19. Kihm, A. J. *et al.* An abundant erythroid protein that stabilizes free α-haemoglobin. *Nature* **417,** 758–763 (2002).
20. Hughes, T. R. *et al.* Functional discovery via a compendium of expression profiles. *Cell* **102,** 109–126 (2000).
21. Carroll, S. B., Grenier, J. K. & Weatherbee, S. D. *From DNA to Diversity* (Blackwell Science, Malden, Massachusetts, 2001).
22. Hodgkin, J. Fluxes, doses and poisons—molecular perspectives on dominance. *Trends Genet.* **9,** 1–2 (1993).
23. Kratz, E., Dugas, J. C. & Ngai, J. Odorant receptor gene regulation: Implications from genomic organization. *Trends Genet.* **18,** 29–34 (2002).
24. Johnston, M. Feasting, fasting and fermenting. Glucose sensing in yeast and other cells. *Trends Genet.* **15,** 29–33 (1999).
25. Hodges, P. E., McKee, A. H., Davis, B. P., Payne, W. E. & Garrels, J. I. The Yeast Proteome Database (YPD): A model for the organization and presentation of genome-wide functional data. *Nucleic Acids Res.* **27,** 69–73 (1999).
26. Gavin, A. C. *et al.* Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* **415,** 141–147 (2002).
27. von Mering, C. *et al.* Comparative assessment of large-scale data sets of protein–protein interactions. *Nature* **417,** 399–403 (2002).
28. Altschul, S. F. *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25,** 3389–3402 (1997).
29. Eisen, M. B., Spellman, P. T., Brown, P. O. & Botstein, D. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl Acad. Sci. USA* **95,** 14863–14868 (1998).
30. Sokal, R. & Rohlf, M. *Biometry* (Freeman, New York, 1995).

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Widespread horizontal transfer of mitochondrial genes in flowering plants

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Horizontal gene transfer—the exchange of genes across mating barriers—is recognized as a major force in bacterial evolution[1,2]. However, in eukaryotes it is prevalent only in certain phagotrophic protists and limited largely to the ancient acquisition of bacterial genes[3–5]. Although the human genome was initially reported[6] to contain over 100 genes acquired during vertebrate evolution from bacteria, this claim was immediately and repeatedly rebutted[7,8]. Moreover, horizontal transfer is unknown within the evolution of animals, plants and fungi except in the special context of mobile genetic elements[9–12]. Here we show, however, that standard mitochondrial genes, encoding ribosomal and respiratory proteins, are subject to evolutionarily frequent horizontal transfer between distantly related flowering plants. These transfers have created a variety of genomic outcomes, including gene duplication, recapture of genes lost through transfer to the nucleus, and chimaeric, half-monocot, half-dicot genes. These results imply the existence of mechanisms for the delivery of DNA between unrelated plants, indicate that horizontal transfer is also a force in plant nuclear genomes, and are discussed in the contexts of plant molecular phylogeny and genetically modified plants.

We first suspected that there is horizontal transfer of mitochondrial genes by finding three striking distributional anomalies in a survey of mitochondrial gene content in angiosperms[13]. Two ribosomal protein genes, *rps2* and *rps11*, were inferred[13] from blot hybridization data to be absent from mitochondrial DNA of all members of a vast eudicot clade comprising, respectively, 180 and 182 of the 280 angiosperms examined, with the exception of one or two highly derived members of this clade (Fig. 1). Three biological models could account for these anomalies. Two models involve the loss of each gene from mitochondrial DNA early in eudicot evolution and their subsequent re-acquisition by mitochondrial DNA much later (Fig. 1), either, by horizontal gene transfer (HGT) from some unrelated plant or, by vertical transmission, by means of intracellular gene transfer (IGT) from the nucleus of the same plant lineage. A third alternative, that these genes could have been transmitted strictly vertically and exclusively through mitochondrial DNA, would mean extraordinarily frequent and pervasive mitochondrial loss throughout all other eudicot clades in which the three 'special retention' cases shown in Fig. 1 are phylogenetically embedded.

To distinguish between these three possibilities, we analysed levels of sequence divergence and the phylogenetic position of 31 *rps2* and 44 *rps11* genes from a broad array of angiosperms, including the three anomalous plants and their close relatives. All three sets of anomalous genes should, if they are the product of vertical transmission (by the second or third models), group in phylogenetic trees with basal eudicots that never lost these genes from their mitochondrial genomes. Instead, however, *rps2* from *Actinidia* (kiwifruit) groups with monocot *rps2* sequences with high support (Fig. 2a). This placement strongly indicates an HGT event from monocots to eudicots.

