Chapter 10

Horizontal Gene Transfer in Eukaryotes: Fungi-to-Plant and Plant-to-Plant Transfers of Organellar DNA

Susanne S. Renner* and Sidonie Bellot
Systematic Botany and Mycology, University of Munich, Menzinger Straße 67, D-80638 Munich, Germany

Summary

This review focuses on horizontal gene transfer (HGT) involving bacteria, fungi, and plants (Viridiplantae). It highlights in particular the persistent challenge of recognizing HGT, which requires a combination of methods from bioinformatics, phylogenetics, and molecular biology. Non-phylogenetic methods rely on compositional structure, such as G/C content, dinucleotide frequencies, codon usage biases, or co-conversion tracts, while phylogenetic methods rely on incongruence among gene trees, one of which is taken to represent the true organismal phylogeny. All methods are handicapped by short sequence lengths with limited or highly uneven substitution signal; the statistical problems of working with taxon-rich alignments of such sequences include low support for inferred relationships, and difficult orthology assessment. Plant-to-plant HGT is known from two dozen mitochondrial genes and species of phylogenetically and geographically widely separated ferns, gymnosperms, and angiosperms, with seven cases involving parasitic plants. Only one nuclear HGT has

*Author for correspondence, e-mail: renner@lrz.uni-muenchen.de

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come to light, and extremely few fungi-to-plant transfers. Plant mitochondrial genomes, especially in tracheophytes, are prone to take up foreign DNA, but evolutionary consequences of this are still unclear.

I. Introduction

Horizontal gene transfer (HGT) refers to movement of genetic material between organisms that does not follow the normal pathway of vertical transmission from parent to offspring. Horizontal gene transfer is sometimes seen as synonymous with lateral gene transfer, a term better restricted to *within*-species sequence copying, such as group II intron retrotransposition or the massive migration of promiscuous cpDNA into mitochondria of seed plants. With the 2003 discoveries of HGT involving eukaryotes (Bergthorsson et al. 2003; Won and Renner 2003), the availability of full genome sequences, and new insights into transposable elements, HGT has become an important issue also in plant science. Recent reviews of the topic include those of Andersson (2005), Richardson and Palmer (2007), Keeling and Palmer (2008), Keeling (2009a, b), and Bock (2010), and the paradigm is rapidly becoming that HGT is "a highly significant process in eukaryotic genome evolution" (Bock 2010).

The present review focuses on glaucophytes, red algae, green algae, and land plants. Besides briefly summarizing recent findings relevant to plant genomes, it will highlight the persistent challenge of recognizing horizontal gene transfer. This challenge stems largely from the still relatively crude methods for finding matching DNA strings in databases and the inability of phylogenetic algorithms to infer correct relationships from short sequences. Especially the latter problem is often underappreciated in the context of HGT. We therefore begin our review by discussing the combination of bioinformatics, phylogenetics, and molecular biology that forms the basis for inferring and evaluating HGT. We then discuss the evidence for gene transfer between bacteria or fungi and plants, plant-to-plant transfer, and transposable element transfer, and follow with a section on problematic or erroneous earlier inferences of HGT. We end by addressing what is known about the mechanisms of HGT among plants and by providing a perspective on ongoing research that aims at unsolved questions in HGT.

Abbreviations: BLAST – Basic local alignment search tool; cpDNA; – Plastid DNA; DNA – Deoxyribonucleic acid; EST – Expressed sequence tag; HGT – Horizontal gene transfer; HTT – Horizontal transposon transfer; mt(DNA) – Mitochondrial (DNA); MULE – *Mu*-like elements (Mu is *mutator* in corn); My – Million years; ORF – Open reading frame; PCR – Polymerase chain reaction; RNA – Ribonucleic acid; T-DNA – Transferred DNA; TE – Transposable element; Ti-plasmid – Tumor-inducing plasmid

II. Detecting and Evaluating Cases of Horizontal Gene Transfer

A. Bioinformatic Approaches for Detecting HGT

Genome-wide studies of eukaryotes typically will involve a BLAST search (Altschul et al. 1990) to identify genes matching bacterial genes or to find unusual (unique) genes that could be of bacterial origin. Another step is to employ known genes as queries and test for consistency of ORFs or to BLAST against a local database containing well-annotated genomic sequences from model organisms. All these steps rely on BLAST results. It is well understood, however, that BLAST e-values are based on the expected background noise, depend on the sequences in the database at any one time, and are not a reliable indicator of evolutionary relatedness (Koski and Golding 2001). Recent genomics studies have used pair-wise syntenic alignments and BLAST score statistical tests (e.g., Ma et al. 2010).

Other non-phylogenetic methods depend on compositional structure, such as G/C content, dinucleotide frequencies or codon usage biases, but the length of a horizontally transferred gene may be too short to reliably reveal these differences. Methods based on atypical nucleotide or amino acid composition also may only detect recent transfers because donor sequence characteristics will gradually become erased. Moreover, the reliability of these methods is difficult to assess statistically (Ragan et al. 2006). Snir and Trifonov (2010) have proposed using an additional approach that involves comparing just two genomes. With two genomes of a given length one can calculate the probabilities of identical regions (under a chosen model of substitution). To detect HGTs, the method makes use of the expectation that the flanking regions of an inserted region will normally be non-homologous and then uses a sliding window algorithm to detect these HGT borders, essentially searching for sharp borders (or walls). The method has been applied to simulated data and real bacterial genomes.

