

NEWS AND COMMENTARY

Gene exchange between cells by grafting

New insights into plant graft hybridization

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Heredity (2010) 104, 1–2; doi:10.1038/hdy.2009.115; published online 26 August 2009

Grafting as a means of vegetative propagation is well known. Less well known is that genes may move from one plant to another by grafting. Recently, Stegemann and Bock (2009) showed that genetic material is transferred between plants across graft junctions. They examined grafts between tobacco plants that express either a transgene encoded by nuclear DNA or a transgene encoded by plastid DNA, and identified resulting cells that express both sets of transgenic markers. They showed that genetic exchange can occur in both directions, but only involves the transfer of plastid DNA between cells and is limited to the site around the graft. They emphasized that their data do not lend support to the doctrine of Lysenkoism that ‘graft hybridization’ would be analogous to sexual hybridization.

Although graft hybridization has become inextricably linked to the name of Lysenko, Darwin was the first to put forward the concept of graft hybridization, and formulated a pangenetic hypothesis to account for it. In his *The Variation of Animals and Plants under Domestication*, Darwin (1868) recorded various cases in which shoots that developed from grafted trees exhibited the characters of both stock and scion. To explain the formation of graft hybrids and many other facts, he proposed that cells are not only able to grow and divide but are also capable of ‘throwing off’ gemmules (molecular carriers of hereditary characters and the embryonic form of our modern genes). These gemmules were considered capable of self-replication and were able to freely ‘diffuse’ from cell to cell. In the cases of grafts, such diffusion was thought to occur between the cells of the stock and scion. Unfortunately, these ideas of Darwin have generally been considered to be wrong because the stock and scion have been thought to not exchange their genetic materials. For more than a century there has been a refusal to accept the existence of graft hybrids, which have been regarded only as a

type of chimera—an individual composed of genetically different tissues and cells, representing a mixing of stock and scion without any true genetic exchange.

Stegemann and Bock (2009) stated that their finding that gene transfer occurs but is restricted to the contact zone between scion and stock indicates that the changes can become heritable only through a lateral shoot formation from the graft site. Rusk (2009) has interpreted this as ‘molecular evidence that the concept of graft hybridization is untenable’. Over the past several decades, however, the concept of graft hybridization has been tested and the existence of graft hybrids has been confirmed by several independent groups of scientists. For example, Ohta and Chuong (1975), working on *Capsicum annuum*, repeatedly showed that genes for fruit color and fruit position could be transferred by grafting. Commenting on this study, Pandey (1985) noted that ‘the work is thoroughly documented and there is absolutely no cause to doubt the genuineness of the results’. Taller *et al.* (1998) showed that some of the characteristics of stock were introduced into the progeny obtained from selfed seeds of the scion and that novel characteristics appeared as a result of graft induction. It should be noted that Anne McLaren, a distinguished British geneticist, believed that graft hybridization was of great interest and importance in genetics and enthusiastically encouraged our research on this subject (Liu, 2006).

It has been stated that ‘The original idea of graft hybridization was that you can change the heritable properties of the plant by grafting’ (Rusk, 2009). Although there is some truth in this statement, it is misleading. Actually, the phenomenon of graft hybridization is conditional on the correct use of the mentor-grafting method, which differs significantly from conventional grafting techniques. Michurin (1949) made significant use of mentor grafting as a means of influencing and improving immature plants, showing that plants

can be altered by grafting if in a sufficiently early phase of development. The most widely adopted mentor-grafting method involving annual plants consists of grafting very young seedlings (from the cotyledon phase to three- to five-leaved stage) onto mature stocks (2–3-months old, having 20–30 leaves). One-way flow of genetic material from stock to scion is affected by removing the leaves of scion (except for two to three at the top) twice a week during the entire time of growth (Liu, 2006). In plants, cell-to-cell channels, called plasmodesmata, connect each plant cell to its neighbors, facilitating the exchange of informative molecules (Lucas and Lee, 2004). In a graft union, cells of scion and stock are interconnected by true plasmodesmata (Kollmann and Glockmann, 1991). Recently, it was shown that traffic of macromolecules (including nucleic acids) between plant cells is promiscuous in young undifferentiated tissues, becoming much more restricted as tissues mature. The younger the plants, the larger their plasmodesmata (Ueki and Citovsky, 2005).

Rusk (2009) claimed that ‘so far there is no molecular evidence for graft hybridization’. This is not the case. Pandey (1976) noticed that there is a striking similarity between gametic transformations and graft hybridization, and proposed a genetic transformation hypothesis to account for the mechanism of graft hybridization. Later, the term ‘graft transformation’ was adopted by several researchers (Ohta, 1991; Hirata *et al.*, 2003). In a microhistological analysis of stock stems, Ohta (1991) noticed that chromatin masses moved through cell walls and intercellular spaces from lignifying and dying cells toward vascular bundles. He proposed that this chromatin must be transferred through the vascular system, across the graft union, to the floral primordia or growing points of the scion. Interestingly, it was their work on graft hybridization that led Stroun and Anker (2006) to suggest that nucleic acids are released by living cells and circulate throughout the whole organism. They proposed that DNA circulated between stock and scion, and that transformation occurs when some circulating DNA from stock enters the somatic and reproductive cells of the scion. There is evidence that plant cells can release newly synthesized DNA that freely circulate in the plant. This DNA enters cells and their nuclei in which it can be integrated and expressed, acting as a messenger DNA (Gahan, 2003).

Thirty years ago, Steele (1979) presented a 'somatic selection hypothesis', which suggested that endogenous retroviral vectors would capture mRNA from somatic cells and transduce them into germline cells. Once inside, the mRNA would be reverse transcribed and spliced into the genome of the cell by recombination. In recent years, long-distance movement of mRNA has been well documented by plant-grafting experiments (Lucas and Lee, 2004). With the establishment that novel mRNA species may move between cells and around the plant, and the ability of retroviruses or retrotransposon-encoded reverse transcriptases to mediate reverse transcription of mRNA into cDNA capable of being integrated into the genome, mechanisms also exist for horizontal gene transfer from stock to scion and vice versa by grafting (Adler, 2001; Hirata *et al.*, 2003).

In the history of genetics, neglecting certain findings and phenomena is not uncommon. For example, both Mendel's laws of heredity and McClintock's study on transposable elements were ignored for decades. Graft hybridization is potentially a simple and powerful means of plant breeding. It is to be hoped that the new results reported by Stegemann and Bock will once more

direct the attention of geneticists to a challenging, yet unaccepted and unexplained, phenomenon. Stegemann and Bock (2009) have confirmed the exchange of large pieces of plastid DNA at the immediate graft site by ordinary grafting. Further experiments are needed to confirm whether both nuclear DNA and plastid DNA transport are possible over longer distances after mentor grafting.

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