Horizontal transfer of chloroplast genomes between plant species

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The genomes of DNA-containing cell organelles (mitochondria, chloroplasts) can be laterally transmitted between organisms, a process known as organelle capture. Organelle capture often occurs in the absence of detectable nuclear introgression, and the capture mechanism is unknown. Here, we have considered horizontal genome transfer across natural grafts as a mechanism underlying chloroplast capture in plants. By grafting sexually incompatible species, we show that complete chloroplast genomes can travel across the graft junction from one species into another. We demonstrate that, consistent with reported phylogenetic evidence, replacement of the resident plastid genome by the alien genome occurs in the absence of intergenomic recombination. Our results provide a plausible mechanism for organelle capture in plants and suggest natural grafting as a path for horizontal gene and genome transfer between sexually incompatible species.

grafting | horizontal gene transfer | plastid transformation | tobacco | lateral gene transfer

Organelle capture occurs in diverse phylogenetic groups, ranging from mitochondrial capture by transmissible cancers of animals (1) to chloroplast capture by sexually incompatible plant species (2–4). Chloroplast capture, the apparent introgression of a chloroplast (plastid) genome from one plant species into another, is commonly suggested as an explanation for inconsistencies between phylogenetic trees based on nuclear and cytoplasmic marker sequences (2–5). This is because, in many of these instances of conflicting gene trees, the position of species on the chloroplast tree is much closer than their position on the nuclear tree. A most remarkable outcome of chloroplast capture is that plastid genotypes are often associated with geographic locations rather than with taxonomic relationships (4). Occasional accidental hybridization between otherwise sexually incompatible species is regarded as the mechanism resulting in the exchange of chloroplast genomes (i.e., chloroplast capture) (2, 3, 5). A puzzling problem with this explanation is that traces of introgression (i.e., DNA sequences from the species that donated the chloroplast genome) are often not found in the nuclear genome of the recipient (3). To resolve this conundrum, we have considered asexual modes of chloroplast genome transfer between species.

Horizontal gene transfer (HGT) (sometimes also referred to as lateral gene transfer) is defined as the movement of genetic material between organisms other than by descent. Initially thought to be largely restricted to prokaryotes, it is now increasingly being appreciated as a significant force also in the evolution of eukaryotic genomes (6–8). Most well-documented examples of plant-to-plant HGT concern the exchange of mitochondrial genes between species (9–12). The high propensity of mitochondrial genomes to engage in HGT could be related to two peculiar biological features of plant mitochondria: (*i*) they possess an active homologous recombination system; and (*ii*) they readily undergo organelle fusion, with many mitochondria being physically connected and forming network-like structures (13, 14). Accumulating evidence suggests that mitochondrial HGT is particularly prevalent between plants that are intimately associated or at least occasionally establish cell-to-cell contacts, for example, by engaging in mutualistic or parasitic relationships (11, 15–18).

Here, we have considered the possibility that chloroplast capture represents a form of HGT. Unlike plant mitochondria, plastids do not normally fuse and recombine (for a rare exception, see, e.g., Ref. 19). Consequently, the outcome of plastid HGT would not be transfer of individual genes but rather transfer of the entire genome. Importantly, the result of such a horizontal genome transfer would be indistinguishable from and equivalent to what has been described as chloroplast capture. Because chloroplast capture often occurs in the absence of any detectable nuclear introgression (reviewed in Ref. 3), horizontal transfer of plastid genomes could provide a much simpler mechanistic explanation for chloroplast capture than interspecific hybrid formation (2, 3, 5).

We have recently shown that plastid DNA can be transferred between cells in plant tissue grafts (20). In addition to being widely used by humans in organ transplantation, agriculture and horticulture, grafting is also very common in nature. Natural grafting occurs at sites where two plant stems or roots contact each other (Fig. 1*A*) (21). To test whether or not grafting permits the horizontal transfer of plastid genomes between sexually incompatible species and, thus, provides an avenue for chloroplast capture, we attempted to observe, in real time, the horizontal transfer of chloroplasts from the cultivated tobacco, *Nicotiana tabacum*, into two other species: the tree tobacco, *N. glauca*, a woody species; and the herbaceous species *N. benthamiana* (Fig. 1 *B* and *C*).

