Journal of Experimental Botany, Vol. 58, No. 1, pp. 1–9, 2007
Intracellular Compartmentation: Biogenesis and Function Special Issue
doi:10.1093/jxb/erl148 Advance Access publication 9 October, 2006

Journal of Experimental Botany

SPECIAL ISSUE PAPER

Horizontal gene transfer in plants

Aaron O. Richardson and Jeffrey D. Palmer*

Department of Biology, Indiana University, 1001 East Third Street, Bloomington, IN 47405, USA

Received 31 March 2006; Accepted 8 August 2006

Abstract

Horizontal gene transfer (HGT) has played a major role in bacterial evolution and is fairly common in certain unicellular eukaryotes. However, the prevalence and importance of HGT in the evolution of multicellular eukaryotes remain unclear. Recent studies indicate that plant mitochondrial genomes are unusually active in HGT relative to all other organellar and nuclear genomes of multicellular eukaryotes. Although little about the mechanisms of plant HGT is known, several studies have implicated parasitic plants as both donors and recipients of mitochondrial genes. Most cases uncovered thus far have involved a single transferred gene per species; however, recent work has uncovered a case of massive HGT in *Amborella trichopoda* involving acquisition of at least a few dozen and probably hundreds of foreign mitochondrial genes. These foreign genes came from multiple donors, primarily eudicots and mosses. This review will examine the implications of such massive transfer, the potential mechanisms and consequences of plant-to-plant mitochondrial HGT in general, as well as the limited evidence for HGT in plant chloroplast and nuclear genomes.

Key words: *Amborella trichopoda*, genome evolution, horizontal (lateral) gene transfer, plant mitochondria.

Introduction

Horizontal gene transfer (HGT), the transfer of genes between non-mating species, is remarkably common and important in prokaryotes. In many prokaryotes, HGT has contributed 10–20% of the genes (Koonin *et al*., 2001; Lawrence and Ochman, 2001; Nakamura *et al*., 2004). HGT often critically influences prokaryotic evolution, leading to acquisition or modification of such important traits as antibiotic resistance, virulence, photosynthesis, and nitrogen fixation. Some authors suggest that HGT 'may be the dominant force [in prokaryotic evolution], affecting most genes in most prokaryotes' (Doolittle *et al*., 2003).

With very rare exception, HGT occurs much less frequently in eukaryotes than in bacteria, although the process may have been more common early in eukaryotic evolution. Several groups have inferred that the eukaryotic nuclear genome derives from HGT through the fusion of archaebacterial and eubacterial genomes (Moreira and Lopez-Garcia, 1998; Rivera *et al*., 1998; Rivera and Lake, 2004), but this interpretation has been called into question (Kurland *et al*., 2006). Following the endosymbiotic origin of mitochondria and chloroplasts, many genes of eubacterial origin migrated to the nucleus from these organelles via intracellular gene transfer (IGT) (reviewed in Lang *et al*., 1999; Adams and Palmer, 2003; Timmis *et al*., 2004). Functional IGT from the mitochondrial genome has, based on current evidence, entirely ceased in animals and virtually ceased in fungi. In contrast, it occurs relatively frequently in flowering plants (Adams *et al*., 2002).

Among the eukaryotes, unicellular eukaryotes generally experience the most HGT (Keeling and Palmer, 2001; Andersson *et al*., 2003; Richards *et al*., 2003; Andersson, 2005), perhaps because they lack a sequestered germline and because they often engulf their prey, releasing DNA near the nucleus (Doolittle, 1998). Most of the foreign genes detected in these protists were acquired from bacterial donors. Although frequent in eukaryotic terms, the amount of HGT in unicellular eukaryotes ranges from a single gene to several dozen, accounting for <1% of the genome (Andersson, 2005).

Nuclear HGT is rare in multicellular eukaryotes (animals, fungi, and plants). Nearly all known cases involve bacteria as donors (Garcia-Vallve *et al*., 2000; Rosewich and Kistler, 2000; Screen and St Leger, 2000; Intrieri and Buiatti, 2001; Veronico *et al*., 2001; Watts *et al*., 2001; Wolf and Koonin, 2001; Kondo *et al*., 2002; Zardoya *et al*.,

* To whom correspondence should be addressed. E-mail: jpalmer@indiana.edu
Abbreviations: HGT, horizontal gene transfer; IGT, intracellular gene transfer; mtDNA, mitochondrial DNA.

2002; Hall *et al*., 2005). Among the plants, *Agrobacterium rhizogenes* has donated genes, some functional, to members of its host genus *Nicotiana* (Furner *et al*., 1986; Aoki and Syono, 1999*a*, *b*; Intrieri and Buiatti, 2001; Suzuki *et al*., 2002). During pathogenesis, *Agrobacterium* transforms its host with several plasmid-encoded genes, with HGT as a natural consequence. Additional putative cases of bacterium-to-plant nuclear genome HGT (outside of organelle-to-nucleus IGT) include the acquisition of aquaglyceroporins from a eubacterium ~1200 million years ago (Zardoya *et al*., 2002) and of glutathione biosynthesis genes from an alpha-proteobacterium (Copley and Dhillon, 2002).

The low levels of horizontal transfer of nuclear genes in multicellular eukaryotes contrasts with evidence that their nuclear transposable elements have moved horizontally on numerous occasions, although relatively few such transfers have yet been documented in plants (Kidwell and Lisch, 2001; Feschotte and Wessler, 2002; Diao *et al*., 2006). Like nuclear genomes and yeast mitochondrial genomes (Goddard and Burt, 1999), plant mitochondria have been subject to horizontal transfer of mobile genetic elements. Most notably, the discovery of high frequency angiosperm-to-angiosperm horizontal transfer of a homing group I intron in the mitochondrial *cox1* gene (Cho *et al*., 1998; Cho and Palmer, 1999) foreshadowed the recent discovery of widespread horizontal transfer of plant mitochondrial genes.

HGT in plant mitochondria

Two papers appearing in 2003 showed that unlike other eukaryotic genomes, plant mitochondrial genomes experience frequent and evolutionarily widespread horizontal transfer of genes acquired from other eukaryotes, plants in particular. Won and Renner (2003) showed that an intron-containing portion of the mitochondrial *nad1* gene had been transferred from an angiosperm to the gymnosperm *Gnetum* (also see Table 1). Their discovery arose from a study of *Gnetum* phylogeny: the authors showed that this transfer was recent (2–5 million years ago) and restricted to a single Asian clade within *Gnetum*. Following the acquisition of the angiosperm-derived sequence in the common ancestor of this Asian clade of *Gnetum*, several species in the clade have lost the foreign sequence, while all have retained the corresponding native sequence.

