Beyond Arabidopsis flowers, evolution of LEAFY and its partnership with UFO in land plants

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Background

LEAFY (LFY) is a transcription factor that originated in algae. In land plants, LFY shows a conserved role in the regulation of pools of stem cells called meristems, while specifying floral organs identity in many angiosperm species (Figure 1A). In *Arabidopsis thaliana*, **UNUSUAL FLORAL ORGANS (UFO)** acts as a cofactor of LFY. UFO redirects LFY to specific DNA binding sites, and thus to a specific panel of transcriptional targets [1]. Phenotypes of *ufo* mutants in various angiosperm species suggest that UFO also has a conserved function in meristem regulation [2].

While the molecular network in which LFY operates for its floral function has been described in angiosperms, little is known outside this group, especially in bryophytes (Figure 1B). Here we question the origin and evolution of the LFY-UFO partnership and we characterize LFY function and downstream network in the liverwort *Marchantia polymorpha*, which does not possess a UFO homolog.

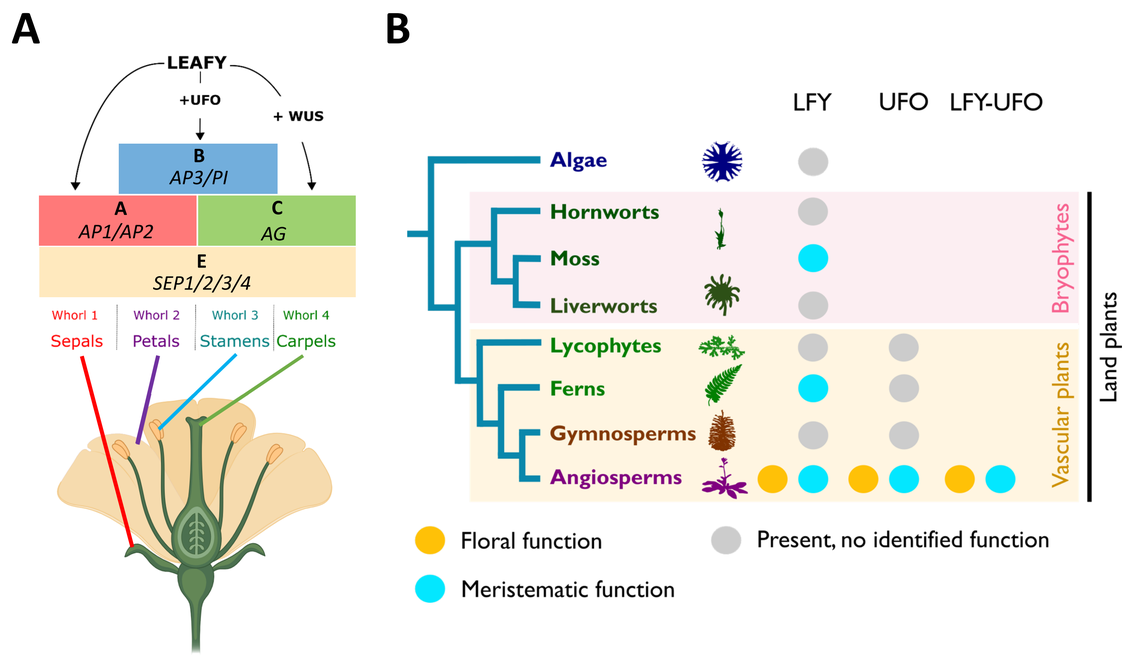
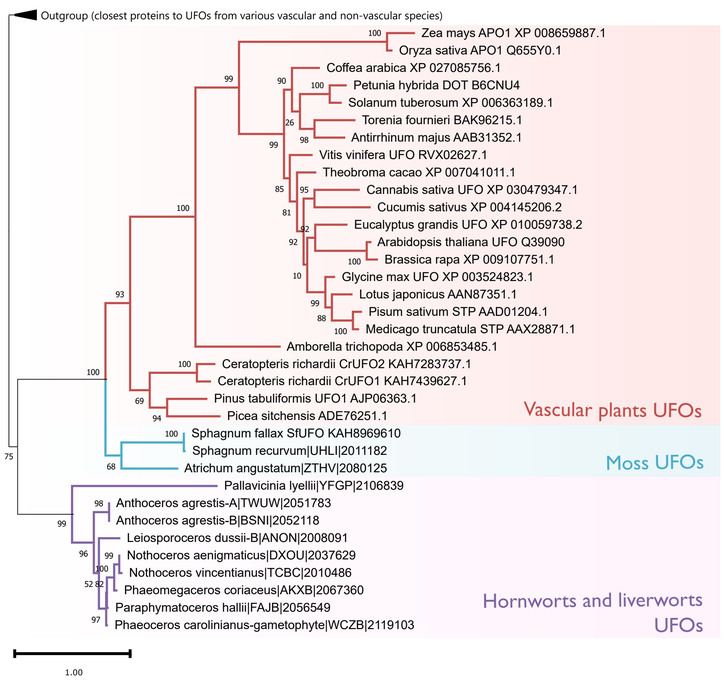


