Chimeras and Variegation: Patterns of Deceit

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Variegation and chimerism are phenomena with both economic and scientific importance for horticulture and for the study of plant development. Yet, there remains considerable confusion in the literature regarding the meaning of these terms and the controlling mechanisms for each phenomenon. Popular magazines, texts, and, less commonly, research journals contain misuses of the terms "variegation" and "chimeras" or make gross generalizations about variegated or chimeral plants using a limited number of examples. Plants are called chimeras when they are not, or are not recognized to be chimeral when they are chimeral. Frequently, it is the exception to the rule that initiates the confusion. In this review, I present a comprehensive treatment on the causes of chimerism and variegation with special emphasis on defining terms and discussing the genetic and anatomical reasons why variegation and chimerism exist in higher plants. The differences and similarities between variegation and chimerism will be considered. Finally, I will introduce some unusual cases where variegation patterns are difficult to interpret, and demonstrate what steps or tests should be performed before assumptions are made about the cause of variegation pattern on a particular plant.

WHAT IS VARIEGATION?

Variegation can be defined as the presence of discrete markings of different colors on an organ or an organism. In animals, zebras and leopards are classic examples. In plants, variegation is most frequently manifested as stripes, blotches, and streaks, or by differences in color between leaf (or petal) margins and the leaf (or petal) midregion. Technically, nutritional disorders such as iron chlorosis, or pest damage such as that caused by spider mite feeding, can lead to a variegated phenotype. In this review, however, I will restrict my comments to variegation that is genetically controlled.

Variegation types can be categorized as either cell lineage type or noncell lineage type (Kirk and Tilney-Bassett, 1978). With few exceptions, cell lineage variegation occurs in genetic mosaics (individuals with cells of different genotypes). The cells of one color are clonally related, so that each colored layer, streak, or patch is descended by successive cell divisions from the original cell in which the color change occurred (Fig. 1, top left and right). Since somatic plant cells do not migrate, cell lineage variegation patterns are related directly to cell division planes, and patch (clone) size and shape is related to the duration and rate of cell division, and, to a lesser extent, to differences in the magnitude of cell elongation.

In plants with noncell lineage variegation, all cells have the same genotype but the genes responsible for the synthesis or destruction of pigments are expressed in only some of the cells. This kind of variegation is very common in both animals (e.g., the black and white regions on panda bears and bald eagles) and plants [e.g., the leaves of *Caladium bicolor* and the stripes on watermelon (*Citrullus lanatus*) rind]. With noncell lineage variegation there is no necessary relationship between the pattern or rate of cell division and the pattern of colors. Instead, the "geographic" location of the cell, rather than the cell's lineage or history, ultimately determines its phenotype (Fig. 1, bottom left and right). With noncell lineage variegation all cells have the genes necessary to make or destroy the pigment but only those in the "correct" location are "instructed" to do so. The patterns seen are the result of differential gene expression between genetically identical cells.

Noncell lineage variegation patterns can be transmitted sexually from one generation to

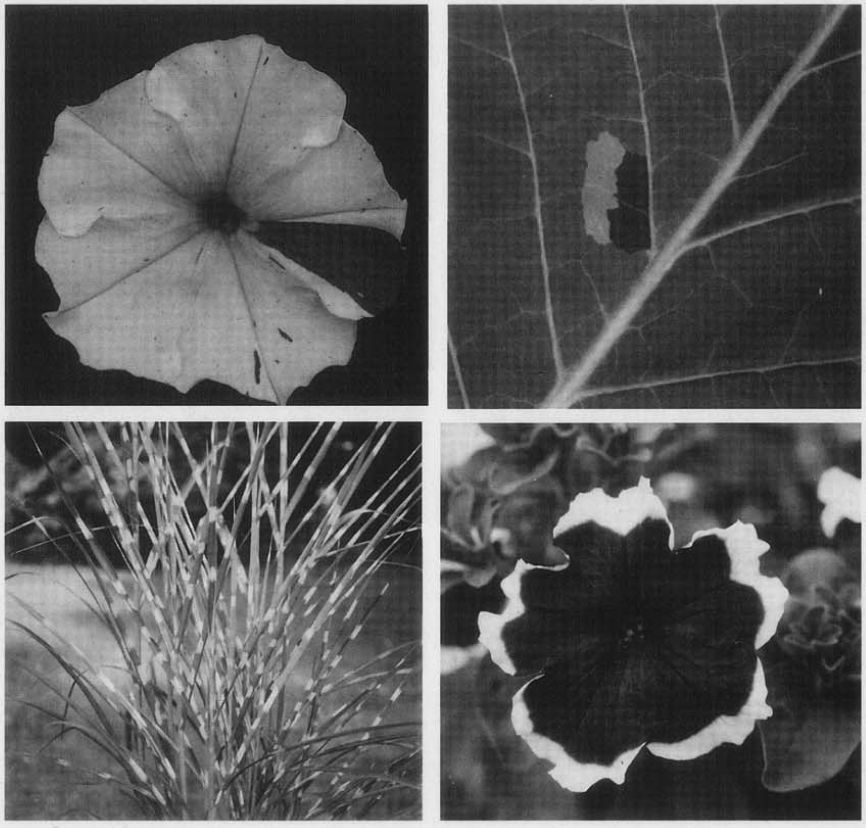

Fig. 1. Types of variegation as they relate to cell position or cell lineage. Cell lineage variegation is displayed in (**top left**) and (**top right**), noncell lineage variegation in (**bottom left**) and (**bottom right**). (**top left**) Genetic mosaic in *Petunia ×hybrida* caused by a transposable genetic element (Doodeman et al., 1984). Red sectors on white corolla are directly descended from cells that have mutated. Larger sectors were initiated earlier in development. (**top right**) Variegation caused by somatic crossing-over. Yellow-leaf *Nicotiana tabacum* (*Su/su*) with a green (*su/su*) and white (*Su/Su*) twin spot. In this photo, the heterozygous tissue appears light gray and the homozygous tissue is white or dark gray. The spots are direct descendants from a cell that underwent somatic crossing-over. (**bottom left**) *Miscanthus sinensis* 'Strictus' (porcupine grass). Cell divisions during leaf development in grasses proceed from meristematic tissue at the leaf base. This horizontal banding does not follow cell lineage. (**bottom right**) Variegation expressed as a floral pattern. *Petunia ×hybrida* 'Hulahoop Blue' possessing purple flowers edged in white. The "white" cells are white because of their position, despite the fact that many are derived from the "purple" cells.

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the next. As with most other traits, variegation patterns vary slightly within a population as interactions with other genes and the environment alter gene expression. Noncell lineage variegation patterns can be inherited simply (e.g., Henny, 1982, 1983; Reisch and Watson, 1984) or complexly (Boye, 1941; Boye and Rife, 1938), and may have evolved when selection pressures generated patterns that either act as camouflage against herbivores or as patterns to attract pollinators (e.g., pigment patterns in flowers). Nonchimeral noncell lineage variegation is the most common cause of variegation.

PATTERN VS. NONPATTERN VARIEGATION

Frequently, variegation is described as patterned or nonpatterned. These terms merely describe phenotype and yield little information. Pattern variegation implies a predictable, regular, repeated pattern between leaves (or flowers, or stems) on the same plant. So, e.g., if all variegated leaves or bracts are green with a white margin (Fig. 2), they would be considered patterned whereas the corolla of the petunia in Fig. 1, top left, is nonpatterned, instead displaying a random splashing of color with the extent of each color being variable and unpredictable for each flower on the plant.

