

Horizontal gene transfer of the algal nuclear gene *psbO* to the photosynthetic sea slug *Elysia chlorotica*

Mary E. Rumpho^{a,1}, Jared M. Worful^a, Jungho Lee^b, Krishna Kannan^a, Mary S. Tyler^c, Debashish Bhattacharya^d, Ahmed Moustafa^d, and James R. Manhart^e

^aDepartment of Biochemistry, Microbiology, and Molecular Biology, University of Maine, Orono, ME 04469; ^bGreen Plant Institute, Seoul National University, Gwonseon, Suwon, Gyeonggi 441-853, Korea; ^cSchool of Biology and Ecology, University of Maine, Orono, ME 04469; ^dDepartment of Biological Sciences and the Roy J. Carver Center for Comparative Genomics, Interdisciplinary Program in Genetics, University of Iowa, Iowa City, IA 52242-1324; and ^eDepartment of Biology, Texas A&M University, College Station, TX 77843

Edited by Lynn Margulis, University of Massachusetts, Amherst, MA, and approved September 17, 2008 (received for review June 9, 2008)

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.

symbiosis | *Vaucheria litorea* | evolution | plastid | stramenopile

Symbiotic associations and their related gene transfer events are postulated to contribute significantly to evolutionary innovation and biodiversity. This comes from extensive analysis of organelles such as plastids (e.g., chloroplasts) that originated via primary endosymbiosis of a free-living cyanobacterium (1, 2). The cyanobacterial genome was greatly reduced by endosymbiotic gene transfer (EGT) to the host nucleus and wholesale gene loss, giving rise to the primary lineages of plants and green algae (streptophytes and chlorophytes), red algae (rhodophytes), and glaucophytes (3–6) [see the scheme in supporting information (SI) Fig. S1]. The diverse group of secondary or "complex" algae (e.g., chromalveolates, euglenids), in turn, arose by secondary endosymbiosis—the uptake of a eukaryotic alga (green or red lineage) by a heterotrophic eukaryotic host. In this case, in addition to EGT, transfer of genes between the unrelated organisms by lateral or horizontal gene transfer (HGT) and loss of genes occurred as a result of the "merger" of the two nuclei (host and endosymbiont) (7). As a result of primary and secondary endosymbiosis, plastid genomes (ptDNAs) encode less than 10% of the predicted 1,000 to 5,000 proteins required to sustain the metabolic capacity of the plastid (8, 9).

Examples of HGT between unrelated or nonmating species are abundant among prokaryotes (10, 11) but less so between prokaryotes and unicellular (12–14) or multicellular eukaryotes (15–

20). Most of these latter examples are associated with parasitism or phagotrophy, including the elegant studies of HGT from the α -proteobacteria *Wolbachia* to insects and nematodes (16–18), and the finding of rhizobial-like genes in plant parasitic nematodes (19, 20). The exchange of genetic material between two eukaryotes is extremely rare, or at least not well documented to date. The best-studied cases include the transfer of mitochondrial DNA from achlorophyllous or epiphytic plants to the mitochondrial genome (mtDNA) of their closely related photosynthetic hosts (21), the exchange of transposons between two animal (22) or two plant (23) species, and the presence of plant genes in plant parasitic nematodes (in addition to the rhizobial genes discussed previously), which are hypothesized to be "defense" genes whose products protect the parasite from host detection (20).

The sacoglossan mollusc (sea slug) *Elysia chlorotica* represents a unique model system to study the potential for interdomain HGT between two multicellular eukaryotes—in this case, from a filamentous secondary (heterokont) alga (*Vaucheria litorea*) to a mollusc. This emerald green sea slug owes its coloring and photosynthetic ability to plastids acquired during herbivorous feeding (24–29). The plastids do not undergo division in the sea slug and are sequestered intracellularly in cells lining the finely divided digestive diverticula. The plastids continue to carry out photosynthesis, providing the sea slug with energy and carbon during its approximately 10-month life span (27, 28). Long-term plastid activity continues despite the absence of algal nuclei (27, 29), and hence a source of nuclear-encoded plastid-targeted proteins. We hypothesize that the algal nuclear genes encoding essential plastid proteins are present in the sea slug, presumably as a result of HGT. Here, we present evidence for such interdomain HGT of *psbO*, a nuclear gene encoding the plastid manganese-stabilizing protein (MSP = PsbO). MSP is a subunit of the photosystem II complex associated with photosynthetic oxygen evolution (30, 31), which is, unquestionably, the most important enzyme complex of oxygenic life.

