

## Papers to choose:

**Genome sequences:** Due to new next-generation sequencing techniques, the number of complete genome sequences is growing rapidly. Therefore, today an innumerable number of bacterial genomes are available. A very interesting sequencing result of a bacterial genome indicates that the alpha-proteobacterium *Rickettsia prowazekii* is closely related to mitochondria and probably served as a progenitor for all mitochondria (S. G. E. Andersson et al., 1998). Compared to bacterial genomes, only a limited number of plant genome sequences are sequenced so far. One of these sequences is the genome of the moss *Physcomitrella patens* which serves as a model plant in molecular biology (Rensing et al., 2008). Another recent complete sequencing of the genome of the early diverging algae *Cyanophora paradoxa* elucidates the single origin of primary photosynthesis in algae and plants (Price et al., 2012).

**Insights and open questions of land plant evolution:** In the last decade major progress was made in revealing phylogenetic relationships of the major groups of land plants using multigene sequences derived from all three genomes. These analyses confirmed non-vascular plants (“bryophytes”) as a paraphyletic group, with the hornworts as the closest relatives of all vascular plants, or Tracheophytes (Groth-Malonek et al., 2005; Y.-L. Qiu et al., 2006). Another unexpected finding was the description of one distinct clade including ferns, horsetails and whisk ferns – the monilophytes (Pryer et al., 2001, 2004). However, other relationships of deeper branching groups are still uncertain. For instance, large data sets up to complete chloroplast genomes were used to resolve the correct placement of deeper branching groups within the monilophytes (Rai & Graham, 2010). Moreover, a huge amount of plant EST data were used for a whole genome level analysis to address plant phylogenetic questions concerning the hierarchical relationships of the major seed plant lineages (de la Torre-Bárcena et al., 2009).

**Peculiarities of land plant mitochondrial genomes:** The number of complete sequenced mitochondrial genomes is growing rapidly. In contrast to the structurally conservative animal mitochondrial genomes, plants exhibit a surprising evolutionary plasticity of their mitochondrial DNA. This year, the so far largest angiosperm mitochondrial genome was completely sequenced which size exceeds 11 Mbp and is larger than most bacterial genomes (Sloan et al., 2012). Complete sequencing of lycophyte mitochondrial DNAs revealed either frequent genomic rearrangements leading to *trans*-splicing events, the insertion of chloroplast or nuclear fragments, and massive amounts of post-transcriptional editing (Grewe et al., 2009, 2011; Hecht et al., 2011) or a very conservative circular genome (Liu et al., 2012). Nuclear encoded PPR proteins were shown to be involved in this post-transcriptional process of RNA editing in plant mitochondria. Almost all nuclear DYW-type PPR proteins were assigned to specific RNA editing sites using KO mutants of the model plant *Physcomitrella patens* (Rüdinger et al., 2011).

**Endosymbiosis within our grasp: The cpDNA of *Paulinella*:** The amoeba *Paulinella chromatophora* were shown to obtain its photosynthetic organelles by a similar but more recent process, which involved a different cyanobacterium, than all other plants did at their primary endosymbiosis. Sequencing approaches of the *Paulinella* plastid genome demonstrated genomic characteristics typical of cyanobacteria, not plastid genomes (Yoon et al., 2006), and phylogenetic analyses of two distinct strains of *Paulinella* indicated a single plastid origin (Yoon et al., 2009). Using next-generation sequencing the interaction of the genome with the newly obtained organelle was traceable and provides the unique

opportunity to observe the cellular and genomic consequences of a recent endosymbiosis (Nowack et al., 2011; Waller, 2012).

**Gene transfer into the nucleus:** After incorporation of an alpha-proteobacterium and a cyanobacterium in plant cells most of the genes of the endosymbiotic organellar DNA were lost or transferred to the nucleus. Molecular work on the mitochondrial *cox2* gene made it possible to elucidate the different steps which are required before proper integration and activation of the gene in the nuclear genome (Daley et al., 2002; Nugent & Palmer, 1991). Nuclear insertions of mitochondrial DNA can be even larger than the mitochondrial genome itself. For instance, the chromosome 2 of the model plant *Arabidopsis thaliana* harbors a 620 kb repetitive insertion of its mitochondrial DNA (Stupar et al., 2001). A different type of nuclear insertions consists of mosaics of organellar DNA, often derived from both plastids and mitochondria. The evolutionary fate of relatively recent integrations in nuclear DNA were characterized (Noutsos et al., 2005). Additionally, it was shown that environmental stress is increasing the transfer of cytoplasmic organellar DNA into the nucleus in plants (D. Wang et al., 2012).

**Horizontal transfer:** One of the most fascinating and discussed aspect of genome evolution is the horizontal transfer of DNA across species borders. Interestingly, the mitochondrial genome of plants is often incorporating foreign fragments acquired via horizontally transfer. This can affect mitochondrial introns (Cho et al., 1998) or result in a duplication of gene regions, e.g. in the early flowering plant *Amborella* (Bergthorsson et al., 2004). Moreover, a transfer from a parasitic flowering plant to their host flowering plants occurred as a result of direct physical contact (Davis & Wurdack, 2004; Mower et al., 2004; Yoshida et al., 2010). The sea slug *Elysia chlorotica* acquires plastids by ingestion of its algal food source and keeps them photosynthesizing for months in the absence of algal nucleocytoplasm. Nuclear genes necessary to maintain the chloroplast's function in *Elysia* were acquired horizontally from the genome of its algal chloroplast donor (Rumpho et al., 2008).

**Provitamin A accumulation in genetically modified rice:** Although the engineering of genetically modified (GM) plants already proved its advantage in several aspects it is still a highly controversial topic. More than ten years ago the "golden rice project" was started producing rice with accumulated Provitamin A to complement vitamin A deficiency in developing countries where predominantly rice is consumed (Paine et al., 2005; Ye et al., 2000). Even though it is a nonprofit project with best intentions many problems hindered a successful distribution and an acceptance by farmers (Al-Babili & Beyer, 2005). The University of Nebraska-Lincoln is contributing a recent project which pursues the same objective enriching Provitamin A in a starchy root crop Cassava (*Manihot esculenta*) more than 250 million Africans rely on (Sayre et al., 2011).

Al-Babili, S., & Beyer, P. (2005). Golden Rice – five years on the road – five years to go? *Trends in Plant Science*, 10(12), 565–573. doi:10.1016/j.tplants.2005.10.006 Provitamin A accumulates in the grain of Golden Rice as a result of genetic transformation. In developing countries, where vitamin A deficiency prevails, grain from Golden Rice is expected to provide this important micronutrient sustainably through agriculture. Since its original production, the prototype

Golden Rice has undergone intense research to increase the provitamin A content, to establish the scientific basis for its carotenoid complement, and to better comply with regulatory requirements. Today, the current focus is on how to get Golden Rice effectively into the hands of farmers, which is a novel avenue for public sector research, carried out with the aid of international research consortia. Additional new research is underway to further increase the nutritional value of Golden Rice.