WHAT ARE THE CAUSES OF VARIEGATION?

1. *Differential gene expression.* The most common and most misunderstood cause of variegation is differential gene expression. Expression of genes only in specific parts of an organ or in specific cell layers can give rise to this phenotype. Although a multicellular organism can be genetically homogeneous, all genes are not turned on at all times in all organs, tissues, or cells. For example, at the organ level, petals can produce pigments not produced by leaves. At the tissue level, the epidermis can produce a pigment not produced by underlying mesophyll. At the cellular level, cells located near the margin of an organ (e.g., a petal edge) can produce or fail to produce a pigment that cells of the same genotype produce in a different location. Therefore, positional signals that regulate gene expression can determine variegation pattern. Most variegated plants that come "true to seed" fall into this category. Examples include striped watermelon rinds, the commercially available petunia flowers with bicolored corollas, the "faces" on pansy (*Viola wittrockiana*) flowers, and the purple rings on leaves of zonal geranium (*Pelargonium ×hortorum*).

2. *Leaf blisters.* In a few species, variegation manifests itself as silver spots or streaks on leaves. This is not caused by achlorophyllous tissue, but rather from an unusual developmental pattern where, on specific regions of the leaf, cells separate from the cells below them, leaving what can best be described as a blister (Fig. 3) (Hara, 1957). This is an inherited pattern variegation (Hoch et al., 1980) and another example of noncell lineage variegation. In some regions, genes responsible for allowing the cells to separate are expressed. A scientifically documented example of this type of variegation is in *Pilea cadieri* (Vaughn and Wilson, 1981), while *Pulmonaria officinalis*, *Zebrina pendula*, and many spotted begonias (e.g., *Begonia maculata*) appear to possess the same phenotype. This type of variegation is generally patterned and, therefore, positional signals may be involved in determining where blisters appear.

Fig. 2. *Euphorbia marginata* ('Snow on the Mountain'), a seed-propagated annual.

Fig. 3. *Pilea cadieri* (aluminum plant). In this species, a pattern of "blisters" appears where the upper cell layers are detached from the lower cell layers. The plant is not a genetic mosaic.

3. *Virus.* Many viruses initiate variegation by causing nonuniform chlorosis. Tobacco mosaic virus, for example, causes a green/chlorotic variegated phenotype on the leaves of many species. Generally, the symptoms are unattractive and the virus is debilitating. Yet, a few viruses add attractive variegation patterns to either foliage or petals and do not severely impair the growth of the plant. Abutilon mosaic virus causes beautiful variegation patterns in the leaves of certain *Abutilon* species. The leaves of *Abutilon pictum* 'Thompsonii' are marked with bright yellow and cream patches delineated by leaf veins (Fig. 4). This virus can be seed or graft transmitted (Fulton, 1964). In some hybrid tulips (*Tulipa*), tepals are variegated because of a virus and the flower is streaked or striped (Fulton, 1964). In certain genotypes, the vegetative portions of the plants may be stunted by this virus, but in others the vegetative portions appear normal. The virus is transmitted to the daughter bulbs and the variegated phenotype is easily maintained by vegetative propagation. Other plants in which the variegation pattern is uncharacterized may be virus-infected. Grafting to nonvariegated plants of the same species may indicate whether or not a virus is the causal agent, as most viruses transmit readily via the graft union.

4. *Genetic mosaicism.* Genetic mosaics are plants or animals in which cells of different genotypes coexist in a single organism. The genetic difference need not be phenotypically obvious, so, e.g., a genetic mosaic could exist for differences in the levels of a biochemical metabolite. It would not be surprising if all long-lived plants (e.g., trees) are genetic mosaics because, in the absence of a flawless system to purge cells that have spontaneously mutated, at least some cells would genetically differ from others. Patches of mutant cells would be scattered randomly throughout the organism. Many bud sports of horticultural importance have arisen in this manner and have been vegetatively maintained as new cultivars. However, most mutations are not phenotypically obvious and go unnoticed. Plants can become mosaics due to the action of transposable genetic elements (Doodeman et al., 1984) or spontaneous or induced muta-

Fig. 4. Leaf of *Abutilon pictum* 'Thompsonii', an ornamental in which the variegation is caused by a virus.

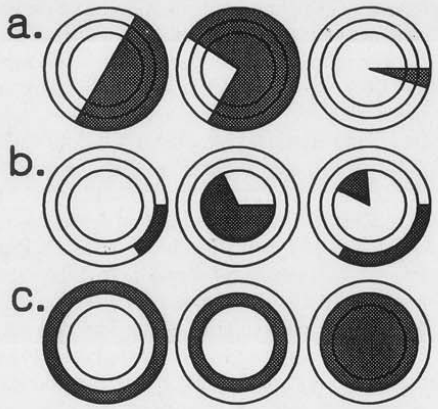

Fig. 5. Diagrammatic representation of cross sections through the shoot apical meristems of chimeral plants showing three independent cell layers for each meristem. Examples are (**a**) sectorial chimeras, (**b**) mericlinal chimeras, and (**c**) periclinal chimeras.

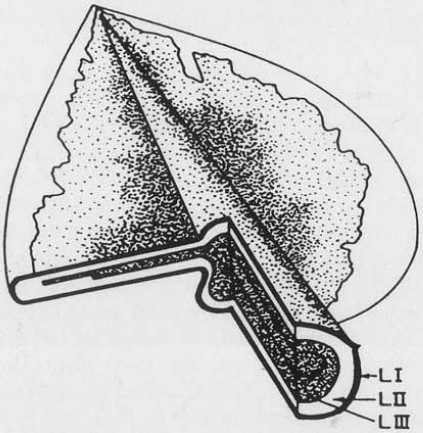

Fig. 6. Leaf of typical GWG dicot chimera. The derivatives of the shoot apical meristem give rise to specific leaf tissue. Since chlorophyll is not expressed in most epidermal cells, the leaf appears to have a white edge. The intensity of green varies because the number of L3-derived cell layers can vary in different positions on the leaf blade (reprinted with permission from Poethig, 1984).

tions in the nuclear or chloroplast genome (Tilney-Bassett, 1986).

CHIMERAS

A plant chimera is a specific type of genetic mosaic in which the genetically dissimilar cells are present in the shoot apical meristem, where they continue to give rise to the cells that form the body of the plant. The arrangement of genetically dissimilar cells in the shoot apex is crucial to the stability of a chimeral state and will dictate the plant's phenotype. Several arrangements are possible because shoot apices in higher plants are multicellular and, with few exceptions, multilayered. Strict anticlinal divisions in the outer shoot apical layer(s) give rise to a stratified meristem with a "tunica-corpus" organization (Schmidt, 1924). Such divisions prevent cells in one layer from invading another layer. Cells in the "tunica" layers divide anticlinally while those in the corpus divide in any plane. Most angiosperms have either two or three well-defined apical cell layers. In most dicots, there are three apical cell layers that remain independent from each other (Tilney-Bassett, 1986), while in monocots, both two- and three-layered apices are common (Stewart and Dermen, 1979).

Although the source of genetic heterogeneity resides in the apical cell layers of the meristem, one can seldom determine if a plant is chimeral by microscopic observation of the shoot apex. The phenotypically evident traits are generally expressed in organs derived from the meristem rather than in the meristem itself. Therefore, the chimeral composition of the shoot apex is most frequently deduced by the phenotype of the organs derived from it. In fact, the verification of the concept of chimeral plants arose from attempts at explaining what caused the white margins on the leaves of variegated geraniums (Baur, 1909). Cytochimeras—chimeras in which different apical layers possess different ploidy levels (Satina et al., 1940)—are the exception, in that longitudinal sections through the apex generally identify chimeras, because apical cell size is frequently augmented with increasing ploidy (Stewart et al., 1972).

