Current Biology

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Review

A roadmap to developing unified streptophyte algal model systems

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https://doi.org/10.1016/j.cub.2025.05.023

SUMMARY

Over the last decade, collaborative efforts in plant evolutionary research have elucidated the phylogenetic relationships in the green plant lineage and provided insights into the emergence of land plants from a group of terrestrial and freshwater streptophyte algae. A foremost finding was that the genetic underpinnings of several key traits emerged much earlier than land plants — they were present in their streptophyte algal progenitors. Currently, the field is at a crossroads, transitioning from genomics-informed descriptions of streptophyte algae to a functional understanding of molecular mechanisms underlying their unique physiology, as well as to understanding their origin and evolution. Major progress has been made in the development of valuable genomic resources, new tools and new model systems in streptophyte algae. In this review, we highlight community-developed resources to study these closest algal relatives of land plants to gain insights into the evolution of land plant traits.

Introduction

Our planet is home to myriad forms of life. Yet, the macroflora on Earth's surface is composed of a single monophylum: the Embryophyta, simply called land plants. Land plants represent a staggering amount of biomass of about 450 gigatons of carbon[1], and the flowering plants alone[2] comprise more than 450,000 species. Land plants have established today's oxygen levels[3] and the web of symbiotic interactions in the soil[4]. Understanding the evolutionary emergence of this monophylum is thus one of the major questions in the field, tackled through the lens of diverse disciplines[5–7]. One of the major revelations — and an important guide for most of this multi-disciplinary work — was brought about through efforts in phylogenomics[8,9] that revealed: firstly, that bryophytes are likely monophyletic, meaning that embryopyhtes are deeply split into vascular and non-vascular plants; and secondly, that the unicellular and filamentous Zygnematophyceae are the closest algal relatives of land plants. To understand the diversity of plant biological processes in the light of this new phylogenetic framework, a community effort aiming at the diversification of the model systems used in laboratory studies has been set in motion.

The development and use of model systems has been one of the triumphs of modern biology. By adopting a common set of organisms, methods, and resources, researchers have made dramatic advances in understanding the biology of a diverse range of prokaryotic and eukaryotic species. Coordination of research among multiple labs facilitates research in many ways: technical challenges, ranging from cultivation and propagation to DNA extraction and genetic manipulation, become less daunting when successful approaches are shared; costs for expensive endeavors such as genome sequencing can be shared among multiple studies; and community sharing of information (including failed experiments) and materials facilitates efficiency. Perhaps most important of all, a large community focused on understanding a single organism means that the combined inputs from multiple investigators serves to solve challenges that would be intractable for any single research group. In this way, the adoption of model systems has facilitated profound advances in the biological sciences.

In the plant science community, modern research has been primarily driven by work on *Arabidopsis thaliana*[10] and several other flowering plants. Comparisons to other members of the green lineage have long been limited to a few select species. When comparisons to algae were made, these largely hinged on 'the green alga' *Chlamydomonas reinhardtii* — which is a powerful system[11] but likely at least a billion years divergent from land plants[12–15]. Important steps forward have been introduced by development of the bryophyte model systems *Physcomitrium patens*[16] and *Marchantia polymorpha*[17]. Now, further diversification of the plant model systems is on the horizon[18,19].

Choosing models

A model organism is a species that combines three main properties: easy handling, including a short life cycle, space and cost efficient cultivation conditions, accessibility to various methods; a feed-forward loop between established resources (protocols, genomic data, genetic lines, etc.), research community efforts and growth, and further development of methods; and the shared occurrence of key biological traits with other organisms. The last point cannot be stressed enough. Just because an organism is convenient does not make it a good model. Model organisms serve the purpose of understanding biological processes that are common across species. For this, the evolutionary framework — the evolutionary distances and trait occurrence mapped onto a phylogenetic tree — needs to be considered. Thus, evolutionary biological framing through phylogenetics can inform the selection of models by providing measures of diversity, genetic distances between genes that underpin traits, and more. The study of biological processes is most successful when a diversity of models is investigated. This means that the peculiarities of any one organism (such as the notorious lack of mycorrhizal associations in *Arabidopsis*) are unlikely to affect the entire field because multiple comparisons are available.

Well-established animal model systems span a wide range of divergence times, which allows them to broadly represent the animal tree of life. There are several advantages to this broad representation. The diversity of model systems means that it is possible to select from a variety of organismal characteristics to identify those that are best suited to the specific problem at hand. Although not an animal, baker's yeast (*Saccharomyces cerevisiae*) has been instrumental in studies of fundamental genetic and cellular mechanisms, whereas zebrafish (*Danio rerio*) is a superior model for developmental studies related to vertebrate body plan. Mouse (*Mus musculus*) is preferred when the system is specific to mammals.

One can select model organisms that are close to the species that is motivating the particular study. However, a more fundamental benefit is that, by intensively studying species that are broadly distributed across a phylogenetic tree, it is possible to reconstruct ancestral character states on that tree (Figure 1A). Evolution acts by a process of iterative modification of a diverging set of lineages. All share a common ancestor, and the traits of that ancestor in effect provide a 'base line' for the entire lineage. Each descendant is modified from that ancestor by a variety of processes, both random and adaptive (or maladaptive). As a result, no organism is identical to its ancestor, but the ancestor's traits are generally widely shared among its descendants. Thus, a knowledge of the ancestral character states provides a good 'first guess' for the traits of its descendants. The broader the phylogenetic sampling of descendants, the more reliable the reconstruction based on it is, which in turn means that it is easier to make reliable inferences.

