

LETTERS

A Land Plant–Specific Multigene Family in the Unicellular *Mesostigma* Argues for Its Close Relationship to Streptophyta

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The search for the unicellular relative of Streptophyta (i.e., land plants and their closest green algal relatives, the charophytes) started many years ago and remained centered around the scaly green flagellate, *Mesostigma viride*. To date, despite numerous studies, the phylogenetic position of *Mesostigma* is still debated and the nature of the unicellular ancestor of Streptophyta remains unknown. As molecular phylogenetic studies have produced conflicting results, we constructed a *M. viride* expressed sequence tags library and searched for sequences that are shared between *M. viride* and the Streptophyta (to the exclusion of the other green algal lineages—the Chlorophyta). Here, we report a multigene family that is restricted to Streptophyta and *M. viride*. The phylogenetic distribution of this complex character and its potential involvement in the evolution of an important land plant adaptive trait (i.e., three-dimensional tissues) argue that *Mesostigma* is a close unicellular relative of Streptophyta.

The origin and further diversification of land plants represented a consequential event with major implications for the evolution of life on Earth. Although it is generally accepted that land plants and their closest green algal relatives, the charophytes, form a monophyletic group (Streptophyta), the evolutionary origin of this group is still debated. Several ultrastructural (e.g., Melkonian 1989) and molecular (e.g., Bhattacharya et al. 1998; Marin and Melkonian 1999; Karol et al. 2001; Delwiche et al. 2002; Martin et al. 2002) studies indicated *Mesostigma viride* (a green flagellate traditionally placed in the paraphyletic Prasinophyceae; Mattox and Stewart 1984) as the closest unicellular relative of Streptophyta. However, other reports challenged *Mesostigma*'s initial placement and proposed a different position for this taxon, at the base of the clade containing both the green algal and land plant lineages (Lemieux, Otis, and Turmel 2000; Turmel, Otis, and Lemieux 2002). To date, despite numerous studies, the phylogenetic position of *Mesostigma* remains uncertain and the nature of the unicellular ancestor of Streptophyta is still unknown (see Lewis and McCourt 2004; McCourt, Delwiche, and Karol 2004 for discussion).

Because data from molecular phylogenetic studies produced conflicting results, more complex characters derived from comparative genomics could provide a robust resolution of the conflicting hypotheses. To this end, we constructed a *M. viride* expressed sequence tags (EST) library and searched for sequences that are shared between *M. viride* and the Streptophyta (to the exclusion of the other green algal lineages—the Chlorophyta) and might be related to traits thought to be important for land plant evolution. This approach is similar to that used to identify the unicellular choanoflagellate *Monosiga brevicollis*, as a close unicellular relative of Metazoa (i.e., by identifying proteins

that predated the origin of Metazoa and were co-opted for animal development) (King and Carroll 2001).

Among the features considered critical to the embryophyte radiation is the presence of a histogenetic apical meristem (i.e., one or more apical cells able to divide asymmetrically and in multiple dimensions) capable of generating a three-dimensional body (Graham 1996; Graham, Cook, and Busse 2000). A simple single-celled histogenetic meristem is believed to have evolved early in plant evolution as it occurs in the earliest diverging land plants, the bryophytes. In the moss, *Physcomitrella patens*, the vegetative development after spore germination involves the formation of multicellular buds that give rise to a leafy three-dimensional gametophore, and recently, six distinct genes, *BIP1* to *BIP6*, specific of bud and gametophore formation have been reported (Brun et al. 2003).

We have searched the available databases for potential *M. viride* *BIP* homologs and identified in both our *M. viride* EST library (strain CCMP2046; aka NIES 296) and GenBank (strain NIES 476), numerous sequences with similarity to one of these bud-induced genes, namely, *BIP2*. The deduced amino acid sequence of the partial *P. patens* *BIP2* was reported to be similar to the C-terminus of a protein family previously thought to be restricted to seed plants (Brun et al. 2003) (fig. 1A); our database searches identified additional *P. patens* *BIP2* ESTs (including several that cover the N-terminus) to support the inclusion of *P. patens* *BIP2* in this family (fig. 1B and C).