Results

Selection System for Chloroplast Capture. We suspected horizontal transfer of plastid DNA (20) to be equivalent to chloroplast capture. Therefore, we sought to test whether horizontal transfer of plastid genomes occurs in grafts between sexually incompatible species. To facilitate identification of such events through selection in laboratory experiments, we generated nuclear-transgenic plants of *N. glauca* and *N. benthamiana* by Agrobacterium-mediated transformation with a construct containing the kanamycin-resistance gene, *nptII*, and the gene for the yellow fluorescent protein, *yfp* (subsequently referred to as KY lines, for lines expressing kanamycin resistance and YFP). These plants were reciprocally grafted onto chloroplast-transformed (transplastomic) *N. tabacum* plants that carry the spectinomycin-resistance gene, *aadA*, and the gene for the green fluorescent protein, *gfp*, in their plastid genome [subsequently referred to as SG lines to indicate expression of spectinomycin resistance and GFP (20)]. After fusion of scion and stock had occurred (Fig. 1*D*), the excised graft sites were exposed to double selection for kanamycin and spectinomycin resistance (Fig. 1 *E* and *F*). Double selection for both antibiotics suppresses regeneration from cells of each of the two grafting partners but would permit division and regeneration of *N. glauca* or *N. benthamiana* cells that have acquired plastid DNA from *N. tabacum* and, thus,

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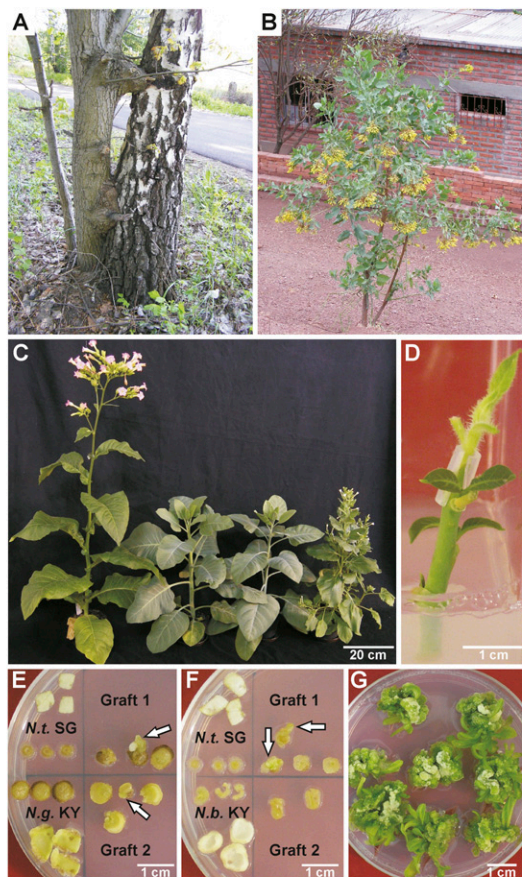

Fig. 1. Grafting and experimental selection for horizontal transfer of chloroplast genomes between different species. (*A*) Natural graft between an oak (*Left*) and a birch (*Right*) in a forest between Potsdam-Golm and Wildpark-West, Germany. The two trees are fused at two different sites. (*B* and *C*) Species used for experimental reconstruction of chloroplast capture by grafting. (*B*) A young tree of *N. glauca*, the tree tobacco, growing in San Francisco, a village in the northern Argentinian Andes. (*C*) Nine-week-old plants of *N. tabacum* (*Left*), *N. glauca* (two plants in the middle), and *N. benthamiana* (*Right*). Note that the herbaceous species *N. tabacum* and *N. benthamiana* flower after 2 mo, whereas the tree tobacco *N. glauca* is still in its early vegetative growth. (*D*) Graft of *N. tabacum* (scion) onto *N. glauca* (stock) growing under aseptic conditions. The silicon sleeve holds scion and stock together before tissue fusion. (*E* and *F*) Selection of putative chloroplast capture lines by exposing stem sections from the graft site to double selection for spectinomycin resistance and kanamycin resistance. Whereas cell division in leaf explants and stem sections from the two grafting partners is fully suppressed (left part of the Petri dish), explants from graft sites frequently give rise to growing calli that are resistant to both antibiotics (right part of the Petri dish; arrows). (*E*) Selection from a *N. tabacum* (*N.t.*)/*N. glauca* (*N.g.*) graft. (*F*) Selection from a *N. tabacum* (*N.t.*)/*N. benthamiana* (*N. b.*) graft. (*G*) Plant regeneration from a putative chloroplast capture line selected from a *N. tabacum*/*N. glauca* graft.