The phylogenetic representation of plant model systems is currently relatively narrow, reflecting the fact that the vast majority of agricultural plants are angiosperms, and in fact, the bulk of human dietary calories derive — directly or indirectly — from a few plant families: the Poaceae (this includes rice, maize, wheat), Solanaceae (including potato, tomato) and Brassicaceae (including cabbages). However, increased understanding of these families alone will not be sufficient to face the challenges that are expected to confront humanity in the next century and beyond. Carbon fixation, which is absolutely crucial to mitigate the current climate crisis, is divided roughly equally between the marine and terrestrial environments, with woody plants, including conifers, playing a key role[20]. Conifers are also of great importance in the lumber industry. Bryophytes dominate the high arctic[21]. The water fern *Azolla*, which establishes symbiotic relationships with nitrogen-fixing cyanobacteria, is used as a biofertilizer in traditional cropping systems in Asia, and has seen some use in sustainable agriculture elsewhere[22]. These all represent properties that cannot easily (or at all) be studied in the 'classical' plant model systems. Furthermore, plants important in the Global South are poorly represented among model systems. A broader representation of model systems would also make available properties that are currently not well captured. For example, because all embryophytes are multicellular, single-celled model systems are poorly represented. However, they offer unique opportunities, as they consolidate many processes — like signaling and biosynthetic pathways, molecular cell biology underpinning growth, and more — into a single cell[19]. The chlorophyte green alga *Chlamydomonas reinhardtii* is a spectacular model system, but the lineage it belongs to, the chlorophytes, diverged from the streptophyte lineage about a billion years ago[8,9,13,15,23,24].

Streptophyte green algae represent targets for the development of new plant model systems. Green algae (Chloroplastida) are an ancient lineage that began its diversification around 1.8 billion years ago, sprouting two distinct lineages — chlorophytes and streptophytes[8,9,13,15,23,24]. Potentially, the prasinodermatophytes are a green sister lineage to both of these[25] (Figure 1B). The bulk of recognized green algal diversity is among the Chlorophyta, which are highly diverse organisms found in marine, freshwater, and subaerial (terrestrial) environments. Streptophytes include the embryophytes (land plants) along with several lineages of green algae, collectively recognized as the grade of streptophyte algae (more on their phylodiversity below). Streptophyte algae are highly diverse and range from single-celled

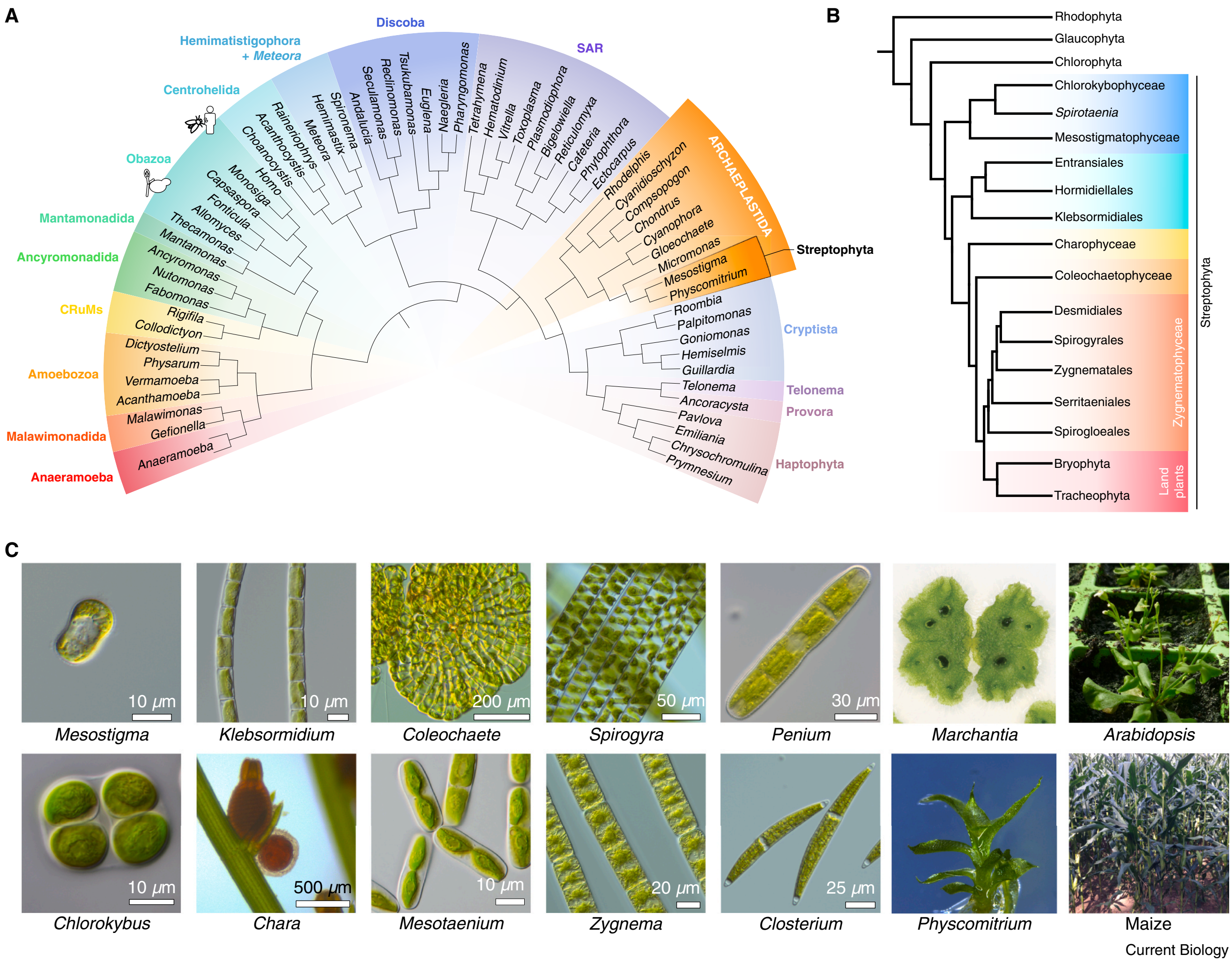

Figure 1. Diversity of streptophytes and scarcity in model phylodiversity.
(A) Cladogram of eukaryotes based on recent phylogenomic data by Williamson *et al.*[140]. Icons label the phylogenetic affinity of classical animal and fungi model systems. Within the diversity of eukaryotes, Streptophyta belong to the group of Archaeplastida (orange highlight). (B) A cladogram of Archaeplastida with a focus on the Streptophyta. The Streptophyta consist of the land plants and six classes of streptophyte algae found in five mono-phyla. The Zygnematophyceae are the closest algal sister lineage to land plants and consist of five orders. (C) Micrographs of streptophyte algae highlighted in the text, along with two bryophytes (*Marchantia polymorpha* and *Physcomitrium patens*) and two angiosperms (*Arabidopsis thaliana* and *Zea mays*); all micrographs of algae are unpublished and were taken by Dr. T. Darienko (de Vries lab, University of Göttingen) except for the picture of *Mesotaenium*, which was published by co-author J. de Vries and team[100] and is here reproduced with permission.