This family (see supplementary fig. 1 [fig_S1.pdf] in Supplementary Material online) comprises a *Picea glauca* sequence expressed in somatic embryos (Dong and Dunstan 1999), two *Zea mays* sequences specifically expressed in root-cap cells (Matsuyama et al. 1999), and several *Oryza sativa* and *Arabidopsis thaliana* sequences whose expression pattern has not been yet reported. The predicted primary sequence of these proteins is characterized by the presence of a conserved domain at their C-terminus, named the root-cap domain; many also feature two additional conserved domains (fig. 1A). Noteworthy, the N-terminus of these proteins is quite variable in size (from 75 amino acids in *P. glauca* to 300 amino acids in one of the *A. thaliana* sequences) and sequence (including the

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Key words: *Mesostigma viride*, land plant evolution, Streptophyta, histogenetic meristem, gene family.

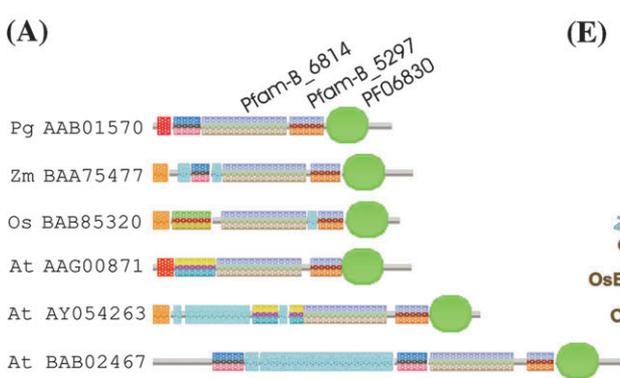
E-mail: anedelcu@unb.ca.

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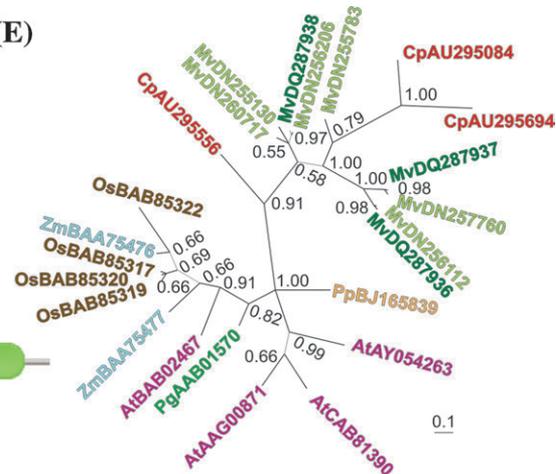
doi:10.1093/molbev/msj108

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(A)



(E)



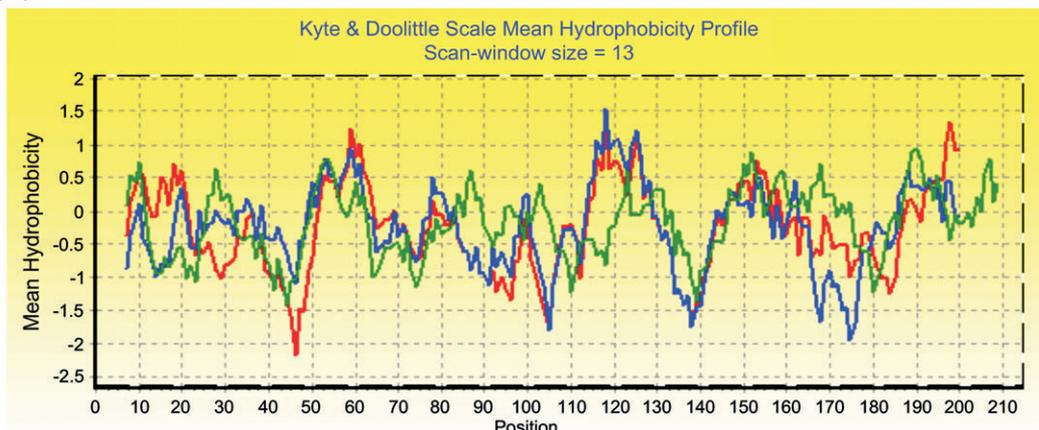
(B)