Results and Discussion

Plastid Genetic Autonomy. The plastids in *E. chlorotica* are not transmitted vertically; rather, they must be acquired with each generation early in development to ensure maturation to the

Author contributions: M.E.R. and J.R.M. designed research; J.M.W., J.L., and K.K. performed research; J.L., M.S.T. contributed new reagents/analytic tools; K.K., D.B., A.M., and J.R.M. analyzed data; and M.E.R. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. EU912438 (*V. litorea* complete ptDNA), EU599581 (*E. chlorotica* complete mtDNA), DQ514337 (*V. litorea psbO* cDNA), EU621881 (*V. litorea psbO* DNA), and EU621882 (*E. chlorotica psbO* DNA)].

¹To whom correspondence should be addressed. E-mail: mrumpho@umit.maine.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0804968105/DCSupplemental.

© 2008 by The National Academy of Sciences of the USA

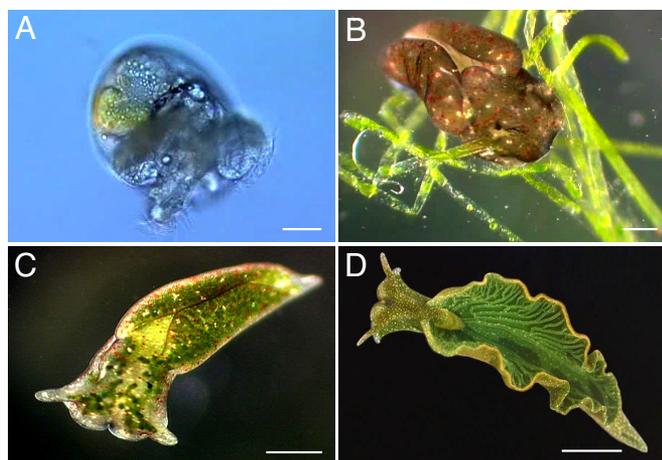


Fig. 1. Laboratory culturing of *E. chlorotica*. (A) Free-swimming *E. chlorotica* veliger larvae. (Scale bar, 100 μm .) Under laboratory conditions, the veliger larvae develop and emerge from plastid-free sea slug–fertilized eggs within approximately 7 days. The green coloring in the digestive gut is attributable to planktonic feeding and not to the acquisition of plastids at this stage. Metamorphosis of the larvae to juvenile sea slugs requires the presence of filaments of *V. litorea*. (B) Metamorphosed juvenile sea slug feeding for the first time on *V. litorea*. (Scale bar, 500 μm .) The grayish-brown juveniles lose their shell, and there is an obligate requirement for plastid acquisition for continued development. This is fulfilled by the voracious feeding of the juveniles on filaments of *V. litorea*. (Also see [Movie S1](#).) (C) Young adult sea slug 5 days after first feeding. (Scale bar, 500 μm .) By a mechanism not yet understood, the sea slugs selectively retain only the plastids in cells that line their highly branched digestive tract. (D) Adult sea slug. (Scale bar, 500 μm .) As the sea slugs further develop and grow in size, the expanding digestive diverticuli spread the plastids throughout the entire body of the mollusc, yielding a uniform green coloring. (Also see [Movie S2](#).) From these controlled rearing studies, we were able to conclude that the only source of plastids in our experimental sea slugs was *V. litorea*.

