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Insight

The symbiosome—a transient organelle in evolution

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Casaes PA, Ferreira dos Santos JM, Silva VC, Rhem MFK, Teixeira Cota MM, de Faria SM, Rando JG, James EK, Gross E. 2024. The radiation of nodulated *Chamaecrista* species from the rainforest into more diverse habitats has been accompanied by a reduction in growth form and a shift from fixation threads to symbiosomes. Journal of Experimental Botany **75**, 3643–3662.

The efficiency of nitrogen-fixing root nodule symbiosis is greatly dependent on the manner in which the symbiont is intracellularly accommodated. Rhizobia can reside either in cell wall-bound fixation threads (FTs) or in membrane-bound, organelle-like structures termed symbiosomes (SYMs). Casaes *et al.* (2024) investigated the evolution of *Chamaecrista* (a legume genus belonging to the *Caesalpinioideae*, a sister subfamily to the *Papilionoideae*), focusing on a possible relationship between the plant's growth habitat and the rhizobial housing mechanism. They identified tree species with FTs, (sub) shrubs with SYMs, and, notably, several shrub species displaying an intermediate FT–SYM phenotype. The presence of multiple rhizobia housing mechanisms within a single evolutionary genus, in combination with the presence of a possibly unique intermediate form of rhizobia housing, opens up the opportunity to unravel the genetic adaptations leading towards SYM release, and could potentially shed more light on organelle evolution.

Symbiosomes: a crucial evolutionary adaptation

Both FT- and SYM-type structures enable nutrient exchange between the host plant and the N_2-fixing rhizobium in its symbiotic form, the bacteroid. Three key features distinguish the FTs from SYMs (Fig. 1A–C). Firstly, the FT is derived from invaginations of the host cell wall of the infection thread (IT) and remains cell wall bound at all times (Brewin, 1998; Fonseca *et al.*, 2012). Conversely, SYM release involves the breakdown of the plant cell wall at the tip of the IT, the release of the rhizobia into the host cytoplasm, but with the retention of the symbiont only in a thin host-derived (peribacteroid) membrane (Gavrin *et al.*, 2016). A second key difference is that SYMs typically contain only one to at most a few rhizobia. Combined with the removal of the host-derived cell wall, this effectively reduces the amount of space occupied by each individual symbiont, and, as a consequence, allows the host to accommodate a far greater number of symbionts per cell (de Faria *et al.*, 2022; Casaes *et al.*, 2024). Akin to chloroplasts, a greater number of smaller organelles is believed to be more efficient than a few larger FTs (Schumpp *et al.*, 2009; Xiong *et al.*, 2017). A third, and probably crucial key difference, is that the SYM fully encloses the symbiont by only the peribacteroid membrane, thereby maximizing the surface area available for nutrient exchange while probably at the same time minimizing any impediment to diffusion of metabolites and nutrients. This more efficient plant–microbe interaction probably confers a competitive advantage to the host plant. It is therefore not surprising that the SYM-type symbiosis is the predominant symbiont housing strategy among all nodulating *Fabaceae* species (de Faria *et al.*, 2022).

The currently prevailing hypothesis on the origin of nodulation is a single gain followed by massive parallel losses (Griesmann *et al.*, 2018; van Velzen *et al.*, 2018). Stable retention of the nodulation trait seemingly correlates with the ability to develop SYM-type nodules (de Faria *et al.*, 2022). Within the N_2-fixing clade, the vast majority of nodulation species are found within the *Fabaceae* family (Doyle, 2011; Griesmann *et al.*, 2018). Yet even here the nodulation trait is unequally distributed. Of the over ~20 000 *Fabacea*e, most nodulating species are members of either the *Papilionoideae* subfamily or

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the mimosoid clade in the *Caesalpinioideae* subfamily (de Faria *et al.*, 2022). With the exception of a few basal *Papilionoideae* clades, all of the aforementioned nodulation-competent species exclusively develop SYM-type nodules. The phylogenetic distribution of these species suggests the FT to be an ancestral trait. Consequently, SYMs must have been acquired independently through convergent evolution (Sprent *et al.*, 2013; Ardley and Sprent, 2021; de Faria *et al.*, 2022) (Fig. 2). It thus appears that the development of SYMs is pivotal, as failure to do so may ultimately lead to the loss of the ability to nodulate.

