexual species and includes the genera *Sciaphila*, *Seychellaria*, *Soridium*, *Andruris* and *Hyalisma*. Finally, the tribe Triurideae, comprises dioecious individuals – with the exception of *L. schismatica*, which is hermaphroditic – and includes the genera *Peltophyllum*, *Triuridopsis*, *Triuris* and *Lacandonia.* The most species-rich tribe is Sciaphileae (with approximately 30 taxa; Maas-van de Kramer and Weustenfeld 1998).

While Triuridaceae was first established as a botanical family in 1843 (Gardner), it is still far from being thoroughly studied. One of the reasons for such situation is that the habitats where species from this group grow consist of: "dense and humid forests hidden under leaf mould at the base of large trees or along the bank of streams" (cited from Maas-van de Kamer and Weustenfeld p. 456, 1998). Another factor is their minute overall size and coloring. These two characteristics make them difficult to spot while conducting a botanical survey. Thus, new species are still being discovered and described as late as 2003 (Cheek 2003; Cheek *et al.* 2003). Hardly anything is known about these taxa patterns of pollination, dispersal and population renewal. In the next section of this review we will summarize the available cytological information for *L. schismatica* and address how findings at this level may affect other aspects of this plant's biology.

PECULIARITIES OF *LACANDONIA SCHISMATICA*

Reproductive and cell biology of *Lacandonia schismatica*

The flowers of this species have a cleistogamous mode of fertilization (Márquez-Guzmán *et al.* 1993) where pollen grains germinate in the closed flower bud and fertilize adjacent carpels through a lateral micropyle opening (Márquez-Guzmán *et al.* 1989, 1993). Anthesis is then thought to be important as a means for seed dispersal, rather than for pollinator interactions. Due to this pre-anthesis fertilization system, fixation of mutations within populations of this species could take place at a relatively higher rate than under random mating; this inference has been initially supported through a study based on 15 isoenzymatic loci undertaken on individuals from the best well-known population of *L. schismatica.* This study indeed showed that *L. schismatica* shows high homozygosity among the assayed loci (Coello *et al.* 1993); and the same pattern is expected for its most recently discovered populations.

Another interesting feature of this plant, is the unique development of the embryo-sac, which presents a new type of megagametophyte development called the *Lacandonia* type (Vázquez-Santana *et al.* 1998). The latter differs from other types described for other Triuridaceae species (Vázquez-Santana *et al.* 1998); in *L. schismatica* the micropylar megaspore, rather than the chalazal one, is the functional cell that gives rise to a monosporic female gametophyte that lacks cytokinesis during the first division of meiosis (Vázquez-Santana *et al.* 1998).

Peculiar nuclear architecture

At the subcellular level, *L. schismatica* has a standard plant cell structure but chloroplast development is arrested (Jiménez-García *et al.* 1998), an expected feature given its mycoheterotrophic habit. However, the nucleus presents abundant extranucleolar ribonucleoprotein particles that have been called "Lacandonia granules" (Jiménez-García *et al.* 1992; Agredano-Moreno *et al.* 1994). Interestingly, these particles vary in their quantity during flower development and life stage: they are abundant in flower buds (pre-anthesis) in relation to open flowers (post-anthesis), (Agredano-Moreno and Jiménez-García 2000) and are also more abundant during fertilization (Márquez-Guzmán *et al.* 1993; Vázquez-Santana *et al.* 1998). These granules are not *L. schismatica*-specific as was formerly believed; they have been found in many other monocot plant species but never in eudicot plants (Jiménez-García pers. comm. to AGA). Based on cytochemical and immunocytochemical features, Agredano-Moreno and Jímenez-García (2000) proposed that *L. schismatica* granules, perichromatin granules and Balbiani ring granules might be functionally similar.

The importance of the perichromatin region as a functional nuclear domain where DNA replication, transcription and probably most steps of pre-mRNA processing take place, have been acknowledged during the last 30 years (Fakan 2004) and might be correlated with the stages when these granules are more abundant. Also, ring-shaped bodies, which are found in both plants and animals, which contain a central core of RNA surrounded by an electron-dense external ring containing snRNP (small nuclear ribo-nucleoproteins) were found in the nucleus of *L. schismatica.* These have also been documented in animals and could be involved in pre-mRNA metabolism (Agredano-Moreno *et al.* 1994; Zavala and Vázquez-Nin 1997; Agredano-Moreno and Jiménez-García 2000; Agredano-Moreno *et al.* 2001).

It would be useful to investigate if these structures carry out the same function in *L. schismatica* as stated for other organisms, in particular, if the mycoheterotrophic habit of this species is correlated with these peculiar nucleic acid bodies. Furthermore, it would be of particular interest to compare these structures among achlorophyllous and chlorophyllous monocot species in order to study if there is any type of correlation between habit and presence or function of these structures.

PHYLOGENETIC POSITION OF *LACANDONIA SCHISMATICA* WITHIN PANDANALES

The original taxonomic placement of *L. schismatica* (Martínez and Ramos 1989) was contended by Maas-van der Kamer (1995), and to date Lacandoniaceae is considered synonymous to Triuridaceae, where it has been placed as a monospecific genus (Maas-van der Kamer 1995; Maas-van de Kramer and Weustenfeld 1998; Angiosperm Phylogeny Group II 2003). Independently, a study based on comparative developmental series of *L. schismatica* and a Mexican triurid representative; *Triuris brevistylis*, concluded that *L. schismatica* should be placed as a monotypic genus within the tribe Triurideae but not as a separate family (Ambrose *et al.* 2006). This classification has been adopted in recent publications (Rudall and Bateman 2006; Rudall 2008).

From a phylogenetic perspective, the position of Triuridaceae with respect to all other monocot taxa has changed markedly in the last 20 years. This family had been placed as sister to Petrosaviaceae on the basis of their morphology and mycoheterotrophic habits (Cameron *et al.* 2003). Cladistic analyses based on a fragment of the *rbcL* sequence grouped a series of families (Pandanaceae, Velloziaceae and

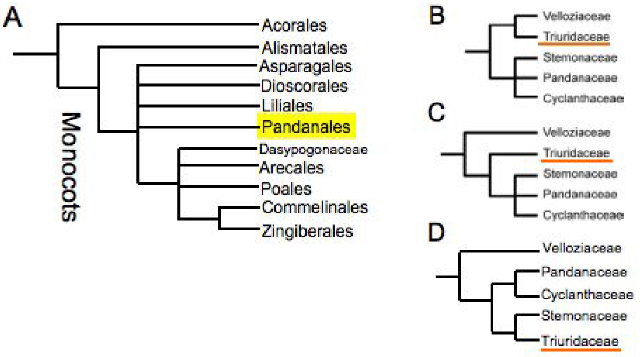

Fig. 2 Position of Pandanales in the Monocot clade and alternative phylogenetic relationships of families comprised within this order. (**A**) Diagramatic representation of the Monocot clade and position of Pandanales (shaded in yellow), based on the cladogram published by The APG II (2003). (**B**), (**C**) Alternative topologies for families within Pandanales (Velloziaceae, Stemonaceae, Cyclanthaceae, Pandanaceae and Triuridaceae) attained using a two-sequence gene analysis based on *atpA* and *Rbcl* sequences; for Triuridaceae only the *atpA* sequence was used (Davis *et al.* 2004; diagram taken from Rudall and Bateman 2006). (**D**) Position of Triuridaceae based on a morphology-based phylogenetic analysis of Pandanales. Simplified diagram drawn after cladogram published by Rudall and Bateman (2006). In (**B**), (**C**) and (**D**) Triuridaceae are underlined in orange. Diagram modified from **Rudall PJ, Bateman RM** (2006) Morphological phylogenetic analysis of Pandanales: Testing contrasting hypotheses of floral evolution. *Systematic Botany* **31**, 223-238, ©2009, with kind permission from the authors and the American Society of Plant Taxonomists, ASPT Publications, Wyoming, USA.