Three kinds of chimeras are defined by the arrangement of genetically different cells in the shoot apical meristem. The first is the sectorial chimera (Fig. 5a). With sectorial chimeras a wedge of genetically unique tissue is present through *all* apical cell layers. Sectorial chimeras are difficult to identify because, in most cases, the phenotype of genetically unique cells is not evident in the descendants of all three cell layers. In addition, neat sectors rarely persist, as cells within a layer tend to get "out of line" with cells in other layers as cell division proceeds. Sectorial chimeras would most likely arise when mutations, either spontaneous or induced, occur during the early stages of embryo development when the number of cells and cell layers in the shoot meristem is at a minimum.

Mericlinal chimeras exist when a fraction of one or more cell layers (but not the entire layer) of a shoot apical meristem is genetically distinct from the remainder of the layer (Fig. 5b). Because the fraction size is variable and the position of the fraction is variable, there are endless potential arrangements for mericlinal chimeras. To the untrained eye, mericlinal chimeras frequently appear as sectorial chimeras, because in some plants the unique phenotype of a deep layer is masked by overlying tissue, or a tissue layer may not express a phenotype in its present position (i.e., positionally dependent gene expression). Mericlinal chimeras are somewhat unstable, and axillary buds on them tend to give rise to either solid shoots or periclinal chimeras. In addition, although positional exchanges between cells in different layers tend to be restricted, within a cell layer there is lateral displacement, and mericlinal chimeras often revert to periclinal chimeras or become nonchimeral.

Periclinal chimeras are chimeras in which one, or more than one, *entire* apical cell layer is genetically distinct from another apical cell layer (Fig. 5c). Periclinal chimeras could not exist in the absence of a tunica corpus meristem organization since it is the orderly division planes in tunica corpus meristems that allow cell layers to remain autonomous. There are six possible arrangements of two genotypes (A and B) for shoot apical layers in periclinal chimeras in species with a two-layered tunica and a corpus (i.e., ABB, BAA, AAB, BBA, ABA, BAB). If each genotype results in a unique phenotype, it is possible for the different periclinal chimeras to have unique phenotypes. However, this is generally not the

case because phenotypic expression of genes may not occur in all layers.

The relationship between apical cell layers and the organs they generate must be clear in order to understand the role of apical cell layers in periclinal chimeras. With little variability, each shoot apical cell layer makes a predictable contribution to the leaf and leaf blade (Fig. 6). In most dicots, leaf epidermis is derived solely from the outer shoot apical layer (L1). The second apical layer (L2) gives rise to palisade parenchyma, as well as all of the spongy parenchyma of the leaf margin. The innermost apical layer (L3) gives rise to the upper and middle layers of the spongy parenchyma, but makes no contribution to the blade margin (Burk et al., 1964).

The relative contribution of each apical layer to developing leaf primordia is generally quite consistent within a plant but somewhat variable in different species or cultivars. There are several factors that influence the extent to which each apical layer contributes to leaves and this influences leaf phenotype in variegated plants. One factor is whether or not the mutation leading to changes in pigment also disables the cell in other ways, making it less fit to compete with its neighboring wild-type cells. If, for example, the cell division rate of albino cells is lower than that of wild-type cells, the latter may make an atypically large contribution to areas of leaf tissue normally developed from cells in the position of the albino cells (Stewart et al., 1974). In addition, some species do not show the same lineage relationships between the shoot apex and mature organs. In some Rosaceous plants, for example, L3 derivatives, while present in the leaf midrib, make little or no contribution to the leaf blade itself, and the blade generally consists of an epidermal cell layer of L1 origin surrounding leaf mesophyll of L2 origin (Dermen and Stewart, 1973). In most monocots, however, L1 derivatives divide periclinally to form the entire edge of the leaf blade, and there may be no independent L3 present in the shoot apex (Stewart and Dermen, 1979). Yet, because the L1 divides periclinally along the leaf margin, leaves of even two-layer monocots can be variegated with a white L1-derived leaf border on an otherwise green leaf (W-G) or the reciprocal green-bordered white leaf (G-W).

In the most common form of chimeral plant, the green and white plastid chimera, all six periclinal chimeras do not possess a unique phenotype (Fig. 7). With the exception of guard cells and, in some plants, trichome glands, chlorophyll is not generally present in epidermal cells. Consequently, the leaves of a WGG (White-Green-Green) plant look dark (normal) green. Microscopic examination of the guard cells would reveal white plastids. In addition, an occasional white fleck might appear on the leaf edge where an unusual periclinal division repositioned an epidermal cell into mesophyll, where it would grow into a small white mesophyll clone. The converse chimera, GWW (which is generally lethal unless it is present as a branch fed by a green or variegated branch), would have what might appear to be all white leaves. However, the presence of chlorophyll in guard cells frequently results in a more yellow leaf, especially when the leaf is not fully expanded and chlorophyll-bearing guard cells are in close proximity. To complicate matters, there are several different mutations that can lead to an achlorophyllous cell phenotype and, in some, the chlorophyll will degrade only as leaves expand and age or the temperature becomes permissive. Thus, mutant tissue appears yellow at first but later becomes white. This condition makes the distinction of a GWW and a WWW leaf possible in many chimeras, since in some GWW shoots the immature leaves appear greenish yellow, rather than white.

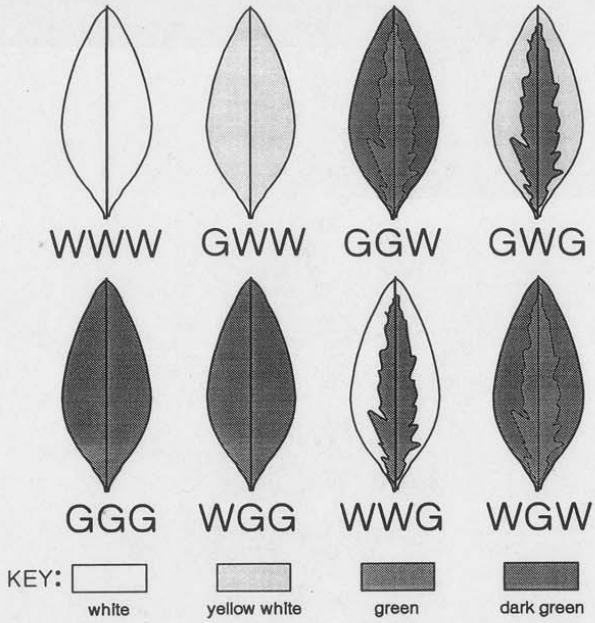

Fig. 7. Leaves from WWW, GGG, and six green (G) and white (W) periclinal chimeras.