B. Phylogenetic Approaches for Detecting HGT

Phylogenetic trees are time-consuming to construct because they require a trustworthy sequence alignment. Nevertheless, many workers consider phylogenetic tree incongruence the best indicator of HGT, perhaps especially ancient HGT. When conflicts are found between two or more gene trees, HGT can be introduced as one possible explanation (for an insightful discussion concerning tree incongruency due to HGT in the microbial world, see Boto 2010). Like the bioinformatics approaches discussed in the previous section, the phylogenetic method for identifying HGT faces several challenges. First, it is incapable of coping with events residing in non-homologous regions since all tree inference methods presume character homology in the underlying sequence alignment. It also requires assumptions about where to seek the HGT events, in other words, assumptions about which tree reflects the true organismal history. There is reason to think that methods that detect HGT using atypical genomic composition ("signatures") are better at finding recent transfers whereas "phylogenetic incongruence" methods may be better at detecting older HGTs because of the increasing mutational signal over time, until saturation (Ragan et al. 2006; Cohen and Pupko 2010). Whether this generalization holds will depend on details of the substitution process since all phylogenetic methods, whether parsimony, maximum likelihood, or Bayesian inference, require sufficient mutational signal.

The statistical cut-off deemed acceptable for particular splits in a tree is a matter of debate. Among phylogeneticists, accepted cut-offs values are >75% under parsimony and likelihood optimization, and 98% under Bayesian tree sampling, values rarely reached in trees used to infer HGT because of taxon-rich alignments and short sequences. A sense of the amount of signal needed for statistical support can be gained from Felsenstein's (1985) demonstration that three non-homoplastic substitutions suffice for a bootstrap support (for a node) at the 95% level. These statistical reasons imply that well-supported phylogenies usually require concatenated multi-locus alignments. One then faces the question of which loci can safely be combined. For plants, one solution has been to accept combined plastid gene phylogenies as "true" and to view phylogenies from mitochondrial genes as HGT-prone (Cho et al. 1989a, b; Bergthorsson et al. 2003; Burger et al. 2003; Hao et al. 2010; Archibald and Richards 2010; compare Sect. VII). This is based on the rationale that no evidence has so far come to light of HGT involving plastid genes of Viridiplantae.

Statistical tests for tree incongruence, such as the Incongruence Length Difference test (Farris et al. 1994), require sufficient mutational signal and usually cannot reliably identify nodes in phylogenies due to HGT as long as the trees are based on single genes. This leaves workers in a bind, and many HGT studies have therefore inferred incongruence by eyeballing more or less unsupported trees or by contrasting an unresolved

gene tree with an organismal tree supported by other evidence, for example, morphological and/or genetic data analyzed in other studies. A software to detect HGT from tree incongruence alone is SPRIT (Hill et al. 2010), but it requires assuming that all splits in the trees being compared are true.

A second difficulty with phylogenetic approaches for detecting HGT is that gene phylogenies may be incongruent because of biases in the sequence data and not (only) because of HGT. Well known biases include uneven nucleotide frequencies (Embley et al. 1993; Foster et al. 2009; Stiller 2011), long-branch attraction (Felsenstein 1978), codon bias, and model over-parameterization. Long branch attraction is a systematic error, corresponding to the inconsistency of a statistical procedure (namely maximum parsimony), and leads to the convergence towards an incorrect answer as more and more data are analyzed. It occurs when two (or more) sequences in a phylogeny have unusually high substitution rates, resulting in their having much longer branches than the remaining sequences. Long-branch attraction cannot be resolved by adding more characters, and it is a severe and under-appreciated problem in HGT detection. (Removing one of the long branches can sometimes eliminate the problem; e.g., Goremykin et al. 2009).

A third difficulty in identifying HGT is to distinguish it from ancestral gene duplication and differential gene loss (Stanhope et al. 2001; Gogarten and Townsend 2005; Noble et al. 2007). Duplication and loss in gene families affects especially nuclear genes, and since relatively few densely sampled and deep (i.e., going back millions of years) phylogenies have been built with nuclear genes, lineage sorting has so far not been a major discussion point in HGT (but see Noble et al. 2007).

A recent study involving fungi and angiosperms, illustrates the problems of detecting HGT. To test for plant/fungi gene exchange, Richards et al. (2009) generated automated gene-by-gene alignments and phylogenies for 4,866 genes identified in analyses of the *Oryza* genome and in BLAST comparisons. Visual inspection of the phylogenies used two criteria for HGT: Either a plant gene sequence branching within a cluster of sequences from fungal taxa (or vice versa) or a phylogeny that demonstrated a diverse plant-specific gene family absent from all other taxa except a narrow taxonomic group of fungi (or vice versa). Using these criteria, Richards et al. detected 38 plant-fungi HGT candidates, of which two were detected using the rice genome-specific analysis, 35 were detected using the BLAST-based survey, and one was detected using both search protocols. However, when these authors added more sequences (taxa) from GenBank and expressed sequence tag (EST) databases, only 14 of the putative HGTs remained because increasing taxon sampling decreased the number of isolated or wrongly placed suspected HGT sequences. The number of suspected HGT events was then further reduced to nine by reconstructing phylogenies with better fitting maximum likelihood substitution models that accounted for rate heterogeneity. The study beautifully illustrates the risk of overestimating the frequency of HGT from insufficient taxon sampling and poorly fitting substitution models, with rate heterogeneity being the single most important model parameter (Yang 1994).

As is generally true for tree inference, also the dynamics of gene gains and losses in gene families are probably better inferred using maximum likelihood than parsimony optimization of the minimal number of gains and losses needed to explain the distribution of a group of orthologous genes in a phylogeny (Mirkin et al. 2003; Richards et al. 2009; Cohen and Pupko 2010). These and other studies (Cusimano et al. 2008; Goremykin et al. 2009; Ragan and Beiko 2009; Ferandon et al. 2010) all caution against inferring rampant HGT from phylogenetic incongruence among gene trees, at least as long as the trees are based on short sequences (analyzed under parsimony or, worse, neighbor-joining) from genetically distant organisms with millions of years of evolution separating them.