Andersson, S. G. E., Zomorodipour, A., Andersson, J. O., Sicheritz-Pontén, T., Alsmark, U. C. M., Podowski, R. M., Nislund, A. K., et al. (1998). The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. *Nature*, 396(6707), 133–140. doi:10.1038/24094 We describe here the complete genome sequence (1,111,523 base pairs) of the obligate intracellular parasite *Rickettsia prowazekii*, the causative agent of epidemic typhus. This genome contains 834 protein-coding genes. The functional profiles of these genes show similarities to those of mitochondrial genes: no genes required for anaerobic glycolysis are found in either *R. prowazekii* or mitochondrial genomes, but a complete set of genes encoding components of the tricarboxylic acid cycle and the respiratory-chain complex is found in *R. prowazekii*. In effect, ATP production in *Rickettsia* is the same as that in mitochondria. Many genes involved in the biosynthesis and regulation of biosynthesis of amino acids and nucleosides in free-living bacteria are absent from *R. prowazekii* and mitochondria. Such genes seem to have been replaced by homologues in the nuclear (host) genome. The *R. prowazekii* genome contains the highest proportion of non-coding DNA (24%) detected so far in a microbial genome. Such non-coding sequences may be degraded remnants of “neutralized” genes that await elimination from the genome. Phylogenetic analyses indicate that *R. prowazekii* is more closely related to mitochondria than is any other microbe studied so far.

Bergthorsson, U., Richardson, A. O., Young, G. J., Goertzen, L. R., & Palmer, J. D. (2004). Massive Horizontal Transfer of Mitochondrial Genes from Diverse Land Plant Donors to the Basal Angiosperm *Amborella*. *Proceedings of the National Academy of Sciences of the United States of America*, 101(51), 17747–17752. doi:10.1073/pnas.0408336102 Several plants are known to have acquired a single mitochondrial gene by horizontal gene transfer (HGT), but whether these or any other plants have acquired many foreign genes is entirely unclear. To address this question, we focused on *Amborella trichopoda*, because it was already known to possess one horizontally acquired gene and because it was found in preliminary analyses to contain several more. We comprehensively sequenced the mitochondrial protein gene set of *Amborella*, sequenced a variable number of mitochondrial genes from 28 other diverse land plants, and conducted phylogenetic analyses of these sequences plus those already available, including the five sequenced mitochondrial genomes of angiosperms. Results indicate that *Amborella* has acquired one or more copies of 20 of its 31 known mitochondrial protein genes from other land plants, for a total of 26 foreign genes, whereas no evidence for HGT was found in the five sequenced genomes. Most of the *Amborella* transfers are from other angiosperms (especially eudicots), whereas others are from nonangiosperms, including six striking cases of transfer from (at least three different) moss donors. Most of the transferred genes are intact, consistent with functionality and/or recency of transfer. *Amborella* mtDNA has sustained proportionately more HGT than any other eukaryotic, or perhaps even prokaryotic, genome yet examined.

- Cho, Y., Qiu, Y.-L., Kuhlman, P., & Palmer, J. D. (1998). Explosive Invasion of Plant Mitochondria by a Group I Intron. *Proceedings of the National Academy of Sciences*, 95(24), 14244–14249. doi:10.1073/pnas.95.24.14244 Group I introns are mobile, self-splicing genetic elements found principally in organellar genomes and nuclear rRNA genes. The only group I intron known from mitochondrial genomes of vascular plants is located in the *cox1* gene of *Peperomia*, where it is thought to have been recently acquired by lateral transfer from a fungal donor. Southern-blot surveys of 335 diverse genera of land plants now show that this intron is in fact widespread among angiosperm *cox1* genes, but with an exceptionally patchy phylogenetic distribution. Four lines of evidence—the intron's highly disjunct distribution, many incongruencies between intron and organismal phylogenies, and two sources of evidence from exonic coconversion tracts—lead us to conclude that the 48 angiosperm genera found to contain this *cox1* intron acquired it by 32 separate horizontal transfer events. Extrapolating to the over 13,500 genera of angiosperms, we estimate that this intron has invaded *cox1* genes by cross-species horizontal transfer over 1,000 times during angiosperm evolution. This massive wave of lateral transfers is of entirely recent occurrence, perhaps triggered by some key shift in the intron's invasiveness within angiosperms.
- Daley, D. O., Adams, K. L., Clifton, R., Qualmann, S., Millar, A. H., Palmer, J. D., Pratje, E., et al. (2002). Gene transfer from mitochondrion to nucleus: novel mechanisms for gene activation from *Cox2*. *The Plant Journal*, 30(1), 11–21. doi:10.1046/j.1365-313X.2002.01263.x The evolutionarily recent transfer of the gene for cytochrome c oxidase subunit 2 (*cox2*) from the mitochondrion to the nucleus in legumes is shown to have involved novel gene-activation steps. The acquired mitochondrial targeting presequence is bordered by two introns. Characterization of the import of soybean *Cox2* indicates that the presequence is cleaved in a three-step process which is independent of assembly. The final processing step takes place only in the mitochondria of legume species, and not in several non-legume plants. The unusually long presequence of 136 amino acids consists of three regions: the first 20 amino acids are required for mitochondrial targeting and can be replaced by another presequence; the central portion of the presequence is required for efficient import of the *Cox2* protein into mitochondria; and the last 12 amino acids, derived from the mitochondrially encoded protein, are required for correct maturation of the imported protein. The acquisition of a unique presequence, and the capacity for legume mitochondria to remove this presequence post-import, are considered to be essential adaptations for targeting of *Cox2* to the mitochondrion and therefore activation of the transferred gene in the nucleus.
- Davis, C. C., & Wurdack, K. J. (2004). Host-to-Parasite Gene Transfer in Flowering Plants: Phylogenetic Evidence from Malpighiales. *Science*, 305(5684), 676–678. doi:10.1126/science.1100671 Horizontal gene transfer (HGT) between sexually unrelated species has recently been documented for higher plants, but mechanistic explanations for HGTs have remained speculative. We show that a parasitic relationship may facilitate HGT between flowering plants. The endophytic parasites Rafflesiaceae are placed in the diverse order Malpighiales. Our multigene phylogenetic analyses of Malpighiales show that mitochondrial (*matR*) and nuclear loci (18S ribosomal DNA and *PHYC*) place Rafflesiaceae in Malpighiales, perhaps near Ochnaceae/Clusiaceae. Mitochondrial *nad1B-C*, however, groups them within Vitaceae, near

their obligate host *Tetrastigma*. These discordant phylogenetic hypotheses strongly suggest that part of the mitochondrial genome in *Rafflesiaceae* was acquired via HGT from their hosts.