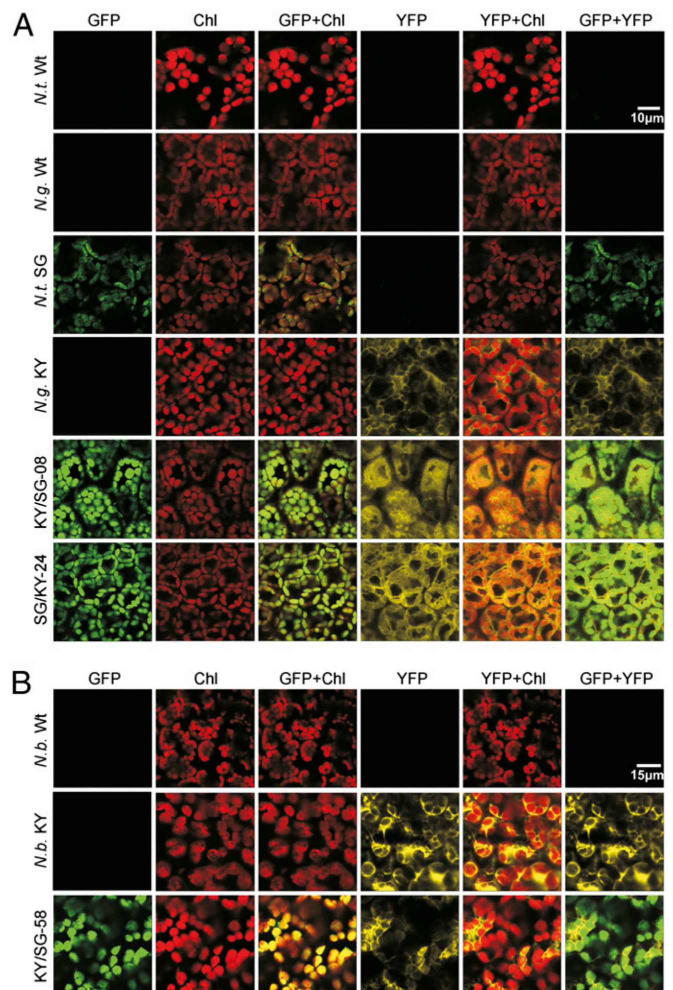

Fig. 2. Expression of both fluorescent reporter proteins in the same cell after horizontal chloroplast DNA transfer from *N. tabacum*. GFP fluorescence, YFP fluorescence, and chlorophyll (Chl) fluorescence and the three pairwise overlays are shown for both wild types, the two grafting partners, and putative chloroplast capture lines. (*A*) Analysis of two independent lines obtained from *N. tabacum* (*N.t.*)/*N. glauca* (*N.g.*) grafts. Line KY/SG-08 was selected from a graft with *N. glauca* as scion and *N. tabacum* as stock, line SG/KY-24 was selected from a reciprocal graft. In both selected lines, GFP-expressing chloroplasts reside in *N. glauca* cells that accumulate YFP in the cytosol and the nucleus. (*B*) Analysis of a putative chloroplast capture line obtained from an *N. tabacum*/*N. benthamiana* (*N.b.*) graft.

harbor both resistance genes (*nptII* in the nuclear genome and *aadA* in the plastid genome). The appearance of such doubly resistant calli was indeed observed at relatively high frequency: 8 independent lines were obtained from 16 grafts between *N. tabacum* and *N. glauca*, and 8 lines from 19 grafts between *N. tabacum* and *N. benthamiana*. Callus tissue samples were then taken and regenerated into plants under continued selection for both antibiotics (Fig. 1*G*).

