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Living with Two Genomes: Grafting and Its Implications for Plant Genome-to-Genome Interactions, Phenotypic Variation, and Evolution

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Abstract

Plant genomes interact when genetically distinct individuals join, or are joined, together. Individuals can fuse in three contexts: artificial grafts, natural grafts, and host–parasite interactions. Artificial grafts have been studied for decades and are important platforms for studying the movement of RNA, DNA, and protein. Yet several mysteries about artificial grafts remain, including the factors that contribute to graft incompatibility, the prevalence of genetic and epigenetic modifications caused by exchanges between graft partners, and the long-term effects of these modifications on phenotype. Host–parasite interactions also lead to the exchange of materials, and RNA exchange actively contributes to an ongoing arms race between parasite virulence and host resistance. Little is known about natural grafts except that they can be frequent and may provide opportunities for evolutionary innovation through genome exchange. In this review, we survey our current

understanding about these three mechanisms of contact, the genomic interactions that result, and the potential evolutionary implications.

INTRODUCTION

What happens when two genomes come in contact? These close encounters of the genomic kind have been widely studied for some classes of organisms. In bacteria, for example, the proximity of two different species—or two different individuals—can lead to the exchange of plasmids or to horizontal gene transfer (HGT). Similarly, viruses interact with the genome of their eukaryotic host, typically integrating into the host genome. The remnants of these integrations eventually became the transposable elements that now dominate eukaryotic genome content.

This is well and good for bacteria and viruses, but what about exchange between eukaryotic genomes? This is a more complex and less studied category of interaction, but the outcomes of these interactions have had enormous effects across the tree of life. One prominent example is secondary endosymbiosis, which refers to the phagocytosis of one plastid-containing eukaryote by another eukaryote. By engulfing a plastid-containing cell, the phagocytosing cell gained the ability to photosynthesize, conferring a huge advantage within competitive, resource-limited environments. These secondary endosymbiotic events have occurred on at least four occasions and are likely responsible for the widespread evolutionary success of several eukaryotic lineages (47). An interesting facet of secondary endosymbioses is that interactions continue to occur between the eukaryotic genome and the engulfed plastid genome because they exchange genes (8).

These brief examples show that close genomic encounters have had profound evolutionary effects. In this review, we consider close encounters between plant genomes and the circumstances that lead to those encounters. Plant genomes from different individuals or species can come in contact through at least three mechanisms. The first mechanism is artificial grafting, in which shoots and leaves (the scion) are grafted to an existing root system (the rootstock). Artificial grafting is among the most important inventions in agriculture because it facilitated the domestication and propagation of a wide variety of crops and ushered in a second wave of plant domestication (72). The second mechanism is natural grafting (or inosculation), which occurs when roots or stems from two individuals (or two species) are in close physical proximity and fuse, often facilitated by some external pressure. The third mechanism is parasitism. Parasitic plants, such as mistletoe (*Viscum* spp.) or witchweed (*Striga* spp.), penetrate the body of their plant hosts with haustoria. The haustoria extend into the phloem or xylem of the host, thereby gaining access to nutrition and also allowing exchange of other materials.

This review is designed to survey our current understanding about these three mechanisms of contact and the resulting interactions between genomes. We begin by discussing artificial grafts, particularly the steps necessary to establish the graft junctions that serve as the conduit for genome-to-genome interactions. We also consider cases in which grafting does not succeed, and discuss how such incompatibilities might arise. Next, we cover new information about these interactions, especially the nucleic acids that flow between grafted roots and scions, thereby leading to the potential for both genetic and epigenetic effects. Finally, we ask whether these interactions have any relevance for understanding the real world, i.e., beyond the horticultural and agronomic applications of artificial grafting. To ponder the real world, we begin by assessing the prevalence and effects of natural grafts and host–parasite interactions and end by considering some potential evolutionary implications of close encounters.

THE SUCCESS AND FAILURE OF ARTIFICIAL GRAFTS

Artificial grafting is the "…deliberate fusion of plant parts so that vascular continuity is established between them" (78, p. 439). Grafting was invented approximately 2,000 years before the Common Era (78) and probably spread quickly thereafter (63). Grafting probably began as a means to replicate a desirable genotype in long-lived, outcrossing perennials, but the development of grafting had other important implications, including the opportunity to (*a*) domesticate several new woody crops, (*b*) use wild plants as rootstocks, (*c*) avoid lengthy juvenile periods, (*d*) create dwarf trees, which can ease harvesting, and (*e*) impart both abiotic and biotic resistance (78, 117). But what makes a successful graft?

Steps to a Successful Union

In many woody plants, artificial grafts begin with an incision of the stem, which severs the vasculature and disrupts long-distance movement of water and solutes. There are two major groups of flowering plants: monocots and eudicots. These two groups are distinguished primarily by having one or two embryonic leaves, but they also differ in the organization of their vasculature. Both monocots and eudicots have xylem, which functions primarily to move water and nutrients from the root to the shoot, and phloem, which transports sugars, nutrients, and other molecules around the plant body. However, the two groups differ in that eudicots contain a third tissue, the vascular cambium. This last tissue is particularly crucial for graft success because the cambium has meristematic cells integral to repair (9). After incision, at least three steps are required to repair the severed vascular strands and resume physiological processes central to plant survival (80, 116, 124). First, the scion and rootstock must adhere. This typically occurs through the excretion of pectin after ruptured cells are cleared from the wound. Second, callus (undifferentiated tissue from which new organs can emerge) develops at both junctions. Third, the callus and surrounding cambium differentiate into xylem and phloem strands. This final step establishes the vascular connection between the scion and the rootstock, allowing the long-distance movement of water and solutes.

Researchers have detailed the precise timing of key events in graft formation by using *Arabidopsis thaliana* seedlings as a model (70, 124). After two genotypes are grafted, the scion and rootstock attach as early as 2 days after grafting (DAG). Attachment is followed by phloem reconnection (3 DAG), resumption of root growth (5 DAG), and finally, xylem reconnection (7 DAG) (70). The sequential activation of genes expressed in cambial tissue, phloem, and then xylem supports the observation that phloem reconnection precedes xylem formation at the graft site (68). In contrast to *Arabidopsis*, in which graft establishment occurs in roughly one week, graft formation can take several months in woody perennial species (27, 87).