The *rps11* genes of *Lonicera* (honeysuckle; Fig. 1a) and other Caprifoliaceae (order Dipsacales) also fail to group in the position expected for vertical transmission, nesting instead within the unrelated order (Ranunculales) with strong support from bayesian analysis and alternative topology tests (see Fig. 2b, Methods and Supplementary Information). Important additional evidence for *rps11* HGT from Ranunculales to Caprifoliaceae comes from a non-coding sequence immediately upstream of *rps11*. The two Caprifoliaceae upstream sequences cluster strongly with the *Berberis* (Ranunculales) sequence in phylogenetic trees to the exclusion of Trochodendraceae (Fig. 2c), the position expected if vertically transmitted.

The phylogenetic position of *rps11* sequences from the third anomalous group, *Betula* (birch; Fig. 1b) and other Betulaceae, is unresolved and is indeed consistent with vertical transmission (Fig. 2b). The phylogenetic evidence for recapture of *rps11* in Betulaceae therefore rests on the phylogenetically anomalous presence of *rps11* in mitochondrial DNA in this family, together with the evidence that both other such anomalies are very likely to reflect gene recapture. Analysis of sequence divergence levels provides important evidence that the putatively recaptured *rps11* gene of Betulaceae is the result of HGT rather than IGT from nucleus to mitochondrion (and further supports a horizontal origin of the *Actinidia rps2* and Capriofoliaceae *rps11* genes). Nuclear substitution rates are far higher than mitochondrial rates in angiosperms[14,15], such that nuclear genes of mitochondrial origin quickly become long branches in mitochondrial gene trees (refs 15 and 16, and Supplementary Fig. 1). Reverse IGT (the second model) therefore predicts a highly divergent mitochondrial *rps11* or *rps2* gene in each plant group. This is clearly not so (Fig. 2a, b, and Supplementary Fig. 1), and thus mitochondrial HGT is the best explanation.

The *rps11* phylogeny serendipitously revealed a fourth, quite remarkable and well-supported case of HGT. Phylogenetic analysis

of full-length *rps11* placed *Sanguinaria canadensis* (bloodroot; Papaveraceae), a basal eudicot, in a basal position of the monocot *rps11* clade with high support (data not shown). On closer examination, *Sanguinaria rps11* turns out to be chimaeric: its 5′ half is of expected eudicot, vertical origin (Fig. 2d), but its 3′ half is indisputably of monocot, horizontal origin (Fig. 2e). A test of recombination[17], using *Bocconia* and *Disporum* to represent Papaveraceae and monocots, respectively, was highly significant ($\chi^2 = 27.5$, $P < 0.0001$) and placed the point of recombination midway in the gene (Fig. 3). Other genera in the Papaveraceae contain only a non-chimaeric, vertically transmitted *rps11* gene (Fig. 2d, e), making this transfer evolutionarily recent.

Finding four cases of HGT for just two mitochondrial genes, each only modestly sampled taxonomically, implies that HGT occurs at an appreciable frequency for plant mitochondrial genes in general. Indeed, perusal of the limited literature on plant mitochondrial phylogenies identified a fifth, strongly supported but previously misinterpreted, case of HGT. *Amborella trichopoda*, the sole extant member of the earliest branch of angiosperm evolution (see, for example, refs 18, 19), was reported by two different groups to contain a mitochondrial *atp1* gene of anomalous phylogenetic placement within eudicots. One study[18] attributed this placement to the *Amborella* gene's 'divergent sequence', implying that it was misplaced as an artefact of long-branch attraction. The other study[19] found an additional mitochondrial *atp1* gene in *Amborella*, of expected basal position; the authors invoked gene duplication, calling the eudicot-like *atp1* gene a 'paralogue' of this basal gene.

We independently isolated the same two *atp1* genes from *Amborella* as those reported in ref. 19, and our phylogenetic analyses strongly support a eudicot placement for one of these genes (Fig. 2f). We are convinced that neither published explanation is correct and that instead the 'misplaced' *atp1* duplicate in *Amborella* is the result of HGT from eudicots. If long-branch attraction were the explanation, then this gene should itself represent a long branch and should group with other long-branched *atp1* genes. This does not happen (Fig. 2f). Moreover, that this gene is more similar to *atp1* genes of eudicots than to *atp1* genes of basal angiosperms also negates long-branch attraction. Paralogy as an explanation fails because, given the basal position of *Amborella* among angiosperms, this implies gene duplication in a common ancestor of angiosperms. If so, the two *Amborella* genes should each branch at the base of two separate clades of angiosperm *atp1* genes, each containing various diverse angiosperms (even allowing differential gene loss), in a pattern recapitulating the generally understood branching pattern of angiosperm phylogeny. This is clearly not so (Fig. 2f), leaving HGT as the only viable explanation.