Cyclanthaceae) into a new order: Pandanales (Chase *et al.* 1993). Further analyses based on the 18S rDNA sequence included Stemonaceae and Triuridaceae into this order and placed Pandanaceae as sister group to Triuridaceae (Chase *et al.* 2000). By 2003, The Angiosperm Phylogeny Group corroborated this placement and integrated Pandanales into its lists of recognized orders. While the families comprising this order have remained consistent since its proposal, the relationships among families within this order, as well as of this group with respect to other orders, have remained unresolved. This lack of resolution within the Pandanales is an important issue: while Stemonaceae and Velloziaceae are groups with true hermaphroditic flowers, Cyclanthaceae and Pandanaceae comprise species with reduced or compressed inflorescences (pseudanthia) in which arrested floral organs are clearly visible in scanning electron micrographs of developing inflorescences (See for example *Freycinetia arborea* (Pandanaceae); Cox 1990). The ontogenetic differences between these two kinds of reproductive units are far from being trivial from the *Evo-Devo* perspective and can give further support to the two competing hypotheses regarding the identity of Triuridaceae reproductive structures.

Several phylogenetic analyses for Pandanales have been conducted based on morphological and/or molecular data and have been summarized by Rudall and Bateman (2006; see **Fig. 2**). Most of the relationships among these groups are not well resolved due to the low number of taxa sampled and the lack of available chloroplast markers for Triuridaceae due to their mycoheterotrophic habit, as well as to additional molecular markers. For instance, in some analyses Triuridaceae is placed as sister to Pandanaceae (Chase *et al.* 2000; Rudall 2003), while in others, it is sometimes placed as sister to Velloziaceae (Davis *et al.* 2004), or as a sister group to all other Pandanales (Davies *et al.* 2004; Davis *et al.* 2004).

The latest phylogenetic study of Pandanales based on 39 morphological characters, comparing 23 genera representing the five families of the order shows an unexpected result with Velloziaceae as sister to all Pandanales, and Cyclanthaceae and Pandanaceae forming a separate clade that is in turn sister to a paraphyletic group in which Triuridaceae nests within Stemonaceae. In this reconstruction, the recently discovered genus *Kupea* was placed as a basal member of Triuridaceae (Rudall and Bateman 2006). Confirmation of this result would likely require a comparison with a phylogenetic analysis based on plastid (*atpA*) and nuclear (18S rDNA) markers using a similar or broader number of taxa.

Results of phylogenetic analyses summarized here have complemented morphological analyses in the discussion concerning the identity of the reproductive axes of *Lacandonia schismatica* and other triurids.

CONTROVERSIES ON THE IDENTITY OF THE REPRODUCTIVE STRUCTURES OF TRIURIDACEAE

Euanthial vs Pseudanthial interpretation of the reproductive axes in Triuridaceae

Two main hypotheses have been advanced regarding the nature of *L. schismatica* reproductive axes. Stevens (1991) and Rudall (2003) proposed that the entire family of Triuridaceae comprised species that bore pseudanthia (reduced inflorescences with aborted floral organs of single flowers), rather than euanthia (true flowers). Rudall based her hypothesis in the position previously assigned to *Schiaphila* in a molecular phylogenetic analysis (Chase *et al.* 2000), where it was sister to Pandanaceae, and supported this placement through comparative morphological analyses of mature reproductive structures of both groups (Rudall 2003). An alternative hypothesis regarded *L. schismatica* reproductive units as true flowers (euanthia) with a homeotic inversion of sexual organs where stamens are interior to carpels (Márquez-Guzmán *et al.* 1989; Martínez and Ramos 1989; Vergara-Silva *et al.* 2003; Ambrose *et al.* 2006).

The pseudanthial hypothesis has been contended based on the observation that no aborted reproductive organs or perianth organs were observed in developmental series of *L. schismatica* and *T. brevistylis* flower development using both histological sections and scanning electron micrographs (Márquez-Guzmán *et al.* 1989; Vergara-Silva *et al.* 2003; Ambrose *et al.* 2006). The most recent of these studies, has provided new structural evidence in support of the euanthial hypothesis. In addition to documenting the absence of aborted floral organs, extra evidence includes the presence of a bract subtending and encapsulating each floral primordium, found in opposition to the perianth organ (tepal) that first arises during flower development; this trait is characteristic of most flowers. Also, the temporal progression of floral organ development is reminiscent of flowers and not inflorescences with perianth organs arising first in the most external whorl, then stamens (irrespective of their position) and finally, carpels.

Another interesting feature documented through SEM was the presence of common primordia for stamens and carpels that developed centrifugally (from the center of the floral meristem to the periphery of the flower). In *L. schismatica*, such common primordia bear stamens in the distal part, while carpels differentiate in the proximal (closest to the perianth) section (**Fig. 3**). Furthermore, carpels differentiate from these common primordia not in successive whorls, as could be expected if *L. schismatica*'s reproductive unit was an inflorescence, but in ridges that radiate from the distal part of each stamen primordia (Ambrose *et al.* 2006; Rudall 2008; see **Fig. 3**, panels C, D, E). This type of floral organ development from a common primordium is relatively rare among angiosperms, but had been documented previously in legume species from the subfamily Papilionoideae, which bear true flowers (Tucker 2003). Interestingly, in the Papilionoid species in which this type of ontogenesis has been reported, the common primordium gives rise to stamens and petals (Tucker 2003); while *L. schismatica* unique compound primordium gives rise to stamens in the