flagellated organisms, such as *Mesostigma*, to complex multicellular organisms, such as *Chara* and *Coleochaete*, and filamentous forms like *Zygnema*, *Mougeotia* and *Spirogyra* (see next section). This diversity of morphology is promising for model systems biology because the diversity of traits can be useful for studying processes as diverse as tissue formation, hormone signalling, cell biology, photosynthesis, and cell wall chemistry[19,26]. In fact, some streptophyte algae have already served as important model systems in the pre-genomic era. *Chara* and *Nitella* have been apt systems for electrophysiology[27–30] and cytoskeleton research[31]; *Micrasterias* for morphogenesis and cell wall research[32]; *Mougeotia* for chloroplast movements and phytochromes[33].

We summarize below some of the successes using streptophyte algae as model systems. Following that, we address challenges that impede their adoption, and then finish with suggestions for a path forward in the development of these promising organisms for broader use by the community represented by the authors of this article, and hopefully, beyond.

Streptophyte algal lineages and species

Following the initial events that transpired during primary endosymbiotic incorporation of the cyanobacterial plastid progenitor[34–37], photosynthetic eukaryotes have diversified over the course of about two billion years of evolution[38]. This gave rise to an astounding biodiversity in forms and functions found in three major lineages of primary plastid-bearing organisms, and various lineages that emerged from secondary endosymbioses[39–41]. The most species-rich of all primary lineages are the green plastid-bearing Chloroplastida.

The lineage of Chloroplastida split into streptophytes and chlorophytes more than one billion years ago[13,38,42,43]. This course of one billion years of evolution has brought forth a diversity of streptophyte algae that are rich in form and function. Currently, we recognize six classes within the paraphylum of streptophyte algae (Figure 1B): a clade of Mesostigmatophyceae plus Chlorokybophyceae, Klebsormidiophyceae, Charophyceae, Coleochaetophyceae, and Zygnematophyceae. These are sometimes grouped into the two grades, called KCM and ZCC[44]. The ZCC grade (Charophyceae, Coleochaetophyceae, and Zygnematophyceae) and the land plants form the monophylum of Phragmoplastophyta. Collectively, the diverse classes of streptophyte algae bear valuable information salient to the evolutionary origin of key traits and thus to the emergence of land plants.

Mesostigmatophyceae

Of all streptophyte algae, the Mesostigmatophyceae are probably the most enigmatic. As far as we know, they only consist of one (maybe two) species. From their plain morphology, *Mesostigma* being a unicellular flagellate (Figure 1C), organisms like *Chlamydomonas* come to mind. Indeed, whether *Mesostigma* might be a streptophyte had been a question in the field, but investigations of its flagellar root (the multi-layered structures, or MLS) already hinted at affinity to streptophytes[45], which was confirmed by early molecular phylogenetics[46]. Now we know that Mesostigmatophyceae and Chlorokybophyceae form a monophyletic sister to all other streptophyte algae[47] (that includes an enigmatic *Spirotaenia*[48,49]). *Mesostigma* strains have peculiar (and divergent) biology worth exploring. Instead of a typical cell wall, their cells are encased by scales, which are biosynthesized intracellularly and trafficked via the Golgi apparatus to the outside[50]. Overall, they show striking morphotypical differences compared with *Chlorokybus*. Both *Chlorokybus* and *Mesostigma* are key for our capacity to understand the root of streptophyte diversity — especially given the inferred secondary loss of coding capacity in the *Mesostigma* genome[51].

Chlorokybophyceae

For almost 100 years, the genus *Chlorokybus* was thought to contain only a single species, *Chlorokybus atmophyticus*, but recent work has determined that it consists of a minimum of five species[49]. The late recognition of their genetic diversity is fitting given the rarity of finding *Chlorokybus* in nature. However, these algae are interesting, as they live in wet soil, and thus a terrestrial habitat, and because they grow as sarcinoid cell packages (Figure 1C), which can be considered a simple type of multicellularity. Thus, a comparative approach that considers Chlorokybophyceae, Klebsormidiophyceae, and Phragmoplastophyta is needed to understand the deep evolutionary origin of streptophyte multicellularity. Although *Chlorokybus* is readily available in culture, it has not been widely used as an experimental system — possibly due to its slow growth and the presence of extracellular polysaccharides, which are nevertheless worth investigating[41,52].

Klebsormidiophyceae

Klebsormidiophyceae include resilient colonizers of challenging terrestrial habitats, and are ubiquitous across the globe. Indeed, it is likely that readers of this paper have encountered *Klebsormidium* growing as a green cover on a façade, rock, or tree bark. The Klebsormidiophyceae are divided into three orders and show growth types ranging from unicellular, to sarcinoid, to filamentous[15]. While a filamentous '1D' body without plasmodesmata (Figure 1C) can be considered the simplest form of multicellularity[53,54], they bear important information on the origins of multicellular growth types in plants[15]. Various members of the Klebsormidiophycae are extremely resilient to terrestrial challenges. Several aspects of this have been studied, such as their ability to withstand desiccation by regulating their photosynthesis and cell wall biology in a controlled manner[55–57]. Therefore, they have interesting physiological properties among streptophyte algae, particularly when challenged with stresses. Research on members of the Klebsormidiophyceae reflects this, with several studies addressing the biochemical basis together with the first transcriptomes to be published in *Klebsormidium*[58] to understand their desiccation tolerance, giving insight into their adaptation to the terrestrial environment and the tolerance of stress[59,60]. Notably, the first streptophyte alga whose genome was fully sequenced was *Klebsormidium nitens*[61]. The genome of *K. nitens* was the first to reveal an unexpectedly large number of genes previously considered land plant-specific. Thus, Klebsormidiophyceae are exciting easy-to-grow organisms that unite key physiological and morphological properties with key insights into plant evolution.