We examined the expression status of two of the five cases of HGT. Fifteen *rps11* complementary DNAs were sequenced from *Sanguinaria* and each was found to be identical to its chimaeric *rps11* gene except for five sites of partial C → U RNA editing (Table 1). This result establishes that the chimaeric *Sanguinaria* gene is both transcribed and RNA edited, and indicates, in concert with its being an intact open reading frame, that it is probably functional. The horizontally acquired *atp1* gene of *Amborella* is also transcribed and RNA edited (U.B., C. Mathews, and J.D.P., unpublished observations). Roughly half of the genes characterized in the three other HGT cases are intact and therefore merit studies on their expression, whereas half show signs of being pseudogenes. The intact open reading frames include four of five Caprifoliaceae *rps11* genes and one of four Betulaceae *rps11* genes (the non-intact genes each contain a single frameshift mutation of four or five nucleotides). The phylogenetic mixture of both intact and probably

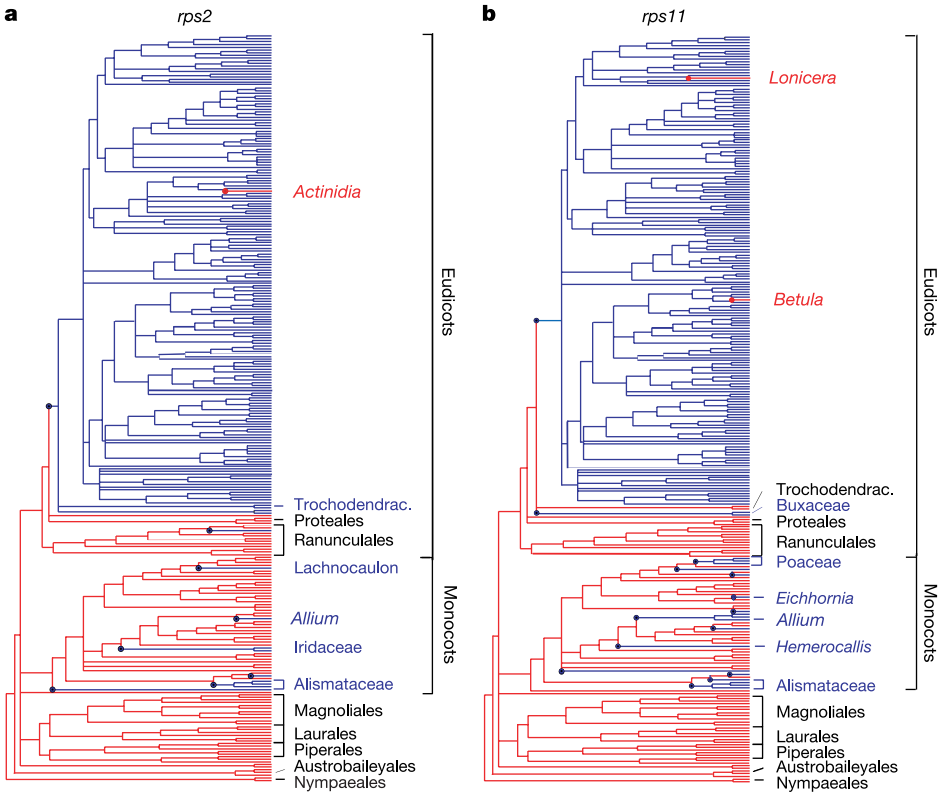

Figure 1 Anomalous presence of ribosomal protein genes in three angiosperm mitochondrial DNAs. A consensus phylogeny of 280 angiosperms is marked according to the presence (red branches) or absence (blue branches) of *rps2* (**a**) and *rps11* (**b**) in mitochondrial DNA (tree topology and gene presence/absence data are from ref. 13). Blue and red bullets mark inferred losses and regains, respectively, of these genes. Names of taxa with gene regain are shown in red lettering, selected taxa with gene loss are in blue, and names of major groups of angiosperms are in black. Trochodendrac., Trochodendraceae.

disabled genes in these two families might mean that these are all non-functional genes, with only some having already been hit by disabling mutations. Alternatively, it might reflect differential usage and fixation of a duplicated gene (relative to a transferred nuclear homologue; see Supplementary Fig. 1), perhaps analogous to the situation reported for the transcompartmental *cox2* gene family created by gene transfer from mitochondrion to nucleus in legumes[20]. Finally, all *Actinidia rps2* genes contain a single NT substitution early in the gene that creates a stop codon, unless remedied by rare U → C RNA editing.