Mv DQ287936	DPHFVAGNGLKFDPMGAPGSIYTVYTDRLRHMMHMI GVRGNS--ERKCTWMD EISIMF
Mv DN256112	DPHFVAGNGLKFDPMGTGPGSIYTVYTDRLRHMMHMI GVRGNS--ERKCTWMD EISIMF
Mv DQ287938	DPHFVAGNGMRYDFMGKAGETTYCLSD ETLHMMHMI GVKSNP--LRKATWMDA ISIMY
Mv DQ287939	DPHFVAGNGLAYDFMGRAGEETYCMRSDQTLHMMHMI GVKSNP--SRKATWMDA ISIMY
Mv DN259133	DPHFVAGNGLAYDFMGYPGETTYCMRSDKTLHMMHMI GVKNNP--TRKSTWMDA ISIMY
Mv DQ287940	DPHFVAGNGLAYDFMGKAGETTYCLSDKTLHMMHMI GVKSNP--LRKATWMDT ISIMY
Pp BJ159300	DPKILGGDGMVYFHYGKDA DFCVLSDSLHNAHFI GMRPEG-RT RDEFTWVQGLGEVY
Pg AAB01570	DRFFIGGDMVYFHYGKRQD DFCVLSDSLHNAHFI GKRGG-MGRDFTWVQSI GVL
Zm BAA75477	DRFFIGGDMVYFHYGRRD DFCVLSDRDLHNAHFI GKHGADGMSRDFTWIQAVL
Os BAB85320	DRFFIGGDNVYFHYGKDDH DFCVLSADLHNAHFI GKRNPT-MSRDFTWIQALGTRF
At BAB02467	DRFFIGGDLTFYFHYGKDSN FCLISDPNLHNAHFI GKRRAG-MARDFTWVQSLAIF
At AY054263	DRFVGGDGMVYFHYGSKGN FAVISDNNLQINAHFI GTRPVG-RIRDFTWVQALNVMF
At AAG00871	DRFFIGGDIVYFHYGKRDEH FALISDVDFQVNAHFI GLRPNG-RA RDEFTWVQSLGLIF

(C)

Mv DQ287936	LNLKISALNATQAVHGVMGOTFRETFDVT ELPVK-GKDGEGVIEGKAEDYIVSGMFAADCKLCTV
Mv DN256112	LNLKISALNATQAVHGVMGOTFRETFDVT ELAVK-GKDGEGVIEGKAEDYIVSGMFAADCKLCSV
Mv DQ260717	LNEKVVSLNVS DSVHGVLGOTFRSSEF DVT DPAAVQGNKEGVL EGSMDYVASDI LAADCKVSCF
Mv DQ287937	LNFKVTALNATD E V HGVMGOTFRETF SVTSLPANAGKDGQGVIEGKPEDYIVSGMYAADCKFCSG
Mv DN257760	LNFKVTALNATD E V HGVMGOTFRETF SVTSLPANAGKDGQGVIEGKPVYIVSGMYAADCKFCSSG
Mv DQ287938	LNEKVVSLNATDNVHGVLGOTFRASEDRTE-KDVAGKNGEGVIEGSMDYVASDI LKADCRVSCF
Mv DN256206	LNFKVVSLNATDNVHGVLGOTFRASEDRTE-KDVSNGKNGEGVIEGSMDYVASDI LKADCRVSCF
Mv DN255783	LNVKIVGLNVTDNVHGVLGOTFRETFDVT E V KDTK-KMGEVTLGKASDYEVSSSLAIDCKFSQ
Mv DN255130	LNEKVVSLNATDNVHGVLGOTFRSSEFGSS-EDVKGNKEGVL EGNMADYVSTIGL LAADCRVSCF
Pp BJ165839	ENMQFRFYSLS THVNGVLGOTYQEGE----KNPVKRGVAMPIMCGA-DRYITSSSLAIDCKVNOY
Pg AAB01570	LELSFKFYSLSPNVS GVLGOTYGAEY----RSPVKMGVAMPIMCGE-SNYVTSNLFAADCKVARE
Zm BAA75477	LDLAFKFGALTA DVHGVVGTYRSY----VNRFDVKASMPMCGD-SNYITSSSLFAADCVARY
Os BAB85320	LDLGFKFYDLS D V HGVLGOTYRSY----VNKLSVSASMPVVMCGA-PSYVASDI FSAADCVARE
At BAB02467	LDLGFKFODLS D V GVLGOTYRSY----VSRVKIGVHMPVVMCGD-REFOTTGLFAFDCSAARE
At AY054263	LETQEKFLDLS E V GVLGTYRDPY----VSSAKTGVPMVPLGGE-DKYQTPSLFSPTCRICRF
At AAG00871	LEVQFRFLKLSNVEGVLGRTYKEDF----KNPAKPGVAMPVVMCGE-DKYRTASLETSCNACVY