adult sea slug (32). Laboratory coculturing studies were carried out to establish that the alga *V. litorea*, a derived heterokont alga that contains secondary plastids of red algal origin (33) ([Fig. S1](#)), was the sole source of plastids in the sea slugs ([Fig. 1](#), [Movie S1](#), [Movie S2](#), and [SI Methods](#)). Subsequent sequencing and mapping of the *V. litorea* ptDNA (only the fifth heterokont ptDNA to be published to date) revealed a very compact 115,341-bp double-stranded circular genome encoding 169 genes, including 139 protein-encoding genes (14 are conserved ORFs [ORF-designated *ycfs*] and 2 are unknown ORFs), 27 tRNA genes, and 3 rRNA genes ([Fig. 2](#)). The genes are densely arranged with an average intergenic region of 74bp and 11.1% noncoding DNA. The overall G + C content is 28%, which is low compared with most plastids, including other related heterokonts (34, 35). The genome is separated into two large single-copy regions (62,002bp and 43,469bp) by two smaller inverted repeats (both 4935bp). All the plastid rRNA genes are found on both copies of the inverted repeat in the highly conserved operon *rrs-trnI-trnA-rrl-rrf*. Unlike the four published heterokont ptDNAs that lack introns (34, 35), *V. litorea* contains one intron in the *trnL* UAA gene—an ancient intron that is also present in cyanobacteria (36). In addition, *V. litorea* has retained the genes for the light-independent chlorophyll biosynthesis pathway: *chlB*, *chlL*, and *chlN*. However, as expected, the *V. litorea* ptDNA shares more similarity with heterokonts and red algae than it does with green plastids (34, 35, 37) (see complete inventory of plastid genes by category in [Table S1](#)).

Examining the genetic autonomy of *V. litorea* ptDNA revealed the absence of the major core protein of the oxygen evolving complex of photosystem II, MSP (encoded by *psbO*). MSP has

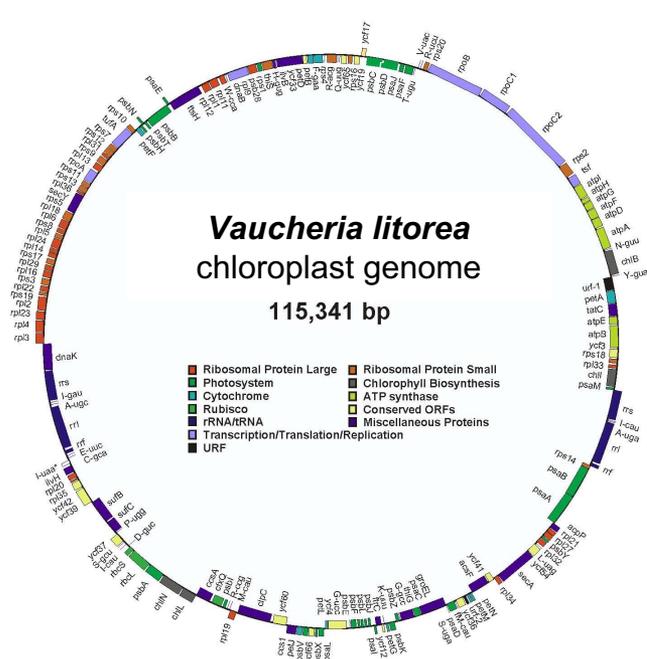


Fig. 2. Map of the ptDNA of *V. litorea*. Genes on the outside are transcribed in the clockwise direction, whereas genes on the inside are transcribed in the counterclockwise direction. Genes are color coded according to their function as shown. tRNAs are listed by the one-letter amino acid code followed by the anticodon. The only gene with an intron (L-uaa) is indicated by an asterisk.

been reported to be critical to the stability of the water-splitting reaction of photosynthesis that generates atmospheric oxygen (30, 31). The evolutionary conservation of this reaction is demonstrated by the presence of MSP in all oxygenic photosynthetic organisms (30). Likewise, animal genomes have never been shown to contain *psbO*; hence, MSP cannot be made by the sea slug in the absence of HGT. We have previously demonstrated that oxygen evolution is linked to photosynthetic electron transport in the sea slug for at least 5 months after being removed from its algal prey (27), and photosystem II is generally highly susceptible to photo-oxidative damage requiring *de novo* synthesis and reassembly of its subunits (38, 39). For these reasons, we targeted *psbO* for HGT from *V. litorea* to *E. chlorotica*.