Figure 1. (A) ABCE model, adapted from Philippe Rieu's thesis. **(B)** Evolution of LFY and UFO functions in plants.

How did LFY molecular network evolve in land plants and what is the role of UFO in this evolution?

UFO originates in land plants last common ancestor



1. Monophyly of UFOs
2. Low copy number of UFOs in land plants
3. UFO originates in **land plants last common ancestor**

Figure 2. Phylogeny of bryophytes and vascular plants UFO proteins. Vascular plants UFO group is not exhaustive, we selected species along the phylogeny to have a representation of diverse groups. Bryophyte UFOs were found using the OneKP project database, except for SfUFO that was found within ncbi databases.

LFY-UFO from various land plants bind the LUBS

Interacting regions of *Arabidopsis* UFO with LFY and DNA are highly conserved across vascular plants evolution (A), and LFY and UFO from various land plant species are able to interact with the LFY-UFO Binding Site (LUBS) from *Arabidopsis* (B).

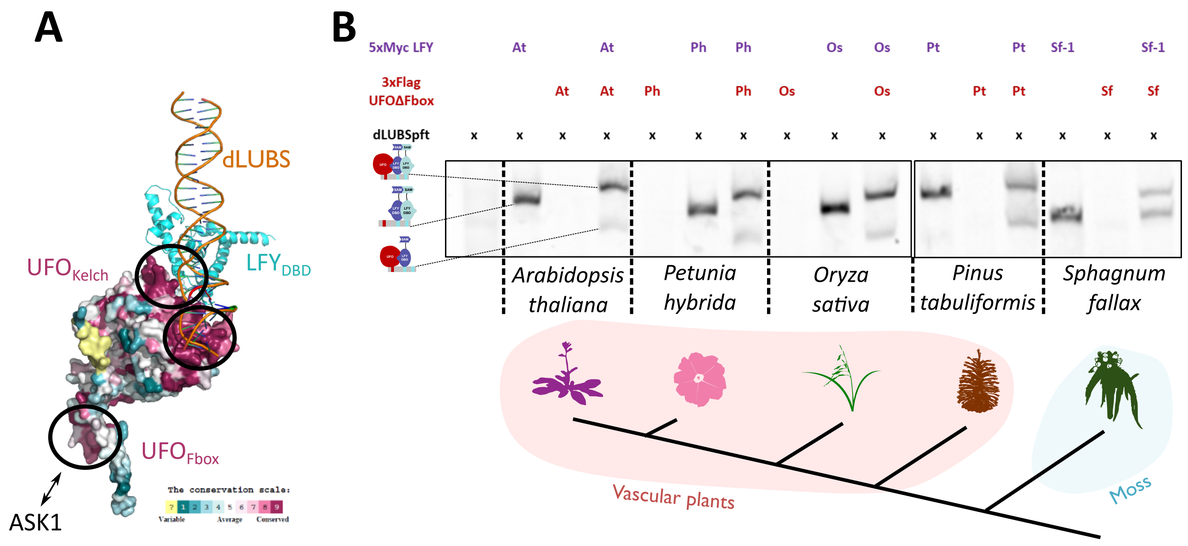
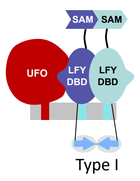


Figure 3. (A) Conservation level of AtUFO mapped on $AtLFY_{DBD}$-AtUFO-DNA cryoEM structure obtained in [1]. **(B)** Electrophoretic Mobility Shift Assay (EMSA) with LFY and UFO from *Arabidopsis thaliana* (At), *Petunia hybrida* (Ph), *Oryza sativa* (Os), *Pinus tabuliformis* (Pt), and *Sphagnum fallax* (Sf) on dLUBSperfect.