Charophyceae

Of all streptophyte algae, Charophyceae have the most complex bodies. Their thallus (plant body) consists of an upright axis (appearing shoot-like), whorls (leaf-like), and multicellular rhizoids for anchorage. Species of *Chara* (and *Nitella*) have body plans that superficially resemble those found in the embryophytes *Equisetum* or *Najas*. Because of this, Charophyceae have been discussed as close relatives of embryophytes since the 19th century[62]. Yet, phylogenomics tells us that they are, among the phragmoplastophytic streptophyte algae (the ZCC grade), the most distant to land plants[8,9]. Now, they stand as an example of how the streptophyte developmental toolkit can bring forth complex bodies in different ways than embryophytes, showing independent expansions of important developmental regulators, such as genes coding homologs of auxin efflux PIN proteins[63]. Studying these body plans holds the promise of learning about parallel evolution in developmental programs.

Charophyceae exhibit intricate sexual reproduction mechanisms, encompassing both monoicous and dioicous species. They bear female oogonia and male antheridia, both of which are structurally complex and surrounded by sterile (non-reproductive) jacket cells; fertilization results in the formation of oospores (zygotes). Oospores, the sole diploid life stage in Charophyceae, are rich in species-specific ultrastructure[29]. Further, there is a good fossil record of Charophyceae.

Not only is the whole thallus large, but so are its cells (Figure 1C). Reaching sizes of several centimeters, internodal cells of *Nitella* are particularly apt for electrophysiological experimentation, such as patch-clamp, and were also widely used in early studies of the actin–myosin cytoskeletal motor system because the large scale of the cells made whole-cell manipulation practical. Thus, *Chara* has been a classical model for decades, uncovering basic mechanisms in wound healing and cytoplasmic streaming[64,65] underpinned by ultrafast myosin[66].

Coleochaetophyceae

One of plant evo-devo's underrated stars is *Coleochaete* (Figure 1C), exhibiting diverse growth forms. The organization of their thallus varies from loose arrangements of filaments to

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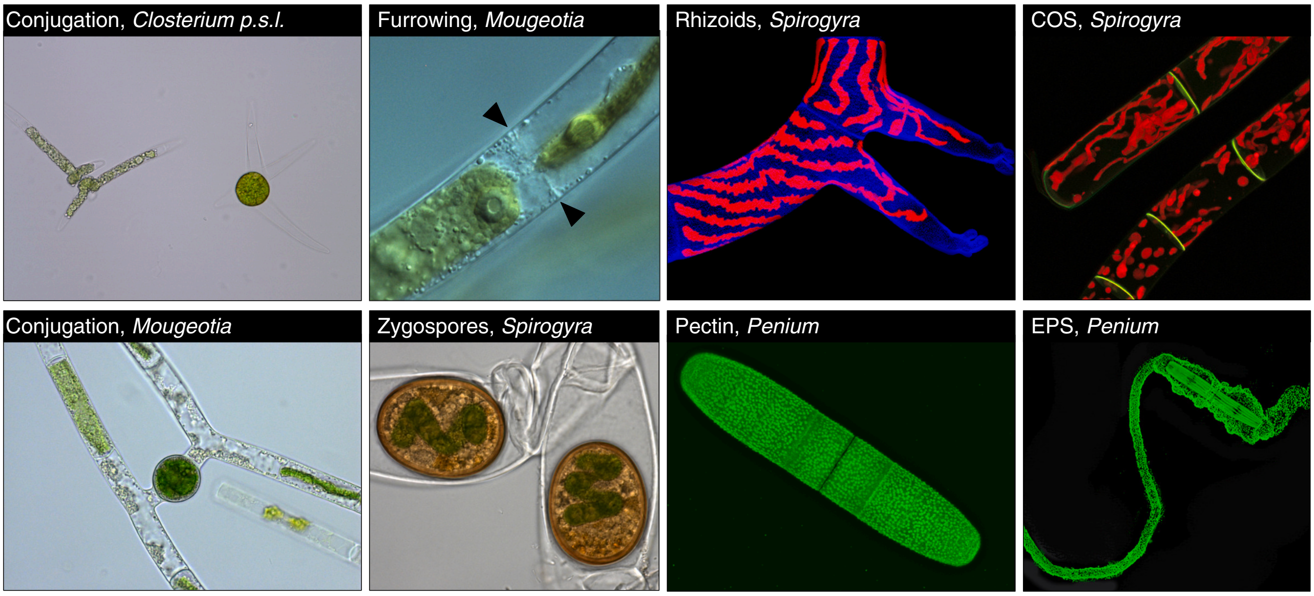

Figure 2. Exciting biology in zygnematophytes.

(Top) From left to right: Conjugation in *Closterium peracerosum–strigosum–littorale*, furrowing in *Mougeotia*, rhizoids developing in *Spirogyra* (red = plastids, blue = cell wall) and FITC-labelled chitooligosaccharides (COS). (Bottom) From left to right: conjugation in *Mougeotia disjuncta*, zygospores in *Spirogyra mirabilis*, anti-pectin (JIM5) labelled *Penium margaritaceum*, and extracellular polysaccharides (EPS) labelled with fluorescent beads. All pictures were taken by the authors and are unpublished except for *Penium* and its EPS, which was published by co-author D. Domozych and team[130] and is here reproduced with permission.

tight, laterally joined patterns resembling parenchyma. This variation is underpinned by distinct cell division patterns, where one can distinguish Y- and T-shaped divisions[67–69]. In the case of the latter, this has profound implications: the species of *Coleochaete* that perform T-shaped division boast both periclinal and anticlinal cell division (that is, cells dividing either parallel or perpendicular to the edge of the radially symmetrical species *C. scutata* and *C. orbicularis*), which has been interpreted as representing the basic toolkit that plants use to form complex bodies[54,70,71]. These species also contain multiple cell types and have a (relatively) rapid life cycle. This makes them excellent candidates for the study of processes underlying plant morphogenesis.