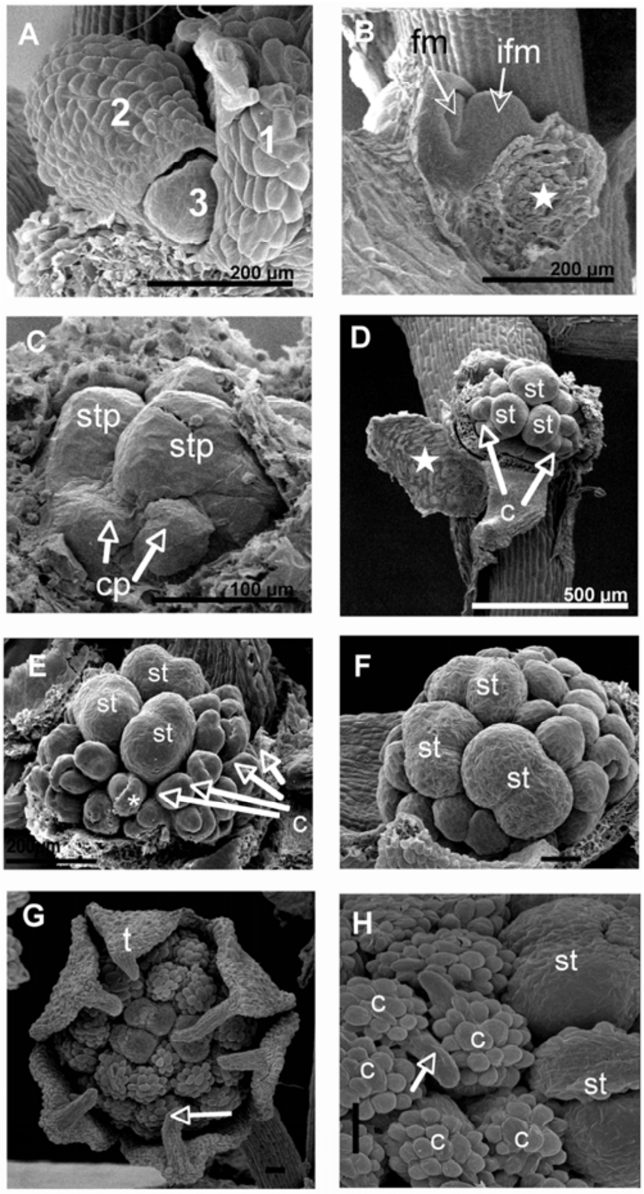

Fig. 3 SEM photographs of *Lacandonia schismatica* flower development. (**A**) Three floral buds before pedicel elongation. Flowers numbered from oldest (1) to youngest (3). (**B**) Inflorescence apex. The inflorescence meristem (ifm), is marked by an arrow. One floral bud has been removed (star) and a flower meristem (fm), covered with a bract is also indicated by an arrow. (**C**) Close-up of a flower bud. Bract and developing tepals have been removed. Common primordia with stamen primordia in the apical part and carpel primordia developing in the distal part are shown. (**D**) Older flower bud. Tepals have been removed and a section of the subtending bract is still apparent (star). Stamen and carpels are shown. (**E**) Close-up of another flower bud where multiple developing carpels are apparent. An asterisk shows a developing carpel wall enclosing an individual nucellus. (**F**) Another flower bud close-up. Bilobulation of developing stamens is apparent. (**G**) Mature flower at anthesis. Note tepals and filamentous tepal tips (arrow). (**H**) Detail of carpels from a mature flower. Note papillous carpel cells and stigmatic extensions (arrow). Figure abbreviations: ifm= inflorescence meristem; stp=stamen primordium; cp= carpel primordia; st= stamen; c=carpel; t= tepal.

most apical position, but instead of producing petals in their periphery, carpels develop. Also, the three stamen primordia develop in the central part of the receptacle since the first stages of organogenesis, thus, refuting the hypothesis of a secondary physical inversion, as already contended in other studies (Márquez-Guzmán *et al.* 1993). Additionally, all floral whorls have a trimerous arrangement that is reminiscent of true flower meristic arrangement proper of other monocot families: three stamen primordia from where three carpel primordia start to differentiate and fasciate, while the perianth is usually composed of six basally fused tepals (Vergara-Silva *et al.* 2003; Ambrose *et al.* 2006).

Although some of the characteristics described above can also be present within species bearing inflorescences (such as a trimerous arrangement of floral organs as well as the temporal progression of floral organ development), while other species with true flowers lack some of these traits, their overall presence in a single species is very suggestive of an euanthial interpretation.

Is there an unclear floral/inflorescence meristem distinction in Triuridaceae? Morphology-based hypotheses

In addition to the summarized developmental biology evidence, the pseudanthial hypothesis was further challenged by a comprehensive cladistic analysis based on morphological characters, which showed that Stemonaceae formed a paraphyletic group with Triuridaceae (Rudall and Bateman 2006). Based on the polyapocarpic flowers of Triuridaceae and given that Stemonaceae has true flowers with single free carpels (except for the case of *Pentastemona*), Rudall and Bateman (2006) speculate a scenario involving carpel multiplication in the origin of Triuridaceae. They argue that these events could have been coupled with a partial loss of determinacy in the flower meristem, that created chimeric axes where the boundary of flower – inflorescence is not clear (Rudall and Bateman 2006).

Morphological evidence of a seemingly blurry boundary between an inflorescence and floral meristem has been put forward for different plant species (see for example, Prenner and Rudall 2007 [Euphorbia]; Rudall *et al.* 2007 [Hydatellaceae]; Rudall 2008 [Triuridaceae]; Rudall 2009 [review of different plant families]; Rudall *et al.* 2009 [Hydatellaceae]) as well as for particular floral architectures, such as racemes, were it has been proposed that what could be interpreted as a terminal flower in these kind of undetermined inflorescences could actually be a very simplified pseudanthium (Sokoloff *et al.* 2006). Overall, a lack of a definitive boundary among meristems has been related to a possible spatio-temporal overlap among domains of expression of inflorescence determination genes and floral meristem genes. (Sokoloff *et al.* 2006; Prenner and Rudall 2007; Rudall *et al.* 2007; Rudall 2008, 2009).

Concerning the blurring of the distinction between the flower and inflorescence meristem, the data by Ambrose and collaborators (2006) showed that in *L. schismatica* these two types of meristems are clearly distinguishable, and while the inflorescence meristem is naked, the flower meristem is covered by a bract.

In the Triuridaceae, further morphological analyses performed by Rudall (2008) address the identity of the reproductive axes of members of this family by comparing the development of these strange polyapocarpic flowers in different species. This study notes several morphological novelties present in flowers of Triuridaceae, including filamentous structures in tepal tips or in central positions of male flowers of *Seychellaria* or *Triuridopsis*, fasciated carpels with stigmatic extensions, and a transition of centripetal to centrifugal carpel development (centrifugal carpel inception), in species within the tribe Triurideae. Based on the presence of these structures, Rudall (2008) considers two alternative scenarios for the origin of the inside-out flowers of *L. schismatica*: the first consists on a homeotic substitution (as previously proposed in Vergara-Silva *et al.* 2003) of the three central carpel primordia from female flowers by stamen primordia (somehow 'predisposed' by centrifugal carpel inception); or alternatively, a heterochronic scenario in which male flowers underwent secondary carpel development between the stamen and perianth whorls. In either case, an inversion in the position of stamens and carpels within hermaphroditic flowers arouse in *L. schismatica*.