Chamaecrista as a novel experimental system to investigate symbiosome evolution

Despite being a crucial adaption, the genetic changes underlying a transition from FT- to SYM-type rhizobia housing have remained largely elusive. Previous studies on SYM evolution predominantly focused on SYM-developing species (Gavrin *et al.*, 2016; Libourel *et al.*, 2023), presumably limited by the large evolutionary gap between FT-type and

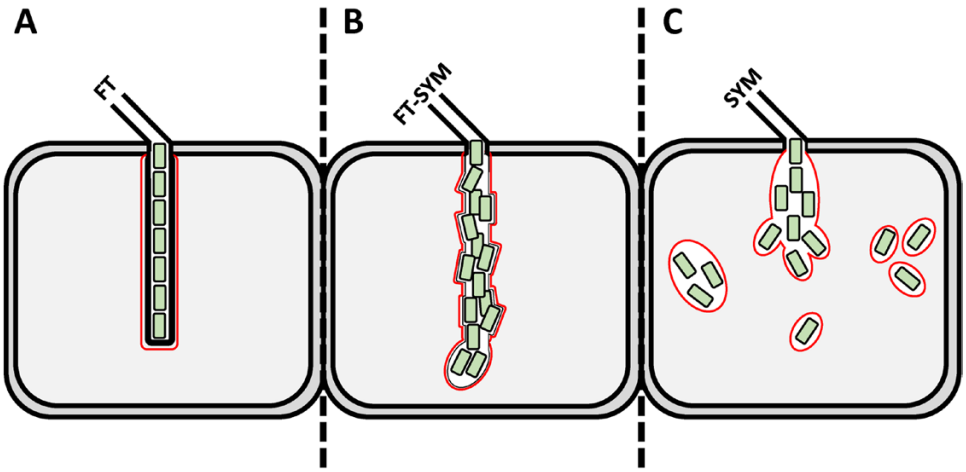

Fig. 1. Schematic overview of the different rhizobial housing mechanisms. (A) A fixation thread (FT) derived from invagination of the plant cell wall (black) and surrounded by a plant-derived membrane (red). (B) An intermediate FT–symbiosome (FT–SYM) housing type. Note the thinning of the cell wall and protrusion of the symbionts. (C) SYMs surrounded by a plant-derived membrane and lacking a plant cell wall.

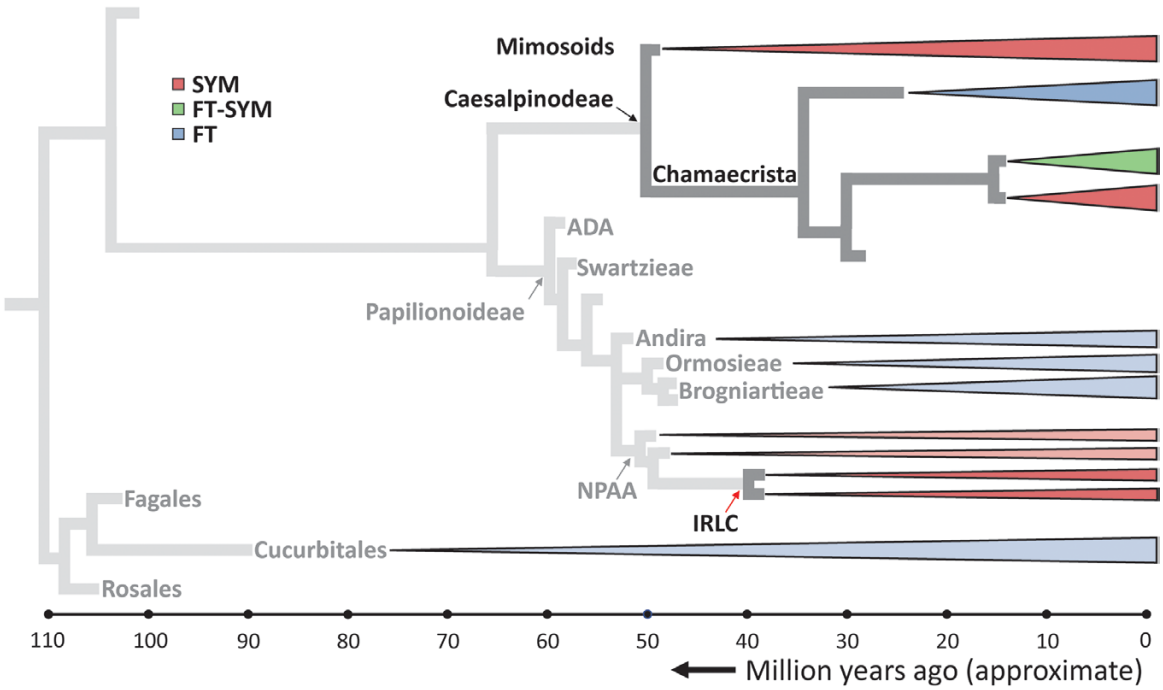

Fig. 2. Simplified phylogeny of the nitrogen-fixing clade with emphasis on the rhizobial housing mechanism and the legume family. Symbiosomes (SYMs; red); fixation threads (FTs; blue); and fixation thread–symbiosome intermediate (FT–SYM; green). IRLC=inverted repeat lacking clade. Time scales are approximate.