Reproductive structures exhibit different forms, with some species featuring zygotes covered by neighboring filaments, forming a cortex after fertilization. In species like *C. orbicularis*, corticating filaments have wall ingrowths resembling bryophyte placental transfer cells[72], suggesting nutrient transfer between haploid and diploid generations. The Coleochaetophyceae include at least three genera: *Coleochaete*, with a dozen well-characterized and several poorly known species; *Chaetosphaeridium*, with three species; and the enigmatic *Awadhiella*. Stark differences in gross morphology notwithstanding, structural features, like a characteristic sheathed hair, unite the Coleochaetophyceae.

Zygnematophyceae

The Zygnematophyceae have gained major attention in the last years: in the last decade, their position as sister lineage to land plants has been cemented[8,9,73,74]. Thus, they harbor key information for inferring the last common ancestor of embryophytes and streptophyte algae. Reconstructing the shared gene set between Zygnematophyceae and embryophytes is, however, not trivial, since they are the most species-rich class of all streptophyte algae spread over five orders[75], and since they may have experienced a reduction in the body plan, likely reflected by genomic streamlining[76], and a loss of all flagellate stages. The answer to these challenges is taking a comparative and complementary approach both across Zygnematophyceae and other streptophyte algae.

Several ecophysiological and structural traits found across the diverse Zygnematophyceae are salient to a terrestrial lifestyle. Foremost, they can withstand terrestrial challenges through physiological adaptations via specific protective pigments[77–80] and photophysiological[81,82] adaptations. Tolerances to light and temperature stress[83], as well as desiccation stress, have been extensively investigated[84–86], and sequencing of the desmid *Penium margaritaceum* (Figure 1C) revealed a substantial evolutionary expansion in the Zynematophyceae of genes associated with the synthesis and restructuring of polysaccharide cell wall polymers needed for protection and developing complex body plans in terrestrial environments[80] (Figure 2).

One striking set of data was generated on field-collected *Zygnema* (Figure 1C) samples, where a combined metabolomic and transcriptomic analysis elucidated the effects of natural stress scenarios challenging the top layers of an algal mat[87,88]. Furthermore, several Zygnematophyceae form highly resilient resting stages, called zygospores, resulting from sexual reproduction by conjugation, with complex multilayered cell walls containing sporopollenin-like material. Over the past years, the zygospores of *Mougeotia*[89], *Spirogyra*[90] and *Zygnema*[91] (Figure 2) were investigated[92].

Contextualized with embryophytes, the Zygnematophyceae hold the promise of revealing shared molecular programs. Zygnematophyceae indeed stand out in the unique sharing of specific genes. These include the GRAS transcription factors[93,94], which, in embryophytes, regulate diverse important processes from fundamental pattern formation through asymmetric cell division[95] to symbiosis[96]. A second clear case is the emergence of genes that code for the PYRABACTIN RESISTANCE 1/PYR1-LIKE/REGULATORY COMPONENTS OF ABA RECEPTOR (PYR/PYL/RCAR) proteins, which in embryophytes act as abscisic acid receptors[97]. Both *GRAS* and *PYL* genes were gained in the last common ancestor of Zygnematophyceae and embryophytes[94,98]. Interestingly, a protein–protein interaction study of Zygnematophyte PYL proteins has shown that, while they inhibit downstream PP2C phosphatases as in the land plant ABA signaling cascade, they do so in an ABA-independent manner[99]. This speaks for shared but also divergent genetic programs. To pinpoint those independent of a priori preconceptions, co-expression network analyses are being used[76,100], but a broader set of tools is needed to delve deeper.

Key challenges for the development of streptophyte algal models

Despite the deep divergences underpinning the diversity of streptophyte algae of interest to the research community, the challenges in developing streptophyte algal species as model systems are the same across the paraphylum. Here, we elaborate on the major challenges and propose directions for tackling them.

Life cycle and cultivation

Developing any organism into a model system starts with sourcing the organism from its natural habitat. In the case of streptophyte algae, the habitat is often shallow water, but the fact that there is spatial stratification of species within ecosystems — or even of the same species[87] — underlines that there is niche adaptation of each species. Thus, each streptophyte species will likely require specific conditions to be cultivated in the laboratory. There are a number of standardized culture media[101,102] that can be used to best match each species. Alternatively, one may endeavour to generate well-defined synthetic media that support growth of a broad range of streptophyte algal species. In nature, streptophyte algae are associated with other (micro)organisms[103], and an important challenge in cultivation in the laboratory is the development of axenic cultures in which non-essential other microbes (‘passengers’) are gradually eliminated. Yet, one should keep in mind that there are also cases where certain microbes are essential — providing important substances for growth, like vitamin B12, which some algae in nature obtain from bacteria[104] and that, in the lab, can be supplemented. Depending on how intimate the relationship between alga and passenger is, and whether growth depends on its presence, this step can be cumbersome, or may not even be possible, in which case the characterization of the microbial association may also help understand algal biology.

An important aspect of rearing organisms for research is the ability of a species to complete its life cycle under laboratory conditions. The streptophytes are characterized by having a sexual cycle: meiosis in somatic cells produces gametes that either conjugate (in Zygnematophyceae) or are motile and fuse to form a new organism[105,106]. In addition, there is a vegetative cycle where unicellular algae divide or multicellular species fragment. Both modes support dispersion. While establishing algal growth in laboratory conditions is usually not a bottleneck, the formation of zygospores, successful mating or conjugation can be a limiting factor. Gamete formation and fusion are induced by external or internal factors in the natural setting[105,107–109], and it can be problematic to identify and/or emulate these in the laboratory. In addition to the fact that the sexual cycle comprises an important aspect of biology, without closing the sexual cycle in the lab, however, there is a risk of accumulating somatic mutations and evolving towards sterility, as shown, for example, in *Physcomitrium*[110,111]. Therefore, a key step in developing streptophyte algae as models is to identify conditions (light, temperature, drought, daylength, etc.) that induce gametogenesis and fertilization.