Roles of Hormones in Graft Success

Relatively little is known about the molecular mechanisms that govern graft formation, but it is known that phytohormones are crucial for both wound healing and vascular differentiation (see 80). Early studies suggested a role for auxin in graft formation by demonstrating that xylem differentiation was blocked by the removal of shoot tissue from the scion, the primary location of auxin synthesis (67, 96, 102). More recent work on tobacco (*Nicotiana tabacum*) has shown that elevated auxin and reduced cytokinin levels enhance rates of grafting success and also reduce overgrowth of the rootstock (61).

To better characterize the role of auxin and cytokinin in graft formation, Melnyk et al. (70) utilized the *Arabidopsis* model to perform micrografting experiments between wild-type seedlings and

41 mutants in auxin and cytokinin biosynthesis, signaling, and perception. Surprisingly, no single mutant completely blocked graft formation, but reconnection of phloem strands was delayed by multiple days in 5 of the 41 mutants. Four of the 5 mutants were involved in auxin perception, and 2 of these were asymmetric in their effect, in that their proteins were required only below the graft junction. Only a single gene involved in cytokinin biosynthesis delayed phloem reconnection, but its effects were not as severe as those of the 4 auxin perception mutants. Since the mutants were insufficient to abolish graft formation, this work suggests that grafting induces a response that is distinct from wound repair or vascularization.

One key difference between grafting and wound healing is that grafting stimulates an asymmetric response in starch and hormone accumulation as well as gene expression. In *Arabidopsis* grafts, both starch and auxin accumulate above the incision shortly after the phloem strands are severed (68). Curiously, the different concentrations of auxin above and below the wound lead to a similar outcome—increased cell division—but by different pathways. Above the incision, auxin accumulation triggers auxin-responsive transcription factors (2, 68, 124). Below the incision, low auxin levels reduce the expression of auxin response factors, which in turn derepress another family of transcription factors (2, 93). The promotion of cell division both above and below the cut site is critical for tissue reunion (2). Given the differential concentrations of auxin, it is perhaps not surprising that gene expression is asymmetric above and below the graft incision. By contrasting gene expression among joined, unjoined, and uncut *Arabidopsis* hypocotyls, Melnyk et al. (68) showed that asymmetric gene expression above and below the cut site was threefold more common after incision than symmetric gene expression was, but this asymmetry reversed by 5 DAG. This foundational study also confirmed that the grafting response differs from the wound response on the basis of gene expression profiles.

Grafts Are Not Always Successful: Incompatibility

One fascinating feature of artificial grafts is that they are not always successful. Eudicots and gymnosperms, a group of nonflowering plants that includes pine trees, tend to form compatible grafts to themselves (i.e., autografts), but monocots such as maize (*Zea mays*) or rice (*Oryza sativa*) typically cannot (69, 97). Inefficient grafting in monocots likely reflects their complex arrangements of vascular bundles and also the fact that monocot species rarely undergo secondary growth owing to their lack of cambium (80).

Within eudicots and gymnosperms, the failure of two individuals to form a successful graft, which is known as graft incompatibility, highlights how close encounters between plant genomes can have unfavorable outcomes. Relatively little is known about the biological processes that underlie graft incompatibility, but it is well known that it correlates with phylogenetic relatedness. Species within a genus are more likely to produce a successful graft union than species across genera (21, 100). Although phylogenetic relatedness can be used as a general rule to predict graft success, there are exceptions. For example, grafts are often incompatible between different species of the genus *Prunus*, which includes almonds (*P. dulcis*), plums, and peaches (*P. amygdalus*) (25). Intraspecific variation in graft incompatibility is also common, suggesting that genetic studies may help identify the molecular basis of graft success.

One fundamental (and surprising) challenge is that there is no universal definition of graft incompatibility (30). Graft failures fall into three major categories, although others have been suggested (75, 99). Graft failure can be due to insufficient formation of callus, to failure of the vasculature to reconnect across the graft junction, and to toxic substances that cause necrosis of cells at the graft junction. In each case, a breakdown in proper cellular function and communication between neighboring cells results in graft failure. These failures lead to a suite of common

symptoms, such as leaf discoloration, defoliation, premature senescence of the graft combination, or a combination thereof (25, 30, 127). Incompatibilities also have a temporal component; in woody plants, graft incompatibility often develops within one year, but some incompatibilities manifest after many years and sometimes even decades (38, 77). Delayed incompatibilities were initially thought to be due to directional movement of a toxic substance at the graft union that could be rescued with a compatible interstock, a section of plant tissue that is grafted between the scion and the rootstock (38, 77). However, incompatibilities also arise from viral infections, from interactions between scion/rootstock combinations, and from environmental factors (38, 91, 99).

The movement of a toxic substance from one graft partner to the other illustrates one adverse effect caused by the close proximity of two unrelated genomes. In pear (*Pyrus communis*)/quince (*Cydonia oblonga*) and peach/almond graft combinations (32, 33), a cyanogenic glucoside, prunasin, is produced in the quince rootstock and then moves across the graft interface to the pear scion, where it is catabolized to free cyanide. Cyanide poisoning of the cells at the graft interface causes localized necrosis and leads to graft failure. Although fascinating, the occurrence of cyanogenic glucosides is relatively rare in woody plants, so it is unlikely a universal mechanism underlying graft failure (99). Necrosis of cells at the graft junction can also be caused by other, mostly phenolic, compounds that can accumulate at the interface of incompatible grafts (25).

Unlike the case of prunasin, the evidence linking insufficient callus formation and vascular differentiation to graft incompatibility is less clear. Surprisingly, defects in callus formation or vascular reconnection are not always associated with ineffective graft unions. Callus proliferation can occur in both compatible and incompatible scion/rootstock combinations, and the amount of callus can vary by combination (75, 87). Similarly, mild to severe defects in the anatomical properties, including the degree of vascular continuity, across the graft union are evident in both compatible and incompatible combinations (30, 99). Scions can also survive in the absence of vascular continuity (38, 79); in an extreme case, citrus scions grafted onto avocado rootstocks survived for nearly a year without forming a graft union (35). Graft incompatibility has also been linked to the inability of the callus to differentiate into vascular tissue, potentially due to defective hormone signaling or perception (75), but *Arabidopsis* studies show that defects in auxin and cytokinin biosynthesis, signaling, and perception do not abolish graft formation (70).