HGT and Expression of *psbO*. Heterologous degenerate primers ([Table S2](#)) were designed based on alignments of published *psbO* sequences to amplify an internal fragment using reverse transcriptase (RT)-PCR. A 452-bp fragment was amplified from both algal and sea slug cDNA (5 months after algal feeding) ([Fig. 3A](#)). The translated product was used to blast the GenBank database, which revealed a relatively high identity (48%–68%) to several secondary red algal–derived MSP amino acid sequences. The entire *V. litorea psbO* cDNA sequence was then obtained using 5'- and 3'-RACE, and this sequence was used to design homologous primers to amplify a 963-bp internal fragment of the *V. litorea psbO* cDNA ([Fig. 3C](#)). These same primers yielded a similar sized PCR product from sea slug cDNA and DNA templates ([Fig. 3C](#)), the sequences of which were identical to the *V. litorea psbO* cDNA sequence beginning with the start codon (there are no introns in the algal gene; [Fig. S2](#)).

Although it had been several months since the sea slugs had been in contact with any algal prey, the possibility of algal nuclei remaining in the gut of the sea slug and contaminating the total genomic DNA preparation was eliminated by carrying out the same PCR on sea slug egg DNA. Because plastids are

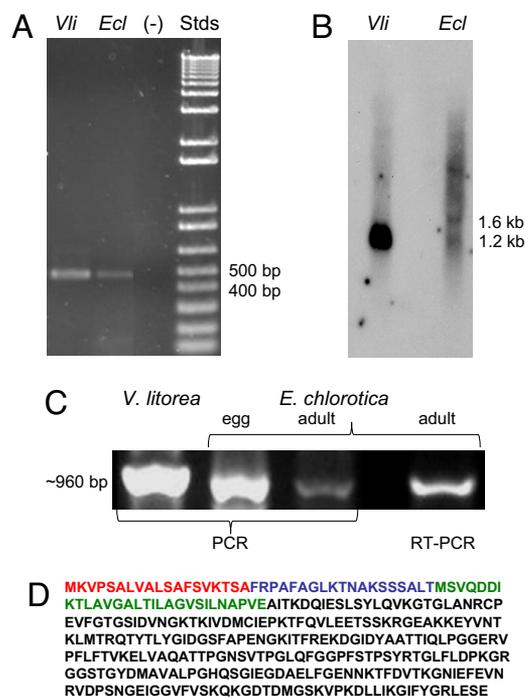


Fig. 3. Expression of *psbO* in *V. litorea* and *E. chlorotica*. (A) RT-PCR using heterologous primers to *psbO* amplified a 452-bp fragment from both algal and adult sea slug cDNA. Water served as the negative control. Standards were a 1-kb Plus DNA ladder (Invitrogen). *Vli*, *Vaucheria litorea*, *Ecl*, *Elysia chlorotica*. (B) Northern blot analysis employing the cloned *V. litorea* 452 bp *psbO* product as probe hybridized with a 1.2-kb transcript for *V. litorea* and *E. chlorotica* as well as a 1.6-kb transcript in the sea slug. RNA Millennium Size Markers (Ambion) were run to estimate transcript size. (C) Homologous primers were designed from the RACE-amplified sequence of the *V. litorea psbO* fragment in A. By PCR, these primers amplified a 963-bp product from genomic DNA of *V. litorea* and *E. chlorotica* eggs and adult tissue as well as *E. chlorotica* adult cDNA by RT-PCR. (D) Translation of the *psbO* sequences obtained from the 963-bp products in B, for both *V. litorea* and *E. chlorotica*, yielded an identical amino acid sequence with a putative tripartite targeting signal for MSP. The signal sequence is in red, the transit peptide is in blue, and the thylakoid targeting domain is in green. Note the highly conserved phenylalanine residue at the cleavage site of the signal sequence.