(D)



presence of very long KE- or P-rich stretches in some *A. thaliana* sequences) (fig. 1A and supplementary fig. 1 [fig_S1.pdf] in Supplementary Material online). The specific function and cellular localization of these proteins are not known; some have potential signal peptides and might be secreted extracellularly (Dong and Dunstan 1999; Matsuyama et al. 1999). The *Mesostigma BIP2*-like sequences are similar to the land plant homologs—especially in the regions corresponding to two of the conserved domains (fig. 1B and C). Despite amino acid sequence differences, the *M. viride* and land plant predicted proteins are very similar in overall amino acid composition (with a high percentage of glycine and alanine) and hydrophobicity profiles (GRAVY index of -0.2 to -0.3) (Kyte and Doolittle 1982) (fig. 1D).

To address the possibility that this protein family is specific to *M. viride* and Streptophyta, we searched the available databases for other *BIP2*-like sequences. We found such sequences in the fern *Ceratopteris richardii* and numerous seed plants as well as in the unicellular charophyte, *Closterium peracerosum-strigosum-littorale* complex. However, no *BIP2*-like sequences were found in any other algal taxa for which extensive sequence information is available (table 1).

Interestingly, the *M. viride BIP2*-like sequences correspond to several (at least nine) different but related genes (supplementary fig. 2 [fig_S2.pdf] in Supplementary Material online). This is consistent with the situation described for the *P. glauca* and maize sequences (thought to belong to multigene families; Dong and Dunstan 1999; Matsuyama et al. 1999) and with the presence in GenBank of several distinct *BIP2*-like ESTs in both *P. patens* and *Closterium* (which suggests that multiple coding regions are also present in these lineages). The large number of functional gene copies and the overall level of divergence among both *Mesostigma* and land plant *BIP2*-like sequences (supplementary figs. 1 and 2 [fig_S1.pdf and fig_S2.pdf] in Supplementary Material online) are consistent with selective pressures for duplication as well as retention and functional divergence of duplicated sequences in both lineages. The latter is also supported by differences in their N-terminus (fig. 1A)—which can add functional specificity to these proteins. Although more data are needed to decipher the evolutionary history of this complex gene family, preliminary analyses of the available sequences (fig. 1E) suggest that (i) some of the duplication events responsible for the diversification of this gene family might have taken place before

the *Mesostigma*/Charophyta divergence and (ii) additional duplication events took place in the land plant lineage.

The evolution of three-dimensional tissues in the earliest land plants is believed to have conferred an adaptive advantage by reducing surface area and, consequently, water loss (Graham, Cook, and Busse 2000). While its precise role is not known, the *P. patens BIP2* is thought to be a morphogenesis gene, and, as no *BIP2* transcripts have been detected in the stages preceding bud formation and in *bud* mutants, this gene appears to be specifically associated with the acquisition of three-dimensional architectures (Brun et al. 2003). Noteworthy, although not located apically, some charophycean algae also possess histogenetic cells dividing asymmetrically and in multiple dimensions (see Graham, Cook, and Busse 2000 for discussion). If *BIP2*-like sequences are relevant to the evolution of the histogenetic meristem in the earliest land plants (and thus are of potential significance to land plant evolution), it is remarkable that such sequences are also found in both the unicellular charophycean *Closterium* as well as the unicellular prasinophyte *Mesostigma* but not in other prasinophyte or chlorophyten taxa.