- Grewe, F., Herres, S., Viehöver, P., Polsakiewicz, M., Weisshaar, B., & Knoop, V. (2011). A Unique Transcriptome: 1782 Positions of RNA Editing Alter 1406 Codon Identities in Mitochondrial mRNAs of the Lycophyte *Isoetes engelmannii*. *Nucleic Acids Research*, *39*(7), 2890–2902. doi:10.1093/nar/gkq1227 The analysis of the mitochondrial DNA of *Isoetes engelmannii* as a first representative of the lycophytes recently revealed very small introns and indications for extremely frequent RNA editing. To analyze functionality of intron splicing and the extent of RNA editing in *I. engelmannii*, we performed a comprehensive analysis of its mitochondrial transcriptome. All 30 groups I and II introns were found to be correctly removed, showing that intron size reduction does not impede splicing. We find that mRNA editing affects 1782 sites, which lead to a total of 1406 changes in codon meanings. This includes the removal of stop codons from 23 of the 25 mitochondrial protein encoding genes. Comprehensive sequence analysis of multiple cDNAs per locus allowed classification of partially edited sites as either inefficiently edited but relevant or as non-specifically edited at mostly low frequencies. Abundant RNA editing was also found to affect tRNAs in hitherto unseen frequency, taking place at 41 positions in tRNA-precursors, including the first identification of U-to-C exchanges in two tRNA species. We finally investigated the four group II introns of the *nad7* gene and could identify 27 sites of editing, most of which improve base pairing for proper secondary structure formation.
- Grewe, F., Viehoever, P., Weisshaar, B., & Knoop, V. (2009). A Trans-Splicing Group I Intron and tRNA-Hyperediting in the Mitochondrial Genome of the Lycophyte *Isoetes engelmannii*. *Nucleic Acids Research*, *37*(15), 5093–5104. doi:10.1093/nar/gkp532 Plant mitochondrial genomes show much more evolutionary plasticity than those of animals. We analysed the first mitochondrial DNA (mtDNA) of a lycophyte, the quillwort *Isoetes engelmannii*, which is separated from seed plants by more than 350 million years of evolution. The *Isoetes* mtDNA is particularly rich in recombination events, and chloroplast as well as nuclear DNA inserts document the incorporation of foreign sequences already in this most ancestral vascular plant lineage. On the other hand, particularly small group II introns and short intergenic regions reveal a tendency of evolution towards a compact mitochondrial genome. RNA editing reaches extreme levels exceeding 100 pyrimidine exchanges in individual mRNAs and, hitherto unobserved in such frequency, also in tRNAs with 18 C-to-U conversions in the tRNA for proline. In total, some 1500 sites of RNA editing can be expected for the *Isoetes* mitochondrial transcriptome. As a unique molecular novelty, the *Isoetes cox1* gene requires trans-splicing via a discontinuous group I intron demonstrating disrupted, but functional, RNAs for yet another class of natural ribozymes.
- Groth-Malonek, M., Pruchner, D., Grewe, F., & Knoop, V. (2005). Ancestors of Trans-Splicing Mitochondrial Introns Support Serial Sister Group Relationships of Hornworts and Mosses with Vascular Plants. *Molecular Biology and Evolution*, *22*(1), 117–125. doi:10.1093/molbev/msh259 Some group II introns in the organelle genomes of plants and algae are disrupted and require trans-splicing of the affected exons from independent transcripts. A peculiar mitochondrial *nad5* gene structure is universally conserved in flowering plants where two trans-splicing introns frame a tiny exon of only 22 nucleotides, and two additional conventional group II introns

interrupt the nad5 reading frame at other sites. These four introns are absent in the liverwort *Marchantia polymorpha*, which carries a group I intron at an unrelated site in nad5. To determine how intron gains and losses have sculptured mitochondrial gene structures in early land-plant evolution, we have investigated the full nad5 gene structures in the three bryophyte classes and the fern *Asplenium nidus*. We find the single *Marchantia* group I intron nad5i753 present as the only intervening sequence in both closely (*Corsinia* and *Monoclea*) and distantly related (*Noteroclada*, *Bazzania*, and *Haplomitrium*) liverwort genera. In a taxonomically wide spectrum of mosses (*Sphagnum*, *Encalypta*, *Timmia*, *Ulota*, and *Rhacocarpus*); however, we additionally identify the angiosperm-type group II introns nad5i230 and nad5i1455. The latter is a cis-arranged homolog to one of the two angiosperm trans-splicing introns, notably the first of its kind in mosses. In the hornwort *Anthoceros*, the “moss and liverwort-type” group I intron nad5i753 is absent, and, besides nad5i230 and nad5i1455, intron nad5i1477 is present as the second ancestral group II intron which has evolved into a trans-splicing arrangement in angiosperms. The influence of highly frequent RNA editing, most notably in the genera *Haplomitrium*, *Anthoceros*, and *Asplenium*, on phylogenetic tree construction is investigated and discussed. Taken together, the data (1) support a sister group relationship of liverworts as a whole to all other embryophytes, (2) indicate loss of a group I and serial entries of group II introns in the nad5 gene during early evolution of the nonliverwort lineage, and (3) propose a placement of hornworts as sister group to tracheophytes.

- Hecht, J., Grewe, F., & Knoop, V. (2011). Extreme RNA Editing in Coding Islands and Abundant Microsatellites in Repeat Sequences of *Selaginella Moellendorffii* Mitochondria: The Root of Frequent Plant mtDNA Recombination in Early Tracheophytes. *Genome Biology and Evolution*, 3, 344–358. doi:10.1093/gbe/evr027 Using an independent fosmid cloning approach and comprehensive transcriptome analysis to complement data from the *Selaginella moellendorffii* genome project, we determined the complete mitochondrial genome structure of this spikemoss. Numerous recombination events mediated mainly via long sequence repeats extending up to 7kbp result in a complex mtDNA network structure. Peculiar features associated with the repeat sequences are more than 80 different microsatellite sites (predominantly trinucleotide motifs). The *S. moellendorffii* mtDNA encodes a plant-typical core set of a twin-arginine translocase (tatC), 17 respiratory chain subunits, and 2 rRNAs but lacks atp4 and any tRNA genes. As a further novelty among plant chondromes, the nad4L gene is encoded within an intron of the nad1 gene. A total of 37 introns occupying the 20 mitochondrial genes (four of which are disrupted into trans-splicing arrangements including two novel instances of trans-splicing introns) make the *S. moellendorffii* chondrome the intron-richest and gene-poorest plant mtDNA known. Our parallel transcriptome analyses demonstrates functional splicing of all 37 introns and reveals a new record amount of plant organelle RNA editing with a total of 2,139 sites in mRNAs and 13 sites in the two rRNAs, all of which are exclusively of the C-to-U type.
- Liu, Y., Wang, B., Cui, P., Li, L., Xue, J.-Y., Yu, J., & Qiu, Y.-L. (2012). The Mitochondrial Genome of the Lycophyte *Huperzia squarrosa*: The Most Archaic Form in Vascular Plants. *PLoS ONE*, 7(4), e35168. doi:10.1371/journal.pone.0035168 Mitochondrial genomes have maintained some bacterial features despite their residence within eukaryotic cells for approximately two billion years. One of these features is the frequent presence of polycistronic operons. In land plants,