Sooner or later, the community needs to converge on the same culture media for consistent lab-to-lab data comparison. These media should ideally omit supplements with ‘soil extract’, which is hard to reproducibly generate as well as apply, and which can interfere with downstream applications, such as proteomics and metabolomics.

Storage

Another aspect of cultivation is the storage of strains. As the research flourishes, many strains will be generated and used: wild-type, mutant, and transformants. These strains need to be kept alive for later experimentation. If a high-frequency inoculation is required to keep the strain alive, it consumes resources, and thus, a method to maintain the line with minimal effort is desired. Slow growth conditions at low temperature is one course, and cryopreservation is optimal. With algae, cryopreservation is generally difficult, and methods for each lineage need to be established. *Klebsormidium nitens* is now kept frozen at the NIES-mcc (https://mcc.nies.go.jp/strainList.do?strainId=2677&strainNumberEn=NIES-2285). Zygospores can be expected to be generally stable for long-term storage, but genetically different offspring are produced for dioicous species.

Transformation

Describing the behavior, physiology, morphology and responses of a species is incredibly powerful in developing hypotheses about organismal function and its genetic or molecular control. Critically testing such hypotheses, however, requires interference with normal regulation to infer effects on function. For this reason, genetic modification is of critical importance. For example, studying a mutant defective in a gene will help place that gene in a biological process. Likewise, modifying a gene of interest, or changing the regulation of the gene or the properties (levels, subcellular localization, etc.) of the protein it encodes, helps fortify links between the properties of the gene or protein and the function studied. Random mutagenesis (e.g., induced by UV or ethyl methanesulfonate treatments) and subsequent forward genetic analysis is an approach that can, in principle, be applied to the study of most species. While avenues of increasing efficiency in these random mutagenesis-based approaches are possible (e.g., TILLING), effectively studying the function of an individual gene requires the manipulation of just that gene. The methods available for this are RNA interference, genome editing (e.g., CRISPR/Cas), overexpression or other kinds of gene modification (promoter–reporter fusion, translational fusion, etc.). All these methods require the availability of protocols to genetically transform cells. Such transformation can be transient, or can involve stable integration of external genetic material in the genome.

Unfortunately, streptophyte algae have proven to be exceptionally challenging in this regard. A stable, *Agrobacterium*-based transformation protocol has been reported for *Penium margaritaceum*[112], but this method has proven difficult to deploy since its initial publication. Transient DNA transfection using intact cells or protoplasts has been reported for a number of species, including *Micrasterias radians var. evoluta*[113], *Mougeotia scalaris*[114], *Closterium*[115], and more. Recently, an electroporation-based protein delivery method has been reported in multiple Zygnematophycean species, as a proof of concept that has the potential to be used for functional and genome editing applications[116]. In the past, microinjection has been used on *Micrasterias*[117]. This variety of methods and species tested and the lack of wide adoption highlight the urgent need for standardized and broadly applicable methods for stable transformation. The most advanced and tried method suite is for the unicellular zygnematophyte *Closterium peracerosum-strigosum-littorale* complex (*Closterium*) (Figure 2). Particle bombardment-based and electroporation-based stable transformation methods are available for *Closterium*, and these have been used to study the function of several genes[118–122]. Now is the time for its broader adoption.

With transformation on its way, the development of a community resource of DNA modules (i.e., Patron *et al.*[123]), including codon-optimized resistance markers and constitutive and inducible promoters, will be essential to facilitate the deployment of these models.

Omics

Molecular and genetic characterization of any organism pivots on the availability of a well-annotated genome, as well as on knowledge of which genes are transcribed where and when, and what proteins these translate into. All this starts with a well-curated genome sequence. In the past years, genome sequences have been released for a number of streptophyte algal species since the initial publication of the *Klebsormidium nitens* genome[61]. This includes the Zygnematophyceae *Penium margaritaceum*[80], *Closterium*[109], *Zygnema circumcarinatum*[76], *Spirogloea muscicola*[94] as well as *Mesotaenium endlicherianum*[94], *Mesostigma viride* (Mesostigmatophyceae)[51], *Chlorokybus atmophyticus* (Chlorokybophyceae)[51] and *Chara braunii* (Charophyceae)[63]. The sizes of these genomes are very different and range from the small ~60 Mbp genomes of *Zygnema circumcariantum*[76] to the enormous *Penium margaritaceum* genome assembly, with 4.7 Gbp. For genome size characterization, classical methods using flow cytometry gave strikingly different results[124], so great caution is necessary in interpreting the data. Given the sparse genome sequencing efforts, the completeness and quality of genome assemblies vary, and there is a need to improve assemblies using increased sequencing and long-read sequencing. It should be noted that such methods require the isolation of high-quality, high molecular weight genomic DNA, which is a serious effort in several of these species, and will benefit from broad sharing of protocols (see next section).

Inferring the functional potential of the genome is strongly facilitated by the availability of RNA sequencing data that can inform about gene structure, transcript isoforms and differential expression. Such efforts have been applied to a range of streptophyte algal species, including those that have a genome assembly, but also beyond[125]. With such efforts — supported by large-scale sequencing efforts[100,126] — the functional annotation, and the ability to predict cellular and organismal function, will likely soon improve.

While much of the RNA population is translated to protein, and is therefore a proxy for protein accumulation, the actual protein accumulation landscape requires different approaches to come to light. There have been very few systematic proteomic studies on streptophyte algae, yet comparative studies on phosphoproteomics have been instrumental in identifying deep conservation of hormone response[127]. The further deployment of proteomics, as well as metabolomics methods[128], in these lineages will be essential for offering in-depth insight into cellular function, physiology and organization.

Most streptophyte algae possess large and complex, polysaccharide-rich extracellular matrices (ECM) consisting of cell walls and often gel-like mucilages[52]. The ECM has widely diverse functions and requires significant contributions of the cell's photosynthate and genetic machinery[76,80,89,129–131]. Yet, our understanding of glycomics of the ECM, including detailed studies of its chemistry, biosynthesis and functions, is only in its infancy and future studies would greatly benefit from the establishment of a collaborative network of chemists, geneticists and cell biologists.