Recently, a new interpretation of the identity of peculiar reproductive axes such as those in Triuridaceae, has been put forward by Rudall and collaborators (2009). In this study, based on the morphological analysis of the reproductive structures of Hydatellaceae, which posses reduced bisexual axes with central stamens, the authors propose the term "nonflower" to describe either "an inflorescence-like structure that is derived from a secondarily modified flower" or alternatively, a pre-floral structure (Rudall *et al.* 2009; p. 79). Importantly, the authors suggest that the first definition of nonflower could possibly apply to Triuridaceae (see also Rudall 2008). Hydatellaceae has been recently recircunscribed as part of the basal angiosperm grade (Saarela *et al.* 2007), which is evolutionarily distant from derived groups such as Triuridaceae. The proposal of a convergent nonflower structure would imply multiple origins of this peculiar morphogenetic path during angiosperm evolution and as such is difficult to reconcile with current angiosperm systematics. Also, the SEM photographs of atypical reproductive structures of specimens of *Trithuria submersa* presented by Rudall and colleagues in their 2009 paper, suggest a closer relationship of this taxon with an inflorescence structure, as phillomes – which could be either subtending bracts of perianth organs – are present in between reproductive organs, while carpels are some times found at the center of the reproductive unit. In the case of *L. schismatica*, these two arrangements have not been documented either when assessing the natural variation of this taxon (Vergara-Silva *et al.* 2003), or through developmental series (Ambrose *et al.* 2006). A comprehensive set of developmental series of Hydatellaceae representatives would be convenient to further address this issue, although morphological evidence available to date (see Rudall *et al.* 2007, 2009), shows that this family has a floral morphology far more divergent than Triuridaceae with regards to the stereotypical floral *bauplan* of other angiosperms (for instance, no clear subtending bracts can be assigned, no clear perianth is visible, emergence of sexual organs is seemingly spiral, etc. see Rudall *et al.* 2007, 2009).

For the case of singular reproductive axes such as the ones found in *L. schismatica* and Hydatellaceae, controversial interpretations on the homology of structures and the understanding of the origin of new characters during flower evolution, require knowledge provided by developmental genetics. Thus, gene expression and functional experiments assessing the role of genes known to be fundamental for the inflorescence to flower meristem transition should be performed in these taxa.

Developmental genetics hypotheses

Much insight can be gained from the data attained in the model species *Arabidopsis thaliana*, where several transcription factors including *LEAFY* (*LFY*), *APETALA1* (*AP1*) (a member of the *SQUAMOSA* lineage) and more recently, *SEPALLATA3* (*SEP3*), have been suggested as crucial for the establishment and spatio-temporal patterning of the flower organ identity (ABC) genes (Bowman *et al.* 1993; Parcy *et al.* 1998; Lohmann *et al.* 2001; Liu *et al.* 2009). In this species, the domains of expression of these genes coincide with the places where flower meristems are being specified. Furthermore, homologous copies of these genes with similar functions have been found in other monocots. In rice, when the *LEAFY* homologue *RFL* is silenced, mutant plants lack flowers and gain-of-function lines promote early flowering (Rao *et al.* 2008), although not all characterized LFY orthologs within diverse angiosperm model species have the same function (see review by Moyroud *et al.* 2009). In the case of wheat, *Leafy Hull Sterile*, a homologue of SEPALLATA proteins, seems to be also involved in the determination of the spikelet meristem (Malcomber and Kellogg 2004). *SQUAMOSA*-like homologues are present in monocots but their role is yet unknown outside eudicots, although AP1 seems to be a more specific flower meristem marker in *A. thaliana* (Bowman *et al.* 1993).

Alternatively, floral regulators such as *AGAMOUS* (*AG*) have been shown to be involved in the determination of the floral meristem by suppressing its activator, *WUSCHEL* (*WUS*), in a negative feedback loop (Lohmann *et al.* 2001). *WUS* is a gene involved in stem cell population maintenance that activates *AG* by interacting with LFY (Lohmann *et al.* 2001). The expression profiles and functional characterization of *AG* orthologs of Triuridaceae could point at whether there are divergences in the determination of the floral meristems in this lineage. Recent studies with the proteins REBOLOTE (RBL), SQUINT (SQN) and ULTRAPETALA1 (ULT1) in *A. thaliana* have shown that they are also involved in floral meristem determination (Prunet *et al.* 2008). Multiple mutants of these genes result in indeterminate flowers with repetitions of whorls of carpels and stamens in their flower center, as well as flowers with extra whorls of stamens such as in *superman* mutants (*sup*; Bowman *et al.* 1992), due to a decrease in *AG* expression in the 4th whorl of the flower. Experiments aimed to resolve the spatio-temporal expression profiles of combinations of these genes in key species spanning the five families of the order Pandanales, with emphasis on the Triuridaceae, would be very informative.

This approach would also be useful for species of Hydatellaceae, as already noted by Rudall and colleagues (2009), who analyzed the gene expression pattern of LFY in developing floral units of *Trithuria submersa* (Rudall *et al.* 2009). While the data presented is informative, other organ specification genes such as the ABC class genes should be assayed; the patterns of expression of the former would yield important data that could aid in clarifying the identity of the reproductive units of this family of plants.

For the remaining of this paper, we will proceed under the assumption that the reproductive axes of the Triuridaceae correspond to true flowers and hence aim at analyzing the case of the inside-out flower of *L. schismatica*, as a case of morphological saltation in the context of flower angiosperm evolution.

THE HOMEOTIC FLOWERS OF *LACANDONIA SCHISMATICA* CONSTITUTE A MORPHOLOGICAL SALTATION: STUDIES AT THE POPULATION LEVEL

Some of the fundamental questions regarding the origin of *L. schismatica* heterotopic flower are related to the nature of the underlying genetic variation implicated in this homeosis. One of the possibilities is that such heterotopy is the outcome of microevolutionary forces affecting the genetic variation of homeotic genes, a phenomenon that has been reported (Barrier *et al.* 2001; Olsen *et al.* 2002), while another alternative is that this homeosis is of a "saltational" nature and as such, it is related to discrete changes in particular homeotic genes (Vergara-Silva *et al.* 2003). A means to approach these alternatives is to assess the natural variation within contemporary populations of *L. schismatica* in order to document if a continuum of forms is present in the flower morphs of this taxon. Furthermore, while the heterotopic flower of *L. schismatica* is regarded as an autapomorphy, this approach can help address another related question: was this homeosis present in the most recent common ancestor of *L. schismatica* and its putative sister taxon, *Triuris brevistylis*?

A study by Vergara-Silva and collaborators (2003), addressed the former questions through the study of the natural variation among inflorescences from contemporary populations of *L. schismatica* and *T. brevistylis*, both of which dwell in the Lacandon rainforest and are sympatric to each other (**Fig. 4**). In this study, Vergara-Silva and colleagues (2003) analyzed over 1000 inflorescences of *L. schismatica*. This study revealed an overall fixation of the inverted floral phenotype, with a few variants in this species. Approximately 2% of the analyzed flowers were unisexual (both male and female); a few others had variation in sexual organ number (stamens and carpels); while an additional few individuals bore fertile and sterile stamens. However, flowers with central carpels in bisexual flowers were not observed. A complementary morphological analysis was