C. Footprints and Signatures of HGT

The third way of identifying HGT is to look for signatures or "footprints" of the HGT events themselves (Adams et al. 1998; Cho

et al. 1998; Cho and Palmer 1999; Sanchez-Puerta et al. 2008). Such footprints might be the co-conversion tracts of group I introns, which are short stretches of flanking exon sequence (>50 bp into the 5′ exon and <25 bp into the 3′ exon) that may be converted to the donor DNA sequence during intron insertion or excision (Lambowitz and Belfort 1993; Lambowitz and Zimmerly 2004). If the flanking exon stretches in the donor and recipient differ, then co-conversion will create a footprint that can stay even after the intron itself is lost again. The first study using the molecular footprint approach focused on a group I intron in the mt *cox1* gene and inferred 3–5 HGT events in a small clade of Araceae (Cho and Palmer 1999). This was inferred although a parsimony reconstruction favored a vertical transmission history with one intron gain, followed by two losses, that is, three evolutionary events, rather than five (Cho and Palmer 1999). Subsequently, reliance on co-conversion tracts as inconvertible footprints led to the extrapolation of at least 1,000 HGTs of the *cox1* intron among living angiosperms, based on a survey of the intron's distribution that suggested 32 separate cases of intron acquisition from unknown donors to account for the intron's presence in 48 of 281 species from 278 genera (Cho et al. 1998b).

Seven cases of chimeric sequences between foreign and native mt gene copies have been described (Vaughn et al. 1995; Adams et al. 1998: *Peperomia polybotrya cox1* intron; Bergthorsson et al. 2003: *Sanguinaria canadensis rps11*; Barkman et al. 2007: *Pilostyles thurberi atp1*; Hao et al. 2010: *Ternstroemia stahlii atp1*; *Hedychium coronarium matR*; *Boesenbergia rotunda matR*; Mower et al. 2010: *Plantago macrorhiza atp1*). In some cases, the chimeric sequences appear functional, in others they are unexpressed pseudogenes. A recent re-analysis of these cases based on a new recombination search algorithm developed specifically for plant mitochondrial genomes showed that detecting HGT-generated chimeras requires dense taxonomic sampling (Hao 2010). Using the new algorithm, Hao and Palmer (2009) also identified nine putative cases of short-patch gene conversion of native, functional plant mt *atp1* genes by homologous *atpA* genes of chloroplast origin. If confirmed, these cases of recombination between mitochondrial and chloroplast genes provide unique evidence for the creation of functional chimeric genes across the ca. one-billion-year divide between chloroplast and mitochondrial genes.

For transposable elements (TEs), detection of horizontal transposon transfer (HTT) may sometimes be possible by comparisons of the rates of synonymous substitution, the Ks values, observed in TEs with those in orthologous genes (Sanchez-Gracia et al. 2005; Schaack et al. 2010). If the presence of a TE in two hosts is due to horizontal transfer, then it will be younger than the hosts and will have accumulated fewer synonymous mutations than the host genes. With many complete genome sequences now available, this approach can be implemented in a robust statistical framework taking into account the Ks value distribution of hundreds of host genes to define the Ks threshold under which the presence of a TE is considered to be the result of HTT. The approach has been applied to closely related species, such as *Drosophila melanogaster* and *Drosophila simulans*, which diverged less than 5 My ago (Schaack et al. 2010).

III. DNA Transfers Among Bacteria or Fungi and Plants

The classic example of HGT from prokaryotes to multi-cellular eukaryotes is the transfer of DNA from the *Agrobacterium* Ti plasmid to plants (reviewed by Gelvin 2009). Other bacterial species, such as *Sinorhizobium meliloti* and *Mesorhizobium loti*, when harboring modified Ti plasmids, can also transfer them to plants (Broothaerts et al. 2005). During transformation, the transferred DNA (T-DNA) is moved through the plasma membrane via a channel formed by a bacterial protein that also participates in coating of the T-DNA during its transfer to the nucleus (Dumas et al. 2001). The extent of natural recent incorporation of prokaryotic genetic material into plants is

unclear, although bacterial chromosomal DNA apparently is introduced into the nuclei of transgenic plants occasionally (Ülker et al. 2008).

So far, there is one reported case of the horizontal acquisition of a group II intron in the plastid *psbA* gene of the green alga *Chlamydomonas sp.* that appears to come from a cyanobacterium (Odom et al. 2004). From red algae, two genes, *rpl36* and an unusual rubisco operon, *rbcLS*, may have been transferred from bacterial donors to the common ancestor of red algae (*rbcLS*) or the common ancestor of cryptophytes and haptophytes (*rpl36*) (Keeling and Palmer 2008, and references therein).

Genetic exchange between plants and fungi is exceedingly rare, particularly in angiosperms (Richards et al. 2009). Richards et al. compared the genomes of six plant species (*Arabidopsis thaliana*, *Populus trichocarpa*, *Sorghum bicolor*, *Oryza sativa*, *Selaginella moellendorffii*, and *Physcomitrella patens*) with those of 159 prokaryotes and non-plant eukaryotes. Comprehensive phylogenetic analyses of the data, using methods that account for site-specific substitution rate heterogeneity, supported only nine HGTs between plants and fungi (methods used in this study were discussed above in Sect. II.B). Five were fungi-to-bryophyte and fungi-lycophyte transfers and four were plant-to-fungi transfers. An older report of the transfer of a group I intron from the angiosperm *Youngia japonica* (Asteraceae) into the 18S rRNA of its pathogenic fungus *Protomyces inouyei* (Nishida and Sugiyama 1995) has yet to be followed-up.