What could be the roles *BIP2*-like sequences have in these unicellular taxa or might have had in the last common ancestor of Streptophyta? The answers to these questions would only be speculative at this time, and have to await further studies. Likely, some of the functions *BIP2*-like proteins currently have or had in *Mesostigma* are different from the ones of their plant homologs. Nevertheless, it is conceivable that the presence of this gene family (or some of its members) in the unicellular ancestor of Streptophyta was a precondition for the appearance of subsequent derived characters that acquired altered functions (exaptations) in land plants (Graham 1996). This scenario is analogous to the evolution of the pherophorin multigene family in the volvoclean green algal group; in this case, pherophorin-like sequences such as those found in the unicellular *Chlamydomonas reinhardtii* (some of which are stress-induced) have been co-opted into the proteins that make up most of the extracellular matrix in the multicellular *Volvox carteri*, as well as into the sexual induction pathway (associated with changes in the developmental program towards the formation of sexual individuals) (Nedelcu 2005).

Large-scale fixation of duplicated genes have accompanied pivotal evolutionary events such as the origin of animals and early vertebrate evolution (Lynch and Conery 2000), and gene co-option events involving duplicated sequences are known to have had a major role in the evolution

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FIG. 1.—Land plant and *M. viride BIP2*-like predicted proteins. (A) Structural features among several land plant *BIP2*-like sequences (as predicted and provided by PFAM at <http://www.sanger.ac.uk/Software/Pfam/>; orange, signal peptide; red, transmembrane; blue, low complexity region; green, the root-cap domain, PF06830; mixed colors, various Pfam_B domains). (B) The Pfam-B_6814 domain and (C) the PF06830 domain of several *M. viride* (Mv), *P. patens* (Pp), *P. glauca* (Pg), *Z. mays* (Zm), *O. sativa* (Os), and *A. thaliana* (At) *BIP2*-like sequences. (D) Hydropathy profiles (Kyte and Doolittle 1982) of *P. glauca* (green) and two *M. viride* (red, DQ287936; blue, DQ287938) *BIP2*-like deduced amino acid sequences (analysis includes only the alignable region corresponding to position 76–290 in *P. glauca*). (E) Bayesian analysis of selected *M. viride* (Mv; sequences from strain CCMP2046 and strain NIES 476 are in dark and light green, respectively), *C. peracerosum* (Cp), *P. patens* (Pp), *P. glauca* (Pg), *Z. mays* (Zm), *O. sativa* (Os), and *A. thaliana* (At) *BIP2*-like amino acid sequences (GenBank accession numbers are also indicated). As (i) full sequences are not available for all taxa and (ii) some regions cannot be aligned with confidence across land plant and algal sequences, only the C-terminus was used here. Unrooted tree (mixed amino acid model; 3,500,000 generations; 100 sample frequency; 5,000 burnin); numbers represent posterior probability distributions of trees (Huelsenbeck and Ronquist 2001); paralogs within a species/strain are in the same color. Most of these relationships are also represented in maximum likelihood analyses (data not shown); however, many nodes are not well supported (bootstrap values <35%).

Table 1
Presence/absence of *BIP2*-like sequences in various lineages

Taxon	<i>BIP2</i> -like	Source	GenBank Accession numbers
Streptophyta			
Mesostigmatophyceae			
<i>Mesostigma viride</i> CCMP20466	+	EST project ^a	DQ287936–DQ287943
<i>Mesostigma viride</i> NIES 476	+	GenBank	DN256112, DN259133, DN260717, DN257760, DN256206, DN255783, DN255130, DN260764, DN263615
Zygnematophyceae			
<i>Closterium peracerosum</i>	+	GenBank	AU295084, AU295694, AU295556
Mosses			
<i>Physcomitrella patens</i>	+	GenBank	BJ165839, BJ968564, BJ159300
Ferns			
<i>Ceratopteris richardii</i>	+	GenBank	BE643267, BE641546, BE641534
Conifers			
<i>Picea glauca</i>	+	GenBank	AAB01570
Flowering plants			
<i>Zea mays</i>	+	GenBank	BAA75476, BAA75477
<i>Oryza sativa</i>	+	GenBank	BAB85319, BAB85320, BAB85322, BAB85317
<i>Arabidopsis thaliana</i>	+	GenBank	CAB81390, AAG00871, AY054263, BAB02467
Chlorophyta			
Prasinophyceae			
<i>Ostreococcus tauri</i>	–	Genome project ^b	
<i>Micromonas</i> sp.	–	EST project ^a	
Chlorophyceae			
<i>Chlamydomonas reinhardtii</i>	–	Genome project ^c	
<i>Chlamydomonas incerta</i>	–	EST project ^a	
<i>Polytomella parva</i>	–	EST project ^a	
<i>Scenedesmus obliquus</i>	–	EST project ^a	
Trebouxiophyceae			
<i>Prototheca wickerhamii</i>	–	EST project ^a	
Ulvophyceae			
<i>Acetabularia acetabulum</i>	–	EST project ^a	
Glaucocystophyta			
<i>Cyanophora paradoxa</i>	–	EST project ^a	
<i>Glaucocystis nostochinearum</i>	–	EST project ^a	
Rhodophyta			
<i>Cyanidioschyzon merolae</i>	–	Genome project ^d	
Bacillariophyta			
<i>Thalassiosira pseudonana</i>	–	Genome project ^e	