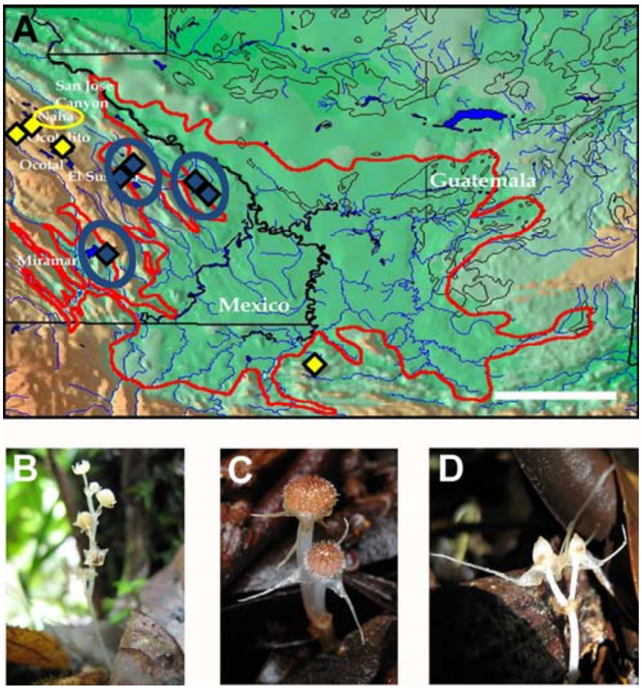

Fig. 4 Lacandon rainforest map with extant *Lacandonia schismatica* and *Triuris brevistylis* populations. (**A**) Former margins of glacial lake *ca*. 7000 years ago (red line), known *L. schismatica* populations (in blue diamonds) and *T. brevistylis* populations (in yellow diamonds). Note how *L. schismatica* populations are settled in close proximity of former lake borders. (**B**) *L. schismatica* inflorescence growing in the Lacandon rainforest. (**C**) *T. brevistylis* female inflorescence. (**D**) *T. brevistylis* male inflorescence growing in the Lacandon rainforest. (map in panel (**A**) modified from Vergara-Silva *et al.* 2003; photos in panels (**B**), (**C**) and (**D**) by Eduardo Flores, Joel McNeal and Esteban Martínez, respectively).

conducted on around 1000 inflorescences of *T. brevistylis* by the same authors (Vergara-Silva *et al.* 2003). In this species, morphological variation was also found: 1% of observed individuals bore male flowers and 0.5% of these were bisexual. Bisexual flowers had varying arrangements of sexual organs, with cases where stamens were present on one side of the androphore while carpels were present in the opposing side; other specimens had stamens surrounded by carpels and this was observed in flowers that seemed both, overall female or male.

The results regarding morphological variation in flowers from *L. schismatica* and *T. brevistylis* do not form a continuum of intermediate forms between extreme phenotypes and as such, strongly suggest that *L. schismatica* heterotrophic flower is the product of a "saltational" event regarding the change in a genetic module or set of modules (Vergara-Silva *et al.* 2003). Furthermore, given the results of *T. brevistylis*, it seems that the most recent common ancestor of these taxa bared unisexual flowers with a "pre-disposition" for homeosis.

Regarding the speciation event that could have originated these triurid species, a biogeographical hypothesis has been put forward based on current knowledge of the distribution of contemporary populations of these two taxa. *L. schismatica* extant populations rest in the Lacandon forest low-lands (around 200 m above sea level; see **Fig. 4**, panels A and B), encircling the perimeter of a glacial lake. *T. brevistylis* populations are localized in the high lands of this forest as well as in the Guatemalan forests, but at higher altitudes (600-800 m above sea level), in more temperate (6 to 8ºC lower than sites for *L. schismatica*) zones. The distribution of *T. brevistylis* also loosely encircles the perimeter of the ancient lake, but at higher elevations (**Fig. 4**, panels A, C and D). This setting, the morphological similitude, the over 40 plant species shared among habitats of *L. schismatica* and *T. brevistylis* contemporary populations, as well as the projected limits of the ancient margins and contemporary margins of the glacial lake, suggest that this two species probably derive from a common Triuris-like ancestor whose populations were isolated after the retraction of the glacial lake approximately five million years ago (Vergara-Silva *et al.* 2003).

The next interesting *Evo-Devo* question that needs to be addressed concerning the morphological saltation of *L. schismatica* floral organ arrangement, concerns the nature of the genetic changes involved, under the hypothesis that relatively small genetic alterations could be at the basis of such a relatively large morphological change.

MOLECULAR GENETIC MECHANISMS UNDERLYING THE SINGULAR SPATIAL ORGAN ARRANGEMENT OF *LACANDONIA SCHISMATICA* FLOWERS

The conserved floral organ arrangement pattern in angiosperms, suggests a restricted and robust developmental mechanism that was broken in the evolution of the Mexican triurids, with a saltation in such floral arrangement having been fixed in *L. schismatica* populations. Indeed, such unique floral morphology has not been found either among natural or experimentally induced mutant phenotypes, thus *L. schismatica* is particularly interesting for studies of the gene regulatory networks underlying flower development, and the constraints that these networks imply, as well as which of their aspects were altered along this species lineage.

We have been studying the molecular genetic basis of *L. schismatica* flower development, based on the knowledge accumulated for model organisms. In particular, we derived our first hypothesis from the ABC model of floral organ specification. The classical ABC model was inferred using *Arabidopsis thaliana* and *Antirrhinum majus* flower homeotic mutants (Bowman *et al.* 1989; Coen and Meyerowitz 1991). This model proposed that the distinct floral organs of a flower are the product of the concerted action or mutual repression of a set of type II MADS-box genes expressed in a precise spatio-temporal order in the floral meristem during flower development. In this model, A class genes alone induce the formation of sepals; A + B class genes specify the formation of petals; B + C class genes induce the development of stamens and C genes alone specify carpel formation. Additionally, A and C genes mutually repress each other (Coen and Meyerowitz 1991).

ABC model-based hypotheses to explain the inside-out flower of *Lacandonia schismatica*

Based on the ABC-model of floral development, the simplest hypothesis to explain the inversion of stamens and carpels in *L. schismatica* flowers, would involve a displacement of B-function gene expression to the center of the floral meristem, while C class gene function would remain in the third and fourth whorls of the developing flower primordium (Vergara-Silva *et al.* 2000; Vergara-Silva *et al.* 2003) (**Fig. 5**).

Such hypothesis is justified because ABC gene orthologs expression patterns and overall function during sexual organ flower development are conserved among angiosperms, particularly in eudicots and grasses (Ambrose *et al.* 2000; Nagasawa *et al.* 2003; Whipple *et al.* 2004, 2007). Overall, B genes have been always associated to stamen specification (Ambrose *et al.* 2000; Nagasawa *et al.* 2003; Rijpkema *et al.* 2006).