IV. Plant-to-Plant DNA Transfers

Exchange of genetic material between mitochondria of land plants has been inferred for diverse taxa. The species involved come from phylogenetically and geographically widely separate clades of ferns, gymnosperms, and angiosperms, suggesting that HGT among plants may be relatively widespread. The known cases involve the following mitochondrial sequences and taxa:

- The *rps2* gene in the dicot *Actinidia arguta* coming from a monocot (Bergthorsson et al. 2003),
- *rps11* in an unidentified *Lonicera* (Caprifoliaceae) coming from Ranunculaceae/Berberidaceae; in the dicot *Sanguinaria canadensis* from a monocot; and in two unidentified Betulaceae from an unidentified non-Betulaceae donor (Bergthorsson et al. 2003),
- *atp1* in *Amborella trichopoda* (Amborellaceae) from an unknown Asteridae (Bergthorsson et al. 2003); in *Ternstroemia* (Pentaphylaceae) from Ericaceae, and in *Bruinsmia* (Styracaceae) from Cyrillaceae (Schönenberger et al. 2005),
- The *nad1* second intron in *Gnetum* (Gymnospermae) coming from an unknown Asteridae, that is, a flowering plant (Won and Renner 2003),
- The *nad1* second intron plus *atp1* in two parasitic species of Rafflesiaceae from their respective host plants (Davis and Wurdack 2004; Barkman et al. 2007),
- The same intron plus *matR* in the fern *Botrychium virginianum* from an unknown Loranthaceae root-parasite (Davis et al. 2005),
- *atp1* in *Pilostyles thurberi* (Apodanthaceae) from its legume host, *Psorothamnus emoryi*; in *Mitrastema yamamotoi* (Mitrastemonaceae) from its host *Quercus subsericea* (Fagaceae; Barkman et al. 2007), and
- *atp1*, *atp6* and *matR* in species of *Plantago* (Plantaginaceae) from parasitic *Cuscuta* (Convolvulaceae) and *Bartsia* (Orobanchaceae; Mower et al. 2004, 2010).

The transferred mitochondrial genes appear to sit in the hosts' mitochondrial genomes, and most are non-functional pseudogenes. Seven cases of chimeric sequences between foreign and native mt gene copies (see especially Mower et al. 2010) were already discussed above (Sect. II). The putative HGT of the mitochondrial *cox1* intron across thousands of flowering plants, either from plant to plant or via unknown fungal donors (Adams et al. 1998; Cho et al. 1998b; Cho and Palmer 1999; Sanchez-Puerta et al. 2008) is discussed in Sects. II and VI.

An additional report about mitochondrial HGT on a massive scale involves the basal angiosperm *Amborella*, which may have acquired one or more copies of 26 mitochondrial protein genes from other land plants. Twenty foreign gene sequences appear to come from other angiosperms, six from moss donors. The transferred genes seem to be intact, but have not been shown to be functional (Bergthorsson et al. 2004). The report has attracted criticism (Martin 2005; Goremykin et al. 2009; see also Sect. VI). Large-scale genome sequencing of *Amborella* is ongoing and may resolve the controversy.

A single HGT event probably can involve multiple mitochondrial genes as made plausible by the results for *Cuscuta* and *Plantago* of Mower et al. (2010). This study also suggests a complicated history of the transferred genes within *Plantago* subsequent to their acquisition via HGT, with additional transfers (including intracellular transfer), gene duplication and differential loss and mutation-rate variation (Mower et al. 2010). Resolving this history will probably require complete mitochondrial and nuclear genome sequencing from multiple individuals.

So far, only one nuclear plant-plant HGT event has come to light. It involves the parasitic Orobanchaceae *Striga hermonthica,* for which BLAST searches between an EST database of *Striga* and plant genome databases, sequencing of a 6,423 bp-long genomic region and Southern blotting collectively imply recent uptake of genetic material from an unknown monocot host (Yoshida et al. 2010). The transferred gene encodes a 448 amino acid-long protein of unknown function, is phylogenetically closer to *Sorghum* than to its *Brachypodium* ortholog, and was acquired recently, that is, after the divergence between *Striga* and *Orobanche* (both in Orobanchaceae) but before the divergence of *S. hermonthica* and *S. gesnerioides*.

From the above it emerges that most plant-to-plant HGT events involve mitochondrial DNA and that close physical association, as exists, for example, between parasitic plants and their hosts, apparently facilitates plant-to-plant HGT. See Sect. VII for possible reasons why plant mitochondria may incorporate foreign DNA more readily than other genomes.

V. Transposable Elements

There are some 200 putative cases of transposable elements (TEs) moving horizontally in eukaryotes, but such events appear to be rare among plants. The first report of the horizontal transfer of a nuclear TE between plants was that of a *Mutator*-like element between the plant genera *Setaria* and *Oryza* (Diao et al. 2006). For clades other than Viridiplantae, it has been argued that introduction of transposable elements by horizontal transfer in eukaryotic genomes has been a major force propelling genomic variation and biological innovation (Sanchez-Gracia et al. 2005; Gilbert et al. 2010; Schaack et al. 2010). Whether there is any correlation between the horizontal transfer of TEs and the horizontal transfer of functional genes is unclear. Although TEs have not yet been shown to transfer host genes between different species in eukaryotes, they are capable of capturing and transducing sequences at high frequency within a species (Schaack et al. 2010). Of 3,000 analyzed TEs in rice, many contained gene fragments of genomic DNA that apparently had been captured, rearranged and amplified over millions of years (Jiang et al. 2004). Other examples of gene duplication and exon shuffling by transposons come from *Zea mays* (Morgante et al. 2005).

VI. Problematic, Controversial, and Erroneous Reports of HGT Involving Plants

Claims of HGT require considerable supporting evidence and caution (Kurland et al. 2003; Martin 2005; Richards et al. 2009), with a case in point being the problems with

the early reports of massive HGT in the draft human genome (Lander et al. 2001) and their later dismissal (Salzberg et al. 2001; Stanhope et al. 2001). It is therefore not surprising that a few reports of HGT have been discussed controversially or turned out to be erroneous. Thus, the report of HGT between unknown Malvaceae and the parasitic species *Pilostyles thurberi* (Nickrent et al. 2004), after re-sequencing of the relevant gene region (18S RNA), turned out to be due to contaminated DNA sequences (Filipowicz and Renner 2010).