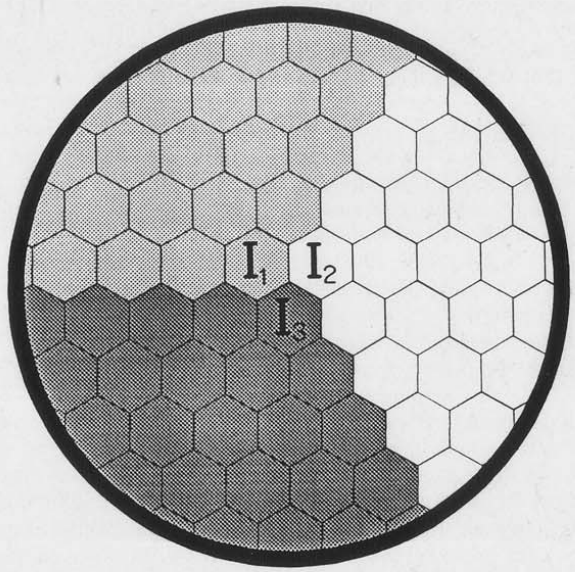

Fig. 8. Diagrammatic representation of the surface of a shoot apical meristem. Within each apical layer there are believed to be apical initials (the most stable number may be three), each of which gives rise to cells within the layer. The condition depicted is not permanent, as the daughter cell of an initial may replace another initial and begin contributing to a major sector of the layer. Although only L1 is depicted, apical initials also exist in the subtending L2 and L3.

The maintenance of a chimeral shoot apical meristem is dependent on the arrangement of cells in the meristem. Periclinal chimeras are the most stable form, as apical layers remain independent and axillary buds possess the same apical organization as the terminal bud from which they were generated (Marcotrigiano, 1991). Periclinal chimeras, therefore, can be propagated by stem cuttings, grafting, leaf-bud cuttings, or division.

The type of chimera observed depends on the position of the mutant cell in the meristem. By analyzing chimeras, the "behavior" of apical cells has been deduced. At the summit of the shoot apex and within each apical cell layer there are "apical initial cells" that give rise to

Fig. 9. *Eleutherococcus sieboldianus* 'Variegatus' (=*Acanthopanax sieboldianus* f. *variegatus*), a periclinal chimera. An atypical cell division in an axillary bud changed the periclinal composition of the meristem from GWG to GWW on the branch indicated by the arrow.

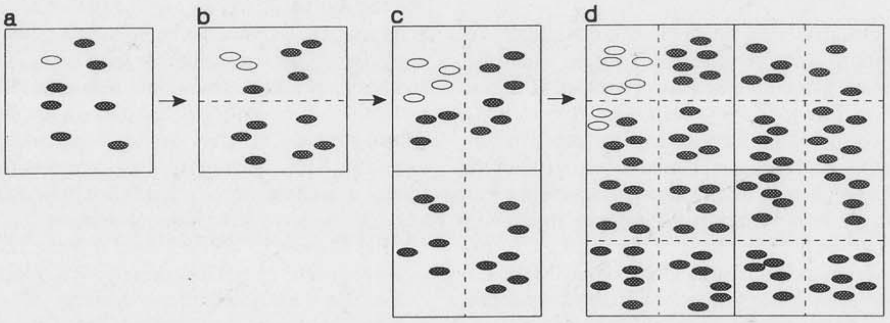

Fig. 10. Plastid mutation leading to variegation. (**a**) A single mutant plastid (unshaded) in a cell results in a heteroplastidic cell. As cells and plastids divide (**b** and **c**), a homogeneously white cell can arise (**d**). Dotted lines represent planes of cell division.

all of the cells of that layer and ultimately the body of the plant. Although considerable controversy exists regarding the number and permanence of these initials (e.g., Klekowski, 1988; Newman, 1965; Stewart and Dermen, 1970a), there seems to be more support for the concept that apical initials, while normally remaining within a layer, are not indefinitely fixed in position and can be periodically, and perhaps frequently, supplanted by their own descendants or by an adjacent apical initial from the same apical layer (Fig. 8). Much more infrequently, cells from one layer can divide periclinally to establish a new cell line in a different layer. When cells from an inner layer take over the position of a cell from an outer layer, "displacement" is said to occur while "replacement" occurs when there is a periclinal division of a cell in an outer layer which invades an inner layer (Stewart and Dermen, 1970a). Displacements and replacements cause "layer switching" in periclinal chimeras, frequently resulting in "bud sports" on chimeral plants (Fig. 9).

In summary, the persistence of a chimeral state in higher plants is dependent on the stability of apical cell layers and the number and persistence of apical initial cells in each apical cell layer. Chimeras occur when different genotypes, rather than merely different patterns of gene expression, occur in independent apical cell layers. Therefore, chimeral variegation is not variegation typical of a species and plays no natural role in camouflage or pollination. Periclinal chimeras are unlikely to persist if they arise in nature because they 1) cannot be seed propagated, and 2) frequently are at a selective disadvantage because (if they are plastid chimeras) they may have a slower growth rate.

ORIGINS OF CHIMERAS

1. *Genetic changes in nuclear or chloroplast genome (either spontaneous or induced).* Any genetic change in one or more cells in a multicellular shoot meristem can result in the generation of a chimera. Pigment mutations are obvious and one could mistakenly conclude that they occur more frequently than other mutations. Mutations can also result in the absence of morphological features. Some "thornless" (i.e., more correctly "prickleless") blackberries are indeed periclinal chimeras with "thornless" L1 surrounding a "thorny" inner core (McPheeters and Skirvin, 1983).

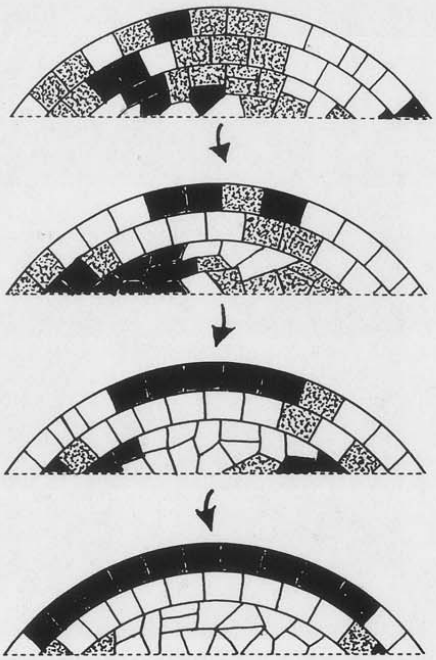

Fig. 11. Longitudinal section through a heteroplastidic shoot meristem. White cells contain all mutant plastids, black cells contain all normal plastids, and stippled cells are heteroplastidic. Eventually, cells with sorted plastids are present in the terminus of the shoot apex and, eventually, a periclinal chimera can result.

The plant appears to be homogeneously thornless because "thorny" genes are not expressed in nonepidermal cells. Adventitious shoots that arise from roots, however, are "thorny" as they originate from internal cell layers.

The most common cause of chimeral variegation is spontaneous mutation in the pathway to chlorophyll synthesis or plastid morphology. These mutations are generally chloroplast mutations rather than nuclear mutations. Nuclear mutation is a less likely cause of variegation in chimeras for two main reasons. First, since most albino mutations are recessive, spontaneous somatic mutations of the nucleus would lead to a heterozygous condition and the affected cell would remain green. Second, if the heterozygous mutant cell eventually gave rise to gametes, the next generation would yield a population of albino seedlings and green seedlings rather than variegated seedlings. In contrast, a mutation in chloroplast DNA would lead to a single albino chloroplast coexisting in a cell dominated by green chloroplasts. A cell having two types of plastids is said to be "heteroplastidic." During cell division, chloroplasts are also dividing and, in time, stochastic processes lead to a "sorting out" that eventually results in the production of a cell line with only one type of chloroplast (Fig. 10). When a cell is completely "sorted out" (i.e., contains all normal or all mutant plastids), all of its descendants will be identical to it and the cells will be "homoplastidic." The plant's phenotype will be a mosaic of green and white or yellow patches of various sizes depending on the time the sorting out occurred (Jagannathan and

Fig. 12. Interspecific mericlinal graft chimera composed of *Nicotiana tabacum* (T) and *N. glauca* (G). The larger lighter leaves are T. The darker leaves have a T epidermis covering G mesophyll (from Marcotrigiano and Gouin, 1984b).