Artefacts of DNA contamination or mislabelled samples, always a concern when invoking HGT, can be ruled out in all five transfer cases because multiple sampling (see Fig. 2b, for example) showed

Table 1 **RNA editing of *Sanguinaria rps11***

Site (nucleotide)	Codon change	Efficiency*
78†	TTC (Phe) → TTT (Phe)	3/15
92†	TCG (Ser) → TTG (Leu)	8/15
143	CCA (Phe) → CTA (Leu)	9/15
146	CCG (Pro) → CTG (Leu)	8/15
351†	TTC (Phe) → TTT (Phe)	7/15

*Efficiency is shown as the fraction of the 15 cDNA clones that have been edited at a particular site; although editing efficiency at any one site did not exceed 60%, 7 of 15 cDNAs were edited at all three sites of non-synonymous editing.
†Sites that are also edited in rice *rps11* (ref. 30); the other two editing changes conserve the amino acids coded at these positions in monocots.

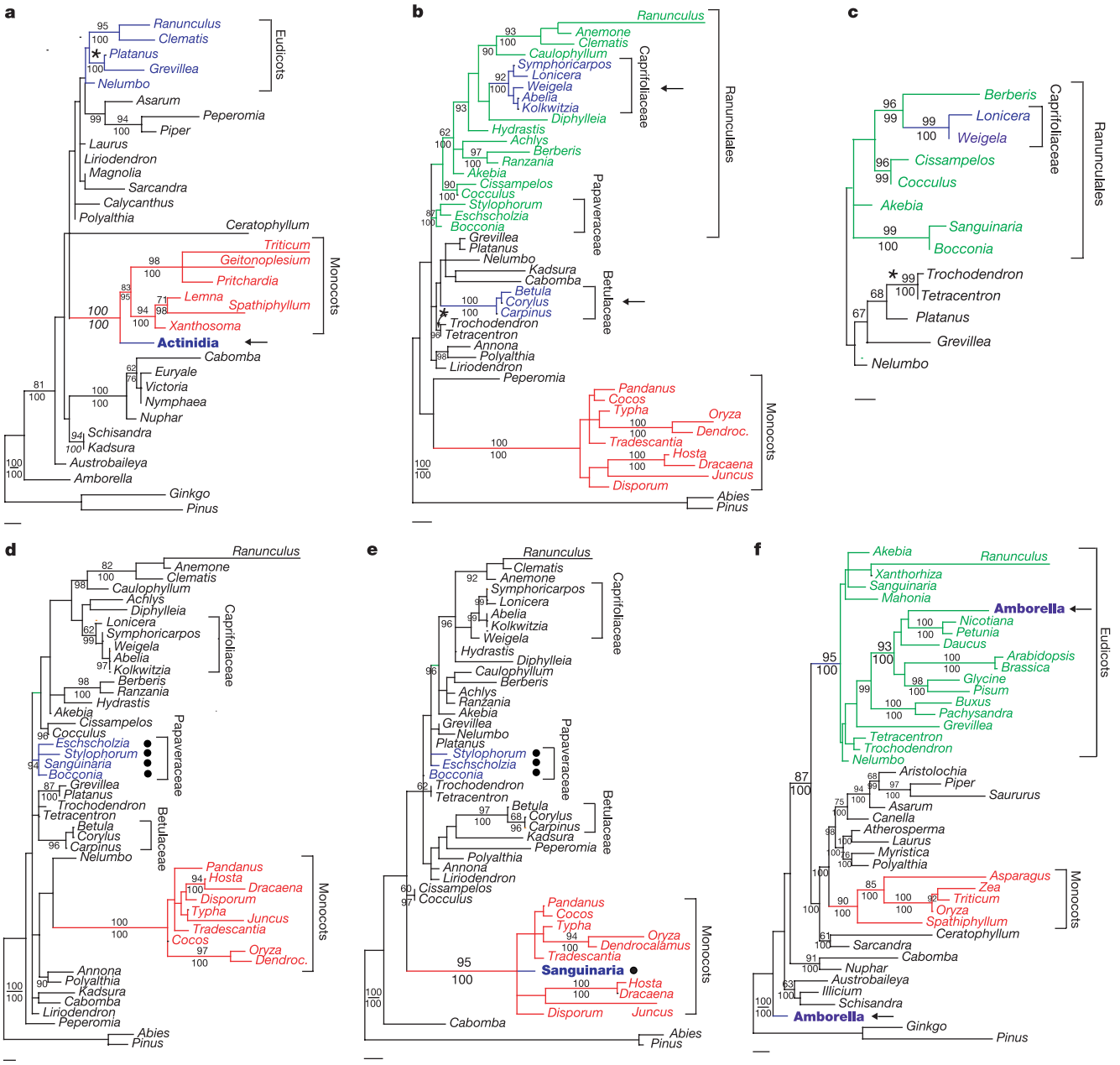

Figure 2 Phylogenetic evidence for HGT in angiosperm mitochondrial DNA. Maximum likelihood trees of *rps2* (474-nucleotide alignment) (**a**), *rps11* (456 nucleotides) (**b**), sequences immediately upstream of *rps11* (457 nucleotides) (**c**), 5′ half of *rps11* (219 nucleotides) (**d**), 3′ half of *rps11* (237 nucleotides) (**e**) and *atp1* (1,254 nucleotides) (**f**). *Dendroc.*, *Dendrocalamus*. Bootstrap support values more than 60% from parsimony analyses are given above nodes, and bayesian posterior probability values more than 90% are given below. All scale bars correspond to 0.01 nucleotide substitutions per site. Asterisks in **a**–**c** indicate the positions of *Actinidia rps2* and Caprifoliaceae and Betulaceae *rps11* expected according to models of vertical transmission.

Figure 3 Chimaeric structure of the *Sanguinaria rps11* gene. Shown are all *rps11* variations in two Papaveraceae (*Bocconia frutescens* (Bf) and *Sanguinaria canadensis* (Sc)) and the monocot *Disporum hookeri* (Dh). Shading marks taxa that have the same nucleotide at a given position, numbered according to its location in *Sanguinaria*. Triangles mark deletions. The six variations (sites 192, 270, and so on) that do not follow the general shaded patterns are uniquely derived among either all 44 *rps11* genes sequenced (positions 192, 344 and 395, see Supplementary Fig. 3), or within the relevant group (Ranunculales for position 270, and monocots for positions 341 and 347; see Supplementary Fig. 3).