An example of putative HGTs being discussed critically is the mt *cox1* intron, which occurs in hundreds of species of flowering plants (Vaughn et al. 1995; Cho et al. 1998a, b; Cho and Palmer 1999; Cusimano et al. 2008; Sanchez-Puerta et al. 2008, 2011). Phylogenetic analysis of the *cox1* intron does not result in statistically supported trees because the intron contains too few phylogenetically informative mutations (Cusimano et al. 2008: sequence similarity among 110 *cox1* introns from throughout angiosperms ranges from 91% to 100%). Even so, the *cox1* tree for the flowering plants matches accepted relationships of orders, families and, in a few cases, genera (Cusimano et al. 2008). A parsimonious explanation is that the *cox1* intron was horizontally acquired once or a few times during the history of flowering plants, followed by vertical inheritance and numerous losses (Cusimano et al. 2008; also Ragan and Beiko 2009; Richards et al. 2009; Inda et al. 2010; Ferandon et al. 2010). Distinct mutations in co-conversion tracts, however, can lead to a scenario of intron insertions from hundreds or thousands of unknown fungal donors (Cho et al. 1998b; Sanchez-Puerta et al. 2008; fungi-to-angiosperm gene transfers are otherwise exceedingly rare: Richards et al. 2009). Resolving the issue will require a better understanding of the mechanisms of intron homing, specifically the creation and decay of co-conversion tracts (Wolf et al. 2001; Belshaw and Bensasson 2006; Ragan and Beiko 2009).

The controversy surrounding Bergthorsson et al.'s report (2004) of rampant HGT of the mtDNA of *Amborella trichopoda* has already been mentioned (Martin 2005; Goremykin et al. 2009). It is clear also from the difficult interpretation of the history of the elongation factor genes in the green algal lineage (Noble et al. 2007; Rogers et al. 2007) that greater taxon sampling can sometimes lead to a scenario more consistent with multiple losses than horizontal gains. Both processes are likely to have played important roles, and knowledge of the function of putatively transferred genes and of the biology of the involved species should help formulate testable hypotheses.

VII. Mechanisms of Plant-to-Plant HGT

The means of DNA exchange between unrelated organisms could theoretically be (1) vectors, such as bacteria, fungi or phloem-sucking bugs; (2) transfer of entire mitochondria through plasmodesmata, when there is plant-to-plant contact; (3) illegitimate pollination followed by elimination of most foreign DNA except for a few mitochondria that might fuse with native mitochondria (below) or (4) natural transformation. Of the 10–36 cases of plant-to-plant HGT (listed in Sect. IV; the numerical range depends on whether the 26 *Amborella* mt genes putatively taken up from other flowering plants and mosses are included; Bergthorsson et al. 2004), at least seven involve parasitic plants (namely Apodanthaceae: *Pilostyles*; Convolvulaceae: *Cuscuta*; unknown root-parasitic Loranthaceae; Mitrastemonaceae: *Mitrastema*; Orobanchaceae: *Bartsia*, *Striga*, *Orobanche*, *Phelipanche*; the common ancestor of the Rafflesiaceae). This ratio suggests that direct contact between donor and recipient facilitates HGT. The host plants can be the donor (Mower et al. 2004, 2010; Davis et al. 2005) or the recipient (Davis and Wurdack 2004; Barkman et al. 2007; Yoshida et al. 2010). The apparent high frequency of HGT involving parasitic plants fits with the experimental demonstration of DNA moving through a graft junction between different lines of tobacco (Stegemann and Bock 2009, although the transferred DNA stayed in the

graft zone). That messenger RNA can pass through plasmodesmata is well documented (Roney et al. 2007; Lucas et al. 2009), but whether paired DNA or entire organelles can pass through plasmodesmata remains to be investigated. Alternatively, vesicle transport of DNA or organelles from cell to cell could be involved in the horizontal transfer of genetic material (Bock 2010).

All but one of the known plant-to-plant HGTs involve mitochondrial DNA, the exception being the nuclear gene taken up by *Striga hermonthica* probably from a monocot host (Yoshida et al. 2010). The propensity of plant mitochondria to incorporate foreign DNA is remarkable, since among thousands of animal mitochondrial genomes sequenced, no convincing evidence of HGT has been found, and embryophyte (land plant) plastid genomes also apparently are devoid of horizontally transferred foreign DNA. So why are plant mitochondrial genomes so open towards foreign DNA? One explanation may be that plant mitochondria are capable of importing RNA and double-stranded DNA (Koulintchenko et al. 2003). Another explanation may be the great propensity of plant mitochondria to fuse with one another (Arimura et al. 2004; Sheahan et al. 2005) and the high recombinational activity of mtDNA throughout tracheophyte evolution (Grewe et al. 2009; Hecht et al. 2011). This may have set the stage for the integration of foreign DNA in plant mt genomes, also amply documented by the frequent integration of chloroplast DNA laterally transferred into seed plant mtDNAs. Interestingly, bryophyte mt genomes lacking similarly active DNA recombination may be sources, but not acceptors for HGTs (Knoop et al. 2011; p. 18).

It is not known whether the horizontally transferred genetic material is DNA or RNA. While it was earlier hypothesized that mitochondrial HGT might largely be an RNA-mediated process (Bergthorsson et al. 2003), transfer of double-stranded DNA, which is much more stable, may be more likely (Henze and Martin 2001; Mower et al. 2010). Whether the transferred mtDNA tends to integrate into the recipients' mitochondrial genomes or, instead, becomes transferred to the nucleus is mostly unclear (Martin 2005; Goremykin et al. 2009; Hao et al. 2010). Keeling and Palmer (2008) have suggested that most transferred genes probably are non-functional and coexist with a native, functional homologue.

In addition to the barriers that can prevent the horizontal transfer and integration of foreign DNA in a recipient, it is worth considering the barriers that prevent its spread in a population. In prokaryotes, and probably also in eukaryotes, one such barrier can be the perturbation of gene dosage and expression in the host. An experimental study of the transferability of thousands of genes within *Escherichia coli* by Sorek at al. (2007) showed that toxicity to the host and changed (increased) gene dosage and expression probably are predominant causes for transfer failure. On the other hand, over-expression of an RNA polymerase experimentally transferred from *Bacillus subtilis* to *E. coli* appeared to entail no immediate fitness costs (Omer et al. 2010).