^a <http://amoebidia.bcm.umontreal.ca/public/pepdb/agrm.php>.

^b <http://bioinformatics.psb.ugent.be/genomes.php>.

^c <http://genome.jgi-psf.org/chlre2/chlre2.home.html>.

^d <http://merolae.biol.s.u-tokyo.ac.jp/>.

^e <http://genome.jgi-psf.org/thaps1/thaps1.home.htm>.

of development (True and Carroll 2002). The predicted large number of *BIP2*-like gene copies in *M. viride* is consistent with a recently proposed model for the origin of new gene functions (Francino 2005). This model associates the exploration of a new ecological niche with an increase in the copy number of a preadapted gene and predicts that new gene functions evolve after punctuated bursts of gene amplification and paralog fixation in response to specific selection pressures. It is thus possible (i) that an initial amplification of *BIP2* sequences was triggered by the selective pressures associated with adapting to a freshwater environment—as prasinophytes are primarily marine species (Graham and Wilcox 2000, pp. 411–412), and (ii) that during the transition to multicellularity and subsequent land plant evolution one or several of the *BIP2*-like genes have been co-opted for new functions (through direct co-option and/or

co-option of a duplicated element; Ganfornina and Sanchez 1999).

The restricted presence of *BIP2*-like sequences to *Mesostigma* and the Streptophyta, the complexity of the character (i.e., a multigene family rather than a single-state character), its evolutionary trajectory (fig. 1E), and its potential involvement in the evolution of an important land plant adaptive trait argue for a close relationship between *Mesostigma* and the Streptophyta—to the exclusion of Chlorophyta. The opposite scenario, envisioning *Mesostigma* as basal to the clade containing both the green algal and land plant lineages, would require that a possibly already diversified gene family be entirely lost before the divergence of the lineages leading to the prasinophytes *Ostreococcus*, *Micromonas*, and the presumed prasinophyte ancestors of Chlorophyta, but after the lineage leading to

Streptophyta was established. Such an event would be highly unlikely taking into account the rapid radiation thought to be associated with the early diversification of the first green flagellates (Chapman and Waters 2002).

Supplementary Material

Material and Methods (Mat_Meth.pdf) and supplementary figures 1 and 2 (fig_S1.pdf and fig_S2.pdf) are available at *Molecular Biology and Evolution* online (<http://www.mbe.oxfordjournals.org/>).