the results to be entirely reproducible (see Supplementary Information for details). Evidence that all five transferred genes are located in the mitochondrial genome and were horizontally acquired from mitochondrial rather than nuclear genomes relates to three factors: their lack of divergence (Fig. 2 and Supplementary Fig. 1), their hybridization intensity[13] and their RNA editing (for at least *Sanguinaria rps11* and *Amborella atp1*), all of which are mitochondrion-like (see Supplementary Information for details).

Here we have identified strong evidence for four cases of plant-to-plant horizontal transfer of mitochondrial genes, and weaker evidence for a fifth. Three transfers involve the recapture of a gene lost early during eudicot evolution owing to functional transfer to the nucleus ('recapture HGT'), whereas *Amborella* contains intact genes of vertical and horizontal transmission ('duplicative HGT') and two such genes have recombined in *Sanguinaria* to create a strikingly chimaeric and expressed gene ('chimaeric HGT'). All five cases involve wide HGT within the context of angiosperm evolution (Fig. 4), including two transfers from monocots to eudicots. On the basis of current sampling and molecular-clock-based divergence

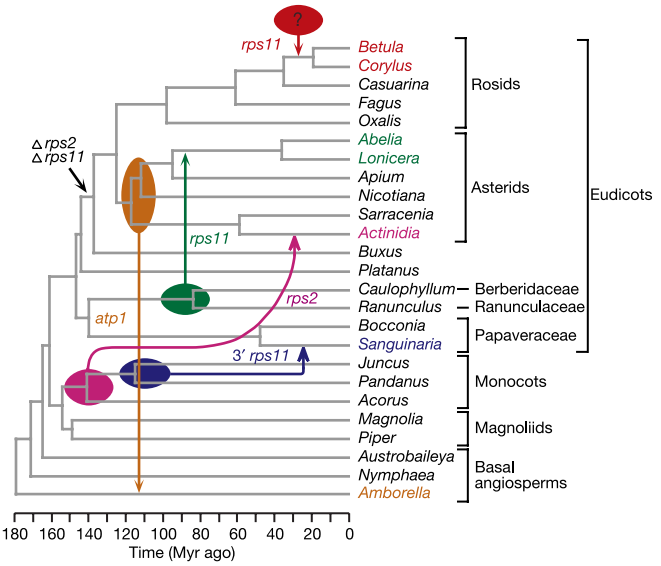

Figure 4 Approximate timing and donor–recipient relationships of five HGT 'events' in angiosperm mitochondrial DNA. Divergence times are from ref. 21. Shadowed ovals indicate rough identity of donor groups (Fig. 2). The exact placement of arrowheads on recipient lineages is arbitrary. If correct, the older ages of donors relative to recipients for the *rps2* and 3′ *rps11* transfers imply the existence of the transferred gene in an intermediate, unidentified vectoring agent or host plant for millions of years, but these discrepancies could easily be due to imprecision in the gene trees (Fig. 2) and/or in molecular-clock-based estimates[21] of divergence times.

times[21], we can very roughly estimate the age(s) of each transfer 'event' (Fig. 4). Further sampling promises to improve the precision of these estimates, to improve the identification of donor and recipient groups and to identify cases of long-term residency of a transferred gene in an intermediate genome, either of a vectoring agent or another plant group (if donors are found to be convincingly older than recipients for any well-dated cases; see Fig. 4 for two potential cases).