Cell biology

Several streptophyte alga taxa have been used to answer similar biological questions[132]. Streptophyte algae have frequently been employed for studies of cell walls and accompanying cell development, which encompass expansive secretory networks and highly coordinated interactions between components of the endomembrane system and cytoskeletal networks[133–135]. There is now a clear need for the development of new, bespoke probes (e.g., chemicals or antibodies) and technologies to illuminate the cell biology of streptophyte algae. Novel technologies that include electron microscopy-based array tomography and focused ion beam-scanning electron microscopy (FIB-SEM) along with novel light-based microscopy systems (e.g., light sheet microscopy, reciprocal oligosaccharide probes), perhaps aided by expansion microscopy[136], should soon contribute significantly to cell biology studies.

A challenge in studying the cell biology of streptophyte algae is the lack of facile genetic transformation methods (see above), which limits the number and diversity of structures that can be visualized. Another challenge, but also an enormous opportunity, is their morphological diversity, which spans small, single-celled organisms to large, filamentous, branched or thalloid shapes. This diversity will require custom-designed methods for individual species. Given the suspended lifestyle of many of these species, microscopic imaging may even require the development and use of dedicated microfluidics devices and traps to immobilize algae and perform long-term or live imaging.

A path forward

Joining forces

Over the past decades, streptophyte algae research targeted a broad variety of species. Individual research groups chose and studied specific phyla or algal groups due to historical reasons, and/or driven by specific biological questions. More recently, the availability of genomic resources has guided the choice of models on which to focus. Still, model choices are not always obvious, as

Table 1. Algal culture collections that rear streptophyte algae.

Name	Acronym	Country	Website
Coimbra collection of algae	ACOI	PT	http://acoi.ci.uc.pt
Culture collection of algae at the University of Prague	CAUP	CZ	https://botany.natur.cuni.cz/algo/caup.html
Central collection of algal cultures	CCAC	DE	https://www.uni-due.de/biology/ccac/
Culture collection of autotrophic organisms	CCALA	CZ	https://ccala.butbn.cas.cz/
Culture collection of algae and protozoa	CCAP	GB	https://www.ccap.ac.uk/
Provasoli-Guillard National Center for Marine Algae and Microbiota	CCMP	US	https://ncma.bigelow.org/
Commonwealth Scientific and Industrial Research Organisation	CSIRO	AU	https://www.csiro.au/
Freshwater algae culture collection at the Institute of Hydrobiology, Chinese Academy of Sciences	FACHB	CN	http://algae.ihb.ac.cn/
Korean collection for type cultures, algae division	KAS	KR	https://kctc.kribb.re.kr/
Microalgae and Zygnematophyceae collection Hamburg	MZCH	DE	http://www.mzch-svck.uni-hamburg.de
National Institute for Environmental Studies microbial culture collection	NIES	JP	https://www.nies.go.jp/biology/mcc/
Roscoff Culture Collection	RCC	FR	https://roscoff-culture-collection.org/
Sammlung von Algenkulturen Göttingen (Culture collection of algae at Göttingen University)	SAG	DE	https://sagdb.uni-goettingen.de/
Culture collection of algae at the University of Texas at Austin	UTEX	US	https://utex.org/

different streptophyte lineages and species have different advantages and difficulties. For a small community, promoting a collective endeavor to coordinate efforts and resources on a specific set of taxa would increase the feasibility of establishing *bona fide* streptophyte algal model systems. This would draw the attention of an increased number of researchers and resources. Coordinated initiatives, such as MAdLand (https://madland.science), exemplify national programs with international outreach that have successfully promoted the use of streptophyte algae, among other systems, to attract funding.

Establishing links to applied algal research and biotechnology could foster the work on models. There is huge economic potential, since streptophyte algae show so many similarities with land plants, not only in pathways but also cell biology[19]. On the other hand, they are algae and have advantages of algae when it comes to biotechnology. The same way that *Brassica* has not replaced *Arabidopsis* as a model, or *Anopheles* has not eradicated *Drosophila* as a field of study (rather they have boosted the research on the models), it seems possible that biotechnology could strengthen the research on streptophyte models in the future.

Standardizing resources and methods

As more scientists from different backgrounds join a research community, topics and disciplines diverge. This comes with the need to develop tools and platforms, in this case from algal culture to complex single-cell manipulation tools. Inevitably, most of the molecular and cell biology expertise comes from well-established angiosperm research methodologies, but streptophyte algae encompass very different lineages that diverged at different times from the stem streptophytes. As such, many of the commonly used techniques for plant cell manipulation, or analysis tools, such as dyes or antibodies, are not directly transferable. Additional efforts are necessary for the translation and adaptation of existing methods to the study of these algal species, possibly in a species-by-species manner in some cases. Furthermore, we encourage the community to extend beyond the prevailing plant methods and tools and consider more distant biology fields, such as mammalian research. This has proven useful in developing innovative solutions for the transfer of exogenous proteins into multiple algal species[116].

Defining our models

As the interest in studying organisms rises, the urge to develop transferable methods and tools and to establish model strains for performing comparative analyses increases. In turn, the increasing availability of tools and systems also attracts new researchers to the topic. This is no less true in the case of streptophyte algae research, and the prospect of tackling important challenges is key to increasing broader interest. The community should prioritize the development of extensive research toolkits for certain taxa, and as a consequence, not for others. This choice does not have to be erratic, but can be guided by considerations such as the feasibility of developing amenable culture protocols, analytical tools, and methods, to eventually turn them into proper genetic systems. Thus, it is necessary to define standardized model strains, in other words, to find a small number of standard accessions, analogous to the Columbia-0 accession in *Arabidopsis*[137]. This would help in updating genomic resources, improve the transferability of protocols and methods, as well as ease data comparison and re-analyses, and prepare for the eventual distribution of genetic material among different groups, boosting research impact.

The availability of a set of determined strains and species routinely used will persuade more researchers to work on them. For this, we want to bring the spotlight to algal culture collections (Table 1), instrumental institutions and entities for phycology research for decades. Culture collection centres allow for the maintenance and conservation of bioresources and are a fundamental way to share not only material but also information about culture conditions given their expertise.