Marcotrigiano, 1986). For example, if a white cell line is established early in leaf development, a large white patch will appear on the leaf. Only after the apical initial cells are sorted out can a stable periclinal chimera be generated (Fig. 11). In some cases, this does not happen before the vegetative meristem becomes a floral meristem and eggs, containing a mixture of mutant and normal proplastids, are generated. These eggs will generate mosaic seedlings with the relative amount of white or green tissues being influenced by the relative proportion of green and white proplastids in the egg cell. In most plants, plastids are maternally inherited (Kirk and Tilney-Bassett, 1978), so crosses using a green female and a nonsorted variegated male yield only green offspring. The reciprocal cross, however, will yield green, white, and variegated seedlings in a non-Mendelian ratio that is influenced by the plastid composition of the individual eggs within the egg population. Less frequently, plastids are biparentally inherited. The most notable horticultural example is *Pelargonium ×hortorum* where the male gamete contributes proplastids during fertilization (Tilney-Bassett and Birky, 1981).

Genetic mosaics are easily induced by ionizing radiation or chemical mutagens if the target area is multicellular, because the cell cycle is generally asynchronous in a given multicellular organ. This fact has led to numerous technical problems for mutation breeders who generally desire to develop nonchimeric nuclear mutants, but instead recover genetic mosaics that may or may not carry the mutation through a sexual cycle (Broertjes and Van Harten, 1978). The use of mutagens can result in complex mosaics that, in time, may become periclinal chimeras. Spontaneous nuclear mutations in the shoot apical meristem can also generate chimeras but, if they are recessive, they may go unnoticed.

2. *Transposable genetic elements*. In certain species and in certain genetic backgrounds, a series of genetic elements can occasionally be transposed from one position on a chromosome to another position (either on the same chromosome or a different chromosome) or cause chromosome breaks, resulting in a genetic mosaic. When the insert interferes with the function of a gene and the gene codes for a pigment, variegation can occur. This phenomenon, described many years ago in maize (*Zea mays)* (Rhoades, 1938) also occurs in petunia (Fig. 1a) (Doodeman et al., 1984), and *Ipomoea* (Epperson and Clegg, 1987), and is probably the cause of many of the irregularly flecked flower types that exist in ornamentals. If the excision occurs late in development, it generally results in the appearance of small flecks. Upon careful inspection, these markings can be seen to follow cell lineage. When transpositon occurs early in development, large clones, also following cell lineage, will develop. A periclinal chimera can be generated from the early action of a transposable element (Doodeman and Bianchi, 1985), assuming that an entire apical cell layer becomes a clonal population of the new genotype.

3. *Graft induced chimeras*. Oddities resulting from the grafting procedure have been noticed during the millennia that plants have been grafted. Adventitious shoots occasionally arise from the region of the graft union. A shoot may originate from more than one cell and cells from both rootstock and scion can contribute to the generation of the shoot apical meristem. Such incidents result in the production of what were once called "graft hybrids" because even such prominent scientists as Charles Darwin (1868) believed that the fusion of nuclear material occurred at the graft union. We now know that the phenotypically unique shoots that occasionally arose from graft unions were actually graft chimeras with shoot apices composed of cells derived from both rootstock and scion. This knowledge has permitted the intentional synthesis of graft chimeras to study basic aspects of plant development (Marcotrigiano, 1985, 1986a; Marcotrigiano and Bernatzky, 1995; Szymkowiak and Sussex, 1993; Tian and Marcotrigiano, 1993, 1994) or to create unique woody plants (Bergann and Bergann, 1984). The procedure involves the removal of all but a thin layer of scion stem after a splice or saddle graft has healed, followed by the removal of all adventitious shoots that are not arising from the region of the graft union. Although most of the shoots generated in this manner are nonchimeral, mericlinal or sectorial chimeras are occasionally recovered (Fig. 12) and eventually give rise to periclinal chimeras.

4. *Semigamy*. Semigamy, a rare phenomenon, is caused by an abnormal fertilization where the male nucleus penetrates the egg cell but does not fuse with the egg nucleus. The female and male nuclei divide independently, resulting in a chimeric embryo with patches of tissue of maternal or paternal origin (Turcotte and Feaster, 1967). The chimeras are sexually derived and are haploid plants composed of cells of both sexual partners. When phenotypically marked lines are used (e.g., a yellow-leafed male and a purple-leafed female) it is

Fig. 13. *Zea mays ij/ij*.

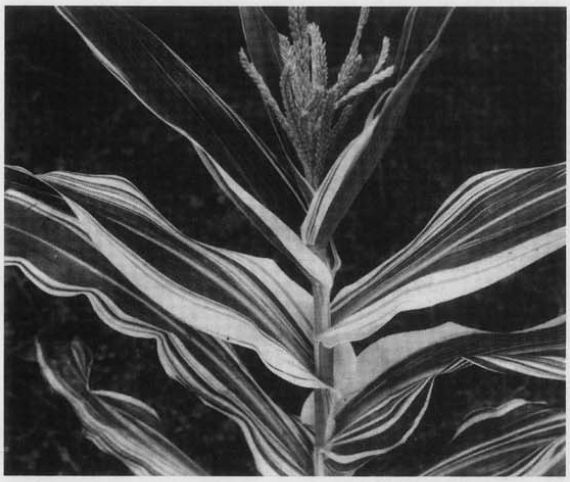

Fig. 14. *Zea mays j1/j1*.

possible to trace the origin of the male-derived and female-derived cells. It is then possible to trace the lineage of embryonic cells to develop a fate map (Christianson, 1983) or to study the role of specific genes in the tissues of developing leaves (Dolan and Poethig, 1991).

5. *Tissue culture synthesis of chimeric meristems*. The intentional synthesis of chimeras in cell culture has met with very limited success (Marcotrigiano, 1990). There is meager evidence that mixed cell suspensions (Carlson and Chaleff, 1974; Marcotrigiano and Gouin, 1984a) or mixed protoplast cultures (Binding et al., 1987) can generate chimeral shoots. A system of in vitro grafting that relies on the generation of adventitious shoots from cultured graft union tissue has been more successful (Noguchi et al., 1992)

CULTIVAR DESIGNATION AND NOMENCLATURE IN VARIEGATED AND CHIMERAL PLANTS

The nomenclature regarding variegation and chimeras can be confusing. With the exception of graft chimeras, a plant name tells us little about the control of variegation and chimerism.

Some species have been named because they possess variegated patterns. For example, *Dracaena marginata* has green leaves with reddish leaf margins. This pattern is typical of the species and is not chimeral. Many variegated chimeras that have been in cultivation for a long time have cultivar designations such as 'Albo-Marginata' that describes white margined leaves, 'Aureo-Maculata' that describes yellow centered leaves, or 'Variegata' that denotes variegation but does not imply location of the pattern. These names, however, do not necessarily indicate genetic mosaicism as a cause for the variegation.