Bergthorsson *et al*. (2003) reported five cases of mitochondrion-to-mitochondrion HGT occurring among flowering plants (Table 1). This study was guided by results of previous work on mitochondrion-to-nucleus IGT that identified *Actinidia arguta*, *Betula nigra*, and *Lonicera* sp. as each possessing a mitochondrial gene that was not present in the mitochondrial genomes of nearly 200 related taxa (Adams *et al*., 2002). For example, *rps11* is absent from all 182 core eudicots examined except *Lonicera* and *Betula* (Fig. 1). Phylogenetic analyses (Bergthorsson *et al*., 2003) provided strong evidence that the *Actinidia* and *Lonicera*/Caprifoliaceae genes were acquired from distantly related angiosperms (Table 1), but are neutral on whether the *Betula* gene is of vertical or horizontal descent. The overall evidence for HGT in *Betula* is thus weaker than in the other cases reported by Bergthorsson *et al*. (2003), resting in the gene's anomalous presence in *Betula* mitochondrial DNA (mtDNA).

The *Actinidia*, *Lonicera*, and *Betula* cases of HGT all represent examples of recapture HGT, where horizontal

Table 1. *Published accounts of horizontally acquired genes shown or thought to be located in plant mitochondrial genomes*

Citation	Recipient[a]	Donor[b]	Gene	State[c]
Bergthorsson *et al*. (2003)	*Actinidia*	Monocot	*rps2*	R
	Amborella	Eudicot	*atp1*	D
	Betulaceae	Unclear	*rps11*	R
	Caprifoliaceae	Ranunculales	*rps11*	R
	Sanguinaria	Monocot	3′ *rps11*	C
Won and Renner (2003)	*Gnetum*	Asterid	*nad1B-C*	D
Davis and Wurdack (2004)	**Rafflesiaceae**	Vitaceae	*nad1B-C*	?
Mower *et al*. (2004)	*Plantago*	**Orobanchaceae**	*atp1*	D
	Plantago	**Convolvulaceae**	*atp1*	D
Nickrent *et al*. (2004)	**Apodanthaceae**	Fabales	*atp1*	?
Woloszynska *et al*. (2004)	*Phaseolus*	Angiosperm	cp *pvs-trnA*	N
Bergthorsson *et al*. (2004)	*Amborella*	Angiosperm[d]	*atp4*, *atp6*, *atp8*, *atp9*, *ccmB*, *ccmC*, $ccmF_{N1}$, *cox2* (2×), *cox3*, *nad1*, *nad2*, *nad4*, *nad5*, *nad7*, *rpl16*, *rps19*, *sdh4*	D
		Moss	*cox2*, *nad2*, *nad3*, *nad4*, *nad5*, *nad6*, *nad7*	D
Schönenberger *et al*. (2005)	*Ternstroemia*	Ericaceae	*atp1*	?
	Bruinsmia	Cyrillaceae	*atp1*	?
Davis *et al*. (2005)	*Botrychium*	**Santalales**	*nad1B-C*, *matR*	D

[a] Recipient lineages are indicated by the genus examined, or when multiple related genera were found to share the same foreign gene, the family name. Parasitic plants are in bold.
[b] Donor lineages as best defined by current data. Parasitic plants are in bold.
[c] State indicates whether the addition of the foreign sequence resulted in duplication (D), recapture (R) of a gene previously lost to the nucleus, chimericism (C), a novel gene (N), or is unknown (?).
[d] All but *atp9*, *nad5*, *nad7*, and *cox3* are from eudicots, a derived group within angiosperms.

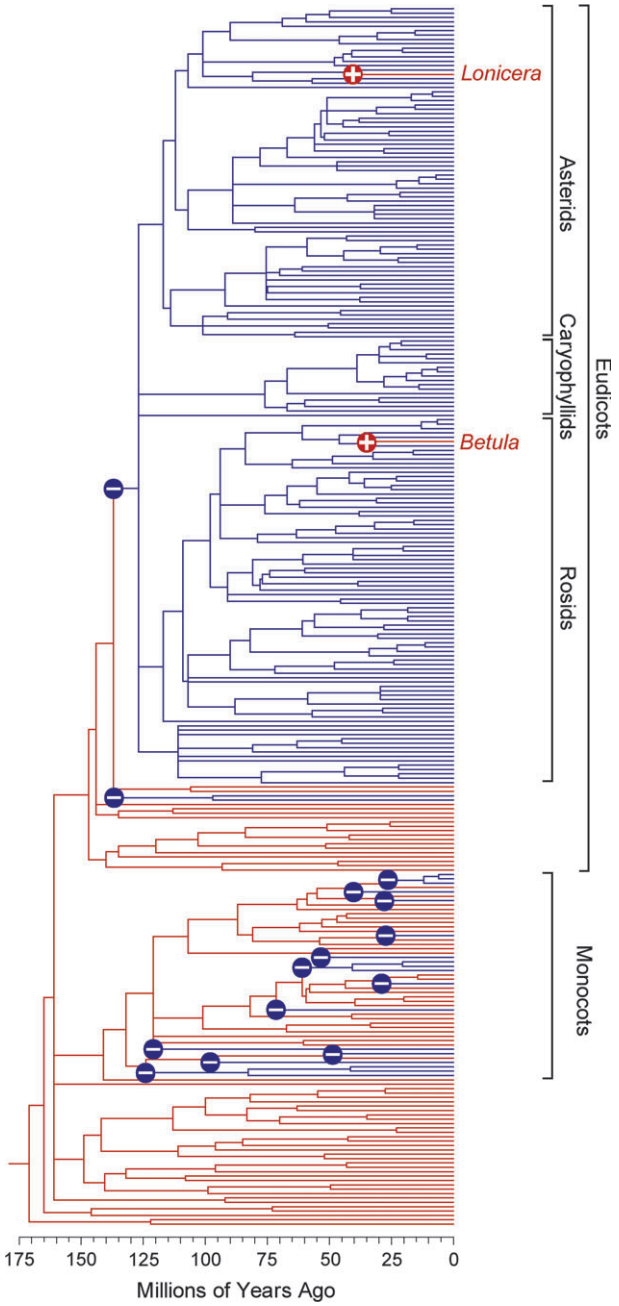

Fig. 1. Distribution of mitochondrial *rps11* among 280 flowering plants. Red and blue branches indicate the presence and absence, respectively, of *rps11* in the mitochondrial genome. Blue circles with minus signs indicate gene losses as inferred by parsimony; red circles with plus signs indicate putative recaptures of *rps11* by mtDNA. Modified from Bergthorsson *et al*. (2003). See Adams *et al*. (2002) for names of all 280 angiosperms represented by the tree.

transfer repopulates a mitochondrial genome with a gene that had previously been lost as a consequence of its functional transfer to the nucleus. The two remaining cases reported in Bergthorsson *et al*. (2003), as well as the case in Won and Renner (2003), represent other outcomes of HGT with respect to the number and structure of members of a mitochondrial gene family. The transfers found in *Amborella* and *Gnetum* represent duplicative HGT, whereby foreign and native copies of a gene co-exist in the same genome (see next section for many such cases in *Amborella* reported in a subsequent study), while chimeric HGT occurred in *Sanguinaria*, giving rise to a hybrid gene that is half native/half foreign (see below for details). No other cases of chimeric HGT have been reported among the many subsequently discovered transfers listed in Table 1 and discussed below; most of the known transfers have created a state of partial to complete gene 'duplication' (Table 1). However, often only a short length of what is potentially a much larger—and possibly chimeric—region of transfer has been characterized, and a recent study has uncovered evidence for substantial chimeric HGT at one particular mitochondrial locus (HC Ong and JD Palmer, unpublished data).