These observations raise many questions regarding the role of cell-to-cell communication in wound healing and vascular differentiation. Genetic studies may be key to unlocking some of these mysteries. Intraspecific variation in graft incompatibility is common, but only a limited number of studies have been conducted to identify the genetic basis of incompatibility (13, 14, 19, 36, 50, 98). The most convincing evidence for the genetic control of this trait comes from Douglas fir (*Pseudotsuga menziesii*) (13, 14), from which 15 segregating F_1 families were used to estimate the heritability of graft incompatibility (14). Not only was graft incompatibility highly heritable (0.81), but it was due predominantly to the additive action of genes (14). In other systems, graft incompatibility seems to be due to only a few genes (19, 98). For example, using segregating F_1 families of interspecific peach/nectarine hybrids, Salesses & Al Kaï (98) determined that genetic control of incompatibility in peach/plum and nectarine/plum graft combinations was caused by the action of two dominant genes. The distribution of graft incompatibility in hoop pine (*Araucaria cunninghamii*) was also bimodal, a characteristic of traits with simple genetic control (19). Similar approaches have been used to genetically map the contribution of rootstocks to scion traits (117). Experimental strategies that leverage segregating families as the rootstock in combination with a fixed scion genotype (or vice versa) are crucial for further genetic characterization of graft incompatibility.

ARTIFICIAL GRAFTS OFFER INSIGHTS INTO GENOME-TO-GENOME INTERACTIONS

Once a graft junction has been established, the genomes of the two grafted partners come in contact through vasculature and plasmodesmata, which are cytoplasmic threads that connect adjacent cells and facilitate cell-to-cell interactions between rootstock and scion cells at the graft junction. Grafted scion phenotypes vary with different rootstock partners (28, 117), which motivates questions about the signals that travel through graft junctions and their potential effects on phenotypes (60, 106). To what extent do cells with different genetic compositions communicate with one another, and what are the implications of this communication for gene expression and ultimately phenotypic variation?

Chimeras Combine Cells of Different Genotypes

Insights into genome-to-genome interactions in grafted plants can be gleaned from systems that contain cells with different genomes, such as genetic mosaics and chimeras (22, 37, 113). A genetic mosaic is an individual that has two or more genetically distinct cell lineages derived from a single zygote; most often this scenario happens when a somatic mutation occurs within an individual and then proliferates within that individual, resulting in mutated and nonmutated cells existing in close proximity (e.g., 94). A chimera is distinguished from a mosaic because the two cell types that cooccur within the chimeric individual are derived from two different zygotes (20). Three categories of chimeras are based on the organization of the different cell types: periclinal chimeras, mericlinal chimeras, and sectorial chimeras (22). Of these, periclinal chimeras have genetically distinct cell layers and have been identified in many plants, including mangoes (*Mangifera* spp.) (53), tobacco (3), citrus (128), *Solanum* (20), and cassava (*Manihot esculenta*) (26), among others.

Chimeric individuals can be generated through grafting, and periclinal chimeras have been particularly useful for dissecting gene expression patterns across genetically distinct cell layers. For example, a periclinal chimera constructed in tomato resulted in an individual with an outermost L1 epidermal layer of cells from the wild tomato *Solanum pennellii* and inner L2 and L3 cell layers derived from cultivated *Solanum lycopersicum* (20). *S. pennellii* cells exhibited patterns of gene expression different from those of *S. lycopersicum* cells, both in the parental lines and in the L1 versus L2 and L3 cell layers (**Figure 1*a***). For genes with cell-layer-specific patterns of expression, L1 expression in the chimera was identical to L1 expression in *S. pennellii*, and L2 expression in the chimera matched L2 expression in *S. lycopersicum*. These data indicate that expression patterns in cell layers reflect allelic variation from the species of origin. But do unique signals move between cell types?

Short-Distance Interactions Among Genetically Distinct Adjacent Cells

Periclinal chimeras of tuber mustard (*Brassica juncea*; T cell type) and red cabbage (*B. oleracea* var. *capitata*; C cell type) have been used to investigate transmission of genetic material between adjacent heterologous cells (60). After using histological and molecular data to confirm the identity of the two distinct cell lineages, the authors used RNA expression analysis to reveal that some small RNAs (sRNAs) detected in the T cell lineage originated in the C cell lineage, most likely via movement between cell types (60). This study provided fine-scale, high-resolution analysis of sRNA movement among adjacent, genetically distinct cells.

Movement of larger materials among adjacent, genetically distinct cells has been documented as well. To explore this dynamic, Stegemann & Bock (106) established a system in which the rootstock and scion of grafted tobacco carried unique marker and reporter genes in either the

