

## PLANT GENOMICS

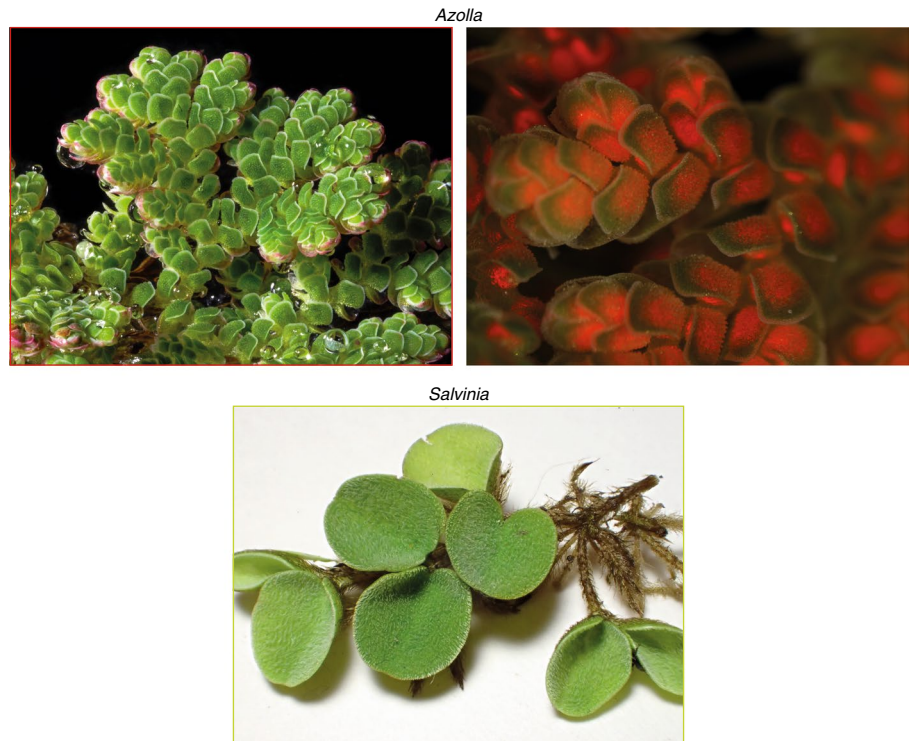
## Fern genomes finally here

The reference genomes of two fern species shed light on fern genome evolution and fern-cyanobacterial symbiosis, paving the way for understanding the unique and interesting biology of ferns.

Jo Ann Banks

Ferns are the second most diverse group of plants on the planet. Phylogenetically, they are sister to the seed plants and therefore are key to understanding vascular plant diversity and evolution, including how seeds, flowers, roots and leaves essential to the human diet, evolved. Until now, no fern genome had been sequenced, making it the only major plant lineage to lack this resource. Reporting in this issue of *Nature Plants*, Li et al.<sup>1</sup> have taken advantage of the relatively small genome sizes of two ferns, *Azolla filiculoides* (750 Mb) and *Salvinia cucullata* (260 Mbp) (Fig. 1), and generated whole genome sequences for both species. In addition to describing the general features of their genes, transposons and proteomes, the authors provide some interesting stories that relate to very different aspects of their biology. These include evidence of a whole genome duplication in *Azolla*; the presence of an exceptionally large pentatricopeptide repeat family that correlates with extensive C-to-U and U-to-C RNA editing within the organelles of these ferns; evidence that the fern insecticidal protein TMA12, recently discovered by Shukla et al.<sup>2</sup>, was acquired in *Salvinia* and other ferns by horizontal gene transfer from bacteria; and that of the 48 transcription factor families related to seed development in angiosperms, 39 are present in ferns but are often less complex, suggesting that the expansion of pre-existing families played a major role in seed evolution.

In addition to providing the plant community with phylogenetically important genomic resources, *Azolla* is particularly noteworthy because it is home to an endosymbiont — the obligate, N<sub>2</sub>-fixing cyanobacterium, *Nostoc azollae*. As well as sequencing two fern genomes, Li et al. re-sequenced five other *Azolla* species and each of their endosymbiont *Nostoc* genomes, using these sequences to address questions regarding the *Azolla*–*Nostoc* symbiotic relationship. Is the common symbiosis pathway, or CSP, which is conserved in plants with arbuscular mycorrhizal (AM) and nitrogen-fixing root nodule (RN) associations, present in these ferns?



**Fig. 1 |** The floating water ferns *Azolla filiculoides* and *Salvinia cucullata*. The *Nostoc* present in *Azolla* fluoresces red under UV light. Photographs courtesy of Laura Dijkhuizen (top left), Jan de Vries (top right) and Pi-Fong Lu (bottom).

Surprisingly, the answer is no. That genes homologous to six essential CSP genes are present in other ferns suggests that they were eliminated in the *Azolla* genomes. So, if not the CSP genes, what genes are essential for the association of *Nostoc* with its host? To identify such genes, Li et al. examined the differential expression of genes from *Azolla* grown in the presence or absence of either nitrogen or its cyanobiont. They found that the greatest changes in gene expression in response to nitrogen occurred when the cyanobiont was absent. Among the genes up-regulated in the absence of nitrogen but in the presence of the cyanobiont — a condition that would presumably promote nitrogen fixation by *Nostoc* — included ammonium and

molybdate transporters and a chalcone synthase required for synthesis of the flavonoid naringenin (which promotes cyanobacterial growth). At this time, the greatest challenge to understanding the *Azolla*–*Nostoc* relationship will be to develop tools in *Azolla* to test the functions of these and other interesting candidate genes.

Being heterosporous, *Azolla* and *Salvinia* are similar to angiosperms but are unlike most other ferns, which are homosporous. Homosporous ferns produce a spore that develops as a male, female or hermaphroditic gametophyte, whereas all heterosporous plants produce mega- and microspores that develop as female or male gametophytes, respectively. Ferns, together with lycophytes, are unique in that they

have both homosporous and heterosporous species. The evolution of heterospory, which has occurred independently in different plant lineages, is one of the most important changes in plant and seed evolution because of its profound effects on reproduction<sup>3</sup>. Many long-standing questions regarding this switch have yet to be addressed. Is the evolution of heterospory a heterochronic shift of sex determination from the gametophyte to the sporophyte generation, as argued by Sussex<sup>4</sup>? Why is gametophyte development endosporic in heterosporous plants and exosporic in homosporous plants? How important was the evolution of heterospory to the evolution of the

seed? And why are homosporous fern genomes large and heterosporous genomes relatively small? Together, the *Azolla* and *Salvinia* genomes are one of two key pieces of genomic information that will be necessary to solve these and other puzzles. The other key piece will be the sequence of a homosporous fern, most likely *Ceratopteris richardii* whose sequence is currently being generated by a group at the University of Florida and the Joint Genome Institute. Together, these genomes will, at long last, provide the plant science community with the resources needed to understand the unique and interesting biology of ferns.

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#### References

1. Li, et al. *Nat. Plants*. <https://doi.org/10.1038/s41477-018-0188-8> (2018).
2. Shukla, A. K. et al. *Nat. Biotechnol.* **34**, 1046–1051 (2016).
3. Bateman, R. M. & Dimichele, W. A. *Biol. Rev.* **69**, 345–417 (1994).
4. Sussex, I. M. in *Trends in Plant Morphogenesis* (ed. Cutter, E.G.) pp 141–152 (Longman, London, 1996).

#### Competing interests

The author declares no competing interests.