These results establish for the first time that conventional genes are subject to evolutionarily frequent HGT during plant evolution and provide the first unambiguous evidence that plants can donate DNA horizontally to other plants (compare refs 12 and 22 on both issues). This is also the best evidence (see also ref. 23) that eukaryotic genomes regularly acquire genes by means of horizontal events that are relatively recent, datable, and definable as to donor and recipient. For several reasons (see Supplementary Information) we believe the five cases reported here are merely the tip of a large iceberg of mitochondrial HGT in plants. Given this and the evolutionarily frequent occurrence of IGT to plant nuclear genomes[13,16,24,25], it seems likely that plant nuclear genomes are also significantly affected by HGT. Indeed, a few cases of horizontal acquisition of bacterial genes by plant nuclear genomes have been reported[23,26]. Despite extensive phylogenetic analysis of chloroplast genes, there is no published evidence for the acquisition of foreign DNA by chloroplasts in any land plant. We therefore predict a much lower incidence of chloroplast HGT than for mitochondrial or nuclear genomes. It is fortunate that the two major sets of genes used to reconstruct plant phylogeny—chloroplast genes and nuclear rRNA genes—seem relatively immune to HGT.

Our findings raise many other questions. Are these results relevant to concerns over the potential escape of transgenes from genetically modified plants by means of HGT? We think not, because although reasonably frequent on an evolutionary time scale of millions of years, HGT is highly unlikely to be a factor on a human time scale. Are certain plants especially susceptible to HGT, as is clearly true for IGT[13]? Does HGT ever occur on a grand scale, leading to the horizontal acquisition of much or all of a mitochondrial genome, and/or of many nuclear genes, as has been seen for IGT[25]? How do genes move from one plant to another, sexually unrelated, plant? Is HGT driven predominantly by potential vectoring agents such as viruses, bacteria, fungi, insects, pollen or even meteorites; or by the transformational uptake of plant DNA released into the soil; or by unrelated plants occasionally grafting together? □

Methods

Gene isolation and characterization

Mitochondrial *rps2*, *rps11* and *atp1* genes were amplified by standard, direct polymerase chain reaction (PCR) in an Idaho Technologies Air Thermocycler. In general, each reaction consisted of 36 cycles of 10 s at 94 °C, 15 s at 50 °C and 45 s at 72 °C. The extension time was 90 s and the annealing temperature was 55 °C for *atp1* amplification and for inverse PCR. A list of PCR primers can be found in Supplementary Information. Sequences flanking *rps2* from *Actinidia arguta* were obtained by inverse PCR: *A. arguta* DNA (2 μg) was digested with either *Apo*I or *Bam*HI plus *Bgl*I (New England Biolabs) and then ligated overnight at 12 °C with T4 ligase (New England Biolabs) in 400 μl. After extraction with chloroform and precipitation with ethanol, the ligated DNA was used for inverse PCR. Primers to conserved sequences immediately upstream of *rps11* in *Lonicera* and *Sanguinaria* (initially amplified by Vectorette PCR; Sigma-Genosys) were used to amplify this region in other species. To verify the authenticity of the *Sanguinaria rps11* sequence, PCR was performed on DNA isolated from three independent sources, and one set of *Sanguinaria* DNA extractions and PCR reactions were done in a different laboratory. *Sanguinaria* RNA was isolated from roots and flower buds using RNeasy Plant Mini Kit (Qiagen) and treated twice with DNAse I (TaKaRa). Reverse transcription was done on 2 μg RNA with random hexamer primers (Invitrogen) and Moloney-murine-leukaemia virus reverse transcriptase (New England Biolabs). Gel-purified RT–PCR products (Qiaquick; Qiagen) of *Sanguinaria rps11* were cloned with TOPO TA Cloning (Invitrogen) in accordance with the manufacturer's directions. Uncloned PCR products and cloned RT–PCR products were sequenced on both strands at the DNA sequencing facility of the Indiana Molecular Biology Institute.

