Heredity (2005) 94, 565–566

www.nature.com/hdy

NEWS AND COMMENTARY

Molecular evolution

Lateral gene transfer and other possibilities

W Martin

Heredity (2005) **94,** 565–566. doi:10.1038/sj.hdy.6800659
Published online 30 March 2005

Two recent reports suggesting that extensive lateral gene transfer occurs among higher plants clash with our view of evolution as Darwin understood it.

The concept of descent with modification has proven exquisitely robust, with only two genuine mechanistic additions to Darwin's principles of natural selection operating on variation among progeny, having emerged over the last 150 years. One is endosymbiosis, where highly divergent lineages merge outright, such as the origin of chloroplasts from cyanobacteria or the origin of mitochondria from proteobacteria. The other is lateral, or horizontal, gene transfer (LGT), where disparate lineages occasionally exchange parts of their genetic fabric. Genome sequences have provided sound evidence that both endosymbiosis among eukaryotes, and LGT – among prokaryotes – are indeed real, although there is still much debate as to just how frequently either has occurred during evolution. That debate now continues.

In 2003, Bergthorsson's team reported sequences homologous to plant mitochondrial DNA (mtDNA) from a variety of species, obtained using polymerase chain reaction (PCR) with conserved primers against protein-coding regions of plant mtDNA. As some sequences in the phylogenies branched in very unusual positions, the authors concluded that frequent lateral transfer of mitochondrial DNA between distantly related plants had caused this pattern. Viruses, bacteria, fungi, insects, pollen, even meteorites and grafting were suggested as vectors for this exchange (Bergthorsson *et al*, 2003). The authors provided a figure showing genes being laterally transferred not only between species but also from ancient lineages in the past to more recent lineages, interpretations that '*imply the existence of the transferred gene in an intermediate, unidentified vectoring agent or host plant for millions of years*' (Bergthorsson *et al*, 2003) – yet without mutation, one should add. Several of the unusually branching sequences involved the shrubby flowering plant *Amborella trichopoda*, prompting a more extensive search among DNA samples from this species.

That follow-up study on *Amborella* has now appeared (Bergthorsson *et al*, 2004) and is no less eyebrow-raising. Conserved primers were designed for the 31 protein-coding genes typical of higher plant mtDNA. In total, 20 of the 31 primer pairs generated two or more different PCR amplification products with *Amborella* DNA as the substrate and these mutiple products branched in disparate parts of the trees. Bergthorsson *et al* (2004) interpreted this as evidence for '*massive*' LGT from a myriad of higher plant donors, and concluded that *Amborella* mtDNA *'has sustained proportionately more HGT than any other eukaryotic, or perhaps even prokaryotic, genome yet examined*'. If true, this would be an unprecedented situation for three reasons. First, plant mitochondrial genome sequences have not yet provided evidence for the acquisition of genes from other species (Bergthorsson *et al*, 2004). Second, the *Amborella* chloroplast genome sequence reveals no acquisitions from other species (Goremykin *et al*, 2003). Third, no reports of widespread lateral acquisition from various higher plant donors have emerged from any plant nuclear genome sequenced so far. Is there really something special about *Amborella* that makes it an LGT-haven? Is higher plant mtDNA hopping among species faster than we can sequence it? Or are there possible alternative interpretations of the observations other than LGT?

If we exclude DNA contamination from other sources as a possible factor in their results, as Bergthorsson's team (2003, 2004) did, we can still ask what positive evidence there is that the sequences in question are indeed incorporated in the *Amborella* mtDNA or nuclear genome to substantiate the LGT case. Specific hybridisation and cloning of the noncoding regions flanking the amplified sequences would have offered the opportunity to see how and where the LGT candidate sequences were integrated in unequivocally endogenous *Amborella* DNA. Flanking sequences as specific probes are needed in the case of plant mtDNA because it evolves at an inexplicably slow rate: across the deepest comparisons of flowering plants, sequences of mtDNA coding regions are typically 95% identical or more at the nucleotide level and hence will crosshybridise. However, these studies only reported the conserved reading frame sequences, without any flanking regions or integration sites, leaving their chromosomal and intracellular location – mitochondrion or nucleus – open, although Bergthorsson and team (2004) favour a mitochondrial localisation. This could have been clarified by a complete mitochondrial genome sequence for *Amborella*, contiguous linkage between the LGT candidates and *bona fide* mtDNA isolated from organelles, or *in situ* hybridisation with specific probes. Indeed, the recently published mitochondrial genome sequence for tobacco (Sugiyama *et al*, 2005) revealed that several genes previously reported to have been lost from that genome and transferred to the nucleus are in fact present in the mtDNA, underscoring the value of complete genome data in assessing subcellular gene localisation.