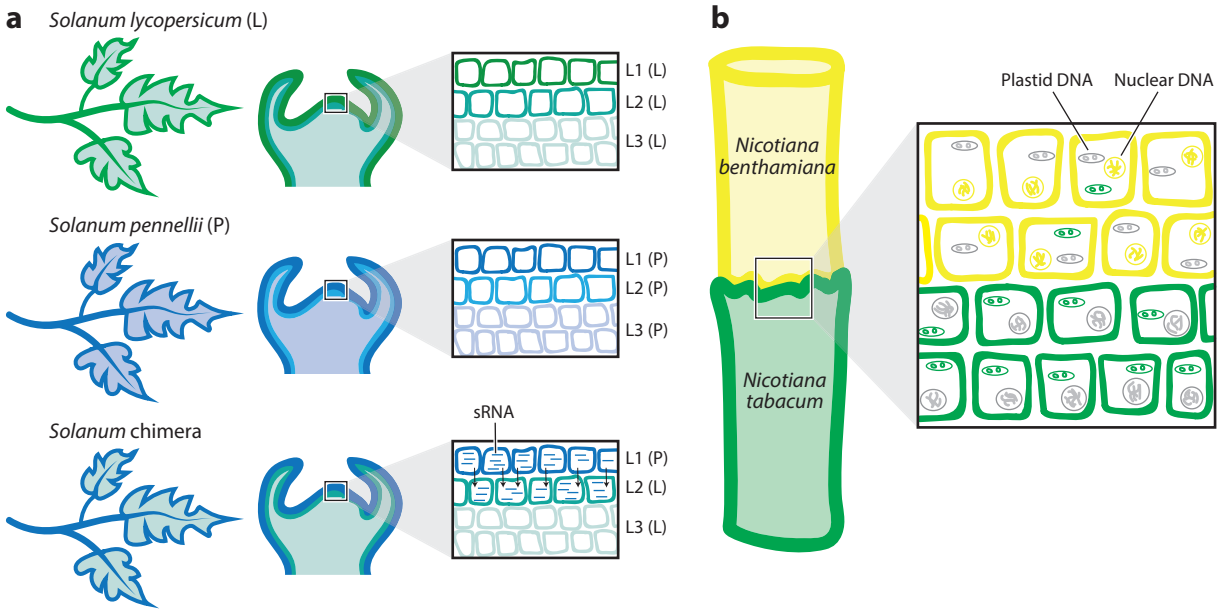


Figure 1

Local genome-to-genome communication. (*a*) The movement of sRNA between meristematic cell layers is detailed for a periclinal chimera derived from *Solanum lycopersicum* and *Solanum pennellii*. The L1 meristematic cell layer in the *Solanum* chimera is derived from the wild species *S. pennellii* (*blue*), while the source of the L2 and L3 cell layers of the meristem is the cultivated species *S. lycopersicum* (*green*). The genomes of the L1 and L2 cell layers differ, and as a result, movement of genetic material between neighboring cells can be investigated. (*b*) *Nicotiana* as a system for examining the movement of chloroplast DNA between neighboring cells at a graft junction. *N. benthamiana* carrying a nucleus-inserted transgene labeled with YFP is grafted onto *N. tabacum* containing a plastid-inserted transgene labeled with GFP. Cells at the graft junction containing both YFP and GFP were identified, indicating that plastid genomes can move from cell to cell. Abbreviations: GFP, green fluorescent protein; sRNA, small RNA; YFP, yellow fluorescent protein.

nucleus or the chloroplast. Transgenic tobacco scions contained a kanamycin-resistant gene and a yellow fluorescent protein gene in the nucleus; the rootstock carried a spectinomycin-resistant gene and a green fluorescent protein gene in the chloroplast. Following grafting, cells at the graft site were selected for resistance to both antibiotics and found to contain both the yellow and green fluorescently labeled proteins. PCR and Northern blots confirmed that the cells contained active marker and reporter genes, indicating DNA movement among cell types. These observations were confined only to the graft site, but other studies offer evidence of longer-distance movement of genetic material.

Building on this observation, Stegemann et al. (107) documented the transfer of complete plastid genomes between cell layers of grafted species. This study detected the movement of the *Nicotiana tabacum* (tobacco) plastid into cells of both *N. glauca*, a woody species, and *N. benthamiana*, an herbaceous species (**Figure 1*b***). Movement was detected with the same system of marker and reporter genes employed previously, and cells carrying both labeled genes were again detected at the graft junction. In this case, however, the authors confirmed through comparative analyses of chloroplast genome sequence data from the donor and recipient chloroplasts that whole organelles moved across the graft junction, and they also showed that transferred *N. tabacum* plastids were stably inherited in regenerated plants. These groundbreaking studies have provided an important foundation for studying horizontal whole plastid genome transfer via grafting, for suggesting a possible mechanism underlying endosymbiotic gene transfer and chloroplast capture (8), and for

introducing transgenes via the chloroplast (e.g., 66, 103). Moreover, whole-genome transfer is not limited to plastids; both mitochondria and nuclear genomes also move from cell to cell and across the graft junction (24, 34).

Long-Distance Transport of Signals and Genetic Material

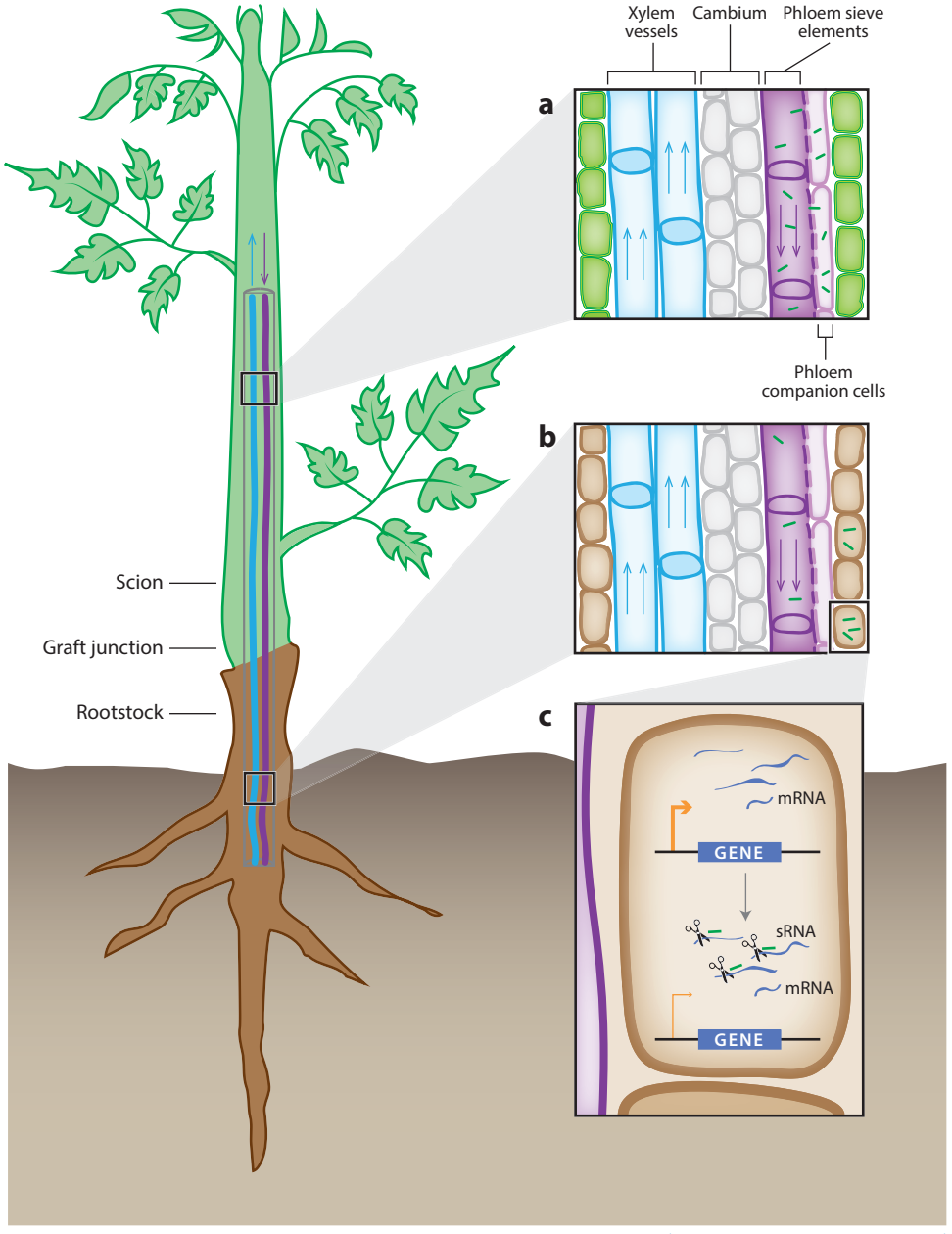
Building on observations of short-distance transport among adjacent cells, a growing body of work has provided evidence for long-distance movement (from shoots to roots and vice versa) of RNA. Messenger RNA (mRNA) can move long distances via the phloem to other regions of the plant (reviewed in 37, 49, 57, 76, 82, 114). Not surprisingly, mRNA movement occurs primarily in the direction of phloem flow, moving from source to sink (shoot to root or leaf to meristem). One example comes from transgenic potato (*Solanum tuberosum*) lines engineered to overexpress *StBEL5*, a transcription factor–encoding gene that is associated with enhanced tuber growth under short-day conditions. Transgenic potato lines overexpressing *StBEL5* were grafted to wild-type rootstock, and the mRNA moved through the phloem into the tips of horizontal stems running across the ground (stolon tips), resulting in a twofold increase in tuber production in grafted plants (5). Additional studies have provided evidence for mRNA movement across graft junctions in pumpkin (*Cucurbita maxima*)/cucumber (*Cucumis sativus*) heterografts (88), Chinese pear/wild pear heterografts (129), grafts of different *Arabidopsis* ecotypes (112) and *Arabidopsis/N. benthamiana* heterografts (83).