Phylogenetic analyses

Phylogenetic analyses were performed with PAUP v. 4.0b10 (ref. 27) and MrBayes v. 2.01 (ref. 28). Sites of known RNA editing were excluded from the analyses. Maximum-likelihood trees were constructed with a HKY85 substitution model; trees in Fig. 2 used a transition-to-transversion ratio of 2.0. For alternative topology tests (see below for Shimodaira–Hasegawa tests, and Supplementary Information for parametric bootstrap analyses), transition-to-transversion ratios and gamma distribution parameters were first estimated from the data. Bootstrap support values are from maximum-parsimony analyses of 1,000 bootstrap replicates and 100 random addition replicates. Posterior probability for clade support was estimated with Markov chain Monte Carlo as implemented in MrBayes. Four Markov chains were run for 10^5 to 10^6 generations after burn-in, using random initial trees and a general time-reversible (GTR) codon-site-specific substitution model for coding sequences and GTR with gamma distribution for non-coding sequences. The Shimodaira–Hasegawa[29] test favoured the horizontal placements shown in the unconstrained trees of Fig. 2 over alternative topologies based on vertical transmission: first, the unconstrained *rps2* tree (Fig. 2a) was favoured over the constrained tree grouping *Actinidia* with *Grevillea*/*Platanus* as a monophyletic group ($P = 0.012$); second, the unconstrained *rps11* tree (Fig. 2b) was favoured over the tree grouping Caprifoliaceae plus Betulaceae plus Trochodendraceae and/or Proteales ($P = 0.036$); third, the unconstrained 3′ *rps11* tree (Fig. 2e) was favoured over the tree grouping *Sanguinaria* with other Papaveraceae ($P < 0.001$); and last, the unconstrained *atp1* tree (Fig. 2f) was favoured over the tree grouping the two *Amborella* sequences ($P < 0.001$). A test of recombination was performed with Maximum Chi-squared for Macintosh, version 1.0, by N. Ross, which implements the original method by J. Maynard Smith[17].

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1. Ochman, H., Lawrence, J. G. & Groisman, E. A. Lateral gene transfer and the nature of bacterial innovation. *Nature* **405,** 299–304 (2000).
2. Koonin, E. V., Makarova, K. S. & Aravind, L. Horizontal gene transfer in prokaryotes: quantification and classification. *Annu. Rev. Microbiol.* **55,** 709–742 (2001).
3. Nixon, J. E. *et al.* Evidence for lateral transfer of genes encoding ferredoxins, nitro-reductases, NADH oxidase, and alcohol dehydrogenase 3 from anaerobic prokaryotes to *Giardia lamblia* and *Entamoeba histolytica*. *Eukaryotic Cell* **1,** 181–190 (2002).
4. Andersson, J. O., Sjögren, Å. M., Davis, L. A. M., Embley, T. M. & Roger, A. J. Phylogenetic analyses of diplomonad genes reveal frequent lateral gene transfers affecting eukaryotes. *Curr. Biol.* **13,** 94–104 (2003).
5. Archibald, J. M., Rogers, M. B., Toop, M., Ishida, K.-I. & Keeling, P. J. Lateral gene transfer and the evolution of plastid-targeted proteins in the secondary plastid-containing alga, *Bigelowiella natans*. *Proc. Natl Acad. Sci. USA* **100,** 7678–7683 (2003).
6. Lander, E. S. *et al.* Initial sequencing and analysis of the human genome. *Nature* **409,** 860–921 (2001).
7. Stanhope, M. J. *et al.* Phylogenetic analyses do not support horizontal gene transfers from bacteria to vertebrates. *Nature* **411,** 940–944 (2001).
8. Salzberg, S. L., White, O., Peterson, J. & Eisen, J. A. Microbial genes in the human genome: lateral transfer or gene loss? *Science* **292,** 1903–1906 (2001).
9. Robertson, H. M. in *Mobile DNA II* (eds Craig, N. L., Craigie, R., Gellert, M. & Lambowitz, A. M.) 1093–1110 (ASM, Washington DC, 2002).
10. Eickbush, T. H. & Malik, H. S. in *Mobile DNA II* (eds Craig, N. L., Craigie, R., Gellert, M. & Lambowitz, A. M.) 1111–1144 (ASM, Washington DC, 2002).
11. Goddard, M. R. & Burt, A. Recurrent invasion and extinction of a selfish gene. *Proc. Natl Acad. Sci. USA* **96,** 13880–13885 (1999).
12. Cho, Y., Qiu, Y.-L., Kuhlman, P. & Palmer, J. D. Explosive invasion of plant mitochondria by a group I intron. *Proc. Natl Acad. Sci. USA* **95,** 14244–14249 (1998).
13. Adams, K. L., Qiu, Y. L., Stoutemyer, M. & Palmer, J. D. Punctuated evolution of mitochondrial gene content: High and variable rates of mitochondrial gene loss and transfer to the nucleus during angiosperm evolution. *Proc. Natl Acad. Sci. USA* **99,** 9905–9912 (2002).
14. Wolfe, K. H., Li, W. S. & Sharp, P. M. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proc. Natl Acad. Sci. USA* **84,** 9054–9058 (1987).
15. Laroche, J., Li, P., Maggia, L. & Bousquet, J. Molecular evolution of angiosperm mitochondrial introns and exons. *Proc. Natl Acad. Sci. USA* **94,** 5722–5727 (1997).
16. Adams, K. L., Daley, D. O., Qiu, Y. L., Whelan, J. & Palmer, J. D. Repeated, recent and diverse transfers of a mitochondrial gene to the nucleus in flowering plants. *Nature* **408,** 354–357 (2000).
17. Maynard Smith, J. Analyzing the mosaic structure of genes. *J. Mol. Evol.* **34,** 126–129 (1992).
18. Qiu, Y. L. *et al.* The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. *Nature* **402,** 404–407 (1999).
19. Barkman, T. J. *et al.* Independent and combined analyses of sequences from all three genomic compartments converge on the root of flowering plant phylogeny. *Proc. Natl Acad. Sci. USA* **97,** 13166–13171 (2000).
20. Adams, K. L. *et al.* Intracellular gene transfer in action: Dual transcription and multiple silencings of nuclear and mitochondrial *cox2* genes in legumes. *Proc. Natl Acad. Sci. USA* **96,** 13863–13868 (1999).
21. Wikstrom, N., Savolainen, V. & Chase, M. W. Evolution of the angiosperms: Calibrating the family tree. *Proc. R. Soc. Lond. B* **268,** 2211–2220 (2001).
22. Feschotte, C. & Wessler, S. R. Mariner-like transposases are widespread and diverse in flowering plants. *Proc. Natl Acad. Sci. USA* **99,** 280–285 (2002).
23. Intrieri, M. C. & Buiatti, M. The horizontal transfer of *Agrobacterium rhizogenes* genes and the evolution of the genus *Nicotiana*. *Mol. Phylogenet. Evol.* **20,** 100–110 (2001).
24. Blanchard, J. L. & Schmidt, G. W. Pervasive migration of organellar DNA to the nucleus in plants. *J. Mol. Evol.* **41,** 397–406 (1995).
25. Stupar, R. M. *et al.* Complex mtDNA constitutes an approximate 620-kb insertion on *Arabidopsis thaliana* chromosome 2: Implication of potential sequencing errors caused by large-unit repeats. *Proc. Natl Acad. Sci. USA* **98,** 5099–5103 (2001).
26. Zardoya, R., Ding, X. D., Kitagawa, Y. & Chrispeels, M. J. Origin of plant glycerol transporters by horizontal gene transfer and functional recruitment. *Proc. Natl Acad. Sci. USA* **99,** 14893–14896 (2002).
27. Swofford, D. L. PAUP*: Phylogenetic analysis using parsimony (*and other methods) Version 4 (Sinauer, Sunderland, Massachusetts, 1998).
28. Hulsenbeck, J. P. & Ronquist, F. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17,** 754–755 (2001).
29. Shimodaira, H. & Hasegawa, M. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* **16,** 1114–1116 (1999).
30. Kadowaki, K., Kubo, N., Ozawa, K. & Hirai, A. Targeting presequence acquisition after mitochondrial gene transfer to the nucleus occurs by duplication of existing targeting signals. *EMBO J.* **15,** 6652–6666 (1996).