Verification of Horizontal Transfer of Plastid DNA Between Species. To test whether doubly resistant plantlets indeed represent horizontal DNA transfer events, we analyzed the expression of the two fluorescent reporter proteins by confocal laser-scanning microscopy (Fig. 2). Indeed, the selected lines showed both YFP accumulation in the nucleocytosolic compartment and GFP accumulation in their chloroplasts, strongly suggesting that transfer of genetic information between species had occurred.

To confirm that the selected lines carry both the two transgenes from the *N. tabacum* plastid genome (*aadA* and *gfp*) and the two transgenes from the *N. glauca* or *N. benthamiana* nuclear genome (*nptII* and *yfp*), we performed a set of PCR analyses. We assayed three independently selected putative chloroplast capture lines from *N. tabacum*/*N. glauca* grafts and three independent lines selected from *N. tabacum*/*N. benthamiana* grafts. The data revealed that all six lines indeed harbored all four transgenes (Fig. 3 *A* and *B*), confirming that chloroplast DNA transfer between species had indeed occurred and suggesting that the transgenic chloroplast genomes from *N. tabacum* (expressing GFP and the spectinomycin resistance) had moved into *N. glauca* and *N. benthamiana*, respectively.

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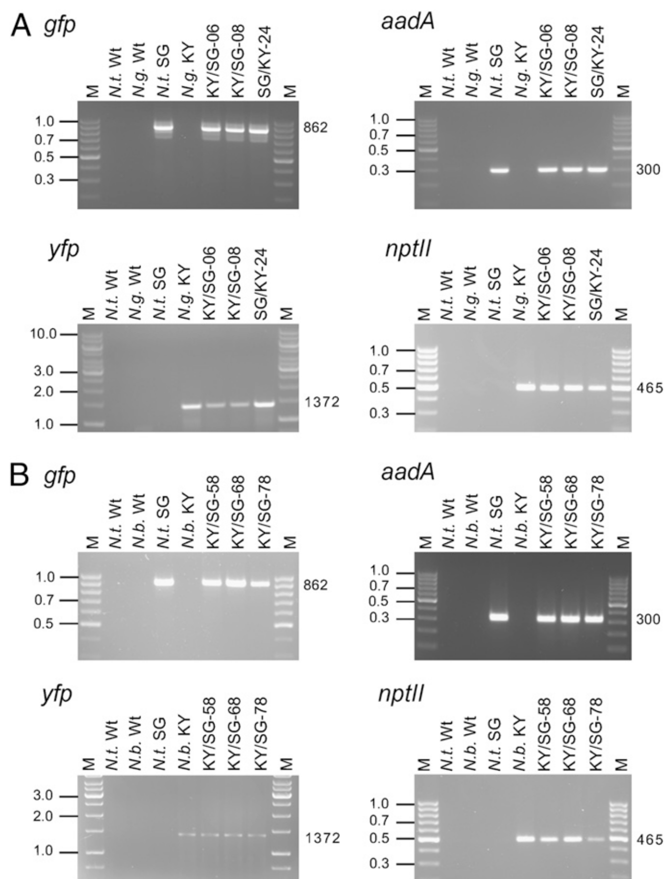

Fig. 3. Detection of the presence of all four transgenes after grafting-mediated chloroplast capture by PCR. (*A*) Analysis of three independent chloroplast capture lines obtained from *N. tabacum* (*N.t.*)/*N. glauca* (*N.g.*) grafts. Presence of the *aadA*, *gfp*, *nptII*, and *yfp* transgenes was tested by PCR assays with gene-specific primer pairs. The two wild types (Wt) and the two grafting partners were included as negative and positive controls, respectively. M, DNA size marker (sizes given in kilobases). (*B*) Analysis of three independent chloroplast capture lines obtained from *N. tabacum* (*N.t.*)/*N. benthamiana* (*N.b.*) grafts. Expected sizes of PCR products are indicated at the right of each gel in base pairs. M, DNA size marker (fragment sizes given at the left of the gel in kilobases).