The Bergthorsson *et al*. (2003) and Won and Renner (2003) papers serve as proof of principle: plants clearly exchange genes via HGT. The last 2 years have seen a small explosion of studies reporting additional cases of horizontal transfer of genes in plants (Bergthorsson *et al*., 2004; Davis and Wurdack, 2004; Mower *et al*., 2004; Woloszynska *et al*., 2004; Nickrent *et al*., 2004; Davis *et al*., 2005; Schönenberger *et al*., 2005) (Table 1). In most, but not all, cases the putative HGTs receive strong support from phylogenetic analysis. In virtually all cases, investigators have taken care to rule out the possibility that a 'misplaced' gene in a phylogeny merely represents experimental error, i.e. DNA contamination or mix-up. That is, results were verified by analysis of multiple DNA extractions, including some conducted in other laboratories.

In all but one of the 40 plant-to-plant HGT cases reported thus far, the transferred gene is a mitochondrial gene (encoding a housekeeping respiratory or ribosomal protein), and thus the dominant mode of HGT in plants reported thus far is mitochondrion to, apparently, mitochondrion. Moreover, the one apparent exception, involving chloroplast *pvs-trnA* in *Phaseolus* (Woloszynska *et al*., 2004), may actually represent mitochondrion-to-mitochondrion transfer too. This is because chloroplast sequences frequently become incorporated into mitochondrial genomes via IGT (Unseld *et al*., 1997; Kubo *et al*., 2000; Notsu *et al*., 2002; Handa, 2003; Clifton *et al*., 2004; Knoop, 2004; Sugiyama *et al*., 2005), and thus the possibility remains that *Phaseolus* acquired this chloroplast sequence via intermediate transfer through the donor's mitochondrial genome.

It is important to emphasize that few of the studies firmly demonstrate the mitochondrial provenance of the foreign sequences, although two provide convincing indirect evidence from patterns of mitochondrial RNA editing, Southern blot hybridization intensity, and substitution rates (Bergthorsson *et al*., 2003, 2004). In cases lacking such data, a cautious interpretation of the results is that the genes,

while of foreign mitochondrial origin, may reside in either the mitochondrial or nuclear genomes (the chloroplast genome is so unlikely that it can be dismissed out of hand; see below).

Most of the published cases of HGT in plants involve transfer from one angiosperm to another. The exceptions involve the angiosperm-to-gymnosperm transfer described above (Won and Renner, 2003), a transfer from angiosperms to a fern (Davis *et al*., 2005), and seven transfers from mosses to an angiosperm (Bergthorsson *et al*., 2004). This apparent bias may simply reflect the fact that most mitochondrial genes sequenced in plants are from angiosperms. Many transfers are quite recent in evolutionary terms, being restricted to a single genus of recipient plants, or even a limited number of species within a genus (Bergthorsson *et al*., 2003; Won and Renner, 2003; Mower *et al*., 2004; Davis *et al*., 2005; Schönenberger *et al*., 2005).

It is clear that plant mtDNA is exceptionally active in HGT. Orders of magnitude more sequence data are available for land plant chloroplast DNA and animal mtDNA than for plant mtDNA, yet no cases of HGT have been found in land plant chloroplast or animal mitochondrial genomes. Although plant mtDNAs usually contain numerous nuclear- and chloroplast-derived sequences (Unseld *et al*., 1997; Kubo *et al*., 2000; Notsu *et al*., 2002; Handa, 2003; Clifton *et al*., 2004; Knoop, 2004; Sugiyama *et al*., 2005), there is no good evidence of a plant chloroplast genome containing DNA from other cellular compartments (Rice and Palmer, 2006). Plant mitochondria possess an active DNA uptake system (Koulintchenko *et al*., 2003), but no such system is known in chloroplasts. This uptake system may be critically important in the incorporation of both foreign and native DNA. A major, well-documented difference between the two organelles that may account, at least in part, for their differential propensity for HGT is their propensity to fuse. Plant mitochondria regularly fuse (Arimura *et al*., 2004; Sheahan *et al*., 2005), promoting recombination between parental mitochondrial genomes in the case of somatic hybrid plants generated by protoplast fusion, whereas chloroplasts virtually never fuse under similar conditions (Kanno *et al*., 1997; Mohapatra *et al*., 1998).

DNA transfer facilitated by direct plant-to-plant contact through parasitism has emerged as a common mechanism of HGT; several of the reported HGT events involve parasitic plants as donors (Mower *et al*., 2004; Davis *et al*., 2005) or as recipients (Davis and Wurdack, 2004; Nickrent *et al*., 2004; Table 1, see taxa in bold). However, this mechanism is unlikely to explain all the transfers, as many of the donor and recipient groups do not have a host–parasite relationship. Parasitic plants could still play a role in HGT, given that a generalist parasite could serve as a vector between two unrelated species. At this point, alternative mechanisms await empirical evidence. Illegitimate pollination, herbivory, bacterial or viral transfer, uptake of naked DNA in the soil, and fungal pathogens or symbionts have all been postulated (Bergthorsson *et al*., 2003; Won and Renner, 2003; Davis *et al*., 2005).

Although some of the transferred genes are obvious pseudogenes, others are intact and potentially functional. Analysis of the expression of these foreign genes has been reported in only one case. This case is striking, because the *rps11* gene of *Sanguinaria* (the only copy present in its mtDNA) is transcribed, RNA-edited, and chimeric: its 5′ half is of eudicot, vertical origin, whereas its 3′ half is of monocot, horizontal origin (Bergthorsson *et al*., 2003; Fig. 2). Whether this and other intact, transcribed, and edited foreign genes actually encode functional protein awaits future studies. Previous work has reported plant mtDNA pseudogenes that are transcribed and edited (Aubert *et al*., 1992; Brandt *et al*., 1993; Sandoval *et al*., 2004; Kim and Kim, 2006), transcribed but not edited (Quinones *et al*., 1996), or not transcribed at all (Dong *et al*., 1998).