Increasing shareability of material

Given the size of the current community working on streptophyte algae and the relatively slow generation of new material aside from genomic data, we stand in a privileged position to unite, standardize, and organize resources, and to make them publicly

Table 2. Model systems and resources.

Physical resources	Strains	Culture collections play a key role by conserving bioresources and can also help with dissemination of information
		Isolation and deposition of new cultures
		Records of strain histories
		Knowledge of how to grow them and complete life cycle in culture (maybe into intellectual resources)
	Molecular genetics	Vectors and plasmids
		Virus sequences
		Parts (promoters, coding sequences, etc.)
	Cell biology	Antibodies
		Selective dyes
		Fixation methods
		Specific tools (microfluidics, baby machines, etc.)
Informatic/digital resources	Interoperable databases	GO terms and others
	Data	Genomes
		Transcriptomes (conditions, tissue types, life cycle, etc.), expression profiles
		Proteomes
		Metabolomes
Intellectual and methodological resources	Protocols	Nucleic acid extractions; coping with polysaccharides
		Transformation ● Electroporation ○ Proteins ○ Plasmids (DNA) ○ RNA ● Agrobacterium ● Microinjection?
		Protoplasting and regeneration
		Cellular fractionation
	Morphological database	
	Annotated literature database	
	The Streptophyte Handbook (by analogy to *The* Chlamydomonas *Sourcebook*)	

available for the community. Considering the challenges discussed, we propose a list of resources that would be necessary to consolidate a thriving streptophyte algae research community (Table 2). The list of resources will benefit from collaboration with commercial partners, as many readily available solutions would ease the lack of widespread and reproducible genetic manipulation methods. A commonly used reagent for cell biology in algal research is antibodies. Antibodies allow for specific detection of certain compounds or proteins, especially when combined with fluorescent probes for subsequent imaging. However, available antibodies might not always react with new organisms, as the composition of molecules may vary, and proteins may not specifically react due to the divergence on their antigenic surfaces. Ideally, the development of new antibodies with algae-specific targets will be furthered in a concerted effort with the biotechnological industry.

As new genetic transformation technologies become available, we expect vectors for genetic manipulation to be constantly generated. Addgene stands as the flagship repository for plasmids, allowing for easy sharing and maintainence of vector collections to be used by a wider community. On top of that, it allows for a small community to easily manage these infrastructure-demanding resources. Vector design also requires the functional validation of biological parts, such as promoters, terminators, or different tags such as fluorescent proteins. Data as such can be also managed digitally, while the sources for these parts can be maintained elsewhere.

Increasing digital data openness and methods approachability

An intrinsic part of promoting algal research is the use of freely and openly accessible data repositories. The streptophyte algae community would benefit from a clear management plan for what can be foreseen as an increasing amount of materials, reagents, resources, and tools. First and most pressing is the need for a common repository for genomic data, as more and more genomes of different taxa are being generated, assembled, and annotated by the day[138]. Genomic data, and related resources such as transcriptomics and epigenomics, need to be made easily reachable to promote comparative analyses, molecular cloning design, or for performing expression meta-analyses, as in the case of transcriptomics, also leading to further evidence-guided hypothesis generation. A repository with an accessible user interface is ideal for such a goal. Indeed, the community would also benefit from emulating eFP browsers[139] for single-cell expression analyses, and multi-stage profiling during the life cycle of different species. As for genomics, similar approaches should be considered for proteomics and metabolomics. These are especially sensitive methodologies given the need to build specific datasets for large-scale untargeted analyses, for which a unified and accessible resource repository would be extremely helpful.

Equally important is the creation of a community-managed data repository with updated and reliable protocols, stemming from culturing techniques, analytical methodologies, and molecular genetics approaches, to any future development regarding novel tools such as microfluidics devices for single-cell analyses or imaging-related techniques. A continuously updating compendium of protocols collected and organized by the community could lead to a 'streptophyte handbook', as an analogous initiative to those in other species (e.g., *The* Chlamydomonas *Sourcebook*). Additional digital resources by and for the community should include morphological databases, as a standardized phenotypically informed gallery of images and descriptions, as well as annotated literature lists. All these databases and repositories should be interoperable and connected, supporting the traceability of sources and literature to any related protocol, image, or dataset.

Concluding remarks

We find ourselves at the dawn of a new era in streptophyte algal research. While we need to establish a path to thrive as a community, we also need to support researchers in finding their fitting model systems and tools to answer their biological questions. Gathering and connecting is easier than ever in the digital era, and so we should take this opportunity to move forward together and develop as a community. With an increasing ease of use of these models, researchers will use streptophyte algae as additional models to support their studies, resulting in a better understanding of an understudied part of the tree of life. Only through comparative work across the streptophyte tree of life can inferences be made for how trait evolution has transpired. But what is more, fundamental principles inherent to any streptophyte cell can be gleaned[19].

ACKNOWLEDGEMENTS

We are grateful to Thomas Friedl and Maike Lorenz of the Department of Experimental Phycology and SAG Culture Collection of Algae, Göttingen for their continuing support of the community working on streptophyte algae. Research on streptophyte algae in D.W.'s team was funded by the Netherlands Organization for Scientific Research (NWO; OCENW.KLEIN.027 to D.W.), Human Frontier Science Program (grant RGP0015/2022 to D.W.), and a Marie Sklodowska-Curie Individual Fellowship (MSCA-IF-2020 ref: 101026004 [REOX]) to J.H.-G., H.B., H.N., S.A.R., and J.d.V. are grateful for support through the German Research Foundation (DFG) within the framework of the Priority Programme 'MAdLand — Molecular Adaptation to Land: Plant Evolution to Change' (SPP 2237; 422691801, 440231723, 440240293, 440540015, 527529748). J.d.V. further thanks the European Research Council for funding under the European Union's Horizon 2020 research and innovation programme (grant agreement no. 852725; ERC-StG 'TerreStriAL').

DECLARATION OF INTERESTS

The authors declare no competing interests.

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