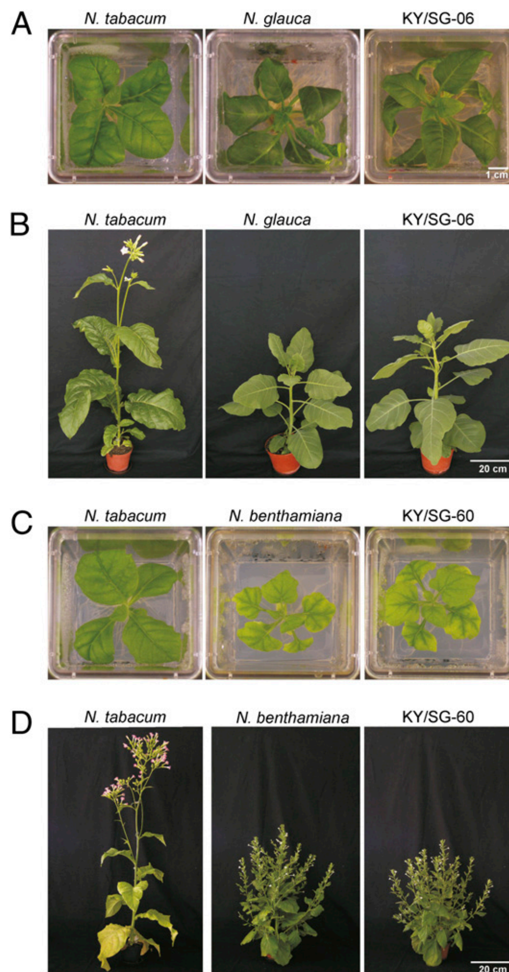

Fig. 4. Morphology of chloroplast capture plants. (*A* and *B*) The phenotype of a chloroplast capture plant selected from a graft between *N. tabacum* and *N. glauca* (KY/SG-06) is compared with a *N. tabacum* plant and a *N. glauca* plant of similar age. The *glauca* phenotype of the chloroplast capture plant is already visible early during growth under aseptic conditions on synthetic medium (*A*) and becomes even more apparent after transfer to soil and growth under greenhouse conditions (*B*). (*C* and *D*) Phenotypic comparison of a chloroplast capture plant selected from a graft between *N. tabacum* and *N. benthamiana* (KY/SG-60), a *N. tabacum* plant and a *N. benthamiana* plant. The *benthamiana* phenotype of the chloroplast capture plant is clearly visible both in in vitro culture (*C*) and upon growth in soil under greenhouse conditions (*D*).

Phenotypes of Putative Chloroplast Capture Lines. To analyze the morphology of the selected HGT lines and compare it with that of the two species they originated from by grafting, regenerated plants were transferred to soil and grown under greenhouse conditions. Interestingly, all lines were phenotypically indistinguishable from the nuclear-transgenic grafting partner (*N. glauca* or *N. benthamiana*) and showed no signs of intermediate morphology with *N. tabacum* (Fig. 4 *A–D*). This suggests that the interspecific transfer of genetic information is largely restricted to chloroplast DNA movement and does not involve the movement of nuclear genome pieces.

Importantly, the horizontal transfer of chloroplast DNA was independent of the orientation of the graft: four KY/SG lines and four SG/KY lines were obtained from reciprocal grafts of *N. glauca* with *N. tabacum* (Fig. 2), and seven KY/SG lines and one SG/KY line were obtained from reciprocal grafts of *N. benthamiana* with *N. tabacum*. This demonstrates that the travel of plastid genetic material across the graft junction has no directionality.

Lack of Intergenomic Recombination upon Horizontal Genome Transfer. Next, we wanted to know whether the transfer of plastid genetic information between species involves recombinational exchange of DNA pieces between the chloroplast genomes of the donor and the recipient species or, alternatively, occurs at the level of entire plastid genomes. We, therefore, sequenced the two polymorphic regions that are most distant from the *aadA* and *gfp* transgenes in the circular plastid genome: the *rpoB*/*psbM* region and the *petA*/*petL* region. For each region, ~3 kb of DNA sequence were obtained for all three species (Figs. S1 and S2). Sequence comparison revealed a large number of polymorphisms (SNPs, as well as insertions/deletions) between species (in total, 67 deviating nucleotide positions between *N. glauca* and *N. tabacum* and 100 deviating positions between *N. benthamiana* and *N. tabacum*; Figs. S1 and S2). We then sequenced these regions from three chloroplast capture lines obtained from *N. tabacum*/*N. glauca* grafts and three independent lines selected from *N. tabacum*/*N. benthamiana* grafts. Interestingly, the sequences of all six putative chloroplast capture lines were 100% identical to the sequence of *N. tabacum*, with not a single *N. glauca* or *N. benthamiana* polymorphism being present in more than 36 kb of DNA sequence. This strongly

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indicated that no recombination between the plastid genomes of the grafting partners had occurred and suggested that entire plastid genomes are horizontally transferred between the species.