There is also evidence for mRNA movement in the opposite direction, from root into scion. In cucumber/pumpkin heterografts, Ruiz-Medrano et al. (95) demonstrated that the transcript *CmNACP*, a component of pumpkin phloem sap, moved readily through phloem into the cucumber scion. (*CmNACP* is a transcript that contains a conserved domain from the NAC domain gene family, some of which have roles in meristem development.) Importantly, *CmNACP* mRNA was absent in ungrafted cucumber controls. Similarly, Xoconostle-Cázares et al. (119) demonstrated movement of the *CmPP16* mRNA from companion cells into sieve elements and through the graft junction into a cucumber scion, where it is not produced endogenously. Grafted grapevines have also contributed to our understanding of long-distance movement of mRNAs. Yang et al. (122) constructed reciprocal grafts of *Vitis girdiana* and *V. palmata*, two native North American wild grapevine species. They found that approximately 12% of protein coding genes produced mRNAs that exhibited either unidirectional or bidirectional movement across the graft junction. The extent of movement of specific mRNAs was influenced by the rootstock and scion genotypes and also by the environment. Other recent advances have linked phloem-mobile mRNA to signaling associated with the abiotic stress response. In one example, phosphate stress in cucumber/watermelon (*Citrullus lanatus*) heterografts resulted in the production of a largely unique suite of mobile mRNA species that originated in one graft partner and were detected in sink tissues of the other partner (see 37, 118).

sRNAs also move long distances through the phloem of plant cells (37, 49, 57, 82, 130). sRNAs range in size from approximately 19 to 25 nucleotides (nt) and include microRNAs (miRNAs), which are typically 21–22 nt in length (41). Recent studies have shown that sRNAs increase in abundance in the phloem under stress conditions (11), move short and long distances in plants (60), and can traverse graft junctions (11, 90). The movement of miRNAs and small interfering RNAs (siRNAs) across graft junctions has been implicated in gene silencing (see 57, 130) (**Figure 2**), either via posttranscriptional silencing, which degrades or destroys mRNA, or via RNA-directed DNA methylation (RdDM), which reduces transcription.

All these mobile molecules have the potential to cause phenotypic changes in one graft partner owing to genes expressed in, and products transported from, the other graft partner. For example,

specific miRNAs move from shoot to root in grafted *Arabidopsis* plants when inorganic phosphate is limited (41); these miRNAs appear to suppress expression of *PHO2*, a regulator of inorganic phosphate uptake (40, 62). Mobile miRNAs may also mediate responses to drought stress in grafted grapevines (89), in which heterografts exhibit altered leaf potential and stomatal conductance relative to autografts and in which specific miRNAs were reduced or enhanced, depending on both the environment and the heterograft genotype. Finally, mRNAs can act as signaling molecules (10, 84).



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

An illustration of long-distance movement of sRNAs from the scion to the root via the phloem. The blue and purple lines in the stem of the plant represent the xylem and phloem, respectively. Inset *a* illustrates the scion vasculature in more detail, showing the xylem vessels (*blue*) predominantly transport materials up the plant, while the phloem (*purple*) transports materials from source to sink. The cambium is also shown, as are the phloem companion cells that actively load materials into the phloem sieve elements. In this example, the companion cells load sRNAs into the phloem sieve elements; these sRNAs are represented in the phloem by small green lines. Once these sRNAs are transported to the rootstock vasculature (inset *b*), they can be unloaded from the phloem to cells in the rootstock tissue. Inset *c* shows one possible effect of the sRNAs, which is to decrease gene expression via posttranscriptional mechanisms. sRNAs can also affect gene expression by recruiting DNA methylation machinery that can transcriptionally silence the target locus. Abbreviations: mRNA, messenger RNA; sRNA, small RNA.

In destination cells, these non-cell-autonomous mRNAs can affect physiology and development through posttranscriptional gene silencing and RNA interference, among other mechanisms (48, 57, 71, 105). One much-discussed example of long-distance transport is a flowering signal initiated in the leaves that moves into meristems and induces flowering (15). The *FLOWERING LOCUS T* (*FT*) is expressed in leaves, but both *FT* mRNA and protein have been recovered from the phloem. The FT protein, but not *FT* mRNA, has been detected in the shoot apical meristem, where it is required for flowering. Reciprocal grafting experiments have shown that FT protein can move from the shoot, over the graft junction, into the root and vice versa in the alternative graft combination. *FT* mRNA has not been detected in the phloem of grafted partners, but subsequent work suggests that *FT* mRNA works as an additional signal to induce flowering (65).