however, it has been shown that all sequenced vascular plant chondromes lack large polycistronic operons while bryophyte chondromes have many of them. In this study, we provide the completely sequenced mitochondrial genome of a lycophyte, from *Huperzia squarrosa*, which is a member of the sister group to all other vascular plants. The genome, at a size of 413,530 base pairs, contains 66 genes and 32 group II introns. In addition, it has 69 pseudogene fragments for 24 of the 40 protein- and rRNA-coding genes. It represents the most archaic form of mitochondrial genomes of all vascular plants. In particular, it has one large conserved gene cluster containing up to 10 ribosomal protein genes, which likely represents a polycistronic operon but has been disrupted and greatly reduced in the chondromes of other vascular plants. It also has the least rearranged gene order in comparison to the chondromes of other vascular plants. The genome is ancestral in vascular plants in several other aspects: the gene content resembling those of charophytes and most bryophytes, all introns being cis-spliced, a low level of RNA editing, and lack of foreign DNA of chloroplast or nuclear origin.

- Mower, J. P., Stefanovi[cacute], S., Young, G. J., & Palmer, J. D. (2004). Plant genetics: Gene transfer from parasitic to host plants. *Nature*, 432(7014), 165–166. doi:10.1038/432165b Plant mitochondrial genes are transmitted horizontally across mating barriers with surprising frequency, but the mechanism of transfer is unclear. Here we describe two new cases of horizontal gene transfer, from parasitic flowering plants to their host flowering plants, and present phylogenetic and biogeographic evidence that this occurred as a result of direct physical contact between the two. Our findings complement the discovery that genes can be transferred in the opposite direction, from host to parasite plant.
- Noutsos, C., Richly, E., & Leister, D. (2005). Generation and Evolutionary Fate of Insertions of Organelle DNA in the Nuclear Genomes of Flowering Plants. *Genome Research*, 15(5), 616–628. doi:10.1101/gr.3788705 Nuclear genomes are exposed to a continuous influx of DNA from mitochondria and plastids. We have characterized the structure of approximately 750 kb of organelle DNA, distributed among 13 loci, in the nuclear genomes of *Arabidopsis* and rice. These segments are large and migrated to the nucleus quite recently, allowing us to reconstruct their evolution. Two general types of nuclear insertions coexist; one is characterized by long sequence stretches that are colinear with organelle DNA, the other type consists of mosaics of organelle DNA, often derived from both plastids and mitochondria. The levels of sequence divergence of the two types exclude their common descent, implying that at least two independent modes of DNA transfer from organelle to nucleus operate. The post-integration fate of organelle DNA is characterized by a predominance of transition mutations, associated with the gradual amelioration of the integrated sequence to the nucleotide composition of the host chromosome. Deletion of organelle DNA at these loci is essentially balanced by insertions of nonorganelle DNA. Deletions are associated with the removal of DNA between perfect repeats, indicating that they originate by replication slippage.
- Nowack, E. C. M., Vogel, H., Groth, M., Grossman, A. R., Melkonian, M., & Glöckner, G. (2011). Endosymbiotic Gene Transfer and Transcriptional Regulation of Transferred Genes in *Paulinella* Chromatophora. *Molecular Biology and Evolution*, 28(1), 407–422. doi:10.1093/molbev/msq209 *Paulinella chromatophora* is a cercozoan amoeba that contains “chromatophores,” which are photosynthetic inclusions of cyanobacterial origin. The recent discovery that chromatophores

evolved independently of plastids, underwent major genome reduction, and transferred at least two genes to the host nucleus has highlighted *P. chromatophora* as a model to infer early steps in the evolution of photosynthetic organelles. However, owing to the paucity of nuclear genome sequence data, the extent of endosymbiotic gene transfer (EGT) and host symbiont regulation are currently unknown. A combination of 454 and Illumina next generation sequencing enabled us to generate a comprehensive reference transcriptome data set for *P. chromatophora* on which we mapped short Illumina cDNA reads generated from cultures from the dark and light phases of a diel cycle. Combined with extensive phylogenetic analyses of the deduced protein sequences, these data revealed that 1) about 0.3–0.8% of the nuclear genes were obtained by EGT compared with 11–14% in the Plantae, 2) transferred genes show a distinct bias in that many encode small proteins involved in photosynthesis and photoacclimation, 3) host cells established control over expression of transferred genes, and 4) not only EGT, but to a minor extent also horizontal gene transfer from organisms that presumably served as food sources, helped to shape the nuclear genome of *P. chromatophora*. The identification of a significant number of transferred genes involved in photosynthesis and photoacclimation of thylakoid membranes as well as the observed transcriptional regulation of these genes strongly implies import of the encoded gene products into chromatophores, a feature previously thought to be restricted to canonical organelles. Thus, a possible mechanism by which *P. chromatophora* exerts control over the performance of its newly acquired photosynthetic organelle may involve controlling the expression of nuclear-encoded chromatophore-targeted regulatory components of the thylakoid membranes.

Nugent, J. M., & Palmer, J. D. (1991). RNA-mediated transfer of the gene *coxII* from the mitochondrion to the nucleus during flowering plant evolution. *Cell*, *66*(3), 473–481. doi:10.1016/0092-8674(81)90011-8 The gene *coxII*, normally present in the mitochondrion, was functionally transferred to the nucleus during flowering plant evolution. *coxII* transfer is estimated to have occurred between 60 and 200 million years ago, whereas loss of *coxII* from the mitochondrion occurred much more recently, being restricted to a single genus of legumes. Most legumes have *coxII* in both the nucleus and the mitochondrion; however, no evidence is found for simultaneous *coxII* expression in both compartments. The nuclear *coxII* sequence more closely resembles edited mitochondrial *coxII* transcripts than the genes encoding these RNAs. Hence, gene transfer appears to have involved reverse transcription of an edited RNA intermediate. The nuclear gene contains an intron at the junction of the transit peptide sequence and the mature protein-coding sequence; exon shuffling may have played a role in assembling a functional *coxII* gene in the nucleus.