To ultimately confirm that complete plastid genomes are exchanged upon grafting, we obtained full plastid genome sequences from altogether seven plants: *N. glauca*, *N. benthamiana*, the transplastomic *N. tabacum* line, two independently selected putative chloroplast capture lines obtained from *N. tabacum*/*N. glauca* grafts, and two independent lines selected from *N. tabacum*/*N. benthamiana* grafts. Using next-generation sequencing, complete draft genomes could be assembled for the plastid DNAs of all seven plant lines. Comparison of the plastid genomes of the three species (*SI Appendix*, Datasets 1 and 2) revealed a high level of overall sequence homology, as expected. Both the *N. glauca* and the *N. benthamiana* plastid genomes display 99.7% overall sequence identity with the *N. tabacum* plastid genome. Sequence alignments revealed that the plastid genomes of all four chloroplast capture lines analyzed were completely identical with the plastid genome of the transplastomic *N. tabacum* line SG (*SI Appendix*, Datasets 1 and 2), confirming that no recombination between the chloroplast DNAs of the two grafting partners had occurred.

Lack of Mitochondrial Genome Transfer. The next-generation sequencing data obtained for the seven lines also allowed us to extract information on the mitochondrial genomes in the three species, as well as the four chloroplast capture lines. Although the sequence coverage was not high enough to allow assembly of complete draft genomes [because of the large size of the nuclear genome in tobacco and the much lower copy number of the mitochondrial genome compared with the plastid genome (22)], three large contigs homologous in all three species and covering more than 42 kb of mitochondrial DNA per species could be assembled and were analyzed in detail (*SI Appendix*, Datasets 3 and 4). Sequence comparison of these contigs between the three species revealed a slightly higher homology of the mitochondrial sequences (99.8% between *N. benthamiana* and *N. tabacum*; 99.7% between *N. glauca* and *N. tabacum*) compared with the plastid sequences, which is in line with the lower mutation rate in the mitochondrial genome (23). Analysis of the three mitochondrial contigs in the four chloroplast capture lines showed that the mitochondrial genomes of *N. glauca* and *N. benthamiana* were not replaced by mitochondrial DNA of *N. tabacum* (*SI Appendix*, Datasets 3 and 4), indicating that genome transfer in these lines was restricted to the plastid genome. This might have been expected, because our selection system was specifically designed to identify plastid DNA transfer events and does not allow selection for mitochondrial genome transfer.

Genetic Stability of the Horizontally Transferred Plastid DNA. Finally, we wanted to confirm that the horizontally captured chloroplast genomes are stably transmitted into the next generation. To this end, we grew plants to maturity, harvested seeds, and germinated them on medium containing spectinomycin, kanamycin, or both antibiotics (Fig. S3). The progeny from chloroplast capture lines was uniformly resistant to spectinomycin [because of maternal inheritance of the plastid genome (24)] and showed the expected Mendelian segregation for the nuclear-encoded kanamycin resistance (Fig. S3). These analyses demonstrated that the *N. tabacum* plastid genome captured by *N. glauca* or *N. benthamiana* is indeed stably inherited across generations and, moreover, confirmed that the transplastomic plastid genome had completely replaced the resident plastid genome.