In periclinal chimeras, different periclinal arrangements can result in different phenotypes. Spontaneous rearrangements in apical cell layers are commonly designated as different cultivars. *Vinca major* 'Elegantissima', for example, is a GWG periwinkle with white leaf margins. It gave rise by layer rearrangement to *V. major* 'Oxoniensis', a GGW chimera possessing green leaf edges and light-green leaf center (Tilney-Bassett, 1986). Many new cultivars of *Sansevieria trifasciata* arise this way. For example, *S. trifasciata* 'Hahnii Favorite', a GWG chimera, will occasionally sport to *S. trifasciata* 'Hahnii Solid Gold', a slow-growing GWW chimera possessing bright-yellow leaves with a narrow green edge (Chahinian, 1986). The two-layered monocot *Chlorophytum elatum* is available in a WG cultivar ('Albomarginatum') and a GW cultivar ('Medio-variegatum') (Collins, 1922; Tilney-Bassett, 1986). In *Dracaena* (Pohlheim, 1982) and in *Rhododendron* (Pogany and Lineberger, 1990) rearrangement of apical layers in chimeras has led to the release of new cultivars. In addition, independent mutations occurring in the same apical layer of different individuals can result in, e.g., two unique GWG cultivars with different "white" genotypes. Two different GWG cultivars may exist within the same species, the only difference being the relative amounts of green and white on the leaves. The contribution of each apical layer to leaf development may depend on the particular mutation. In many cases, the genetic background of the plants giving rise to the mutation is noticeably different. For example, many *Pelargonium ×hortorum* cultivars of independent origin are GWG chimeras, yet differ for traits such as flower color or height. There are GWG chimeras in *Sansevieria trifasciata* that differ in cell fitness and growth habit (Marcotrigiano and Morgan, 1988).

When periclinal chimeras are synthesized via grafting and are released as ornamentals, a "+" sign is given to indicate that the plant is not a true hybrid. The "+" is placed before the genus in intergeneric chimeras and before the species in interspecific chimeras. For example, the interspecific chimera *Camellia* +'Daisy Eagleson' is a graft chimera between two species, *Camellia sasanqua* and *C. japonica* (Stewart et al., 1972), while the cactus *+Hylocalycium singulare* is an intergeneric graft chimera probably composed of *Hylocereus undatus* and *Gymnocalycium mihnaovichii* var. *friedrichii* f. *rubra* hort. (Heath and Histed, 1987; Rowley, 1989). *+Laburnocytisus adamii* is an intergeneric graft chimera composed of *Cytisus purpureus* and *Laburnum anagyroides* (Tilney-Bassett, 1986). In the past, some graft chimeras were designated as new genera (as in the two intergeneric chimeras listed above), and in some situations each periclinal arrangement was given a new specific epithet (Table 1.3 in Tilney-Bassett, 1986). Synthesized graft chimeras that are used for research purposes, however, are not generally given new species names but instead are identified by the constitution of the shoot meristem (e.g., Chimera TTG = *N. tabacum*, *N. tabacum*, *N. glauca*, L1, L2, and L3, respectively) (Marcotrigiano and Gouin, 1984b).

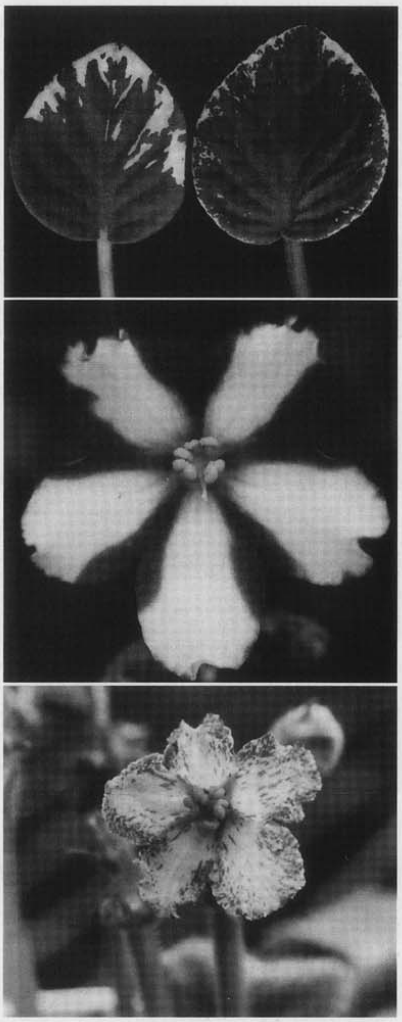

Fig. 15. *Saintpaulia ionantha*. (**top**) Variegated leaves of 'Tommi Lou'. Note how variegation pattern is inconsistent with a pattern that follows cell lineage and appears as a coalescence of white regions. (**center**) 'Mauna Kea' has a "pinwheel" type of variegated flower. (**bottom**) 'Fantasy Beauty' has so-called "fantasy" markings, which are similar in phenotype to those of a petunia with variegation known to be caused by the action of a transposable genetic element (see Fig. 1, top left).

PATTERNS OF DECEIT

The following examples are a sampling of scientifically studied variegated plants with variegation patterns that are easily misinter-

Fig. 16. (**top**) *Euonymous japonicus* 'Mediopictus' and (**bottom**) *Pelargonium* ×*hortorum* 'A Happy Thought', GGW periclinal chimeras with "unmasked" L3 derivatives.

preted. The purpose here is to caution anyone prone to gross generalization to be careful when confronted with a variegated plant that has yet to be investigated scientifically.

1. *Plastid mutations controlled by the nucleus (e.g.,* Zea mays iojap*).* The *iojap* gene is a recessive nuclear gene in maize that, in homozygous plants (*ij/ij*), causes green and white stippled and striped leaves (Fig. 13). This gene impairs the development of some of the plastids (Coe et al., 1988; Jenkins, 1924), resulting in a ribosomeless condition (Walbot and Coe, 1979). The *ij/ij* plants breed as if they were unsorted chloroplast chimeras under cytoplasmic control, so that when *ij/ij* plants are either self-pollinated or used as females in crosses to wild-type males, the offspring that result will fall into three classes—white, green, and variegated—in no particular ratio. The *ij/ij* plants not only carry a nuclear gene that can cause the *iojap* phenotype in subsequent generations, but have the breeding behavior of a plastid mutant when crossed to wild-type males.

2. *Mimicry of chimeral patterns (e.g.,* Euphorbia marginata, Zea mays japonica, *and* Saintpaulia ionantha). In some species, the pattern of variegation can mimic a periclinal chimera. At first glance, the uniquely colored areas appear to be clonal in origin, following cell lineage. *Euphorbia marginata* leaves and bracts look just like those of typical variegated periclinal chimeras (Fig. 2). However, the pattern is seed transmitted and is expressed only in the upper part of the plant. In this nonchimeral species, pattern genes are responsible for noncell lineage variegation. In the *japonica* mutants of *Zea mays* (*japonica-1* and *japonica-2*), the leaves appear to be striped, as in many periclinal chimeras in monocots (Fig. 14). However, while the pattern appears to be one of clonal origin, there is no evidence that this variegation results from unstable mutations or that heritable changes occur in chloroplasts as in *iojap* (Poethig, personal communication). The *japonica* trait is inherited as a single recessive gene and can be moved into any maize line. Its mode of action is not known.