Do different genes or different plant lineages differ in rates of HGT? Work on IGT has shown that mitochondrial ribosomal protein genes are much more likely to be functionally transferred to the nucleus than those involved in bioenergetics (Adams *et al*., 2002). There does not appear to be any such bias in genes subject to HGT (Table 1), perhaps because most of the transfers may be neutral events of no functional consequence. Substantial lineage-specific rate variation in HGT does exist among flowering plant mitochondria. There is no evidence of HGT for the protein-coding genes of the completely sequenced mitochondrial genomes of *Arabidopsis*, *Brassica*, rice, corn, or sugar beet (Unseld *et al*., 1997; Kubo *et al*., 2000; Notsu *et al*., 2002; Handa, 2003; Bergthorsson *et al*., 2004; Clifton *et al*., 2004). Although most published accounts of HGT involve a single gene from any given plant (Table 1), few additional genes have been examined in most of these cases, and thus the possibility of more extensive HGT must be considered. At the extreme, the mitochondrial genome of *A. trichopoda* is enormously rich in foreign DNA; in fact, it may contain more foreign than native DNA.

HGT in *Amborella trichopoda*: promiscuity of unprecedented scale

Amborella trichopoda has acquired, via HGT, partial or full-length copies of 20 of its 31 mitochondrial genes recovered by PCR (Bergthorsson *et al*., 2004). For 15 genes, at least one extra copy was acquired horizontally, while at least two extra copies were acquired for four genes (e.g. Fig. 3), and three copies for one gene. For all of the 20 genes with a foreign copy, *Amborella* mtDNA also contains a copy positioned at the base of angiosperms, as expected for vertical transmission. Therefore, HGT in these cases has created a state of duplication, or triplication, or even quadruplication, for a total of 26 transferred genes.

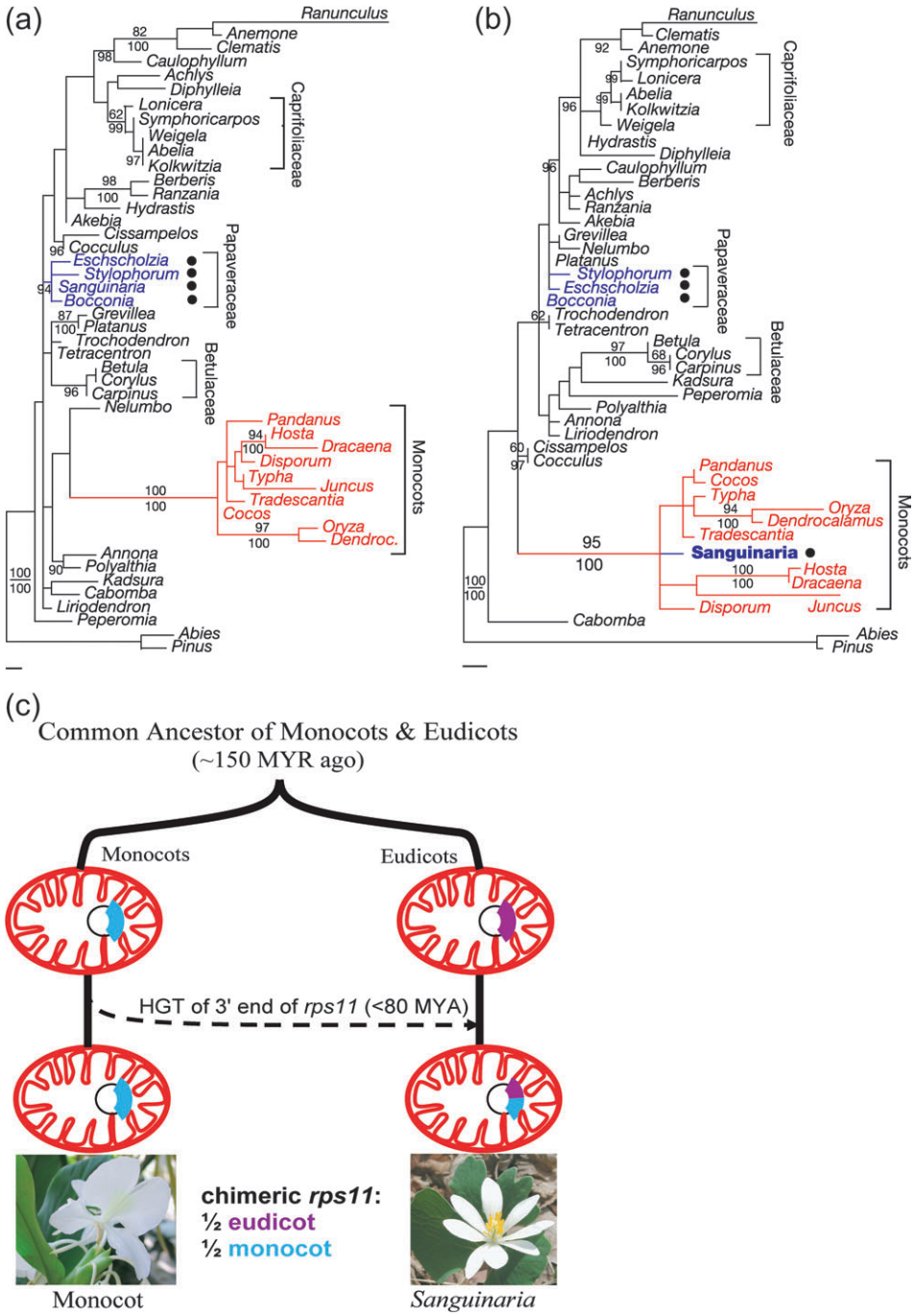

Fig. 2. Chimeric *rps11* in *Sanguinaria* derived in part by HGT. Maximum likelihood trees reproduced from Bergthorsson *et al*. (2003) of (a) the 5′ half (219 nucleotides) and (b) the 3′ half (237 nucleotides) of *rps11*. Bootstrap values >60% from parsimony analysis are given above nodes, Bayesian posterior probability values >90% below. Members of the Papaveraceae are in blue (with black bullets), while monocots are in red. Note that although most nodes in these trees are not well supported owing to the short length of sequence and low substitution rate of plant mitochondrial genes (see references in text), there are several well supported nodes separating the 3′ half of *Sanguinaria rps11* from other members of the Papaveraceae family and placing it firmly with monocots. Scale bars represent 0.01 nucleotide substitutions per site. *Dendroc*., *Dendrocalamus*. (c) Model for the origin of the chimeric *rps11*, with approximate dates from Wikström *et al*. (2001). Photo credits: U Bergthorsson.

Eighteen of the horizontally inherited genes are intact (in the regions sequenced, some genes are much longer than typical PCR products) and potentially functional, while all 31 vertically inherited genes in *Amborella* are intact (Bergthorsson *et al*., 2004; and our unpublished data).