Epigenetic Effects

One of the most significant advances in recent years is the discovery that non-cell-autonomous RNAs moving through phloem may cause epigenetic modifications in destination cells (4, 74, 110). Working on *Arabidopsis*, Molnar et al. (74) demonstrated that 21-nt and 24-nt sRNAs synthesized in source cells can move into sink cells. Greater movement was detected from shoot to root than vice versa, and 23-nt and 24-nt sRNAs moved more than 21-nt sRNA species. Importantly, 24-nt sRNAs are associated with DNA methylation because they act to recruit components of the RdDM pathway (55).

To explore methylation effects, Molnar et al. (74) performed grafting experiments with wild-type and mutant genotypes. The mutant genotypes exhibited reduced production of 22-, 23-, and 24-nt sRNAs as well as reduced methylation relative to the wild type. The authors compared two shoot/root graft combinations: wild type/mutant and mutant/mutant. Roots of the mutant/mutant graft exhibited low levels of methylation, as expected given their reduced capacity to produce sRNAs associated with DNA methylation. In comparison, roots of wild type/mutant plants were hypermethylated, which indicates that wild type–produced mobile sRNAs are mediating epigenetic changes in the mutant root through graft junction movement. Further work used a similar experimental approach but utilized genome-wide methylation data; it identified thousands of methylated DNA bases within the roots of methylation mutants (59). These results strongly suggest that sRNAs not only traverse graft junctions but also affect methylation in destination tissues. Similar work on *N. benthamiana* has demonstrated gene silencing via de novo DNA methylation (4). sRNAs produced in the *N. benthamiana* rootstock silenced expression in the scion tissue adjacent to the leaf vein, but sRNAs produced in the scion resulted in systemic expression silencing in the root (4). These results indicate that RdDM is bidirectional across the graft junction but also suggest that activity follows the flow of phloem, with stronger effects from source to sink.

The next pressing questions are whether these changes affect phenotypes and are heritable. Regarding the latter, evidence is based on a periclinal chimera generated by grafting tuber mustard (cell layer T) and red cabbage (cell layer C), which produced an individual with cell layers L1, L2, and L3 as TTC (12). Selfed progenies of the periclinal chimera are derived from the *B. juncea* (T) L2 layer of the chimera, which was adjacent to the *B. oleracea* (C) L3 layer in the chimera. These selfed progenies have a genotype of TTT_s because the gametes are derived from L2 but with T cells that had been potentially influenced by C cells. The resulting TTT_s progenies were surveyed for phenotypic variation and methylation patterns, and they were compared with TTT individuals derived from an autografted, nonchimeric TTT parent (TTT_{nc}). Both the leaf shape and the shoot apical meristem of the TTT_s progenies differed from that of the TTT_{nc} individuals, and leaf shape differences persisted over multiple selfed generations. Importantly, DNA methylation patterns in the selfed TTT_s offspring differed slightly from the patterns in TTT_{nc} plants, exhibiting a slight trend towards less methylation, with approximately 2–4% of sites demethylated in TTT_s relative to TTT_{nc}. Approximately 1–2% of methylated sites also varied among selfed offspring. This study also reported shifts in gene expression associated with methylation changes, further suggesting that heritable changes in methylation can influence phenotype.

A follow-up study used a similar experimental design but had the advantage of whole-genome methylation data (126). This study found that cells derived from a periclinal chimera exhibited higher levels of methylation relative to genetically identical cells without a chimeric history; the shifts in methylation patterns were accompanied by corresponding shifts in sRNAs (126). Overall, these studies are consistent with graft-transmissible sRNAs directing DNA methylation and the maintenance of that methylation over at least four generations.

Graft-transmissible epigenetic modification has been highlighted as an important, underexplored mechanism of crop improvement, particularly for clonally propagated perennial crops (29). DNA methylation differences in common scions grafted to genetically distinct rootstocks have been documented for a diversity of taxa. For example, in rubber (*Hevea brasiliensis*), grafted scions exhibited epigenetic changes following grafting to genetically distinct rootstocks (115). In experiments with grafted citrus under drought stress, methylation either increased or decreased in scions depending on the rootstock (81). Given the capacity of individuals to affect methylation patterns in their graft partners, and the heritability of many of these epigenetic marks, understanding the drivers and maintenance of epigenetic changes in perennial crops is an important area for future research.

NATURAL OPPORTUNITIES FOR GENOME INTERACTIONS

The preceding sections document extensive progress toward understanding genome-to-genome interactions in grafted partners, with new insights into the mobility of RNAs, their epigenetic effects, and potential effects on phenotypes. This information is fundamentally important for crop improvement and breeding, but it also prompts questions about whether these phenomena exist beyond crop and model species. To explore this issue, we review the phenomena of natural grafts and plant parasitism.

Natural Grafts

Natural grafts may have served as the inspiration for artificial grafts (78), but the steps to their formation are not well understood. The most common idea is that these grafts take place when roots (or stems) come in contact under pressure due to growth and physical restrictions in the soil. As the roots grow, pressure increases until the bark wears away, the cambia come in contact, and

repair leads to a functional graft union that merges the vascular tissue of previously distinct roots (31).

Like artificial grafts, natural grafts can be either intraspecific or interspecific. Intraspecific grafts are thought to be more common than interspecific grafts, but examples of the latter include grafts between oak (*Quercus*) species, between pine (*Pinus*) species, and between maple (*Acer*) species (31). Natural grafts appear to be most common in trees, but they also occur in nontree species. For example, ivy (*Hedera helix*) seems to be particularly prone to interspecific grafts (73). Altogether, natural grafts occur in approximately 200 species (31), but the phenomenon is understudied (58) and may be much more widespread.

Few studies have estimated the frequency of natural grafts, but these suggest that grafts can be quite common (7). For example, Jelínková et al. (42) excavated three stands of *Populus* trees and surveyed for root grafts. They found that, on average, half the trees had a graft across the three stands. A similar excavation of *Pinus banksiana* roots revealed that up to 71% of trees had intraspecific grafts within an excavated plot (111). It is commonly thought that grafts occur more easily between closely related individuals, i.e., that graft compatibility ranges with genetic identity. However, in both *Populus* and *Pinus* there was no compelling relationship between grafting and genetic distance. In *Populus*, for example, grafts were equally likely to occur between two genetically identical (clonal) trees as between two genetically distinct trees. Instead, intraspecific grafts better reflect physical rather than genetic proximity (42, 111).