SYM-type *Papilionoideae*. The comparison between basal FT-type *Papilionoideae* clades and SYM-forming *Papilionoideae* is complicated by >50 million years of evolutionary separation, thereby introducing significant noise within the analysis (Sprent *et al.*, 2017). However, the recent study by Casaes *et al.* (2024) positions the non-mimosoid *Caesalpinioideae* *Chamaecrista* genus as a particularly attractive system to study SYM evolution. *Chamaecrista* stands out as having independently acquired SYMs and being the only known genus to date to contain both SYM- and FT-type nodules. Within the *Chamaecrista* genus, tree species of the basal *Apoucouita* section develop FTs, whereas shrubby members of the *Absus* and *Chamaecrista* sections develop SYMs (Naisbitt *et al.*, 1992; Casaes *et al.*, 2024). Notably, Casaes *et al.* (2024) pinpoint several species seemingly in transition from FT- to SYM-type nodules, further suggesting an ongoing evolutionary development towards SYM acquisition in the *Chamaecrista* genus (Fig. 1B). The most recent FT–SYM to SYM transition event in this genus occurred relatively recently, ~17 million years ago, while a comparison between *Chamaecrista* species employing FT-type and SYM-type strategies is separated by ~35 million years of evolution (Fig. 2). Combined, this means that a comparative analysis within the *Chamaecrista* genus holds promise for identifying the crucial adaptations that could be causal in facilitating a transition towards SYM housing mechanisms of N_2-fixing rhizobia.

The aim of such comparative analyses would be to derive a blueprint of the genetic constraints needed to enable SYM formation. The ultimate proof of concept, and validation, of such a blueprint is to engineer SYMs on an FT-type species. Moving beyond *in silico* analyses towards practical application necessitates developing a diversity of *Chamaecrista* species as suitable models for *in planta* research. The SYM-type shrubby species *Chamaecrista fasciculata* and *C. mimosoides* have already been used in laboratory settings, their genomes have been sequenced, and they are amenable to hairy root transformations via *Rhizobium rhizogenes* (formerly *Agrobacterium rhizogenes*) (Griesmann *et al.*, 2018; Wardhani, 2020). However, the

Box 1. Terminally differentiated bacteroids—a temporary organelle

Following symbiosome release, certain species impose terminal differentiation upon their symbiont. While primarily observed in members of the inverted repeat lacking clade (IRLC), a form of terminal differentiation is also observed in stem nodules of the *Aeschynomene* genus (Mergaert *et al.*, 2006; Czernic *et al.*, 2015). Thus, like symbiosomes, this trait too appears to be an example of convergent evolution.

Terminal differentiation is characterized by the enlargement of the symbiont, endoreplication of its genomic content, increased membrane permeability and the irreversible loss of autonomy (Fig. 3).

In *Medicago truncatula* (an IRLC member), the induction of terminal differentiation is dependent on host-produced nodule-specific cysteine-rich (NCR) peptides (Van de Velde *et al.*, 2010). The NCR family of peptides comprises >600 members, few of which have been characterized. The mature peptides are transported towards the symbiosome membrane where they induce terminal differentiation (Wang *et al.*, 2010; Montiel *et al.*, 2017; Yang *et al.*, 2023). Although the genetic regulation of terminal differentiation in the *Aeschynomene* genus is unknown, it too appears to utilize NCR peptides (Czernic *et al.*, 2015). Terminal differentiation is believed to further enhance the efficiency of the nitrogen-fixing symbiosis (Oono and Denison, 2010), a claim corroborated by the apparent convergent evolution of the trait.