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Topography and synaptic shaping of direction selectivity in primary auditory cortex

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The direction of frequency-modulated (FM) sweeps is an important temporal cue in animal and human communication. FM direction-selective neurons are found in the primary auditory cortex (A1)[1,2], but their topography and the mechanisms underlying their selectivity remain largely unknown. Here we report that in the rat A1, direction selectivity is topographically ordered in parallel with characteristic frequency (CF): low CF neurons preferred upward sweeps, whereas high CF neurons preferred downward sweeps. The asymmetry of 'inhibitory sidebands', suppressive regions flanking the tonal receptive field (TRF) of the spike response, also co-varied with CF. *In vivo* whole-cell recordings showed that the direction selectivity already present in the synaptic inputs was enhanced by cortical synaptic inhibition, which suppressed the synaptic excitation of the non-preferred direction more than that of the preferred. The excitatory and inhibitory synaptic TRFs had identical spectral tuning, but with inhibition delayed relative to excitation. The spectral asymmetry of the synaptic TRFs co-varied with CF, as had direction selectivity and sideband asymmetry, and thus suggested a synaptic mechanism for the shaping of FM direction selectivity and its topographic ordering.

Extracellular multiunit spike responses to sweeps of various speeds and intensities were recorded in the mid-layers of the adult rat A1. Responses from a representative low CF site are shown in Fig. 1. Sweeps of different speeds evoked distinct responses (Fig. 1a). The onset and duration of each response mostly reflected the timing of the sweep's intersection with the TRF of the spike response (Fig. 1b). A direction selectivity index (DSI) was calculated for