Paine, J. A., Shipton, C. A., Chaggar, S., Howells, R. M., Kennedy, M. J., Vernon, G., Wright, S. Y., et al. (2005). Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nature Biotechnology*, *23*(4), 482–487. doi:10.1038/nbt1082 “Golden Rice” is a variety of rice engineered to produce  $\beta$ -carotene (pro-vitamin A) to help combat vitamin A deficiency<sup>1</sup>, and it has been predicted that its contribution to alleviating vitamin A deficiency would be substantially improved through even higher  $\beta$ -carotene content<sup>2</sup>. We hypothesized that the daffodil gene encoding phytoene synthase (*psy*), one of the two genes used to develop Golden Rice, was the limiting step in  $\beta$ -carotene accumulation. Through systematic testing of other plant

psys, we identified a psy from maize that substantially increased carotenoid accumulation in a model plant system. We went on to develop “Golden Rice 2” introducing this psy in combination with the *Erwinia uredovora* carotene desaturase (crtl) used to generate the original Golden Rice1. We observed an increase in total carotenoids of up to 23-fold (maximum 37 g/g) compared to the original Golden Rice and a preferential accumulation of  $\beta$ -carotene.

Price, D. C., Chan, C. X., Yoon, H. S., Yang, E. C., Qiu, H., Weber, A. P. M., Schwacke, R., et al. (2012).

*Cyanophora Paradoxa* Genome Elucidates Origin of Photosynthesis in Algae and Plants. *Science*, 335(6070), 843–847. doi:10.1126/science.1213561 The primary endosymbiotic origin of the plastid in eukaryotes more than 1 billion years ago led to the evolution of algae and plants. We analyzed draft genome and transcriptome data from the basally diverging alga *Cyanophora paradoxa* and provide evidence for a single origin of the primary plastid in the eukaryote supergroup Plantae. *C. paradoxa* retains ancestral features of starch biosynthesis, fermentation, and plastid protein translocation common to plants and algae but lacks typical eukaryotic light-harvesting complex proteins. Traces of an ancient link to parasites such as Chlamydiae were found in the genomes of *C. paradoxa* and other Plantae. Apparently, Chlamydia-like bacteria donated genes that allow export of photosynthate from the plastid and its polymerization into storage polysaccharide in the cytosol.

Pryer, K. M., Schneider, H., Smith, A. R., Cranfill, R., Wolf, P. G., Hunt, J. S., & Sipes, S. D. (2001).

Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. *Nature*, 409(6820), 618–622. doi:10.1038/35054555 Most of the 470-million-year history of plants on land belongs to bryophytes, pteridophytes and gymnosperms, which eventually yielded to the ecological dominance by angiosperms 90 Myr ago<sup>1, 2, 3</sup>. Our knowledge of angiosperm phylogeny, particularly the branching order of the earliest lineages, has recently been increased by the concurrence of multigene sequence analyses<sup>4, 5, 6</sup>. However, reconstructing relationships for all the main lineages of vascular plants that diverged since the Devonian period has remained a challenge. Here we report phylogenetic analyses of combined data—from morphology and from four genes—for 35 representatives from all the main lineages of land plants. We show that there are three monophyletic groups of extant vascular plants: (1) lycophytes, (2) seed plants and (3) a clade including equisetophytes (horsetails), psilotophytes (whisk ferns) and all eusporangiate and leptosporangiate ferns. Our maximum-likelihood analysis shows unambiguously that horsetails and ferns together are the closest relatives to seed plants. This refutes the prevailing view that horsetails and ferns are transitional evolutionary grades between bryophytes and seed plants<sup>7</sup>, and has important implications for our understanding of the development and evolution of plants<sup>8</sup>.

Pryer, K. M., Schuettpelz, E., Wolf, P. G., Schneider, H., Smith, A. R., & Cranfill, R. (2004). Phylogeny and

Evolution of Ferns (monilophytes) with a Focus on the Early Leptosporangiate Divergences.

*American Journal of Botany*, 91(10), 1582–1598. doi:10.3732/ajb.91.10.1582 The phylogenetic structure of ferns (= monilophytes) is explored here, with a special focus on the early divergences among leptosporangiate lineages. Despite considerable progress in our understanding of fern relationships, a rigorous and comprehensive analysis of the early leptosporangiate divergences was lacking. Therefore, a data set was designed here to include critical taxa that were not included in earlier studies. More than 5000 bp from the plastid (rbcl,

atpB, rps4) and the nuclear (18S rDNA) genomes were sequenced for 62 taxa. Phylogenetic analyses of these data (1) confirm that Osmundaceae are sister to the rest of the leptosporangiates, (2) resolve a diverse set of ferns formerly thought to be a subsequent grade as possibly monophyletic ((Dipteridaceae, Matoniaceae), Gleicheniaceae), Hymenophyllaceae), and (3) place schizaeoid ferns as sister to a large clade of “core leptosporangiates” that includes heterosporous ferns, tree ferns, and polypods. Divergence time estimates for ferns are reported from penalized likelihood analyses of our molecular data, with constraints from a reassessment of the fossil record.

- Qiu, Y.-L., Li, L., Wang, B., Chen, Z., Knoop, V., Groth-Malonek, M., Dombrowska, O., et al. (2006). The Deepest Divergences in Land Plants Inferred from Phylogenomic Evidence. *Proceedings of the National Academy of Sciences*, 103(42), 15511–15516. doi:10.1073/pnas.0603335103
- Phylogenetic relationships among the four major lineages of land plants (liverworts, mosses, hornworts, and vascular plants) remain vigorously contested; their resolution is essential to our understanding of the origin and early evolution of land plants. We analyzed three different complementary data sets: a multigene supermatrix, a genomic structural character matrix, and a chloroplast genome sequence matrix, using maximum likelihood, maximum parsimony, and compatibility methods. Analyses of all three data sets strongly supported liverworts as the sister to all other land plants, and analyses of the multigene and chloroplast genome matrices provided moderate to strong support for hornworts as the sister to vascular plants. These results highlight the important roles of liverworts and hornworts in two major events of plant evolution: the water-to-land transition and the change from a haploid gametophyte generation-dominant life cycle in bryophytes to a diploid sporophyte generation-dominant life cycle in vascular plants. This study also demonstrates the importance of using a multifaceted approach to resolve difficult nodes in the tree of life. In particular, it is shown here that densely sampled taxon trees built with multiple genes provide an indispensable test of taxon-sparse trees inferred from genome sequences.
- Rai, H. S., & Graham, S. W. (2010). Utility of a large, multigene plastid data set in inferring higher-order relationships in ferns and relatives (monilophytes). *American Journal of Botany*, 97(9), 1444–1456. doi:10.3732/ajb.0900305 • Premise of the Study: The monilophytes (ferns and relatives)—the third largest group of land plants—exhibit a diverse array of vegetative and reproductive morphologies. Investigations into their early ecological and life-history diversification require accurate, well-corroborated phylogenetic estimates. We examined the utility of a large plastid-based data set in inferring backbone relationships for monilophytes. • Methods: We recovered 17 plastid genes for exemplar taxa using published and new primers. We compared results from maximum-likelihood and parsimony analyses, assessed the effects of removing rapidly evolving characters, and examined the extent to which our data corroborate or contradict the results of other studies, or resolve current ambiguities. • Key Results: Considering multifamily clades, we found bootstrap support comparable to or better than that in published studies that used fewer genes from fewer or more taxa. We firmly establish filmy ferns (Hymenophyllales) as the sister group of all leptosporangiates except Osmundaceae, resolving the second deepest split in leptosporangiate-fern phylogeny. A clade comprising Ophioglossaceae and Psilotaceae is currently accepted as the sister group of other monilophytes, but we recover Equisetum in this