There is also the possibility that the unusual PCR products from *Amborella* stem from substrates somewhere in its genome, but that the unusual trees are not due to LGT. Those who study animal mitochondrial DNA have long known that nuclear pseudogenes of mitochondrial DNA often have unusual sequences (Bensasson *et al*, 2001). Such nuclear pseudogenes of mtDNA are called 'numts' and have been found in large numbers in most sequenced eukaryotic genomes, particularly among plants (Richly and Leister, 2004). Thalmann *et al* (2004) recently showed that numts in primates are a serious problem because they are readily, sometimes even preferentially, amplified over genuine mtDNA, even though the organelle copy is present in larger template numbers. Numts are extremely difficult to distinguish from *bona fide* mtDNA (Bensasson *et al*, 2001; Thalmann *et al*, 2004, 2005) and can produce very unusual branching patterns in phylogenetic trees causing primate species to apparently intermingle (Thalmann *et al*, 2004) in a pattern that would suggest rampant LGT, were the identity of the *bona fide* mtDNA and the numt not known.

The levels of sequence differences between higher plant mtDNA from different orders are low, less than that observed between human and chimp mtDNAs. For example, the *rps2* sequences representing the deeply diverging dicot orders Laurales and Magnoliales – separated by roughly 150 million years (Bergthorsson *et al*, 2003) – have only two nucleotide differences across 474 sites: G→T transversions at positions 89 and 263. The 1.2 kb-long *atp1* sequences from the gymnosperm *Ginkgo* and the angiosperm *Illicium*, separated by about 300 million years (Kim *et al*, 2004), have only 6% nucleotide differences (Bergthorsson *et al*, 2004). By comparison, human and chimp sequences for the 1.8-kb long *nad5* gene from mtDNA have nearly twice as much (11%) differences. The extremely low rate of substitution in higher plant mtDNA makes it amenable to using conserved primers (Bergthorsson *et al*, 2003, 2004), but low numbers of substitutions alone does not distinguish whether the PCR products obtained are mtDNA or numts (Bensasson *et al*, 2001; Thalmann *et al*, 2004). Adding to these uncertainties is the issue of RNA editing, the C→T changes of which affect over 20 codons each in 10 different reading frames of *Arabidopsis* mtDNA (Geige and Brennicke, 1999). Little is known about the prevalence and among-gene distribution of mitochondrial editing in other higher plant lineages. Other reports for plant-to-plant LGT have also appeared recently (Won and Renner, 2003) but they, too, involved exclusively mtDNA and very small numbers of remarkably distributed sequence differences. There is also the issue of current phylogenetic methods themselves, which are anything but error-free (Holland *et al*, 2004).

None of this is to say that the mechanisms and amounts of LGT inferred in the recent findings from plant mtDNA cannot be true. However, the inferences of LGT via meterorites, LGT from the past to the present, and more frequent LGT among shrubs than among prokaryotes are rather surprising. It is prudent, therefore, to consider possible alternative explanations. After all, dinosaur bone DNA once caused quite a stir, but it turned out to be a numt (Zischler *et al*, 1995). Thus, it will be of interest to see this new LGT evidence corroborated by independent experimental approaches that circumvent PCR and to see its biological significance for the process of heredity among the organisms in question.

W Martin is at the The Institute of Botany, University of Düsseldorf, Germany.

e-mail: w.martin@uni-duesseldorf.de

Bensasson D *et al* (2001). *Trends Ecol Evol* **16**: 314–321.
Bergthorsson U *et al* (2003). *Nature* **424**: 197–201.
Bergthorsson U *et al* (2004). *Proc Natl Acad Sci USA* **101**: 17747–17752.
Geige P, Brennicke A (1999). *Proc Natl Acad Sci USA* **96**: 15324–15329.
Goremykin VV *et al* (2003). *Mol Biol Evol* **20**: 1499–1505.
Holland BR *et al* (2004). *Mol Biol Evol* **21**: 1459–1461.
Kim S *et al* (2004). *Am J Bot* **91**: 2102–2118.
Richly E, Leister D (2004). *Mol Biol Evol* **21**: 1081–1084.
Sugiyama Y *et al* (2005). *Mol Genet Genom* **272**: 603–615.
Thalmann O *et al* (2004). *Mol Ecol* **13**: 321–335.
Thalmann O *et al* (2005). *Mol Ecol* **14**: 179–188.
Won H, Renner SS (2003). *Proc Natl Acad Sci USA* **100**: 10824–10829.
Zischler H *et al* (1995). *Science* **268**: 1192–1193.

Further reading

Hay JM, Sarre SD, Daugherty CH (2004). Nuclear mitochondrial pseudogenes as molecular outgroups for phylogenetically isolated taxa: a case study in Sphenodon. *Heredity* **93**: 468–475.
A rebuttal by Palmer *et al* is available at http://www.nature.com/hdy.