Discussion

Our findings reported here demonstrate that simple grafting allows genes and entire organellar genomes to cross species barriers. Considering that natural grafting is common in plants (Fig. 1*A*) (21) and that grafting also can occur naturally in animals (including mammals) (25), this suggests an intriguing path for the horizontal transfer of genetic information between sexually incompatible species. We propose that natural grafting may also account for at least some of the recently reported frequent horizontal transfer of plant mitochondrial DNA (9, 10, 16), especially in all those cases that obviously do not involve parasitic interactions between plants (8). Whereas chloroplasts do not normally undergo recombination, plant mitochondria recombine very frequently. Therefore, the outcome of horizontal transfer is different between the two DNA-containing organelles: whole-genome transfer in chloroplasts versus recombined mosaic genomes in mitochondria. Although HGT via grafting initiates as a somatic process, the genetic changes can subsequently become heritable by lateral shoot formation from the graft site, which is not uncommon. Our finding that grafting allows plastid genomes to cross species boundaries, therefore, also provides a plausible explanation for chloroplast capture (2–4), a curious evolutionary phenomenon that so far has eluded a satisfactory mechanistic explanation. It is important to note that this type of horizontal transfer of plastid genomes is likely restricted to relatively closely related species. It is well established that the transfer of organelles between species can result in nucleocytoplasmic incompatibilities (26–28). These incompatibilities represent natural barriers to chloroplast capture and probably prevent the horizontal transfer of plastid genomes between many (especially distantly related) species.

An intriguing question is how entire plastids and/or their genomes pass between cells across a graft junction. The cellular events leading to establishment of a graft union include cell wall thinning (through induced enzymatic degradation) and de novo formation of plasmodesmatal connections between opposing stock and scion cells (29). Thus, two mechanisms of plastid transfer across a graft junction seem possible: transfer through plasmodesmata or intercellular transfer of small amounts of cytoplasm following local enzymatic removal of the cell wall separating opposing stock and scion cells. Plasmodesmata are normally far too narrow to allow the passage of large DNA molecules or even entire organelles. However, it is known that under certain conditions, plasmodesmata can be widened and permit the intercellular transfer of large macromolecular complexes, such as entire virus particles (30, 31). Whether or not de novo formation of plasmodesmata at the graft union involves a phase, in which the plasmatic connections between stock and scion cells have a sufficiently large diameter to allow the passage of entire plastids (e.g., in the form of small proplastids) remains to be investigated.

Inconsistencies between phylogenetic trees based on nuclear and chloroplast marker sequences (2–5) represent a persistent problem in molecular phylogeny and have remained difficult to reconcile in many cases. Our finding that chloroplast genomes can be readily transferred between species by natural grafting also provides a possible explanation for why chloroplast sequences, although favored for phylogenetic analyses and DNA barcoding by many researchers (32), frequently provide trees that disagree with canonical phylogeny and/or trees constructed with nuclear markers. Finally, because the genotype of the cytoplasmic organelles can contribute substantially to plant fitness (28, 33), the possibility to transfer chloroplast genomes between sexually incompatible species opens possibilities in plant breeding and also facilitates genetic-engineering approaches in species that are recalcitrant to plastid genome transformation.

Materials and Methods

Plant Material and Generation of Transgenic Plants. Three tobacco species (*N. tabacum* cv. Petit Havana, *N. glauca* cv. Canary Islands, and *N. benthamiana* cv. TW16/NSCU) were used in this study. Test crosses between plants grown under standard greenhouse conditions confirmed that the three species are sexually incompatible. For transformation and grafting experiments, plants were grown under aseptic conditions (from surface-sterilized seeds) on agar-solidified synthetic medium containing 30 g/L sucrose (34). The DNA constructs for nuclear transformation and the generation of transplastomic *N. tabacum* plants have

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been described previously (20). Nuclear transformation of *N. glauca* and *N. benthamiana* was performed by *Agrobacterium tumefaciens*-mediated transformation and selection on kanamycin-containing plant regeneration medium using standard protocols for Solanaceous plants.

Grafting and Selection for Intercellular Gene Transfer. Transplastomic *N. tabacum* plants and transgenic *N. glauca* and *N. benthamiana* plants were raised on synthetic medium under aseptic conditions (34) to exclude possible influences of pathogens or endophytic microbes. Grafting experiments were performed reciprocally by transplanting transplastomic scions onto transgenic stocks and vice versa. Successful grafting was evidenced by establishment of a physical connection between scion and stock and continued growth of the scion. The graft site was then excised and subjected to double selection for kanamycin and spectinomycin resistance as described previously (20). Doubly resistant shoots and calli were transferred to fresh medium and regenerated again under antibiotic selection, to eliminate possible cross-protected cells. Finally, regenerated shoots were rooted on phytohormone-free culture medium (34), followed by transfer to soil and growth to maturity under standard greenhouse conditions.