not inherited in *E. chlorotica*, eggs provide a source of animal DNA and RNA that is free of algal contamination (27). Amplification of the sea slug egg DNA with the same primers resulted in a 963-bp fragment (Fig. 3C) with a sequence identical to the algal and sea slug *psbO* fragments (Fig. S2). As further PCR controls, primers complementary to the *V. litorea* internal transcribed spacer region (*ITS1*; GenBank EF441743) were used as a positive algal nuclear control (27); no product was observed from sea slug or sea slug egg DNA templates. Likewise, attempts to amplify *psbO* from negative controls (pufferfish and *Dictyostelium* DNA) using the same primers and reagents did not yield a positive PCR product. Finally, identical PCR results have been obtained from sea slugs collected from the same site on multiple occasions over a 3-year period (results not shown). Expression of the foreign gene in the sea slug was further supported by Northern blot analysis. The *V. litorea psbO* probe cross-reacted with a 1.2-kb transcript for both *V. litorea* and *E. chlorotica* RNA, along with a slightly larger transcript (1.6 kb) in the sea slug (Fig. 3B).

The identical translated MSP amino acid sequences for both *V. litorea* and *E. chlorotica* (Fig. 3D) were analyzed by phylogenetic methods (40, 41) incorporating 23 published MSP se-

quences. This revealed nesting of the sequences in the red algal lineage in a clade containing other heterokonts (Fig. S3). As expected for this secondary lineage, the *V. litorea* MSP preprotein contains a tripartite targeting sequence (Fig. 3D; as predicted by refs. 42, 43). This reflects the presence of chloroplast endoplasmic reticulum around the complex plastids, which must be traversed by proteins targeted to the plastid (44). What is interesting is that the MSP preprotein in the sea slug has retained the entire tripartite targeting sequence (Fig. 3D), especially in light of the observation that the chloroplast endoplasmic reticulum does not appear to be retained around the engulfed chloroplasts (28).

Recently, it was reported that nuclear genes encoding plastid-localized light harvesting complex proteins (*fcp*, *lhcv1*, and *lhcv2*) have also been transferred from *V. litorea* to *E. chlorotica* (45). Using a similar PCR approach, identical nucleotide sequences were reported for sea slug and algal *fcp* and *lhcv1*, and only a single base substitution was found between larval *lhcv2* and adult sea slug or alga *lhcv2*. Although evidence from Southern blotting has not been achieved in the study reported here or for the light harvesting complex protein genes (45), we were able to obtain sequence information using genome walking for the 3' untranslated flanking region of the *psbO* gene from both algal DNA and sea slug egg DNA. A nested gene-specific primer coupled with an adapter-specific primer (Table S2) yielded a 3' flanking sequence from both organisms that was identical for the first 81bp corresponding to the 3' end of the *psbO* gene and ending with the stop codon (Fig. S4). This sequence was followed by a highly diverged sequence corresponding to the 3' untranslated region in each genome. These results support the interdomain transfer of an algal gene to a mollusc, its expression in the foreign host, and also that the gene has been inserted into the germline, even though the plastids are not yet transmitted vertically in the sea slug.