position. We also recover marattioid and leptosporangiate ferns as sister groups. Maximum-likelihood rate-class estimates are somewhat skewed when a long-branch lineage (*Selaginella*) is included, negatively affecting bootstrap support for early branches. • Conclusions: Our findings support the utility of this gene set in corroborating relationships found in previous studies, improving support, and resolving uncertainties in monilophyte phylogeny. Despite these advances, our results also underline the need for continued work on resolving the very earliest splits in monilophyte phylogeny.

Rensing, S. A., Lang, D., Zimmer, A. D., Terry, A., Salamov, A., Shapiro, H., Nishiyama, T., et al. (2008). The *Physcomitrella* Genome Reveals Evolutionary Insights into the Conquest of Land by Plants. *Science*, 319(5859), 64–69. doi:10.1126/science.1150646 We report the draft genome sequence of the model moss *Physcomitrella patens* and compare its features with those of flowering plants, from which it is separated by more than 400 million years, and unicellular aquatic algae. This comparison reveals genomic changes concomitant with the evolutionary movement to land, including a general increase in gene family complexity; loss of genes associated with aquatic environments (e.g., flagellar arms); acquisition of genes for tolerating terrestrial stresses (e.g., variation in temperature and water availability); and the development of the auxin and abscisic acid signaling pathways for coordinating multicellular growth and dehydration response. The *Physcomitrella* genome provides a resource for phylogenetic inferences about gene function and for experimental analysis of plant processes through this plant's unique facility for reverse genetics.

Rüdinger, M., Szövényi, P., Rensing, S. A., & Knoop, V. (2011). Assigning DYW-type PPR proteins to RNA editing sites in the funariid mosses *Physcomitrella patens* and *Funaria hygrometrica*. *The Plant Journal*, 67(2), 370–380. doi:10.1111/j.1365-313X.2011.04600.x The plant-specific pentatricopeptide repeat (PPR) proteins with variable PPR repeat lengths (PLS-type) and protein extensions up to the carboxyterminal DYW domain have received attention as specific recognition factors for the C-to-U type of RNA editing events in plant organelles. Here, we report a DYW-protein knockout in the model plant *Physcomitrella patens* specifically affecting mitochondrial RNA editing positions *cox1eU755SL* and *rps14eU137SL*. Assignment of DYW proteins and RNA editing sites might best be corroborated by data from a taxon with a slightly different, yet similarly manageable low number of editing sites and DYW proteins. To this end we investigated the mitochondrial editing status of the related funariid moss *Funaria hygrometrica*. We find that: (i) *Funaria* lacks three mitochondrial RNA editing positions present in *Physcomitrella*, (ii) that *F. hygrometrica* cDNA sequence data identify nine DYW proteins as clear orthologues of their *P. patens* counterparts, and (iii) that the “missing” 10th DYW protein in *F. hygrometrica* is responsible for two mitochondrial editing sites in *P. patens* lacking in *F. hygrometrica* (*nad3eU230SL*, *nad4eU272SL*). Interestingly, the third site of RNA editing missing in *F. hygrometrica* (*rps14eU137SL*) is addressed by the DYW protein characterized here and the presence of its orthologue in *F. hygrometrica* is explained through its simultaneous action on site *cox1eU755SL* conserved in both mosses.

Rumpho, M. E., Worful, J. M., Lee, J., Kannan, K., Tyler, M. S., Bhattacharya, D., Moustafa, A., et al. (2008). Horizontal Gene Transfer of the Algal Nuclear Gene *psbO* to the Photosynthetic Sea Slug *Elysia Chlorotica*. *Proceedings of the National Academy of Sciences*, 105(46), 17867–17871.

doi:10.1073/pnas.0804968105 The sea slug *Elysia chlorotica* acquires plastids by ingestion of its algal food source *Vaucheria litorea*. Organelles are sequestered in the mollusc's digestive epithelium, where they photosynthesize for months in the absence of algal nucleocytoplasm. This is perplexing because plastid metabolism depends on the nuclear genome for >90% of the needed proteins. Two possible explanations for the persistence of photosynthesis in the sea slug are (i) the ability of *V. litorea* plastids to retain genetic autonomy and/or (ii) more likely, the mollusc provides the essential plastid proteins. Under the latter scenario, genes supporting photosynthesis have been acquired by the animal via horizontal gene transfer and the encoded proteins are retargeted to the plastid. We sequenced the plastid genome and confirmed that it lacks the full complement of genes required for photosynthesis. In support of the second scenario, we demonstrated that a nuclear gene of oxygenic photosynthesis, *psbO*, is expressed in the sea slug and has integrated into the germline. The source of *psbO* in the sea slug is *V. litorea* because this sequence is identical from the predator and prey genomes. Evidence that the transferred gene has integrated into sea slug nuclear DNA comes from the finding of a highly diverged *psbO* 3' flanking sequence in the algal and mollusc nuclear homologues and gene absence from the mitochondrial genome of *E. chlorotica*. We demonstrate that foreign organelle retention generates metabolic novelty ("green animals") and is explained by anastomosis of distinct branches of the tree of life driven by predation and horizontal gene transfer.