The 26 foreign genes in *Amborella* mtDNA were acquired from a broad range of plant donors (Table 1; Fig. 3). Seven genes were acquired from mosses and the other 19 from angiosperms. Whether some transfer events may have introduced two or more foreign genes is currently

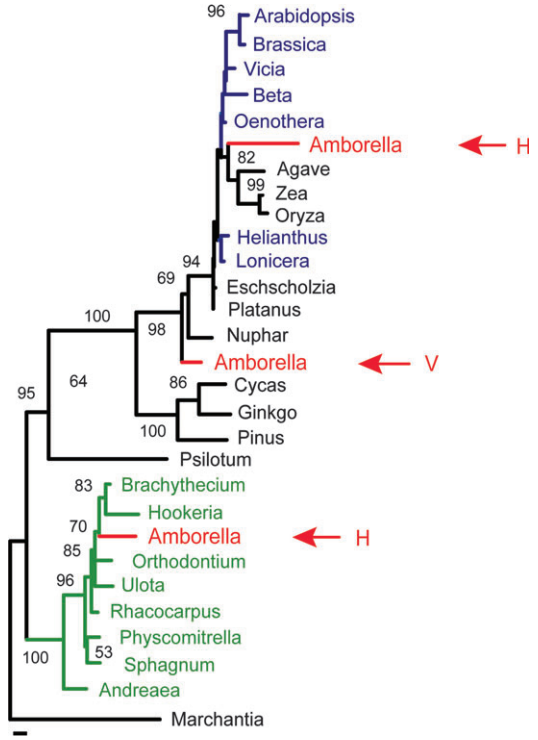

Fig. 3. Two transfers of *nad5* to *Amborella* from disparate plant donors. Maximum likelihood tree of *nad5* exons [reproduced from Bergthorsson *et al*. (2004), Copyright 2004 National Academy of Sciences, USA]. *Amborella* genes are shown in red, core eudicots genes in blue, and moss genes in green. Bootstrap values (100 ML replicates) >50% are shown. H, horizontally acquired gene; V, vertically inherited gene. The scale bar represents 0.01 nucleotide substitutions per site. The number of nucleotides in the alignment varies across genes as not all exons amplified for all sequences; vertically transmitted copy, 1238 nucleotides; angiosperm-derived copy, 601 nucleotides; moss-derived copy, 1062 nucleotides.

unclear. Most of the 19 angiosperm-derived genes are from eudicots. In two cases, a foreign gene has a strong affiliation with a particular eudicot clade (*nad1* with *Corylus*, a rosid, and $ccmF_{N1}$ with *Daucus*, an asterid), but the limited sampling (~10 of 175 000 species of eudicots) for most other genes does not permit identification of the donor lineage.

The 26 HGTs inferred by the PCR study of Bergthorsson *et al*. (2004) is a lower bound; the entire *Amborella* mitochondrial genome sequence will undoubtedly contain additional cases of HGT, particularly those too short or too divergent to be amplified by primers designed for full-length flowering plant genes. Indeed, current sequencing of the *Amborella* mitochondrial genome (our unpublished data) reveals many previously undocumented foreign sequences. The available genome sequence contains all of the foreign mitochondrial genes identified in our published PCR study (Bergthorsson *et al*., 2004), thus putting to rest the concern that the PCR products may have been derived from foreign mtDNA integrated into the nuclear rather than the mitochondrial genome (Martin, 2005). The massive amount of HGT found in *Amborella* mtDNA contrasts with the absence of HGT in *Amborella* chloroplast DNA (Goremykin *et al*., 2003; Stefanovic *et al*., 2004; Rice and Palmer, 2006).

The unique geographic location of *Amborella* provides special opportunities for unravelling the factors contributing to its spectacular amounts of HGT. *Amborella* is a monotypic genus (and family) of subcanopy shrubs and small trees endemic to the South Pacific island of New Caledonia, where it grows on steep slopes in mid-elevation (300–900 m high) montane tropical rain forests (Feild *et al*., 2001). In this dank, dark environment, *Amborella* leaves, branches, trunks, and even fruits are often covered with diverse epiphytes, including mosses and other bryophytes. This could readily promote direct, plant-to-plant HGT, especially given the potential for herbivory to introduce epiphytic tissue and exudates within wounded *Amborella* tissue. Substantial published evidence for direct plant-to-plant HGT occurring in the context of parasitism was discussed above. Epiphytism may offer similar opportunities for HGT.

Nearly 80% of the ~3400 vascular plants native to the island are endemic to New Caledonia (Lowry, 1998), as are an appreciable fraction of the New Caledonian bryophytes. Therefore, molecular examination of this largely endemic flora growing on and in association with *Amborella* should prove crucial in efforts to (i) elucidate the factors promoting such extensive HGT; (ii) uncover other cases of extensive HGT; (iii) pinpoint donor identities; (iv) estimate the timing of transfer; and (v) estimate the number of transfers. A major collecting trip to New Caledonia was mounted in late 2005 and DNA from hundreds of species that grow on and in association with *Amborella* is now being analysed to address these and other issues. In preliminary studies, promising levels of population-level polymorphism in *Amborella* mtDNA has also been uncovered, including two cases of apparent polymorphism for the presence/absence of horizontally acquired genes. The extraordinary abundance and presumed frequency of HGT in *Amborella* may well provide special opportunities for studying the evolutionary dynamics of HGT at the population level. Finally, in unpublished studies, it is found that many of the foreign mitochondrial genes in *Amborella* mtDNA are expressed at the level of detectable steady-state transcripts, some of which are RNA edited. This raises the possibility that some of these genes may actually be functional, although it should be pointed out that numerous instances of transcribed and RNA-edited pseudogenes are known in plant mitochondrial genomes (Aubert *et al*., 1992; Brandt *et al*., 1993; Sandoval *et al*., 2004; Kim and Kim, 2006), as well as even two cases of expressed intact genes that are nonetheless suspected on the basis of their aberrant editing patterns to be 'cryptic' pseudogenes (Mundel and Schuster, 1996; Handa, 2003).

Concluding remarks

Although PCR-based studies have revealed much about HGT, clearly the next major step is to sequence whole mitochondrial genomes of plants known to have experienced HGT. Genome sequencing will uncover transfers that are too short or from such evolutionarily distant donors that they fail to amplify by PCR with flowering plant primers. It should also be established whether multiple genes can be transferred simultaneously, or at least from the same donor lineage. It may even be found that whole donor mitochondria are transferred and fuse with recipient mitochondria to create a state of massive HGT following recombination (as described above, plant mitochondria readily fuse in general). Another clear need is to uncover very recent transfers that could provide further insight into the transfer process and answer several outstanding questions: how long are tracts of HGT; does DNA move back and forth between donors and recipients, or is transfer unidirectional; and do lineages experiencing higher levels of HGT have common ecological or physical characteristics that suggest a transfer mechanism? Addressing these questions will require both broad surveys and dense sampling.