Perhaps no other species has sparked so much debate with regard to the underlying causes of variegation as has *Saintpaulia ionantha*. African violets can have variegated leaves with a pattern reminiscent of a periclinal chimera. Upon close inspection, however, although leaf margins are white, the white border is somewhat dappled (Fig. 15, top). The proportion of white to green tissue is variable and appears to be dependent on genetic background and growing conditions. The phenotype is maintained in adventitious shoots originating on leaf cuttings and can be transmitted sexually to develop new variegated cultivars (Ralph Robinson, personal communication). African violet flowers can also be variegated, either in a pattern termed "pinwheel" (Lineberger and Druckenbrod, 1985; Fig. 15, middle) or with random patches and streaks called "fantasy markings" (Fig. 15, bottom). "Pinwheel" violets must be propagated from axillary shoots. Plants propagated from leaf cuttings normally have nonvariegated flowers. In contrast, fantasy violets will usually maintain their phenotype when propagated from adventitious shoots (i.e., leaf cuttings).

Based on histological observations, Naylor and Johnson (1937) thought that the adventitious shoots that develop on petioles of African violet leaf cuttings originate from the epidermis of the leaf, a fact which would make the maintenance of periclinal chimeras by leaf cuttings impossible. As plant chimeras are useful subjects for elucidating the origin of adventitious shoots (e.g., Marcotrigiano, 1986b), this histologically derived conclusion gained support from the experimental dissociation of true periclinal chimeras. Pohlheim (1981), using mutagenesis, recovered a WGG periclinal chimera (as evidenced by white plastids in guard cells). Leaf cuttings from this phenotypically green-leafed plant gave rise to all WWW (albino) plantlets. Peary et al. (1988) investigated a radiation-induced variant of African violet that had white leaves and was maintained in vitro. Guard cells of the plant contained green chloroplasts, indicating that the L1 was genetically green and, therefore, the plant was a GWW chimera. In vitro propagation from leaf cuttings of the "white" leaves produced all green plants, further supporting the epidermal origin of adventitious shoots.

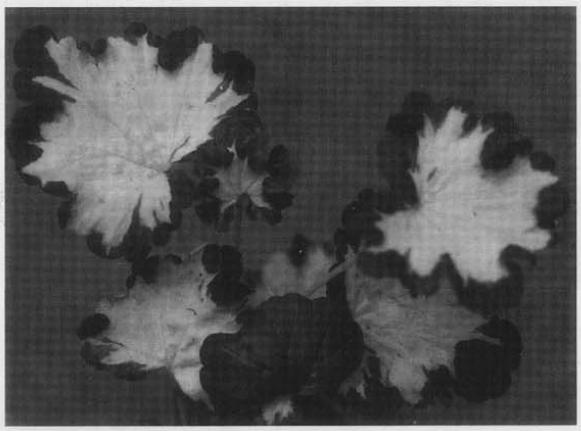

Fig. 17. *Pelargonium* ×*hortorum* 'Freak of Nature', a viable GWW periclinal chimera.

Fig. 18. (**left**) *Juniperus davurica* 'Expansa Aureospicata', a WG periclinal chimera. Green portions are WG with white epidermis having no effect on phenotype. Yellow-white regions are derived from WW apices, which arose when a periclinal division in L1 displaced L2 cells out of the shoot apex. (**right**) *Spiraea* ×*bumalda* 'Anthony Waterer' displaying a similar unstable phenotype.

However, Smith and Norris (1983) and Sunblade and Meyer (1982) demonstrated that commercially available cultivars with variegated leaves could be micropropagated from leaf segments without loss of variegation pattern. This experiment was later repeated and interpreted by Norris et al. (1983) to indicate that regenerated plants were of multicellular origin, with meristems organized from the derivatives of all three shoot apical cell layers, not just the epidermis. This analysis was based on an assumption that the plants were periclinal chimeras possessing cell layers of different genotype. This conclusion was challenged because this variegated leaf trait can be inherited sexually and the presence of isolated white spots indicates that the pattern does not follow cell lineage (Marcotrigiano and Stewart, 1984). In addition, if the plant were a periclinal chimera, it would be improbable for the original periclinal chimera to be generated from organizing meristems to the exclusion of the other five possible periclinal arrangements. Subsequently, Peary et al. (1988) demonstrated that when a cultivar possessing both variegated pinwheel flowers and variegated leaves was propagated by adventitious shoots, the regenerants all had variegated leaves, but the flowers of most plants were no longer variegated, segregating into two solid color types. Clearly, therefore, Peary et al. (1988) are correct in concluding that all cells in variegated leaves of African violets have the genetic potential to form variegated-leafed plants. Leaf variegation in the commercially propagated variegated violets that have been studied is not the result of the plants being periclinal chimeras. Plants with pinwheel flowers, however, are periclinal chimeras with genetically distinct cell layers coexisting in the shoot apices. The chimerism is manifested as axillary buds develop into flowers composed of genetically and phenotypically different cell layers. Pinwheel violets can be propagated "true to type" only from side shoots developing from axillary buds. Whether floral "fantasy" markings represent the action of pattern genes or unstable genetic elements has yet to be determined. The markings cannot be explained by frequent layer invasion in a periclinal chimera, as the fantasy phenotype is fairly stable via adventitious shoots (i.e., leaf cuttings) and the phenotype can be seed transmitted (Ralph Robinson, personal communication), both traits inconsistent with the behavior of periclinal chimeras.

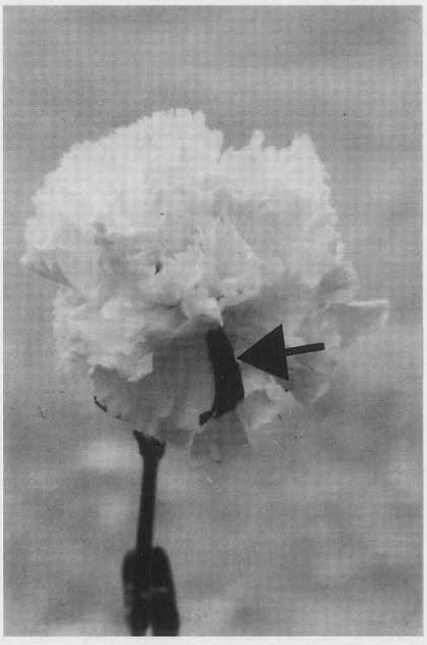

Fig. 19. 'White Sim' carnation, *Dianthus caryophyllus*, a White-Red-Red periclinal chimera. Petals in this species do not contain descendants of L3. If, during petal development, white cells of L1 origin are displaced by genetically red L2 derivatives, the anthocyanins will be expressed in L2-derived epidermis (arrow marks red sector).

3. *Unmasked L3 (e.g.,* Euonymous japonicus *'Mediopictus').* Since GGW chimeras possess a green layer of cells surrounding the white cells, variegation is not strongly manifested. However, in some chimeras (Fig. 16), the L3 may produce a translocatable compound that bleaches out the wild-type L2-derived tissue that is in direct contact with L3 derivatives. This has been proposed for *Euonymous japonicus* 'Mediopictus' (Dermen, 1947) and *Pelargonium* ×*hortorum* 'A Happy Thought' (Tilney-Bassett, 1986). Patterns such as this usually show diffuse boundaries between green and nongreen regions. The bleaching out of L2 "unmasks" the L3, making for a reverse variegation pattern with a light center surrounded by a darker border. Such "dark outside/light inside" patterns are normally only possible in plants (e.g., many monocots) where a green L1 divides periclinally and contributes to the mesophyll of the leaf border, but does not mask the central part of the leaf where the L1 contribution is only one cell thick and does not express chlorophyll except in guard cells.