VIII. Perspective

There are many unsolved questions regarding the transfer of genetic material among phylogenetically distinct clades or species of plants. How can genetic material arrive in a new genome and function there if it lacks active promoters and appropriate downstream sequences for RNA 3' processing and stabilization? Does most transferred DNA consist of complete gene cassettes including functional expression elements? Unless a transferred gene has a homolog in the recipient, it should function only if expression can be properly regulated by the recipient or if it is an "independent gene" as appears to be true of a horizontally transferred antifreeze protein in fish (Graham et al. 2008). Gene conversion between foreign and native genes could have deleterious consequences, for example by perturbing the function of the encoded protein (Ragan and Beiko 2009).

Whether inter-specific HGT has an important role in the evolution of plants is still unclear. Plausible examples of positive evolutionary impacts are the inferred HGTs from fungi to the lycophyte *Selaginella moellendorffii* of a putative membrane transporter gene and from fungi to the moss *Physcomitrella patens* of a putative sugar transporter gene (Richards et al. 2009). Otherwise, beneficial impacts of HGT have been demonstrated or proposed mainly for prokaryotes, unicellular eukaryotes, and animals (Graham et al. 2008; Marchetti et al. 2009; Danchina et al. 2010).

More molecular-biological investigations and better experimental systems in the lab are sorely needed to understand the role(s) of HGT in plants. Horizontal gene transfer in Viridiplantae may be especially difficult to detect because most events seem to involve mtDNA, which at the substitution level evolves extremely slowly, creating a challenge for the phylogenetic approach of inferring events from contradictory gene trees. The warning of Keeling and Palmer (2008) that the picture may be getting more complex with increasingly denser sampling of taxa, genes and genomes so far is borne out (for plants at least), and we are still far from a satisfactory understanding of the mechanisms, vectors and evolutionary significance of natural horizontal gene transfer.

References

Adams KL, Clements MJ, Vaughn JC (1998) The *Peperomia* mitochondrial *coxI* group I intron: timing of horizontal transfer and subsequent evolution of the intron. J Mol Evol 46:689–696

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Andersson JO (2005) Lateral gene transfer in eukaryotes. Cell Mol Life Sci 62:1182–1197

Archibald JM, Richards TA (2010) Gene transfer: anything goes in plant mitochondria. BMC Biol 8:147

Arimura S, Yamamoto J, Aida GP, Nakazono M, Tsutsumi N (2004) Frequent fusion and fission of plant mitochondria with unequal nucleoid distribution. Proc Natl Acad Sci USA 101:7805–7808

Barkman TJ, McNeal JR, Lim S-H, Coat G, Croom HB, Young ND, dePamphilis CW (2007) Mitochondrial DNA suggests at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. BMC Evol Biol 7:248

Belshaw R, Bensasson D (2006) The rise and falls of introns. Heredity 96:208–213

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*. Proc Natl Acad Sci USA 101:17747–17752

Bock R (2010) The give-and-take of DNA: horizontal gene transfer in plants. Trends Plant Sci 15:11–22

Boto L (2010) Horizontal gene transfer in evolution: facts and challenges. Proc R Soc B 277(1683): 819–827

Broothaerts W, Mitchell HJ, Weir B, Kaines S, Smith LMA, Yang W, Mayer JE, Roa-Rodrıguez C, Jefferson RA (2005) Gene transfer to plants by diverse species of bacteria. Nature 433:629–633

Burger G, Gray MW, Lang BF (2003) Mitochondrial genomes: anything goes. Trends Genet 19:709–716

Cho Y, Palmer JD (1999) Multiple acquisitions via horizontal transfer of a group I intron in the mitochondrial *coxl* gene during evolution of the Araceae family. Mol Biol Evol 16:1155–1165

Cho Y, Adams KL, Qiu Y-L, Kuhlman P, Vaughn JC, Palmer JD (1998a) A highly invasive group I intron in the mitochondrial *cox1* gene. In: Moller I-M, Gardestrom P, Glimelius K, Glaser E (eds) Plant mitochondria: from gene to function. Backhuys, Leiden, pp 19–23

Cho Y, Qiu Y-L, Kuhlman P, Palmer JD (1998b) Explosive invasion of plant mitochondria by a group I intron. Proc Natl Acad Sci USA 95:14244–14249

Cohen O, Pupko T (2010) Inference and characterization of horizontally transferred gene families using stochastic mapping. Mol Biol Evol 27:703–713

Cusimano N, Zhang L-B, Renner SS (2008) Reevaluation of the *cox1* group I intron in Araceae and angiosperms indicates a history dominated by loss rather than horizontal transfer. Mol Biol Evol 25:1–12

Danchina EGJ, Rossoa M-N, Vieiraa P, de Almeida-Englera J, Coutinhob PM, Henrissat B, Abad P (2010) Multiple lateral gene transfers and duplications have promoted plant parasitism ability in nematodes. Proc Natl Acad Sci USA 107:17651–17656

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

Davis CC, Anderson WR, Wurdack KJ (2005) Gene transfer from a parasitic flowering plant to a fern. Proc R Soc B 272:2237–2242

Diao X, Freeling M, Lisch D (2006) Horizontal transfer of a plant transposon. PLoS Biol 4:e5

Dumas F, Duckely M, Pelczar P, Van Gelder P, Hohn B (2001) An *Agrobacterium* VirE2 channel for transferred-DNA transport into plant cells. Proc Natl Acad Sci USA 98:485–490

Embley TM, Thomas RH, Williams RAD (1993) Reduced thermophilic bias in the 16S rDNA sequence from *Thermus ruber* provides further support for a relationship between *Thermus* and *Deinococcus*. Syst Appl Microbiol 16:25–29

Farris JS, Kallersjo M, Kluge AG, Bult C (1994) Testing significance of incongruence. Cladistics 10:315–319

Felsenstein J (1978) Cases in which parsimony or compatability methods will be positively misleading. Syst Zool 27:401–410

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791

Ferandon C, Moukha S, Callac P, BenedettoJ-P CM, Barroso G (2010) The *Agaricus bisporus cox1* gene: the longest mitochondrial gene and the largest reservoir of mitochondrial group I introns. PLoS ONE 5:e14048