Mechanism and Site of Integration of Transferred Genes. Similar to many other phagocytic or parasitic relations that lead to presumptive HGT events, the *E. chlorotica/V. litorea* plastid endosymbiosis involves intimate physical contact between predator and prey. During the sea slug's phagocytic feeding, the algal nuclei come into direct physical contact with the sea slug digestive epithelium. Upon nuclear rupture in the gut, pieces of algal chromosomal DNA (and possibly transcripts) may have been randomly transferred by "bulk transfer" or viral transmission (46) to the sea slug. Two potential sites for insertion of foreign genes in the sea slug are the nuclear genome and the mtDNA. Mitochondrion-to-mitochondrion gene transfer is now recognized as a dominant mode of HGT in plants because of the larger and more plastic mtDNAs in these taxa (21). The smaller compact animal or metazoan mitochondrion genome is generally believed to be a poorer target for foreign gene insertion. However, some basal metazoans do exhibit greater variation in mtDNA size and gene content (47). This includes multiple examples of HGT of group I intron sequences (normally not found in animals) into the mtDNA of a sponge (48), a sea anemone (49), and a coral (50).

To determine if the mtDNA of *E. chlorotica* serves as a target for any foreign genes, including *psbO*, we used PCR and primer walking to obtain the complete sequence and map the 14,132-bp mtDNA from sea slug eggs (Fig. 4). The genome was found to encode the standard 37 genes found in other typical animal mitochondria (see ncbi.nlm.nih.gov/genomes/ORGANELLES/33208). No introns were identified, and only 0.0125% of the DNA was noncoding. By measuring the G + C content over adjacent windows of 500 nt with 200-nt overlaps, the values were found to be uniformly distributed across the windows, suggesting homogeneity in GC content of the mtDNA and not supporting