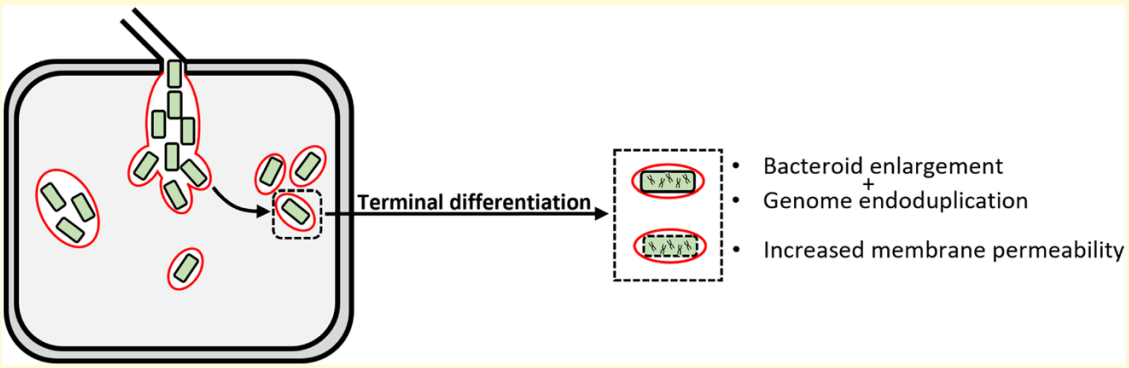

Fig. 3. Terminal differentiation of the nitrogen-fixing symbiont. Following symbiosome release, the symbiont undergoes cell enlargement, genome endoduplication, and its membrane permeability is increased. The symbiont is no longer capable of survival outside the host plant cell.

biggest challenge probably lies with the FT-type *Chamaecrista*. FT-type *Chamaecrista* species are all tropical trees, which generally suffer from relatively long, sexually incompetent, juvenile stages. Consequently, utilizing tree species in laboratory conditions is a non-trivial task. Nevertheless, the successful utilization of the nodulating tree species *Parasponia andersonii* indicates that establishing a model tree species is feasible (Wardhani *et al.*, 2019). Comparative analyses involving *P. andersonii* have already provided valuable insights into the evolution of nodulation (van Velzen *et al.*, 2018; Libourel *et al.*, 2023; Zhang *et al.*, 2023). The *Chamaecrista* genus holds similar potential to provide crucial insights into the evolution of SYMs. However, to truly establish *Chamaecrista* as a model system for SYM evolution, significant efforts must be directed towards establishing FT, SYM, and FT–SYM species as additional experimental models.

The evolution of a transient organelle

SYMs not only boost the efficiency of the symbioses, but also enable the host plant to exert greater levels of control over its symbiont. Consider the inverted repeat lacking clade (IRLC) within the *Papilionoideae* subfamily of legumes for instance. Here, following SYM release, the symbiont is terminally differentiated during the nodulation process, which further optimizes—exploits—the symbiotic interaction. During terminal differentiation, host-produced peptides trigger the symbiont to lose its capacity to function as a free-living organism, essentially becoming a transient organelle (Box 1; Fig. 3) (Mergaert *et al.*, 2006; Van de Velde *et al.*, 2010). While members of the *Mimosoid* clade in the *Caesalpinioideae* do not appear to impose terminal differentiation upon their symbionts (Marchetti *et al.*, 2011; Libourel *et al.*, 2023), it is currently unknown if such terminal differentiation could occur within the SYM-type nodules of *Chamaecrista* species.

During terminal differentiation, the symbiont seems to have relinquished all control to the host. However, one outstanding question that remains is whether the host or the symbiont is in control of SYM formation. The current hypothesis is that the host plant controls the party (Ferguson *et al.*, 2019).

The cross-nodulation experiments performed by Casaes and colleagues (2024) identified strains capable of nodulating on a SYM-type shrub species, though their primary host is an FT-type tree. An investigation on the structure of these nodules would be warranted. Such an analysis of SYM-type nodules with a rhizobial strain generally associated with FT-type nodules, and vice versa, would be able to test the current dogma on host control of bacterial release.

Terminally differentiated or not, SYMs bear a significant resemblance to an organelle; they are enclosed in host-derived membranes, an import–export mechanism is established, and in some cases the symbiont is stripped of its autonomy. This study by Casaes and colleagues (2024) now positions the *Chamaecrista* genus as an interesting system for comparative evolutionary analyses to study SYM evolution. This will not only provide valuable insights for nodule engineering efforts but will also shed light onto the acquisition of a new organelle.

Conflict of interest

The authors declare no conflicts of interest.

Keywords: *Chamaecrista*, evolution, fixation thread, symbiosome.

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