From conservation to variation

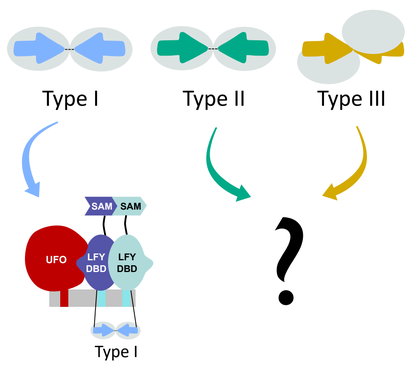
Various LFY type I can bind dLUBS with their UFO.

dLUBS
LFY binding site type I
+ UFO Recruiting Motif



But not all LFYs are type I: are LFY-UFO from some species able to bind alternative LUBS?

In *Anthoceros agrestis* (Hornworts), AaLFY is promiscuous, it can bind type I, II, and III sequences.



The LFY-UFO complex present functional variation in angiosperms.

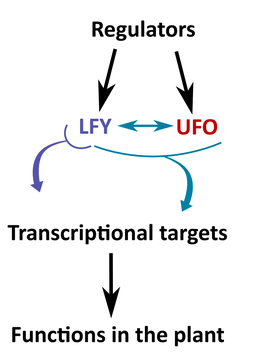
Variations in:

- Floral whorls affected in mutants

- Meristematic functions

- Vegetative develoment functions

How can we explain those variations despite a conserved DNA binding specificity?



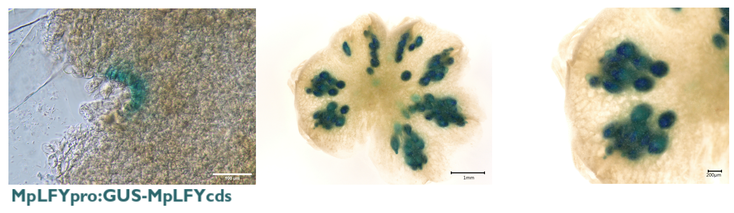
What happens in species that lost UFO?

M. polymorpha

Establishment of a new model species in the lab

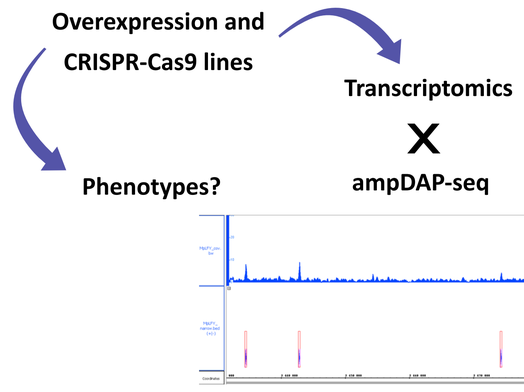
1. MpLFY expression pattern

MpLFY is expressed in gemmae apical meristems (left) and in antheridia (center and right).

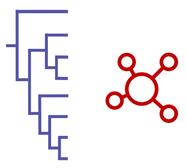


MpLFYpro:GUS-MpLFYcds

2. MpLFY function and targets



3. Integration with function and targets identified in other species to have an integrated view of LFY evolution



Conclusion & perspectives

The DNA binding specificity of LFY-UFO is conserved in various land plant groups: bryophytes, gymnosperms, eudicots, monocots. With regards to this conservation, we are testing if alternative LFY-UFO DNA binding specificities exist.

Taking into account functional, genomic, and *in vitro* data, we explore how the panel of LFY and LFY-UFO targets evolved in land plants.

References

[1] Rieu Philippe *et al.* "The F-Box Protein UFO Controls Flower Development by Redirecting the Master Transcription Factor LEAFY to New Cis-Elements". *Nature Plants* (2023)

[2] Rieu Philippe, Arnoux-Courseaux Moïra *et al.* "Thinking outside the F-box: how UFO controls angiosperm development". *New Phytologist* (2023)

[3] Sayou Camille *et al.*, "A promiscuous intermediate underlies the evolution of LEAFY DNA binding specificity". *Science* (2014)