Reported cases of HGT in plants have thus far involved evolutionarily distant donors and recipients, with times since common ancestry between the donor and recipient lineages ranging from 60 million years in the case of the Orobanchaceae-to-*Plantago* transfer (Mower *et al*., 2004) to 480 million years in the case of moss-to-*Amborella* transfer (Wikström *et al*., 2001; Bergthorsson *et al*., 2004). Does the preponderance of distant HGT reflect biological reality, or is it a bias in the ability to detect HGT with confidence? Studies to date have relied on the phylogenetic signal of the donated DNA being discordant with that of the host. Given the generally low rate of sequence evolution in plant mitochondrial genes (Wolfe *et al*., 1987; Palmer and Herbon, 1988; Laroche *et al*., 1997), if mtDNA were transferred within a plant family, gene sequences may not be divergent enough to provide strong evidence for such HGT. This limitation may present a significant barrier to obtaining a comprehensive view of the tempo and pattern of plant-to-plant HGT if any of the dominant modes of transfer involve mechanisms, such as illegitimate pollination, that favour closely related donors and recipients.

Now that it is clear that plant mitochondria exchange genes relatively frequently, caution is necessary when interpreting plant phylogenies from one or even a few mitochondrial genes as they may not reflect the underlying organismal phylogeny. Fortunately, the majority of past and planned molecular phylogenetic studies in plants (including DNA barcoding; Chase *et al*., 2005; Rubinoff *et al*., 2006) have used chloroplast sequences, which seem essentially immune to HGT. Also, in most cases where mitochondrial genes have been used for phylogenetic purposes, this has been done in conjunction with chloroplast and/or nuclear genes.

Although HGT in plant mitochondria is unlikely to confer radically new phenotypes (as it often does in bacteria), the evolutionary consequence of these new genes remains entirely unknown. Finally, if foreign DNA can make it into the mitochondria of one plant from another so readily, might it do the same for the plant nucleus? After all, plant nuclear genomes frequently take up foreign DNA via IGT; they typically contain dozens to hundreds of recently transferred pieces (or sometimes even entire genomes) of endogenous mitochondrial and chloroplast DNA (Arabidopsis Genome Initiative, 2000; Goff *et al*., 2002; Yu *et al*., 2002). The recent findings of the horizontal transfer of a nuclear transposable element in grasses (Diao *et al*., 2006) and of a *Poa*-like nuclear gene in *Festuca* (Ghatnekar *et al*., 2006) suggest that nuclear genomes are the next frontier in plant HGT research.

Acknowledgements

We thank two anonymous reviewers for insightful comments, as well as Eric Baack, Ulfar Bergthorsson, Matt Hahn, Nolan Kane, Eric Knox, Jeff Mower, Loren Rieseberg, and Greg Young for critical reading of the manuscript and helpful discussion. Research in our laboratory on HGT is supported by NIH grant RO1-GM-70612 (to JDP) and an NSF graduate research fellowship (to AOR).

References

Adams KL, Palmer JD. 2003. Evolution of mitochondrial gene content: gene loss and transfer to the nucleus. *Molecular Phylogenetics and Evolution* **29,** 380–395.

Adams KL, Qiu YL, Stoutemyer M, Palmer JD. 2002. Punctuated evolution of mitochondrial gene content: high and variable rates of mitochondrial gene loss and transfer to the nucleus during angiosperm evolution. *Proceedings of the National Academy of Sciences, USA* **99,** 9905–9912.

Andersson JO. 2005. Lateral gene transfer in eukaryotes. *Cellular and Molecular Life Sciences* **62,** 1182–1197.

Andersson JO, Sjogren AM, Davis LAM, Embley TM, Roger AJ. 2003. Phylogenetic analyses of diplomonad genes reveal frequent lateral gene transfers affecting eukaryotes. *Current Biology* **13,** 94–104.

Aoki S, Syono K. 1999*a.* Function of N*grol* genes in the evolution of *Nicotiana glauca*: conservation of the function of NgORF13 and NgORF14 after ancient infection by an *Agrobacterium rhizogenes*-like ancestor. *Plant and Cell Physiology* **40,** 222–230.

Aoki S, Syono K. 1999*b.* Horizontal gene transfer and mutation: N*grol* genes in the genome of *Nicotiana glauca. Proceedings of the National Academy of Sciences, USA* **96,** 13229–13234.

Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana. Nature* **408,** 796–815.

Arimura S, Yamamoto J, Aida GP, Nakazono M, Tsutsumi N. 2004. Frequent fusion and fission of plant mitochondria with unequal nucleoid distribution. *Proceedings of the National Academy of Sciences, USA* **101,** 7805–7808.

Aubert D, Bisanzseyer C, Herzog M. 1992. Mitochondrial *rps14* is a transcribed and edited pseudogene in *Arabidopsis thaliana. Plant Molecular Biology* **20,** 1169–1174.

Bergthorsson U, Adams KL, Thomason B, Palmer JD. 2003. Widespread horizontal transfer of mitochondrial genes in flowering plants. *Nature* **424,** 197–201.

Bergthorsson U, Richardson AO, Young GJ, Goertzen LR, Palmer JD. 2004. Massive horizontal transfer of mitochondrial genes from diverse land plant donors to the basal angiosperm *Amborella. Proceedings of the National Academy of Sciences, USA* **101,** 17747–17752.

Brandt P, Unseld M, Eckertossenkopp U, Brennicke A. 1993. An *rps14* pseudogene is transcribed and edited in *Arabidopsis* mitochondria. *Current Genetics* **24,** 330–336.

Chase MW, Salamin N, Wilkinson M, Dunwell JM, Kesanakurthi RP, Haidar N, Savolainen V. 2005. Land plants and DNA barcodes: short-term and long-term goals. *Philosophical Transactions of the Royal Society B: Biological Sciences* **360,** 1889–1895.

Cho YR, Palmer JD. 1999. Multiple acquisitions via horizontal transfer of a group I intron in the mitochondrial cox1 gene during evolution of the Araceae family. *Molecular Biology and Evolution* **16,** 1155–1165.

Cho Y, Qiu YL, Kuhlman P, Palmer JD. 1998. Explosive invasion of plant mitochondria by a group I intron. *Proceedings of the National Academy of Sciences, USA* **95,** 14244–14249.

Clifton SW, Minx P, Fauron CMR, *et al.* 2004. Sequence and comparative analysis of the maize NB mitochondrial genome. *Plant Physiology* **136,** 3486–3503.

Copley SD, Dhillon JK. 2002. Lateral gene transfer and parallel evolution in the history of gluathione biosynthesis genes. *Genome Biology* **3,** 0025.1–0025.16.