Isolation of Nucleic acids, PCRs, and DNA Sequencing. Total plant DNAs were isolated from fresh tissue by a cetyltrimethylammonium bromide (CTAB)-based method (35). DNA samples were amplified in an Eppendorf thermal cycler using GoTaq Flexi DNA Polymerase (Promega) and gene-specific primer pairs. The standard PCR program was 30–40 cycles of 1 min at 94 °C, 40 s at 56–58 °C, and 1–2 min at 72 °C with a 5 min extension of the first cycle at 94 °C and a 5 min final extension at 72 °C. To detect the selectable marker genes and reporter genes, the following synthetic oligonucleotides were used as primers: *aadA*: PaadA forward, 5′-CGCCGAAGTATCGACTCA-3′; PaadA reverse, 5′-TCGCGCTTAGCTGGATAAC-3′; *nptII*: Pnpt forward, 5′-GAGGCAGCGCGGCTATC-3′; Pnpt reverse, 5′-GCGGTCCGCCACACCCA-3′; *gfp*: Pgfp forward, 5′-AAAGAGCTCGCTCCCCCGCCGTCG-3′; Pgfp reverse, 5′-TTTTCTAGATTAGTTCATCCATGCCAT-3′; and *yfp*: P35S forward, 5′-GACCAAAGGGCTATTGAGAC-3′; P35S reverse, 5′-CGGGGGATCTGGATTTTAGTAC-3′. For amplification and DNA sequencing of the *rpoB*/*psbM* region of the plastid genomes of the three tobacco species and the chloroplast capture lines, the following primers were used: PrpoB forward, 5′-CAGGTATTGTAGATATTCCCTC-3′; PtrnC reverse, 5′-AGGCGACTCCCGGATTTGAAC-3′; PtrnC forward, 5′-GAGTGGTAAGGCAGAGGAC-3′; Pycf6 reverse, 5′-CTAGAGTCCACTTCTTCCCC-3′; Pycf6 forward 5′-ATAGTAAGTCTTGCTTGGGC-3′; and PpsbM reverse, 5′-AAACAGTCAGTCAAAACGATTAA-3′. For amplification and DNA sequencing of the *petA*/*petL* region, the following primers were used: PpetA forward, 5′-CAATTGGCCGAAATGAATTTCTA-3′; PpsbJ reverse, 5′-ATGGCCGATACTACTGGAAG-3′; PpsbJ3 reverse, 5′-CATTACTCTTTCGTTTCGACAC-3′; PpsbJ forward, 5′-TAGAGGGATGAACCCAATCC-3′; PpsbE reverse, 5′-ATGTCTGGAAGCACAGGAGA-3′; PpsbE forward, 5′-GTAATGCTATGAATGACCCAGT-3′ and PpetL reverse, 5′-GCCGCTAATAGAAAACCGAAATA-3′.

Complete plastid genomes and mitochondrial DNA contigs were assembled from next-generation sequencing data obtained with the Illumina HiSeq2000 platform using chemistry v3 and total plant DNA for the library construction. Between 5.75 and 7.92 Gbp of sequence information were obtained for each plant line sequenced (single reads; average read length: 100 bp; Eurofins MWG Operon). Contigs were assembled using the SeqMan NGen 3.1.1 software package (DNASTAR). Sequence evaluation and construction of alignments were performed with the Lasergene Core Suite 9.1.1 (DNASTAR).

Confocal Laser-Scanning Microscopy. Subcellular localization of GFP fluorescence, YFP fluorescence, and chlorophyll fluorescence was determined by confocal laser-scanning microscopy (TCS SP2; Leica) using an argon laser for excitation (at 488 nm) and a 500–510-nm filter for detection of GFP fluorescence, a 514–527-nm filter for detection of YFP fluorescence, and a 610–700-nm filter for detection of chlorophyll fluorescence.

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