1. Martin W, Kowalik KV (1999) Annotated English translation of Mereschkowsky's 1905 paper Über Natur und Ursprung der Chromatophoren im Pflanzenreiche. *Eur J Phycol* 34:287–295.
2. Margulis L, Sagan D (2003) *Acquiring genomes: A theory of the origins of species*. (Basic Books, New York, NY).
3. Martin W (2003) Gene transfer from organelles to the nucleus: Frequent and in big chunks. *Proc Natl Acad Sci USA* 100:8612–8614.
4. Reyes-Prieto A, Weber AP, Bhattacharya D (2007) The origin and establishment of the plastid in algae and plants. *Annu Rev Genet* 41:147–168.
5. Margulis L (1970) *Origin of Eukaryotic Cells*. (Yale Univ Press, New Haven, CT).
6. Mulikidjanian AY, et al. (2006) The cyanobacterial genome core and the origin of photosynthesis. *Proc Natl Acad Sci USA* 103:13126–13131.
7. Lane CE, Archibald JM (2008) The eukaryotic tree of life: Endosymbiosis takes its TOL. *Trends Ecol Evol* 23:268–275.
8. Martin W, et al. (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.
9. Richly E, Leister D (2004) An improved prediction of chloroplast proteins reveals diversities and commonalities in the chloroplast proteomes of *Arabidopsis* and rice. *Gene* 329:11–16.
10. Koonin EV, Makarova KS, Aravind L (2001) Horizontal gene transfer in prokaryotes: Quantification and classification. *Annu Rev Microbiol* 55:709–742.
11. Dagan T, Martin W (2007) Ancestral genome sizes specify the minimum rate of lateral gene transfer during prokaryote evolution. *Proc Natl Acad Sci USA* 104:870–875.
12. Andersson JO (2005) Lateral gene transfer in eukaryotes. *Cell Mol Life Sci* 62:1182–1197.
13. Loftus B, et al. (2005) The genome of the protist parasite *Entamoeba histolytica*. *Nature* 33:865–868.
14. Nosenko T, Bhattacharya D (2007) Horizontal gene transfer in chromalveolates. *BMC Evol Biol* 7:173.
15. Starcevic A, et al. (2008) Enzymes of the shikimic acid pathway encoded in the genome of a basal metazoan, *Nematostella vectensis*, have microbial origins *Proc Natl Acad Sci USA* 105:2533–2537.
16. Nikoh N, et al. (2008) *Wolbachia* genome integrated in an insect chromosome: evolution and fate of laterally transferred endosymbiont genes. *Genome Res* 18:272–280.
17. Fenn K, et al. (2006) Phylogenetic relationships of the *Wolbachia* of nematodes and arthropods. *PLoS Pathog* 2:e94.
18. Dunning-Hotopp JC, et al. (2007) Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. *Science* 317:1753–1756.
19. Scholl EH, Thorne JL, McCarter JP, Bird DMCK (2003) Horizontally transferred genes in plant-parasitic nematodes: A high-throughput genomic approach. *Genome Biol* 4:R39.
20. Wasmuth J, Schmid R, Hedley A, Blaxter M (2008) On the extent and origins of genic novelty in the Phylum Nematoda. *PLoS Negl Trop Dis* 2:e258.
21. Richardson AO, Palmer JD (2007) Horizontal gene transfer in plants. *J Exp Bot* 58:1–9.
22. Lampe DJ, Witherspoon DJ, Soto-Adames FN, Robertson HM (2003) Recent horizontal transfer of Mellifera subfamily mariner transposons into insect lineages representing four different orders shows that selection acts only during horizontal transfer. *Mol Biol Evol* 20:554–562.
23. Roulin A, Piegou B, Wing RA, Panaud O (2008) Evidence of multiple horizontal transfers of the long terminal repeat retrotransposon RIRE1 within the genus *Oryza*. *Plant J* 53:950–959.
24. Trench RK (1975) Of 'leaves that crawl': Functional chloroplasts in animal cells. *Symposia of the Society for Experimental Biology*, ed Jennings DH (Cambridge Univ Press, London), pp 229–265.
25. Rumpho ME, Summer EJ, Manhart JR (2000) Solar-powered sea slugs. Mollusc/algal chloroplast symbiosis. *Plant Physiol* 123:29–38.
26. Rumpho ME, Dastoor FP, Manhart JR, Lee J (2006) in *Advances in Photosynthesis and Respiration—The Structure and Function of Plastids*, eds Wise RR, Hooper JK (Springer, Berlin), pp 451–473.
27. Green BJ, et al. (2000) Mollusc-algal chloroplast endosymbiosis: Photosynthesis, thylakoid protein maintenance, and chloroplast gene expression continue for many months in the absence of the algal nucleus. *Plant Physiol* 124:331–342.
28. Rumpho ME, Summer EJ, Green BJ, Fox TC, Manhart JR (2001) Mollusc/algal chloroplast symbiosis: How can isolated chloroplasts continue to function for months in the cytosol of a sea slug in the absence of an algal nucleus? *Zoology* 104:303–312.
29. Mujer CV, Andrews DL, Manhart JR, Pierce SK, Rumpho ME (1996) Chloroplast genes are expressed during intracellular symbiotic association of *Vaucheria litorea* plastids with the sea slug *Elysia chlorotica*. *Proc Natl Acad Sci USA* 93:12333–12338.