Davis CC, Anderson WR, Wurdack KJ. 2005. Gene transfer from a parasitic flowering plant to a fern. *Proceedings of the Royal Society B: Biological Sciences* **272,** 2237–2242.

Davis CC, Wurdack KJ. 2004. Host-to-parasite gene transfer in flowering plants: phylogenetic evidence from malpighiales. *Science* **305,** 676–678.

Diao XM, Freeling M, Lisch D. 2006. Horizontal transfer of a plant transposon. *Plos Biology* **4,** 119–128.

Dong FG, Wilson KG, Makaroff CA. 1998. Analysis of the four *cox2* genes found in turnip (*Brassica campestris*, Brassicaceae) mitochondria. *American Journal of Botany* **85,** 153–161.

Doolittle WF. 1998. You are what you eat: a gene transfer ratchet could account for bacterial genes in eukaryotic nuclear genomes. *Trends in Genetics* **14,** 307–311.

Doolittle WF, Boucher Y, Nesbo CL, Douady CJ, Andersson JO, Roger AJ. 2003. How big is the iceberg of which organellar genes in nuclear genomes are but the tip? *Philosophical Transactions of the Royal Society B: Biological Sciences* **358,** 39–57.

Feild T, Brodribb T, Jaffré T, Holbrook N. 2001. Acclimation of leaf anatomy, photosynthetic light use and xylem hydraulics to light in *Amborella trichopoda* (Amborellaceae). *International Journal of Plant Science* **162,** 999–1008.

Feschotte C, Wessler SR. 2002. *Mariner*-like transposases are widespread and diverse in flowering plants. *Proceedings of the National Academy of Sciences, USA* **99,** 280–285.

Furner IJ, Huffman GA, Amasino RM, Garfinkel DJ, Gordon MP, Nester EW. 1986. An *Agrobacterium* transformation in the evolution of the genus *Nicotiana. Nature* **319,** 422–427.

Garcia-Vallve S, Romeu A, Palau J. 2000. Horizontal gene transfer of glycosyl hydrolases of the rumen fungi. *Molecular Biology and Evolution* **17,** 352–361.

Ghatnekar L, Jaarola M, Bengtsson BO. 2006. The introgression of a functional nuclear gene from *Poa* to *Festuca ovina. Proceedings of the Royal Society B: Biological Sciences* **273,** 395–399.

Goddard MR, Burt A. 1999. Recurrent invasion and extinction of a selfish gene. *Proceedings of the National Academy of Sciences, USA* **96,** 13880–13885.

Goff SA, Ricke D, Lan TH, *et al.* 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp *japonica*). *Science* **296,** 92–100.

Goremykin VV, Hirsch-Ernst KI, Wolfl S, Hellwig FH. 2003. Analysis of the *Amborella trichopoda* chloroplast genome sequence suggests that *Amborella* is not a basal angiosperm. *Molecular Biology and Evolution* **20,** 1499–1505.

Hall C, Brachat S, Dietrich FS. 2005. Contribution of horizontal gene transfer to the evolution of *Saccharomyces cerevisiae. Eukaryotic Cell* **4,** 1102–1115.

Handa H. 2003. The complete nucleotide sequence and RNA editing content of the mitochondrial genome of rapeseed (*Brassica napus* L.): comparative analysis of the mitochondrial genomes of rapeseed and *Arabidopsis thaliana. Nucleic Acids Research* **31,** 5907–5916.

Intrieri MC, Buiatti M. 2001. The horizontal transfer of *Agrobacterium rhizogenes* genes and the evolution of the genus *Nicotiana. Molecular Phylogenetics and Evolution* **20,** 100–110.

Kanno A, Kanzaki H, Kameya T. 1997. Detailed analyses of chloroplast and mitochondrial DNAs from the hybrid plant generated by asymmetric protoplast fusion between radish and cabbage. *Plant Cell Reports* **16,** 479–484.

Keeling PJ, Palmer JD. 2001. Lateral transfer at the gene and subgenic levels in the evolution of eukaryotic enolase. *Proceedings of the National Academy of Sciences, USA* **98,** 10745–10750.

Kidwell MG, Lisch DR. 2001. Perspective: transposable elements, parasitic DNA and genome evolution. *Evolution* **55,** 1–24.

Kim DH, Kim BD. 2006. The organization of mitochondrial *atp6* gene region in male fertile and CMS lines of pepper (*Capsicum annuum* L.). *Current Genetics* **49,** 59–67.

Knoop V. 2004. The mitochondrial DNA of land plants: peculiarities in phylogenetic perspective. *Current Genetics* **46,** 123–139.

Kondo N, Nikoh N, Ijichi N, Shimada M, Fukatsu T. 2002. Genome fragment of *Wolbachia* endosymbiont transferred to X chromosome of host insect. *Proceedings of the National Academy of Sciences, USA* **99,** 14280–14285.

Koonin EV, Makarova KS, Aravind L. 2001. Horizontal gene transfer in prokaryotes: quantification and classification. *Annual Review of Microbiology* **55,** 709–742.

Koulintchenko M, Konstantinov Y, Dietrich A. 2003. Plant mitochondria actively import DNA via the permeability transition pore complex. *EMBO Journal* **22,** 1245–1254.

Kubo T, Nishizawa S, Sugawara A, Itchoda N, Estiati A, Mikami T. 2000. The complete nucleotide sequence of the mitochondrial genome of sugar beet (*Beta vulgaris* L.) reveals a novel gene for tRNA (Cys) (GCA). *Nucleic Acids Research* **28,** 2571–2576.

Kurland CG, Collins LJ, Penny D. 2006. Genomics and the irreducible nature of eukaryote cells. *Science* **312,** 1011–1014.

Lang BF, Gray MW, Burger G. 1999. Mitochondrial genome evolution and the origin of eukaryotes. *Annual Review of Genetics* **33,** 351–397.

Laroche J, Li P, Maggia L, Bousquet J. 1997. Molecular evolution of angiosperm mitochondrial introns and exons. *Proceedings of the National Academy of Sciences, USA* **94,** 5722–5727.

Lawrence JG, Ochman H. 2001. Reconciling the many faces of lateral gene transfer. *Trends in Microbiology* **10,** 1–4.

Lowry PI. 1998. *Proceedings of the international symposium on rare, threatened and endangered floras of Asia and the Pacific: Diversity, endemism and extinction in the flora of New Caledonia.* Academia Sinica.

Martin W. 2005. Molecular evolution—lateral gene transfer and other possibilities. *Heredity* **94,** 565–566.

Mohapatra T, Kirti PB, Kumar VD, Prakash S, Chopra VL. 1998. Random chloroplast segregation and mitochondrial genome recombination in somatic: hybrid plants of *Diplotaxis catholica*+ *Brassica juncea. Plant Cell Reports* **17,** 814–818.