Natural grafts facilitate communication between individuals through the transfer of nutrients, hormones, and other compounds. One frequently cited piece of evidence for these effects is the persistence of stumps in the forest, which are dead with respect to photosynthesis but can nonetheless persist as living root systems for several decades (e.g., 30, 42). Experimental modifications have confirmed this effect; in one case, a researcher interrupted the phloem of a pine tree and found that it survived for more than 18 years owing to root grafts (108). Additional studies have traced the transport of compounds from one tree to another through the use of radioactive tracers and other markers (31), and some of this transport can be confidently attributed to grafts (23). Altogether, there is overwhelming evidence that root grafts contribute to ongoing, belowground communication of stands of trees. As such, they comprise part of the wood wide web, in which plants communicate through soil-based networks of mycorrhizae (52, 104), soil exudates, and root grafts. To our knowledge, however, there have been no molecular studies of natural grafts. Do they facilitate the movement of RNA and DNA between graft partners, as clearly occurs in artificial grafts? Do natural grafts affect the epigenetic profiles of grafting partners? If so, what might be the immediate and long-term effects of these interactions?

Host–Parasite Interactions

Like artificial grafts, parasitic plants provide ample opportunities to investigate plant genome-to-genome interactions. There are, however, at least two substantive differences between artificial and natural grafts and host–parasite interactions. The first difference is the anatomical connection. Parasitic plants connect to their host through their haustoria, a structure that invades the host tissue and eventually forms an interface between the parasite and its host. The interface typically includes connection to xylem, resulting in vascular continuity between plants, but it can also include connection to the host phloem (125). For cases in which there is no direct phloem connection, parasites retrieve nutrients through plasmodesmata via cell-to-cell connections at the host–parasite interface (69, 125). The second difference is that host–parasite interactions can form more evolutionarily diverse partnerships than artificial grafts can. This is due in part to the diversity of parasitic plants themselves. Parasitism has evolved at least 12 times in plants (118), and

these 12 origins have diversified to encompass approximately 4,500 parasitic plant species that are members of 28 families and represent approximately 1% of extant angiosperms (125).

The physical connection between host and parasite is a conduit that transports compounds, including nutrients, minerals, water, signaling molecules, viruses, DNA, and RNA, between species. Recent work has focused on RNA movement. For example, Kim et al. (51) infected *Arabidopsis* and tomato hosts with *Cuscuta pentagona* to examine interspecific transport of mRNAs. They found that 44% of the mRNAs produced by *Arabidopsis* stems crossed the haustorial junction into *C. pentagona*. These mRNAs represent a biased set of genes that were highly expressed in *Arabidopsis*, and they tended to have specific functions (e.g., responses to stimuli) (51). mRNA movement was also bidirectional; 24% of *C. pentagona* transcripts were detected in *Arabidopsis* stems. At least some of these mRNAs can travel over long distances (56), with some host mRNAs detectable in parasite stems approximately 20 cm away from the haustorial connection (17). Although 44% and 24% of mRNAs moved from and to *Arabidopsis*, only 1.6% and 1.1% of mRNA moved from and into tomatoes infected with *C. pentagona* (51). The reason for these differences between *Arabidopsis* and tomato hosts is not clear, but it may reflect that tomato has active mechanisms to resist infection, including the secretion of compounds at the infection site (45).

Overall, the foundational studies of *C. pentagona* raise as many questions as answers. Do trans-specific mRNAs have a function, e.g., in protein production or signaling? The potential for function is bolstered by the observation that degradation of trans-specific mRNAs is not immediate; some host mRNAs have half-lives of several hours in the *C. pentagona* stem (56). Another important question is whether a selective process filters mRNA movement. Biases in the mobility of mRNAs suggest some type of selective mechanism (56), as do mobility differences between hosts (51). Although these and other questions remain unanswered (76), it is clear that mRNAs commonly move between hosts and parasites, similar to RNA movement based on artificial grafts (112, 122, 131).

sRNAs also move between parasites and their hosts but with the important difference that at least some sRNAs are known to function trans-specifically. An early illustration of trans-specific sRNA function came from transgenic work (1). In this work, a construct was designed to produce an sRNA that targeted a *C. pentagona* gene. When the construct was transformed into tobacco, the sRNA effectively silenced the *C. pentagona* target, thereby affecting the efficacy of infection (1). More recently, Shahid et al. (101) demonstrated that 22-nt miRNAs encoded by *C. pentagona* target at least six genes in its *Arabidopsis* host, ultimately causing mRNA cleavage and reduced expression for five of the six genes. At least two of these genes function to restrict *C. pentagona* growth on *Arabidopsis*, which strongly suggests the miRNAs have evolved to modulate host–parasite interactions. These same miRNAs have potential targets in another host (*N. benthamiana*) and act to cleave at least one *N. benthamiana* mRNA. Remarkably, these same miRNAs are not predicted to efficiently target homologous genes within *C. pentagona*, suggesting that they have evolved to avoid self-modifications. Given these data, the inescapable conclusion is that genome-to-genome interactions, specifically interactions facilitated by sRNAs, shape the dynamics of the arms race between hosts and parasites.

POTENTIAL EVOLUTIONARY IMPLICATIONS OF GENOME-TO-GENOME INTERACTIONS

Genome-to-genome interactions have the potential for evolutionary implications, as evidenced by arms race dynamics between hosts and parasites. However, the broad evolutionary implications of genome-to-genome interactions have rarely been addressed. Here, we briefly consider three questions of evolutionary interest.

Why Do Plants Graft in Nature?

Natural grafts contribute to a remarkable dynamic in which individual trees compete against each other for both above- and belowground resources but at the same time are not wholly independent of one another (64). Even though the extent of natural grafting has been greatly understudied, grafts occur frequently enough that one wonders why. A prosaic explanation is that root grafts happen randomly due to chance physical contacts in the species-rich soil matrix (43). However, some scholars (54) argue that natural grafts often take place in the absence of physical irritation caused by random contact, suggesting a more deliberate mechanism of graft formation.

Another possibility is that natural grafts are beneficial. But are they adaptive and, if so, what is the benefit? The benefit to a dead stump is obvious; it can remain alive by parasitizing nutrients from photosynthesizing neighbors. Some work even suggests that the support of otherwise dead trees by living trees could retain genetic diversity (42), thereby maintaining evolutionary potential within the population. In return, living trees gain access to water and minerals from the root system of the stump (46). However, the idea that natural grafts evolved to support dead trees is an unsatisfactory evolutionary argument because the selective pressure to graft occurs only after the death of one partner.