Filipowicz N, Renner SS (2010) The worldwide holoparasitic *Apodanthaceae* confidently placed in the Cucurbitales by nuclear and mitochondrial gene trees. BMC Evol Biol 10:219

Foster PG, Cox CJ, Embley TM (2009) The primary divisions of life: a phylogenomic approach employing composition-heterogeneous methods. Philos Trans R Soc Lond B 364:2197–2207

Gelvin SB (2009) *Agrobacterium* in the genomics age. Plant Physiol 150:1665–1676

Gilbert C, Schaack S, Pace JK, Brindley PJ, Feschotte C (2010) A role for host-parasite interactions in the horizontal transfer of transposons across phyla. Nature 464:1347–1352

Gogarten JP, Townsend JP (2005) Horizontal gene transfer, genome innovation and evolution. Nat Rev 3:679–687

Goremykin VV, Salamini F, Velasco R, Viola R (2009) Mitochondrial DNA of *Vitis vinifera* and the issue of rampant horizontal gene transfer. Mol Biol Evol 26:99–110

Graham LA, Lougheed SC, Ewart KV, Davies PL (2008) Lateral transfer of a lectin-like antifreeze protein gene in fishes. PLoS One 3:e2616

Grewe F, Viehoever P, Weisshaar B, Knoop V (2009) A trans-splicing group I intron and tRNA-hyperediting in the mitochondrial genome of the lycophyte *Isoetes engelmannii*. Nucleic Acids Res 37: 5093–5104

Hao W (2010) OrgConv: detection of gene conversion using consensus sequences and its application in plant mitochondrial and chloroplast homologs. BMC Bioinformatics 11:114

Hao W, Palmer JD (2009) Fine-scale mergers of chloroplast and mitochondrial genes create functional, transcompartmentally chimeric mitochondrial genes. Proc Natl Acad Sci USA 106:16728–16733

Hao W, Richardson AO, Zheng Y, Palmer JD (2010) Gorgeous mosaic of mitochondrial genes created by horizontal transfer and gene conversion. Proc Natl Acad Sci USA 107:21576–21581

Hecht J, Grewe F, Knoop V (2011) Extreme RNA editing in coding islands and abundant microsatellites in repeat sequences of Selaginella moellendorffii mitochondria: the root of frequent plant mtDNA recombination in early tracheophytes. Genome Biol Evol 3:344–358

Henze K, Martin W (2001) How do mitochondrial genes get into the nucleus? Trends Genet 17: 383–387

Hill T, Nordström KJV, Thollesson M, Säfström TM, Vernersson AKE, Fredriksson R, Schiöth HB (2010) SPRIT: identifying horizontal gene transfer in rooted phylogenetic trees. BMC Evol Biol 10:42

Inda LA, Pimentel M, Chase MW (2010) Contribution of mitochondrial *cox1* intron sequences to the phylogenetics of tribe Orchideae (Orchidaceae): do the distribution and sequence of this intron in orchids also tell us something about its evolution? Taxon 59:1053–1064

Jiang N, Bao Z, Zhang X, Eddy SR, Wessler SR (2004) Pack-MULE transposable elements mediate gene evolution in plants. Nature 431:569–573

Keeling PJ (2009a) Functional and ecological impacts of horizontal gene transfer in eukaryotes. Curr Opin Genet Dev 19:613–619

Keeling PJ (2009b) Role of horizontal gene transfer in the evolution of photosynthetic eukaryotes and their plastids. In: Gogarten MB et al (eds) Horizontal gene transfer: genomes in flux, vol 532, Methods in molecular biology., pp 501–515

Keeling PJ, Palmer JD (2008) Horizontal gene transfer in eukaryotic evolution. Nat Rev Genet 9:605–618

Knoop V, Volkmar U, Hecht J, Grewe F (2010) Mitochondrial genome evolution in the plant lineage. In: Kempken F (ed) Plant mitochondria, vol 1, Advances in plant biology., pp 3–29

Koski LB, Golding B (2001) The closest BLAST hit is often not the nearest neighbor. J Mol Evol 52: 540–542

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

Kurland CG, Canback B, Berg OG (2003) Horizontal gene transfer: a critical view. Proc Natl Acad Sci USA 100:9658–9662

Lambowitz AM, Belfort M (1993) Introns as mobile genetic elements. Annu Rev Biochem 62:587–622

Lambowitz AM, Zimmerly S (2004) Mobile group II introns. Annu Rev Genet 38:1–35

Lander ES, The International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome. Nature 409: 860–921

Lucas WJ, Ham B-K, Kim J-Y (2009) Plasmodesmata – bridging the gap between neighboring plant cells. Trends Cell Biol 19:495–503

Ma L-J, van der Does HC, Borkovich KA, Coleman JJ, Daboussi M-J, Di Pietro A, Dufresne M, Freitag M, Grabherr M, Henrissat B, Houterman PM, Kang S, Shim WB, WoloshukC XX, Xu J-R, Antoniw J, Baker SE, Bluhm BH, Breakspear A, Brown DW, Butchko RAE, Chapman S, Coulson R, Coutinho PM, Danchin EGJ, Diener A, Gale LR, Gardiner DM, Goff S, Hammond-Kosack KE, Hilburn K, Van Hua A, Jonkers W, Kazan K, Kodira CD, Koehrsen M, Kumar L, Lee Y-H, Li L, Manners JM, Miranda-Saavedra D, Mukherjee M, Park G, Park J, Park SY, Proctor RH, Regev A, Ruiz-Roldan MC, Sain D, Sakthikumar S, Sykes S, Schwartz DC, Turgeon BG, Wapinski I, Yoder O, Young S, Zeng Q, Zhou S, Galagan J, Cuomo CA, Kistler HC, Rep M (2010) Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. Nature 464:367–373

Marchetti A, Parker MS, Moccia LP, Lin EO, Arrieta AL, Ribalet F, Murphy MEP, Maldonado MT, Armbrust EB (2009) Ferritin is used for iron storage in bloom-forming marine pennate diatoms. Nature 457(22):467–470