Not withstanding the former evidence, a number of publications using different basal angiosperms, non-model eudicot and monocot species as experimental systems have appeared in recent years, with important implications on the applicability and variation of the ABC model in members of this latter group and in *L. schismatica* (Kanno *et al.* 2007; Mondragón-Palomino and Theißen 2009). Some of the most relevant findings on ABC gene evolution and expression

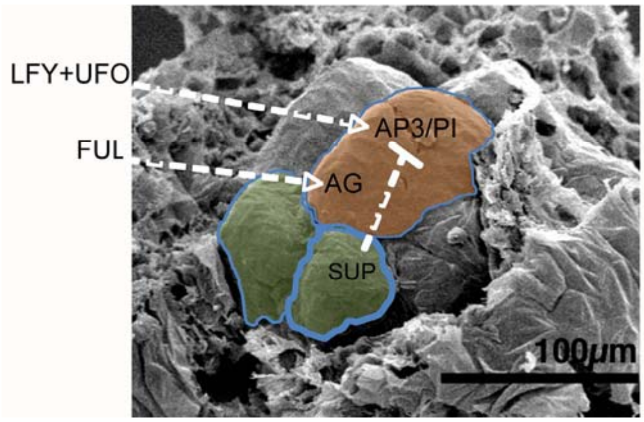

Fig. 5 SEM photograph of a *Lacandonia schismatica* flower bud artificially colorized to show hypothetic domains of expression of different transcription factors. One stamen primordium is colorized in orange; two carpel primordia colorized in yellow. Limits among organs depicted with blue lines. Dotted line with arrowhead implies activation of *AP3/PI* by LFY+ UFO and *AG* by FUL in stamen primordia. Dotted line with bar implies repression of *AP3/PI* by SUP. It should be noted that *AG* expression is present throughout stamen and carpel primordia (Álvarez-Buylla *et al.* unpublished). Figure abbreviations: LFY – LEAFY; FUL – FRUITFULL; UFO – UNUSUAL FLORAL ORGANS; AP3 – APETALA3; PI – PISTILLATA; AG - AGAMOUS; SUP – SUPERMAN.

patterns in different angiosperm lineages are: the existence of pervasive duplication events of MADS-box genes, particularly of the *PI* and *AP3* lineages; these duplications have translated into anomalous expression patterns of B class genes (Stellari *et al.* 2004) or C class genes (Kramer *et al.* 2004), with respect to the ABC model predictions (in basal angiosperms; see Soltis *et al.* 2007; for monocots, see, among others: Kanno *et al.* 2003; Hama *et al.* 2004; Tsai *et al.* 2004; Kanno *et al.* 2007; Xu and Kong 2007; for an eudicot, *Petunia hybrida*, see Vandenbussche *et al.* 2004; Rijpkema *et al.* 2006). Furthermore, such duplication events have led in several cases, to the subfunctionalization and/or neofunctionalization of such gene paralogs and to particular interactions between *PI*-lineage and *AP3*-lineage duplicates in different angiosperm groups (Kramer *et al.* 1998; Irish and Litt 2005; Rijpkema *et al.* 2006; Mondragón-Palomino and Theißen 2009).

It is important to note, however, that the divergences documented to date have never been associated with a homeotic inversion on the position of stamens and carpels caused by a displacement of the B function to the flower center (Kramer and Irish 1999; Kanno *et al.* 2003; Vandenbussche *et al.* 2004; Rijpkema *et al.* 2006; Xu *et al.* 2006, 2007).

Molecular genetics data on *Lacandonia schismatica* floral development

In our laboratory, the B and C gene orthologues of *L. schismatica LsAPETALA3* [LsAP3] and *LsPISTILLATA* [LsPI] and *LsAGAMOUS* [LsAG] have been cloned and characterized (Álvarez-Buylla *et al.* unpublished). Bayesian phylogenetic analyses supported the orthology of these genes as they group with the rest of the B genes and posses characteristic motifs characterized from other angiosperms (Kramer *et al.* 1998, 2003; Álvarez-Buylla *et al.* unpublished).

Based on gene expression analyses, we have observed that the expression domain of the B+C functions is clearly restricted to the most apical or central areas of the compound primordia (Ambrose *et al.* 2006), where stamens develop. Furthermore, B genes of *L. schismatica* are able to rescue or specify stamen development in transgenic complementation and overexpression *A. thaliana* lines which showed very similar phenotypes to corresponding transgenic plants harbouring *A. thaliana* B function genes (Jack *et al.* 1994; Krizek and Meyerowitz 1996; Álvarez-Buylla *et al.* unpublished). However, petals are not completely recovered in these lines (Álvarez-Buylla *et al.* unpublished), which suggests the need for protein complexes involving additional *L. schismatica* native proteins in order to form the multimers which would properly recover both stamens and petals (Honma and Goto 2001).

Alterations of *cis* versus *trans* factors in the regulation of B genes underlying the inside-out flower of *Lacandonia schismatica*

The genetic-molecular data obtained up to date (Álvarez-Buylla *et al.* unpublished) provide a sufficient molecular genetic explanation for the unique spatial inversion of stamens and carpels in *L. schismatica* flowers. However, we still do not know to what extent are other aspects of the GRN in which ABC genes are embedded altered in this species with respect to model organisms. For example, is the atypical expression pattern of *LsAP3* due to changes in the *cis*- regulatory sequences of this gene, while the rest of the GRN is conserved, or are alterations in other *trans*-regulatory components also involved? We provide here some specific hypotheses concerning the possible molecular mechanisms and the involvement of other GRN components in the origin of the *L. schismatica* flower.

Changes in *cis* involve particular mutations in promoter and other regulatory sequences like introns, which could affect the binding affinities of transcription factors that regulate the expression of *LsAP3*. In plants, comparative studies using phylogenetic footprinting of the type to be undertaken in *LsAP3-like* promoters, have shown that the particular inputs of *cis*-motifs can be dissected by comparing promoters or introns of developmental genes of closely related species (Hong *et al.* 2003; Lee *et al.* 2005). In the case of the transcription factor *CRABS CLAW* (*CRC*), comparisons of 5′ upstream regulatory regions between *Arabidopsis*, *Lepidium* and *Brassica* showed conserved modules that have both positive and negative regulatory elements. Noteworthy, each particular motif has the ability to drive reporter gene expression in specific sites as if the overall summation of inputs gave rise to the wild type expression profile (Lee *et al.* 2005). In a related paper, the comparison of the second intron of *AG* between 29 species of *Brassica* allowed dissection of two conserved binding sites for the binding of WUS and LFY; these sites seem to be specific to the Brassicales (Hong *et al.* 2003).

In a conceptually connected but still alternative explanation, divergences in the types, interactions or binding capacities of *trans* activating factors could also render the atypical expression pattern of *LsAP3*. In this scenario, alternative compositions of MADS-domain protein tetramers – the putative functional transcriptional units in *A. thaliana* flower development – could have different affinities for specific regulatory sequences in the *LsAP3* transcriptional regulatory regions. Additionally, proteins forming those complexes could have also experienced spatial or temporal modifications in their expression domains. One such floral regulatory gene that could be concomitantly affected with a change in LFY domain of expression could be *UFO* (UNUSUAL FLORAL ORGANS), an F-box protein that in *A. thaliana*, is a necessary cofactor of LFY required to activate *AP3* expression in the primordia of floral whorls 2 and 3 of this plant. This is achieved by a physical interaction between LFY and UFO in a complex that binds directly to the *AP3* promoter, seemingly tagging a yet unknown *AP3* repressor for degradation (Lee *et al.* 1997; Chae *et al.* 2008). In this paper, we propose that the displacement of *LsAP3* towards the center of the flower meristem of *L. schismatica* could be related to *UFO* being only expressed in the central whorl of this flower, resembling the pattern of *UFO* observed during early *A. thaliana* flower development. Furthermore, the fact that both LFY and *UFO* orthologs from other angiosperms show divergent expression patterns, suggest that alterations in the domain of expression of these genes could be involved in the inversion of stamens and carpels in *L. schismatica* (Souer *et al.* 2008; Moyroud *et al.* 2009).