30. De Las Rivas J, Barber J (2004) Analysis of the structure of the PsbO protein and its implications. *Photosynth Res* 81:329–343.
31. Roose JL, Wegener KM, Pakrasi HB (2007) The extrinsic proteins of photosystem II. *Photosynth Res* 92:369–387.
32. West HH, Harrigan J, Pierce SK (1984) Hybridization of two populations of a marine opisthobranch with different development patterns. *Veliger* 26:199–206.
33. Lee RE (1989), in *Phycology*, ed Lee RE (Cambridge Univ Press, New York), pp 507–522.
34. Oudot-Le Secq MP, et al. (2007) Chloroplast genomes of the diatoms *Phaeodactylum tricorutum* and *Thalassiosira pseudonana*: Comparison with other plastid genomes of the red lineage. *Mol Genet Gen* 277:427–439.
35. Cattolico RA, et al. (2008) Chloroplast genome sequencing analysis of *Heterosigma akashiwo* CCMP452 (West Atlantic) and NIES293 (West Pacific) strains. *BMC Genomics* 9:211.
36. Paquin B, Kathe SD, Nierzwicki-Bauer SA, Shub DA (1997) Origin and evolution of group I introns in cyanobacterial tRNA genes. *J Bacteriol* 179:6798–6806.
37. Reith M, Munholland JA (1993) High-resolution gene map of the chloroplast genome of the red alga *Porphyra purpurea*. *Plant Cell* 5:464–475.
38. Roose JL, Kashino Y, Pakrasi HB (2007) The PsbQ protein defines cyanobacterial photosystem II complexes with highest activity and stability. *Proc Natl Acad Sci USA* 104:2548–2553.
39. Andersson B, Aro E-M (2001) Photodamage and D1 protein turnover in photosystem II. *Regulation of Photosynthesis*, eds Andersson B, Aro E-M (Kluwer, Dordrecht, The Netherlands), pp 377–393.
40. Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783–791.
41. Swofford DL, et al. (2001) Bias in phylogenetic estimation and its relevance to the choice between parsimony and likelihood methods. *Syst Biol* 50:525–539.
42. Westerlund I, von Heijne G, Emanuelsson O (2003) LumenP—A neural network predictor for protein localization in the thylakoid lumen. *Protein Sci* 12:2360–2366.
43. Emanuelsson O, Brunak S, von Heijne G, Nielsen H (2007) Locating proteins in the cell using TargetP, SignalP, and related tools. *Nat Protoc* 2:953–971.
44. Chaal BK, Green BR (2005) Protein import pathways in 'complex' chloroplasts derived from secondary endosymbiosis involving a red algal ancestor. *Plant Mol Biol* 57:333–342.
45. Pierce SK, Curtis NE, Hanten JJ, Boerner SL, Schwartz JA (2007) Transfer, integration and expression of functional nuclear genes between multicellular species. *Symbiosis* 43:57–64.
46. Pierce SK, Mangel TK, Rumpho ME, Hanten JJ, Mondy WL (1999) Annual viral expression in a sea slug population: Life cycle control and symbiotic chloroplast maintenance. *Biol Bull* 197:1–6.
47. Lavrov DV (2007) Key transitions in animal evolution: A mitochondrial DNA perspective. *Integr Comp Biol* 47:734–743.
48. Rot C, Goldfarb I, Ilan M, Huchon D (2006) Putative cross-kingdom horizontal gene transfer in sponge (Porifera) mitochondria. *BMC Evol Biol* 6:71.
49. Beagley CT, Okada NA, Wolstenholme DR (1996) Two mitochondrial group I introns in a metazoan, the sea anemone *Metridium senile*: One intron contains genes for subunits 1 and 3 of NADH dehydrogenase. *Proc Natl Acad Sci USA* 93:5619–5623.
50. van Oppen MJH, et al. (2002) The mitochondrial genome of *Acropora tenuis* (Cnidaria: Scleractinia) contains a large group I intron and a candidate control region. *J Mol Evol* 55:1–13.
51. Palmer JD (1991) Plastid chromosomes: Structure and evolution. *The Molecular Biology of Plastids*, eds Bogorad L, Vasil IK (Academic, San Diego), pp 5–53.
52. Lehman RL, Manhart JR (1997) Preliminary comparison of restriction fragment patterns in the genus *Caulerpa* (Chlorophyta) and the unique structure of the chloroplast genome of *Caulerpa sertularioides*. *J Phycol* 33:1055–1062.
53. Lee J, Manhart JR (2002) Four embryophyte introns and *psbB* operon indicate *Chlorokybus* as a basal streptophyte lineage. *Algae* 17:53–58.
54. Folmer O, Black M, Hoeh R, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* 3:294–299.
55. Wyman SK, Jansen RK, Boore JL (2004) Automatic annotation of organellar genomes with DOGMA. *Bioinformatics* 20:3252–3255.
56. Lohse M, Drechsel O, Bock R (2007) OrganellarGenomeDRAW (OGDRAW)—A tool for the easy generation of high-quality custom graphical maps of plastid and mitochondrial genomes. *Curr Genet* 52:267–274.