Several authors have argued that root grafts serve an adaptive purpose by sharing nutritional resources among individuals, by providing additional physical support against mechanical forces, such as wind and floods, or both. For example, black gum (*Nyssa sylvatica*) has more root grafts in swampland soils than in uplands and floodplain soils, perhaps because the extra support and stability from grafted roots are more beneficial in swamps (46). Others find the argument for physical support to be underwhelming (64), and arguments for the benefits of shared nutrition may be similarly weak (46). Other intriguing possibilities are that natural grafts somehow contribute to the formative features of trees such as high genetic diversity and evolvability (92), or that they comprise networks for RNAs that act as signaling molecules to mobilize responses to environmental cues, such as disease or stress. Altogether, however, the potential benefits of natural grafts remain puzzling (58). Given this uncertainty, there is a pressing need to better characterize the frequency and pattern of grafts in nature and to gather insights into the evolutionary dynamics that promote and maintain natural grafts.

Are Genome-to-Genome Interactions a Source of Evolutionary Innovations?

Artificial grafts can cause heritable phenotypic variation, which is sometimes called graft-induced genetic variation (GIGV), in the scion, rootstock, or both, suggesting that grafting can lead to evolutionary innovations. Although reports of GIGVs have been discounted historically (63), credible examples exist, such as work on grafted peppers that led to the stable inheritance of altered fruit shape, bushiness, and other phenotypes (85, 120, 121). Later investigations have shown that chromatin masses migrated between the pepper rootstock and the scion (86) and that DNA markers have been altered in the scion (39, 109).

This and other information about GIGVs are consistent with the idea that grafts can lead to evolutionary innovations, but the mechanism(s) of altered genotypes is not yet clear. Two pieces of evidence point to the possibility of DNA-mediated exchange between interacting partners. The first, as mentioned above, is experimental work demonstrating the migration of complete mitochondrial, plastid, and nuclear genomes between grafted partners (see above and 8). The second is the study of HGTs between hosts and parasites. HGT has been found in 10 of the 12 parasitic plant lineages (18), and careful study of a single lineage (Orobanchaceae) has detected 106 HGT events between 5 parasitic species and 14 hosts (44). Most of the transferred genes retained their introns, strongly suggesting horizontal transfer via DNA-mediated exchange rather

than mRNA retrotransposition (44, 123). Many of the HGT genes retained open reading frames, and they tended to be expressed in the haustorium, suggesting that they may contribute to innovations of this feeding structure (123). HGT appears to be more common in obligate parasites than in facultative parasites for reasons that are not yet clear but may reflect different modes and patterns of infection (123).

Another distinct possibility is that GIGVs are caused (at least in part) by epigenetic modifications. This explanation gains credibility from the recent work on *Arabidopsis* and *Brassica* (12, 74). To our knowledge, however, no direct link between GIGVs and epigenetic effects has been made. Epigenetic modifications can contribute to heritable phenotypic variation (6) and may thus play a role in evolution. However, these modifications are often unstable over evolutionary timescales and may thus have only short-term effects.

Can Grafting Lead to New Species?

In theory, evolutionary innovations can lead to new species. Charles Darwin (16) believed that grafting provides an opportunity for speciation through asexual hybridization, and he posited a model of graft hybridization, whereby an artificial graft leads to a distinct species through the hybridization of scion and rootstock. He based his model in part on Adam's laburnum (*Laburnocytisus adamii*), a graft of purple broom (*Chamaecytisus purpureus*) onto the common laburnum (*Laburnum anagyroides*) that produces a shoot with flowers intermediate between parental types. Lacking any knowledge of genetics, Darwin explained these results by his theory of pangenesis, which supposes that individual cells release gemmules and that these gemmules migrate and combine in reproductive structures. With respect to grafting, Darwin presumed that gemmules released in the rootstock migrate to the scion and alter the offspring, forming a new species.

We now know that the gemmule theory is incorrect, that Adam's laburnum is a periclinal chimera (and therefore does not contain hybrid cells per se), and that Darwin's hypothesis of graft hybridization is an unlikely mechanism for the asexual origin of species. However, a recent study has reported that an artificial graft led to a new, true-breeding species (24). This study was based on a graft of two *Nicotiana* species, and it made the foundational observation that nuclear genomes can move between rootstocks and scions. As a result of this movement, some cells became allopolyploid, and these allopolyploid cells were regenerated into shoots bearing fertile flowers, i.e., a new allopolyploid species. This interesting study presents a possible approach to construct polyploid plants with substantial potential for breeding and crop improvement (8).

In this context, however, the broader question is whether the apparent asexual formation of a new species via grafting can be extrapolated to nature. This particular study required selective media and plant regeneration techniques to generate the polyploid (24), conditions that are not found in nature. Another obstacle to creating a new species from a graft in nature is that the newly mutated cells must be incorporated into the germline (8). On the basis of these arguments, it seems reasonable to conclude that speciation via grafting is exceedingly unlikely and at best vanishingly rare in nature. That said, even rare events, such as secondary endosymbioses, can have lasting evolutionary impacts. It is never wise to bet against nature's ability to innovate.

CONCLUSIONS AND PERSPECTIVES

Artificial grafts, natural grafts and plant parasites provide opportunities for interactions between genetically distinct genomes. Although artificial grafting has been practiced for millennia, there are surprisingly wide gaps in our knowledge about these genome-to-genome interactions. One gap centers on the interactions that lead to graft success and especially graft failure. We suggest

that there is much to learn about the dynamics of genome interactions by determining why grafts fail. Another gap is defined by epigenetics. Recent evidence suggests that scion–rootstock interactions can lead to epigenetic shifts, but the prevalence of these shifts, their links to heritable phenotypes, and their potential evolutionary implications remain largely unexplored. Fortunately, the introduction of tractable models such as *Arabidopsis* and the parasite *Cuscuta* promises to lead to more insights about the mode and prevalence of RNA transfer and their epigenetic effects. Finally, natural grafts remain a compelling mystery. We know little about how often they occur, the interactions that result from their occurrence, the evolutionary pressures that may lead to their maintenance, and their potential to lead to evolutionary innovation.

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Errata

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