It is important to notice that with respect to type II MADS-box genes, the *SQUAMOSA*-like *FRUITFUL* (*FUL*) and *AP3* seem to have the greatest degree of conservation in their promoter sequences (Koch *et al.* 2001). Whether this responds to a particular developmental or historical constraint is yet to be tested. Overall, the structure of the promoters of *AP3* in angiosperms should be related to the following factors: initial recognition by UFO and LFY and later-developmental state recognition by the AP3-PI heterodimer (coupled with AG and possibly SEP3) which keeps the *AP3* active in a positive feed back loop. Molecular techniques such as Yeast One Hybrid analysis that allow identification of proteins interacting with a specific DNA sequence, or Chip on Chip, that allow identification of DNA sequences to which transcription factors bind to, should be used once the *LsAP3* promoter and genetic sequences are characterized in order to dissect the genetic-regulatory basis of the inside-out floral phenotype of *L. schismatica*.

L. schismatica SEP3 orthologs may also be involved in mechanisms related to the inversion and partial indeterminacy of the carpel whorl. In *A. thaliana*, SEP3, as stated above, is a regulator of floral meristem patterning and may induce ectopic B and C gene expression when ectopically expressed (Castillejo *et al.* 2005). It is a key factor for the co-localization of AP3 and PI proteins to the cell nucleus, forming ternary complexes with these proteins *in planta* (Immink *et al.* 2009). *SEP3* seems to be also important during the transition from inflorescence to flower meristem (Liu *et al.* 2009), and it could be a useful marker to establish the boundary and the distinction between these two types of meristems in *L. schismatica* and other triurids. Furthermore, SEP3 has been shown to bind to the *AP3* promoter and enhance its expression, although loss of function mutants of *SEP3* do not seem to affect stamen formation (but lack petals, Kaufmann *et al.* 2009), maybe due to redundancy with *SEP1,2-4* genes (Kaufmann *et al.* 2009). Another gene that can be implicated in the molecular mechanism related to the homeosis of *L. schismatica*, is *SUP*. This gene encodes a transcription factor with a C2H2-type zinc finger protein important for delimiting the stamen and carpel whorls and controlling cellular proliferation of both sexual organ types, but specially stamens (Bowman *et al.* 1992; Sakai *et al.* 2000). This gene could be particularly relevant during the specification of the stamen-carpel boundary within the common primordia: stamens in the most apical portion and carpels in the most basal one. Interestingly, it is the second whorl from the inside towards the periphery of the flower the one that has a proliferative stage in *L. schismatica.* In *A. thaliana*, *sup* mutants show supernumerary organs, but in this species, stamens rather than carpels are differentiated in this position (see **Fig. 5** for a representation of proposed genetic interactions).

DEVELOPMENTAL CONSTRAINTS NOT BROKEN DURING FLOWER EVOLUTION IN *L. SCHISMATICA:* THE TEMPORAL PATTERN OF FLORAL ORGAN FORMATION AND THE PRESENCE OF COMPOUND PRIMORDIA

It is interesting to note that despite the fact that *L. schismatica* flowers present a unique spatial sexual organ arrangement, the temporal sequence in which floral organs arise during flower development is not altered with respect to the stereotypical temporal pattern found in the large majority of angiosperms. Namely, perianth organs are specified and appear first, and then stamens and carpels are formed next (see Section **CONTROVERSIES ON THE IDENTITY OF THE REPRODUCTIVE STRUCTURES OF TRIURIDACEAE**). The fact that the temporal progression of floral organ formation is conserved, while the spatial pattern is altered in *L. schismatica*, suggests that the genetic mechanisms that underlie such developmental restrictions can be altered in the course of evolution independently of each other.

Recent efforts in GRN modeling grounded on experimental data of model organisms (Espinosa-Soto *et al.* 2004; Álvarez-Buylla *et al.* 2008) have revealed that a robust network leads to stable gene configurations that characterize each of the four types of primordial cell lineages found at early stages of flower development in *A. thaliana*, which later yield the mature floral organs. Interestingly, the conserved temporal progression of floral organ formation seems to emerge also from the dynamics of such gene regulatory module (Alvarez-Buylla *et al.* 2008). Present modeling efforts are geared towards addressing if alterations of the same GRN may independently change the temporal and spatial patterns of floral organ arrangement. Such approaches provide insights of the specific components of the GRN under study that could have been altered in *L. schismatica* floral evolution. Remarkably, models based on *A. thaliana* genetic data have been able to recuperate stable gene activation profiles that give way to the wild type for this plant and also, infer the genetic configuration of known mutants. An interesting outcome of the study by Espinosa-Soto and collaborators (2004) was that a regulatory module or submodule can be altered independently of others and generate mutant phenotypes. This observation further supports the suggestion made by Vergara-Silva and collaborators (2003) after studying inflorescences of *L. schismatica* and *T. brevistylis*, where they concluded that, given the discontinuous nature of the morphological variations documented in different extant populations of these taxa, the heterotopic flower of *L. schismatica* was most probably a consequence, at the genetic level, of a discrete change in one or several modules of the GRN of this plant.

In order to fully assess the former proposal, it will be instrumental to document the ontogeny of a hermaphrodite *Sciaphila* species, through developmental series. If the development of sexual organs in hermaphrodite triurids follows the common ontogenetic path of angiosperms, this could hint to the possibility that only taxa that posses secondary carpel development (or centrifugal carpel inception; all within tribe Triurideae; Rudall 2008), are the ones where a separation between the spatial and temporal patterns of development could have been favored during the course of evolution. While we were working on a revised version of this paper, an article by Paula Rudall (2009) was published that gives additional examples of angiosperm taxa where a spatio-temporal decouplement of flower development has taken place. This paper analyses this issue from the perspective of the centripetal vs centrifugal floral organ progression.

CHANGES IN FLOWER MERISTEM IDENTITY GENES UNDERLYING COMMON PRIMORDIA AS AN ADDITIONAL EXPLANATION OF *LACANDONIA SCHISMATICA* ONTOGENY

An additional explanation of the peculiar development of *L. schismatica* sexual organs could be related to the underlying developmental genetics network of a common primordium. Common primordia have been documented in different plant species within monocots, in members of the Zingiberaceae and Costaceae families (i.e. *Alpinia ceorulea* and *Costus igneus*; Endress 1995), as well as eudicots, such as some legume species (Tucker 2003). In *L. schismatica* the common primordium from where stamens and carpels develop, can be interpreted as being apically determinate and distally –somewhat-indeterminate (proliferative).

The underlying genetic mechanisms that could be involved in this singular primordium could imply not only a shift to the flower center of the B class genes but also, a similar displacement of the domain of expression of floral pre-patterning genes also involved in meristem determinacy such as *LFY* (or UFO, depending on which of these two proteins is displaced). We advance this hypothesis based on findings in *Pisum sativum* and *Lotus japonicus*, two legume species that bare a common primordium from where petal and stamen whorls develop (Taylor *et al.* 2001; Tucker 2003; Dong *et al.* 2005).