Martin W (2005) Lateral gene transfer and other possibilities. Heredity 94:565–566

Mirkin BG, Fenner TI, Galperin MY, Koonin EV (2003) Algorithms for computing parsimonious evolutionary scenarios for genome evolution, the last universal common ancestor and dominance of horizontal gene transfer in the evolution of prokaryotes. BMC Evol Biol 3:2

Morgante M, Brunner S, Pea G, Fengler K, Zuccolo A, Rafalski A (2005) Gene duplication and exon shuffling by helitron-like transposons generate intraspecies diversity in maize. Nat Genet 37:997–1002

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

Mower JP, Stefanovic S, Hao W, Gummow JS, Jain K, Ahmed D, Palmer JD (2010) Horizontal acquisition of multiple mitochondrial genes from a parasitic plant followed by gene conversion with host mitochondrial genes. BMC Biol 8:150

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 Evol Biol 4:40

Nishida H, Sugiyama J (1995) A common group I intron between a plant parasitic fungus and its host. Mol Biol Evol 12:883–886

Noble GP, Rogers MB, Keeling PJ (2007) Complex distribution of EFL and EF-1alpha proteins in the green algal lineage. BMC Evol Biol 7:82

Odom OW, Shenkenberg DL, Garcia JA, Herrin DL (2004) A horizontally acquired group II intron in the chloroplast *psbA* gene of a psychrophilic *Chlamydomonas*: in vitro self-splicing and genetic evidence for maturase activity. RNA 10:1097–1107

Omer S, Kovacs A, Mazor Y, Gophna U (2010) Integration of a foreign gene into a native complex does not impair fitness in an experimental model of lateral gene transfer. Mol Biol Evol 27:2441–2445

Ragan MA, Beiko RG (2009) Lateral genetic transfer: open issues. Philos Trans R Soc Lond B 364: 2241–2251

Ragan MA, Harlow TJ, Beiko RG (2006) Do different surrogate methods detect lateral genetic transfer events of different relative ages? Trends Microbiol 14:4–8

Richards TA, Soanes DM, Foster PG, Leonard G, Thornton CR, Talbot NJ (2009) Phylogenomic analysis demonstrates a pattern of rare and ancient horizontal gene transfer between plants and fungi. Plant Cell 21:1897–1911

Richardson AO, Palmer JD (2007) Horizontal gene transfer in plants. J Exp Bot 58:1–9

Rogers MB, Watkins RF, Harper JT, Durnford DG, Gray MW, Keeling PJ (2007) A complex and punctate distribution of three eukaryotic genes derived by lateral gene transfer. BMC Evol Biol 7:89

Roney JK, Khatibi PA, Westwood JH (2007) Cross-species translocation of mRNA from host plants into the parasitic plant dodder. Plant Physiol 143:1037–1043

Salzberg SL, White O, Peterson J, Eisen JA (2001) Microbial genes in the human genome: lateral transfer or gene loss? Science 292:1903–1906

Sanchez-Gracia A, Maside X, Charlesworth B (2005) High rate of horizontal transfer of transposable elements in *Drosophila*. Trends Genet 21:200–203

Sanchez-Puerta MV, Cho Y, Mower JP, Alverson AJ, Palmer JD (2008) Frequent, phylogenetically local horizontal transfer of the *cox1* group I intron in flowering plant mitochondria. Mol Biol Evol 25:1762–1777

Sanchez-Puerta MV, Abbona CC, Zhuo S, Tepe EJ, Bohs L, Olmstead RG, Palmer JD (2011) Multiple recent horizontal transfers of the cox1 intron in Solanaceae and extended coconversion of flanking exons. BMC Evol Biol 11:277

Schaack S, Gilbert C, Feschotte C (2010) Promiscuous DNA: horizontal transfer of transposable elements and why it matters for eukaryotic evolution. Trends Ecol Evol 25:537–546

Schönenberger J, Anderberg AA, Sytsma KJ (2005) Molecular phylogenetics and patterns of floral evolution in the ericales. Int J Plant Sci 166:265–288

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. Plant J 44:744–755

Snir S, Trifonov E (2010) A novel technique for detecting putative horizontal gene transfer in the sequence space. J Comput Biol 17:1535–1548

Sorek R, Zhu Y, Creevey CJ, Francino MP, Bork P, Rubin EP (2007) Genome-wide experimental determination of barriers to horizontal gene transfer. Science 318:1449–1452

Stanhope MJ, Lupas A, Italia MJ, Koretke KK, Volker C, Brown JR (2001) Phylogenetic analyses do not support horizontal gene transfers from bacteria to vertebrates. Nature 411:940–944

Stegemann S, Bock R (2009) Exchange of genetic material between cells in plant tissue grafts. Science 324:649–651

Stiller JW (2011) Experimental design and statistical rigor in phylogenomics of horizontal and endosymbiotic gene transfer. BMC Evol Biol 11:259

Ülker B, Li Y, Rosso MG, Logemann E, Somssich IE, Weisshaar B (2008) T-DNA–mediated transfer of *Agrobacterium tumefaciens* chromosomal DNA into plants. Nat Biotechnol 26:1015–1017

Vaughn JC, Mason MT, Sper-Whitis GL, Kuhlman P, Palmer JD (1995) Fungal origin by horizontal transfer of a plant mitochondrial group I intron in the chimeric *coxI* gene of *Peperomia*. J Mol Evol 41: 563–572

Wolf YI, Kondrashov FA, Koonin EV (2001) Footprints of primordial introns on the eukaryotic genome: still no clear traces. Trends Genet 17:499–501

Won H, Renner SS (2003) Horizontal gene transfer from flowering plants to *Gnetum*. Proc Natl Acad Sci USA 100:10824–10829

Yang Z (1994) Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. J Mol Evol 39: 306–314

Yoshida S, Maruyama S, Nozaki H, Shirasu K (2010) Horizontal gene transfer by the parasitic plant *Striga hermonthica*. Science 328:1128