Sayre, R., Beeching, J. R., Cahoon, E. B., Egesi, C., Fauquet, C., Fellman, J., Fregene, M., et al. (2011). The BioCassava Plus Program: Biofortification of Cassava for Sub-Saharan Africa. *Annual Review of Plant Biology*, 62(1), 251–272. doi:10.1146/annurev-arplant-042110-103751 More than 250 million Africans rely on the starchy root crop cassava (*Manihot esculenta*) as their staple source of calories. A typical cassava-based diet, however, provides less than 30% of the minimum daily requirement for protein and only 10%–20% of that for iron, zinc, and vitamin A. The BioCassava Plus (BC+) program has employed modern biotechnologies intended to improve the health of Africans through the development and delivery of genetically engineered cassava with increased nutrient (zinc, iron, protein, and vitamin A) levels. Additional traits addressed by BioCassava Plus include increased shelf life, reductions in toxic cyanogenic glycosides to safe levels, and resistance to viral disease. The program also provides incentives for the adoption of biofortified cassava. Proof of concept was achieved for each of the target traits. Results from field trials in Puerto Rico, the first confined field trials in Nigeria to use genetically engineered organisms, and ex ante impact analyses support the efficacy of using transgenic strategies for the biofortification of cassava.

Sloan, D. B., Alverson, A. J., Chuckalovcak, J. P., Wu, M., McCauley, D. E., Palmer, J. D., & Taylor, D. R. (2012). Rapid Evolution of Enormous, Multichromosomal Genomes in Flowering Plant Mitochondria with Exceptionally High Mutation Rates. *PLoS Biol*, 10(1), e1001241. doi:10.1371/journal.pbio.1001241 Genome size and complexity vary tremendously among eukaryotic species and their organelles. Comparisons across deeply divergent eukaryotic lineages have suggested that variation in mutation rates may explain this diversity, with increased mutational burdens favoring reduced genome size and complexity. The discovery that mitochondrial mutation rates can differ by orders of magnitude among closely related

angiosperm species presents a unique opportunity to test this hypothesis. We sequenced the mitochondrial genomes from two species in the angiosperm genus *Silene* with recent and dramatic accelerations in their mitochondrial mutation rates. Contrary to theoretical predictions, these genomes have experienced a massive proliferation of noncoding content. At 6.7 and 11.3 Mb, they are by far the largest known mitochondrial genomes, larger than most bacterial genomes and even some nuclear genomes. In contrast, two slowly evolving *Silene* mitochondrial genomes are smaller than average for angiosperms. Consequently, this genus captures approximately 98% of known variation in organelle genome size. The expanded genomes reveal several architectural changes, including the evolution of complex multichromosomal structures (with 59 and 128 circular-mapping chromosomes, ranging in size from 44 to 192 kb). They also exhibit a substantial reduction in recombination and gene conversion activity as measured by the relative frequency of alternative genome conformations and the level of sequence divergence between repeat copies. The evolution of mutation rate, genome size, and chromosome structure can therefore be extremely rapid and interrelated in ways not predicted by current evolutionary theories. Our results raise the hypothesis that changes in recombinational processes, including gene conversion, may be a central force driving the evolution of both mutation rate and genome structure.

Stupar, R. M., Lilly, J. W., Town, C. D., Cheng, Z., Kaul, S., Buell, C. R., & Jiang, J. (2001). Complex mtDNA Constitutes an Approximate 620-Kb Insertion on Arabidopsis Thaliana Chromosome 2: Implication of Potential Sequencing Errors Caused by Large-Unit Repeats. *Proceedings of the National Academy of Sciences*, 98(9), 5099–5103. doi:10.1073/pnas.091110398 Previously conducted sequence analysis of Arabidopsis thaliana (ecotype Columbia-0) reported an insertion of 270-kb mtDNA into the pericentric region on the short arm of chromosome 2. DNA fiber-based fluorescence in situ hybridization analyses reveal that the mtDNA insert is  $618 \pm 42$  kb,  $\approx 2.3$  times greater than that determined by contig assembly and sequencing analysis. Portions of the mitochondrial genome previously believed to be absent were identified within the insert. Sections of the mtDNA are repeated throughout the insert. The cytological data illustrate that DNA contig assembly by using bacterial artificial chromosomes tends to produce a minimal clone path by skipping over duplicated regions, thereby resulting in sequencing errors. We demonstrate that fiber-fluorescence in situ hybridization is a powerful technique to analyze large repetitive regions in the higher eukaryotic genomes and is a valuable complement to ongoing large genome sequencing projects.

de la Torre-Bárcena, J. E., Kolokotronis, S.-O., Lee, E. K., Stevenson, D. W., Brenner, E. D., Katari, M. S., Coruzzi, G. M., et al. (2009). The Impact of Outgroup Choice and Missing Data on Major Seed Plant Phylogenetics Using Genome-Wide EST Data. *PLoS ONE*, 4(6), e5764. doi:10.1371/journal.pone.0005764 Genome level analyses have enhanced our view of phylogenetics in many areas of the tree of life. With the production of whole genome DNA sequences of hundreds of organisms and large-scale EST databases a large number of candidate genes for inclusion into phylogenetic analysis have become available. In this work, we exploit the burgeoning genomic data being generated for plant genomes to address one of the more important plant phylogenetic questions concerning the hierarchical relationships of the several major seed plant lineages (angiosperms, Cycadales, Ginkgoales, Gnetales, and Coniferales),

which continues to be a work in progress, despite numerous studies using single, few or several genes and morphology datasets. Although most recent studies support the notion that gymnosperms and angiosperms are monophyletic and sister groups, they differ on the topological arrangements within each major group. We exploited the EST database to construct a supermatrix of DNA sequences (over 1,200 concatenated orthologous gene partitions for 17 taxa) to examine non-flowering seed plant relationships. This analysis employed programs that offer rapid and robust orthology determination of novel, short sequences from plant ESTs based on reference seed plant genomes. Our phylogenetic analysis retrieved an unbiased (with respect to gene choice), well-resolved and highly supported phylogenetic hypothesis that was robust to various outgroup combinations. We evaluated character support and the relative contribution of numerous variables (e.g. gene number, missing data, partitioning schemes, taxon sampling and outgroup choice) on tree topology, stability and support metrics. Our results indicate that while missing characters and order of addition of genes to an analysis do not influence branch support, inadequate taxon sampling and limited choice of outgroup(s) can lead to spurious inference of phylogeny when dealing with phylogenomic scale data sets. As expected, support and resolution increases significantly as more informative characters are added, until reaching a threshold, beyond which support metrics stabilize, and the effect of adding conflicting characters is minimized.