In *L. japonicus*, a plant species with a clear flower-like morphology, the ABCE genes plus *LFY* and *UFO* orthologs have been functionally characterized through loss of function mutants. Dong and collaborators (2005) show that in *L. japonicus* the LFY ortholog is not necessary for A and C-function gene initial expression, given that both *LFY* and *UFO* loss of function mutants in this species have flowers with carpel and sepal whorls, but have no distinctive second and third whorls. Rather, a single whorl is present in the place normally occupied by petals and stamens where repetitive floral meristems arise. This data suggest that the functions of LFY and UFO can diverge significantly in plant species with a common primordium (maybe due to extra *LFY* copies that have undergone subfunctionalization) and also, could imply that in *L. schismatica* the domain of expression of its *LFY* (and/or *UFO*) orthologs could be displaced to the flower center (where determinate stamens arise) and only weakly expressed or completely repressed in the area surrounding the stamens, that is partially indeterminate, and where multiple carpels develop. This hypothesis could also help explain the partial indeterminacy of the carpel whorl, while being congruent with the pattern of expression of the B and C genes observed through *in situ* hybridizations in *L. schismatica* (Alvarez-Buylla *et al.* unpublished). An additional characteristic that further supports this scenario is the fact that *L. schismatica* flowers only have three floral whorls comprised of stamens, carpels and a sepal-like perianth (tepal), which is also the number of whorls present in the *L. japonicus lfy* mutant. The underlying mechanism in *L. schismatica* could imply additional genes, but there are relevant structural similarities concerning the common primordia and the *lfy* mutant of the legume with respect to *L. schismatica.* Furthermore, the LFY ortholog present in triurids could have a divergent function from that characterized in *A. thaliana*; in several angiosperm species this has proven to be the case, where LFY orthologs have been documented to be involved in compound leaf development, as well as plant and inflorescence architecture (Moyroud *et al.* 2009).

It could be possible that changes in the spatial and/or temporal expression of certain flower meristem determinacy genes can be a unifying theme of common primordia in distantly related species that independently evolved this type of organ meristem. This proposal is partially supported by an interesting suggestion made by Moyroud and collaborators (2009) who hint to the idea that *trans* regulation of LFY orthologs within different flowering plants can be fundamental in determining the function of this evolutionary conserved transcription factor, as diverse LFY orthologs used to rescue *lfy* mutants in *A. thaliana* fulfill this task, regardless of having different roles in their species of origin.

Alternatively, a de-coupling of the spatial and temporal pattern of organ progression could be a common trait in compound primordia, as in *L. japonicus* the temporal progression of organs is: sepals, carpels, petals and stamens (Dong *et al.* 2005). Further studies en *L. schismatica* and related species, as well as members of the Zingiberaceae and Costaceae will be instrumental to shed light on this subject.

CONCLUSIONS

In this review we have addressed different lines of research that have complemented or given alternative explanations of the heterotopic flower of *L. schismatica*. A common theme to such research lines is that they have used various strategies at hand to investigate the ontogeny, genetics, development and overall evolutionary history of this taxon, with special emphasis on and *Evo-Devo* perspective committed to an integrative approach to biological reality.

As such, evidence on the morphological variation present in contemporary populations of *L. schismatica* (Vergara-Silva *et al.* 2003), developmental data documenting the spatial and temporal pattern of floral organ development (Ambrose *et al.* 2006; Rudall 2008), and developmental genetics data (Álvarez-Buylla *et al.* unpublished), as well as phylogenetic analyses based in morphology and/or molecular markers (Chase *et al.* 2000; Davis *et al.* 2004; Rudall and Bateman 2006), have generated a body of data that substantiate the importance of studying homeosis and heterotopy as phenomena that can underlie “saltational” events during evolution, such as the one that probably occurred in the *L. schismatica* flower.

The studies undertaken in this unique flowering plant contribute to further our understanding of the nature and extent of developmental constraints during morphological evolution and as such, will help address the possible changes in the overall topology and basins of the epigenetic landscape of *L. schismatica*. In line with this conceptual approach, the data at hand suggest that the regulatory mechanisms underlying the spatial and temporal morphogenetic patterns of floral organ formation seem to be decoupled and can be altered independently from each other during the course of evolution. This is based on the fact that while *L. schismatica* spatial floral arrangement is altered and unique, its temporal pattern of floral organ emergence is conserved with respect to most other angiospems. Previous studies suggest that both processes may be controlled by the same GRN (Alvarez-Buylla *et al.* 2008) and thus suggest that the same network may be altered in such a way that independently alters the spatial and the temporal patterns of floral organs.

New and exciting questions are now ready to be addressed with a broader comparative approach and additional developmental genetics molecular tools. On the evolutionary front, one of the most exciting questions concerns how discrete developmental changes arise and become fixed in a subset of individuals in a population. Addressing this interesting issue will require population genetics data of highly variable molecular loci analyzed in a geographical context, paleogeographic reconstructions and natural history research. The ongoing debate regarding the structural nature of Triuridaceae flowers and inflorescences, as well as the mechanisms beyond the ABC model, that underlie the heterotopic *LsAP3* expression, continue to pose intriguing questions. These will contribute to unravel the developmental mechanisms of the phenotypes of *L. schismatica* and other Triuridaceae, but may also contribute to our general understanding of the gene regulatory networks at play during flower development. In the context of the 150th anniversary of the publication of The Origin of Species, *L. schismatica* is keeping yet another old debate in evolutionary biology alive; the gradualistic versus saltational nature of major morphological transitions during evolution and the genetic mechanisms underlying such transitions, as well as the origin of morphological novelties.

ACKNOWLEDGEMENTS

Special thanks to B. Ambrose, S. Espinosa, J. Márquez, E. Martínez, S. Vázquez-Santana, and specially to F. Vergara-Silva, for a long-standing active collaboration in several aspects that have made the triurid project possible, as well as for specific and important suggestions, corrections and additions for this manuscript, and for providing some of the pictures used here (J. McNeal). We also acknowledge M. Benítez-Keinrad for comments on the *Evo-Devo* and modeling approaches discussed in this paper, as well as C. Espinosa-Soto for insightful discussions concerning some of the alternative molecular genetic mechanisms involved in the unique floral structure of *L. schismatica.* Rigoberto Vicencio Pérez-Ruíz and Adriana Corvera-Poiré helped with various laboratory tasks and Diana Romo with logistical support. The authors thank the three anonymous reviewers who made important suggestions that improved this paper. Financial support was from a Human Frontiers Science Program Grant to E.M and E.R.A.B; from “Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica”, Universidad Nacional Autónoma de México (UNAM; IN230002 and IX207104), University of California-MEXUS ECO IE 271, and Consejo Nacional de Ciencia y Tecnología (CONACYT) CO1.41848/A-1, CO1.0538/A-1 and CO1.0435.B-1 grants to E.R.A.B. and 90565 to A.G.A. PhD scholarships from Consejo Nacional de Ciencia y Tecnología and Universidad Nacional Autónoma de México to A.P-N and E.F-S. A.P-N.'s PhD research is done at the Posgrado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (Instituto de Ecología).

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