Moreira D, Lopez-Garcia P. 1998. Symbiosis between methanogenic archaea and delta-proteobacteria as the origin of eukaryotes: the syntrophic hypothesis. *Journal of Molecular Evolution* **47,** 517–530.

Mower JP, Stefanovic S, Young GJ, Palmer JD. 2004. Gene transfer from parasitic to host plants. *Nature* **432,** 165–166.

Mundel C, Schuster W. 1996. Loss of RNA editing of *rps1* sequences in *Oenothera* mitochondria. *Current Genetics* **30,** 455–460.

Nakamura Y, Itoh T, Matsuda H, Gojobori T. 2004. Biased biological functions of horizontally transferred genes in prokaryotic genomes. *Nature Genetics* **36,** 760–766.

Nickrent DL, Blarer A, Qiu YL, Vidal-Russell R, Anderson FE. 2004. Phylogenetic inference in Rafflesiales: the influence of rate heterogeneity and horizontal gene transfer. *BMC Evolutionary Biology* **4,** 40.

Notsu Y, Masood S, Nishikawa T, Kubo N, Akiduki G, Nakazono M, Hirai A, Kadowaki K. 2002. The complete sequence of the rice (*Oryza sativa* L.) mitochondrial genome: frequent DNA sequence acquisition and loss during the evolution of flowering plants. *Molecular Genetics and Genomics* **268,** 434–445.

Palmer JD, Herbon LA. 1988. Plant mitochondrial-DNA evolves rapidly in structure, but slowly in sequence. *Journal of Molecular Evolution* **28,** 87–97.

Quinones V, Zanlungo S, Moenne A, Gomez I, Holuigue L, Litvak S, Jordana X. 1996. The *rpl5–rps14–cob* gene arrangement in *Solanum tuberosum*: *rps14* is a transcribed and unedited pseudogene. *Plant Molecular Biology* **31,** 937–943.

Rice DW, Palmer JD. 2006. An exceptional horizontal gene transfer in plastids: gene replacement by a distant bacterial paralog and evidence that haptophyte and cryptophyte plastids are sisters. *BMC Biology* (in press).

Richards TA, Hirt RP, Williams BAP, Embley TM. 2003. Horizontal gene transfer and the evolution of parasitic protozoa. *Protist* **154,** 17–32.

Rivera MC, Jain R, Moore JE, Lake JA. 1998. Genomic evidence for two functionally distinct gene classes. *Proceedings of the National Academy of Sciences, USA* **95,** 6239–6244.

Rivera MC, Lake JA. 2004. The ring of life provides evidence for a genome fusion origin of eukaryotes. *Nature* **431,** 152–155.

Rosewich UL, Kistler HC. 2000. Role of horizontal gene transfer in the evolution of fungi. *Annual Review of Phytopathology* **38,** 325–363.

Rubinoff D, Cameron S, Will K. 2006. Are plant DNA barcodes a search for the Holy Grail? *Trends in Ecology and Evolution* **21,** 1–2.

Sandoval P, Leon G, Gomez I, Carmona R, Figueroa P, Holuigue L, Araya A, Jordana X. 2004. Transfer of *RPS14* and *RPL5* from the mitochondrion to the nucleus in grasses. *Gene* **324,** 139–147.

Schönenberger J, Anderberg AA, Sytsma KJ. 2005. Molecular phylogenetics and patterns of floral evolution in the Ericales. *International Journal of Plant Sciences* **166,** 265–288.

Screen SE, St Leger RJ. 2000. Cloning, expression and substrate specificity of a fungal chymotrypsin—evidence for lateral gene transfer from an actinomycete bacterium. *Journal of Biological Chemistry* **275,** 6689–6694.

Sheahan MB, McCurdy DW, Rose RJ. 2005. Mitochondria as a connected population: ensuring continuity of the mitochondrial genome during plant cell dedifferentiation through massive mitochondrial fusion. *The Plant Journal* **44,** 744–755.

Stefanovic S, Rice DW, Palmer JD. 2004. Long branch attraction, taxon sampling and the earliest angiosperms: *Amborella* or monocots? *BMC Evolutionary Biology* **4,** 35.

Sugiyama Y, Watase Y, Nagase M, Makita N, Yagura S, Hirai A, Sugiura M. 2005. The complete nucleotide sequence and multipartite organization of the tobacco mitochondrial genome: comparative analysis of mitochondrial genomes in higher plants. *Molecular Genetics and Genomics* **272,** 603–615.

Suzuki K, Yamashita I, Tanaka N. 2002. Tobacco plants were transformed by *Agrobacterium rhizogenes* infection during their evolution. *The Plant Journal* **32,** 775–787.

Timmis JN, Ayliffe MA, Huang CY, Martin W. 2004. Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. *Nature Reviews Genetics* **5,** 123–135.

Unseld M, Marienfeld JR, Brandt P, Brennicke A. 1997. The mitochondrial genome of *Arabidopsis thaliana* contains 57 genes in 366 924 nucleotides. *Nature Genetics* **15,** 57–61.

Veronico P, Jones J, Di Vito M, De Giorgi C. 2001. Horizontal transfer of a bacterial gene involved in polyglutamate biosynthesis to the plant-parasitic nematode *Meloidogyne artiellia. FEBS Letters* **508,** 470–474.

Watts RA, Hunt PW, Hvitved AN, Hargrove MS, Peacock WJ, Dennis ES. 2001. A hemoglobin from plants homologous to truncated hemoglobins of microorganisms. *Proceedings of the National Academy of Sciences, USA* **98,** 10119–10124.

Wikström N, Savolainen V, Chase MW. 2001. Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society B: Biological Sciences* **268,** 2211–2220.

Wolf YI, Koonin EV. 2001. Origin of an animal mitochondrial DNA polymerase subunit via lineage-specific acquisition of a glycyl-tRNA synthetase from bacteria of the *Thermus-Deinococcus* group. *Trends in Genetics* **17,** 431–433.

Wolfe KH, Li WH, Sharp PM. 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast and nuclear DNAs. *Proceedings of the National Academy of Sciences, USA* **84,** 9054–9058.

Woloszynska M, Bocer T, Mackiewicz P, Janska H. 2004. A fragment of chloroplast DNA was transferred horizontally, probably from non-eudicots, to mitochondrial genome of *Phaseolus. Plant Molecular Biology* **56,** 811–820.

Won H, Renner SS. 2003. Horizontal gene transfer from flowering plants to *Gnetum. Proceedings of the National Academy of Sciences, USA* **100,** 10824–10829.

Yu J, Hu SN, Wang J, *et al.* 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp *indica*). *Science* **296,** 79–92.

Zardoya R, Ding XD, Kitagawa Y, Chrispeels MJ. 2002. Origin of plant glycerol transporters by horizontal gene transfer and functional recruitment. *Proceedings of the National Academy of Sciences, USA* **99,** 14893–14896.