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Literature Cited

- Bhattacharya, D., K. Weber, S. S. An, and W. Berning-Koch. 1998. Actin phylogeny identifies *Mesostigma viride* as a flagellate ancestor of the land plants. *J. Mol. Evol.* **47**:544–550.
- Brun, F., M. Gonneau, M. Laloue, and F. Nogue. 2003. Identification of *Physcomitrella patens* genes specific of bud and gametophore formation. *Plant Sci.* **165**:1267–1274.
- Chapman, R. L., and D. A. Waters. 2002. Green algae and land plants—an answer at last? *J. Phycol.* **38**:237–240.
- Delwiche, C. F., K. G. Karol, M. T. Cimino, and K. J. Sytsma. 2002. Phylogeny of the genus *Coleochaete* (Coleochaetales, Charophyta) and related taxa inferred by analysis of the chloroplast gene *rbcL*. *J. Phycol.* **38**:394–403.
- Dong, J. Z., and D. I. Dunstan. 1999. Cloning and characterization of six embryogenesis-associated cDNAs from somatic embryos of *Picea glauca* and their comparative expression during zygotic embryogenesis. *Plant Mol. Biol.* **39**:859–864.
- Francino, M. P. 2005. An adaptive radiation model for the origin of new gene functions. *Nat. Genet.* **37**:573–577.
- Ganfornina, M. D., and D. Sanchez. 1999. Generation of evolutionary novelty by functional shift. *Bioessays* **21**:432–439.
- Graham, L. E. 1996. Green algae to land plants: an evolutionary transition. *J. Plant Res.* **109**:241–251.
- Graham, L. E., M. E. Cook, and J. S. Busse. 2000. The origin of plants: body plan changes contributing to a major evolutionary radiation. *Proc. Natl. Acad. Sci. USA* **97**:4535–4540.
- Graham, L. E., and L. W. Wilcox. 2000. *Algae*. Prentice Hall, Upper Saddle River, N.J.
- Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**:754–755.
- Karol, K. G., R. M. McCourt, M. T. Cimino, and C. F. Delwiche. 2001. The closest living relatives of land plants. *Science* **294**:2351–2353.
- King, N., and S. B. Carroll. 2001. A receptor tyrosine kinase from choanoflagellates: molecular insights into early animal evolution. *Proc. Natl. Acad. Sci. USA* **98**:15032–15037.
- Kyte, J., and R. F. Doolittle. 1982. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157**:105–132.
- Lemieux, C., C. Otis, and M. Turmel. 2000. Ancestral chloroplast genome in *Mesostigma viride* reveals an early branch of green plant evolution. *Nature* **403**:649–652.
- Lewis, L. A., and R. M. McCourt. 2004. Green algae and the origin of land plants. *Am. J. Bot.* **91**:1535–1556.
- Lynch, M., and J. S. Conery. 2000. The evolutionary fate and consequences of duplicated genes. *Science* **290**:1151–1155.
- Marin, B., and M. Melkonian. 1999. Mesostigmatophyceae, a new class of streptophyte green algae revealed by SSU rRNA sequence comparisons. *Protist* **150**:399–417.
- Martin, W., T. Rujan, E. Richly, A. Hansen, S. Cornelsen, T. Lins, D. Leister, B. Stoebe, M. Hasegawa, and D. Penny. 2002. Evolutionary analysis of *Arabidopsis*, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. *Proc. Natl. Acad. Sci. USA* **99**:12246–12251.
- Matsuyama, T., N. Yasumura, M. Funakoshi, Y. Yamada, and T. Hashimoto. 1999. Maize genes specifically expressed in the outermost cells of root cap. *Plant Cell Physiol.* **40**:469–476.
- Mattox, K. R., and K. D. Stewart. 1984. Classification of the green algae: a concept based on comparative cytology. Pp. 29–72 in D. E. G. Irvine and D. M. John, eds. *The systematics of green algae*. Academic Press, London.
- McCourt, R. M., C. F. Delwiche, and K. G. Karol. 2004. Charophyte algae and land plant origins. *Trends Ecol. Evol.* **19**:661–666.
- Melkonian, M. 1989. Flagellar apparatus ultrastructure in *Mesostigma viride* (Prasinophyceae). *Plant Syst. Evol.* **164**:93–122.
- Nedelcu, A. M. 2005. Sex as a response to oxidative stress: stress genes co-opted for sex. *Proc. R. Soc. B* **272**:1935–1940.
- True, J. R., and S. B. Carroll. 2002. Gene co-option in physiological and morphological evolution. *Annu. Rev. Cell Dev. Biol.* **18**:53–80.
- Turmel, M., C. Otis, and C. Lemieux. 2002. The complete mitochondrial DNA sequence of *Mesostigma viride* identifies this green alga as the earliest green plant divergence and predicts a highly compact mitochondrial genome in the ancestor of all green plants. *Mol. Biol. Evol.* **19**:24–38.

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