Waller, R. F. (2012). Second Genesis of a Plastid Organelle. *Proceedings of the National Academy of Sciences*, 109(14), 5142–5143. doi:10.1073/pnas.1202904109 One of the more remarkable discoveries of 20th century biology is that all eukaryotes are chimeras of two or more different cells (1). The tree of life does not just bifurcate through successive speciation events; its branches also graft, one onto another, merging lineages to produce novel fruit. Our mitochondria, and the photosynthetic plastids of plants and algae, are proof of the success of these new combinations. Both organelles were acquired from separate bacterial lineages, where a useful bacterium was internalized and maintained within the host cell—a process known as endosymbiosis. These organelles are no mere hitchhikers. They have been elaborately integrated with their hosts, so intimately that even their genes have been relinquished to the host cell to allow these partnerships to truly operate as one. To achieve this, the host cell learned to take responsibility for expressing the organelle genes and delivering the protein products back into the organelle according to its requirements. Our understanding of how these seminal achievements of organellogenesis occurred, however, is obscured by their antiquity, both organelles arising ~1+ billion years ago (2). It is akin to studying modern jet aircraft in the hope of reconstructing the Wright brothers' first daring attempts at flight. Iterative leaps of technology often mask the formative innovations. However, now we have a chance to revisit this process, as Nowack and Grossman in PNAS (3) show that the new photosynthetic endosymbiont of *Paulinella chromatophora* has also commenced this journey of genetic integration.

Wang, D., Lloyd, A. H., & Timmis, J. N. (2012). Environmental Stress Increases the Entry of Cytoplasmic Organellar DNA into the Nucleus in Plants. *Proceedings of the National Academy of Sciences*, 109(7), 2444–2448. doi:10.1073/pnas.1117890109 Mitochondria and chloroplasts (photosynthetic members of the plastid family of cytoplasmic organelles) in eukaryotic cells

originated more than a billion years ago when an ancestor of the nucleated cell engulfed two different prokaryotes in separate sequential events. Extant cytoplasmic organellar genomes contain very few genes compared with their candidate free-living ancestors, as most have functionally relocated to the nucleus. The first step in functional relocation involves the integration of inactive DNA fragments into nuclear chromosomes, and this process continues at high frequency with attendant genetic, genomic, and evolutionary consequences. Using two different transplastomic tobacco lines, we show that DNA migration from chloroplasts to the nucleus is markedly increased by mild heat stress. In addition, we show that insertion of mitochondrial DNA fragments during the repair of induced double-strand breaks is increased by heat stress. The experiments demonstrate that the nuclear influx of organellar DNA is a potentially a source of mutation for nuclear genomes that is highly susceptible to temperature fluctuations that are well within the range experienced naturally.

- Ye, X., Al-Babili, S., Klöti, A., Zhang, J., Lucca, P., Beyer, P., & Potrykus, I. (2000). Engineering the Provitamin A ( $\beta$ -Carotene) Biosynthetic Pathway into (Carotenoid-Free) Rice Endosperm. *Science*, 287(5451), 303–305. doi:10.1126/science.287.5451.303 Rice (*Oryza sativa*), a major staple food, is usually milled to remove the oil-rich aleurone layer that turns rancid upon storage, especially in tropical areas. The remaining edible part of rice grains, the endosperm, lacks several essential nutrients, such as provitamin A. Thus, predominant rice consumption promotes vitamin A deficiency, a serious public health problem in at least 26 countries, including highly populated areas of Asia, Africa, and Latin America. Recombinant DNA technology was used to improve its nutritional value in this respect. A combination of transgenes enabled biosynthesis of provitamin A in the endosperm.
- Yoon, H. S., Nakayama, T., Reyes-Prieto, A., Andersen, R. A., Boo, S. M., Ishida, K., & Bhattacharya, D. (2009). A single origin of the photosynthetic organelle in different *Paulinella* lineages. *BMC Evolutionary Biology*, 9(1), 98. doi:10.1186/1471-2148-9-98 Background Gaining the ability to photosynthesize was a key event in eukaryotic evolution because algae and plants form the base of the food chain on our planet. The eukaryotic machines of photosynthesis are plastids (e.g., chloroplast in plants) that evolved from cyanobacteria through primary endosymbiosis. Our knowledge of plastid evolution, however, remains limited because the primary endosymbiosis occurred more than a billion years ago. In this context, the thecate “green amoeba” *Paulinella chromatophora* is remarkable because it very recently (i.e., minimum age of  $\approx$  60 million years ago) acquired a photosynthetic organelle (termed a “chromatophore”; i.e., plastid) via an independent primary endosymbiosis involving a *Prochlorococcus* or *Synechococcus*-like cyanobacterium. All data regarding *P. chromatophora* stem from a single isolate from Germany (strain M0880/a). Here we brought into culture a novel photosynthetic *Paulinella* strain (FK01) and generated molecular sequence data from these cells and from four different cell samples, all isolated from freshwater habitats in Japan. Our study had two aims. The first was to compare and contrast cell ultrastructure of the M0880/a and FK01 strains using scanning electron microscopy. The second was to assess the phylogenetic diversity of photosynthetic *Paulinella* to test the hypothesis they share a vertically inherited plastid that originated in their common ancestor. Results Comparative morphological analyses show that *Paulinella* FK01 cells are smaller than M0880/a and differ with respect to the number of scales per column. There are more

distinctive, multiple fine pores on the external surface of FK01 than in M0880/a. Molecular phylogenetic analyses using multiple gene markers demonstrate these strains are genetically distinct and likely comprise separate species. The well-supported monophyly of the Paulinella chromatophora strains analyzed here using plastid-encoded 16S rRNA suggests strongly that they all share a common photosynthetic ancestor. The strain M0880/a is most closely related to Japanese isolates (Kanazawa-1, -2, and Kaga), whereas FK01 groups closely with a Kawaguchi isolate. Conclusion Our results indicate that Paulinella chromatophora comprises at least two distinct evolutionary lineages and likely encompasses a broader taxonomic diversity than previously thought. The finding of a single plastid origin for both lineages shows these taxa to be valuable models for studying post-endosymbiotic cell and genome evolution.

Yoon, H. S., Reyes-Prieto, A., Melkonian, M., & Bhattacharya, D. (2006). Minimal plastid genome evolution in the Paulinella endosymbiont. *Current Biology*, *16*(17), R670–R672. doi:10.1016/j.cub.2006.08.018

Yoshida, S., Maruyama, S., Nozaki, H., & Shirasu, K. (2010). Horizontal Gene Transfer by the Parasitic Plant *Striga Hermonthica*. *Science*, *328*(5982), 1128–1128. doi:10.1126/science.1187145  
Horizontal gene transfer has been postulated to occur between crops to co-occurring parasitic plants, but empirical evidence has been lacking. We present evidence that an HGT event moved a nuclear monocot gene into the genome of the eudicot parasite witchweed (*Striga hermonthica*), which infects many grass species in Africa. Analysis of expressed sequence tags revealed that the genome of *S. hermonthica* contains a nuclear gene that is widely conserved among grass species but is not found in other eudicots. Phylogenetically, this gene clusters with sorghum genes, the monocot host of the parasitic weed, suggesting that nuclear genes can be captured by parasitic weeds in nature.