4. *Atypical cell division planes initiated during leaf development (e.g.,* Filipendula ulmaria *'Aureo-Variegata' and* Pelargonium

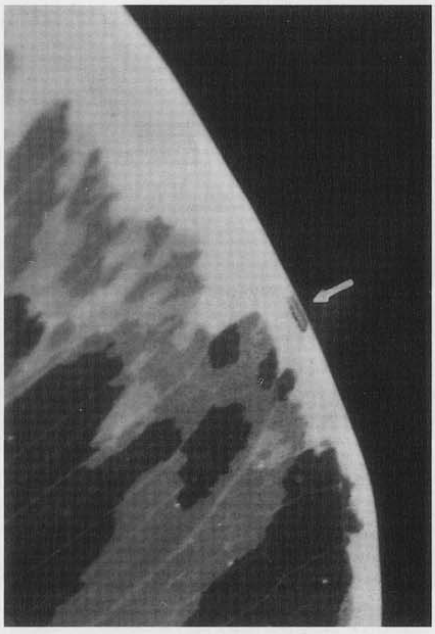

Fig. 20. *Ficus elastica* 'Tricolor', a chimera with a green epidermis overlying white L2-derived mesophyll. Rare replacement of L2-derived by L1-derived cells leads to the genesis of green mesophyll clones on the leaf edge (arrow).

Fig. 21. Plants with multiple causes of variegation. (**left**) *Sansevieria trifasciata* 'Laurentii', a monocot possessing pattern genes that result in horizontal banding of dark green and metallic light green (nonchimeral). The yellowish leaf edge is caused by a chlorophyll-deficiency in L1 (WGG periclinal chimera). (**right**) *Pelargonium ×hortorum* 'Mr. Henry Cox' with pattern genes resulting in a zonal ring of anthocyanin (nonchimeral), and a yellow leaf edge caused by chlorophyll deficiency in L2 (GWG chimera).

'Freak of Nature'). In many green and white variegated plants the albino tissue is disadvantaged so that cells either do not divide as frequently as green tissue or stop dividing sooner. Sometimes this is manifested by twisted or puckered leaves, especially in mericlinal chimeras where the contribution of apical layers is dissimilar on either side of the midrib. In some periclinal chimeras unusual contributions of cell layers to organs can occur (Pohlheim, 1983). For example, GWW chimeras in dicots should be expected to die, as the L1 contribution to chlorophyll is restricted to guard cells, but in some species they do not. In *Filipendula ulmaria* 'Aureo-Variegata', growth inhibition of the chlorotic L2 component results in an increase in periclinal divisions in the epidermis of young leaves, with the leaf edge being composed exclusively of L1 derivatives (Pohlheim and Kaufhold, 1985). This also occurs in *Pelargonium* 'Freak of Nature' (Pohlheim, 1973), where L1 descendants, which normally form only the epidermis, divide periclinally along the leaf margin and make a substantial contribution to the leaf mesophyll (Fig. 17). This does not occur on stem tissue, and stems appear pure white. In both of these plants we see an unusual phenotype in that the white center is "unmasked."

5. *Single-layered tunica with frequent periclinal divisions occurring in L1 (e.g., chimeras in the Cupressaceae and Rosaceae)*. Although most conifers do not possess a tunica corpus meristem, and therefore, cannot exist as periclinal chimeras, some members of the Cupressaceae possess stratified shoot apices with a single-layer tunica surrounding the corpus (Pohlheim, 1971). These WG periclinal chimeras appear green because the L1-derived epidermis is a single cell layer subtended by green tissue. In many species, however, the L1 has the tendency to divide periclinally, thereby invading the mesophyll. When this occurs in the apex, a large WW sector develops on the shoot (Fig. 18, left). Sectors of GG could also be generated if L1 cells were replaced by L2 cells. However, such sectors would look identical to WG sectors and would go unnoticed except for the fact that white sectors would never again spontaneously appear from plants propagated from this type of green shoot. Similarly, some Rosaceous plants appear to have a one-layer tunica. Actually, they have a two-layered tunica but the L3, although sometimes present in the midrib, makes no contribution to the leaf blade as, for example, in peach (Dermen and Stewart, 1973). Therefore WGW and WGG leaves would appear identical. *Spiraea japonica* 'Anthony Waterer', a periclinal chimera, possesses mostly green leaves. However, when L1 divides into L2, the leaf blade can appear white (Fig. 18, right). This phenotype has been interpreted as a case where L1 periclinal divisions replace both L2 and L3. However, it is entirely possible that the L3 rarely makes a contribution to the blade mesophyll and, therefore, L1 cells dividing periclinally can yield white leaves derived from L1 only. The appearance is similar to chimeras in the Cupressaceae that have only one tunica layer.

6. *Differential gene expression within a periclinal chimera (e.g.,* Dianthus caryophyllus *'White Sim' and most GWG periclinal chimeras)*. Some carnations are excellent examples of how positional effects on gene expression can complicate the interpretation of a variegated organ. Sagawa and Mehlquist (1957) performed classic experiments to demonstrate that some carnation clones that appeared to be mutating frequently were actually periclinal chimeras that were undergoing cell-layer displacements rather than mutation. As anthocyanins are not expressed in the subepidermal layers in carnation petals, flowers of red carnations such as 'William Sim' have petals with colored epidermal cells and colorless internal mesophyll. WRR periclinal chimeras, such as 'White Sim', have pure white flowers, even though they breed as if they were red flowers (L2-derived gametes are "red"). Flower color chimeras, therefore, can exist without being phenotypically expressed. Only when genetically red cells from inner layers displace white L1 cell layers are red patches observed (Fig. 19).

Other examples of gene expression influencing chimeral phenotype are periclinal chimeras with green and white leaves. As previously stated, epidermal cells, with the exception of guard cells, do not contain significant amounts of chlorophyll. However, occasionally a periclinal division of the epidermis displaces an L2 derivative and a patch of L1-derived palisade mesophyll will develop. Once positioned in the mesophyll, genes responsible for plastid development and chlorophyll synthesis become active, resulting in a green patch (Fig. 20). In some cultivars of some species this displacement occurs more frequently, resulting in a multitude of green islands on the leaf margin of an otherwise white leaf.

7. *Multiple variegation patterns (e.g., Sanseviera trifasciata 'Laurentii', Pelargonium ×hortorum 'Mr. Henry Cox')*. When more than one pattern of variegation is present on a leaf or flower, there are usually separate causes for each pattern. A chlorophyll mutation can occur in an apical layer of a species that already possesses a nonchimeral variegation pattern. For example, in the monocot *Sansevieria trifasciata*, leaves possess a heritable dark-green horizontal banding pattern that is typical for the entire species. The cultivar 'Laurentii' is a chimera possessing a yellow L1 that results in a yellow leaf edge (Fig. 21, left). Interestingly, the epidermis is also polyploid, resulting in a ridge-like thickening of the yellow tissue on the leaf edge (Chahinian, 1993). The "zonal" *Pelargonium ×hortorum* possesses a ring of anthocyanin on the leaf that is controlled by pattern genes. When a mutation to white or yellow occurs in the L2 the leaf also has a light border (Fig. 21, right).

Less frequently, a chimera that has been in cultivation for a long period of time will undergo an additional mutation and become mutant in more than one layer (e.g., Bergann and Bergann, 1959; Stewart and Dermen, 1970b). Such plants have been coined "trichimeras" (Tilney-Bassett, 1986) to indicate that all three apical layers are a different genotype. In these plants, as in other periclinal chimeras, layer instabilities can result in novel periclinal arrangements. If two cell layers in a three-layered shoot apex have different mutations, there are 24 different possibilities for periclinal chimeras, although it is doubtful that each would have a unique phenotype.

CONCLUSIONS

Before attempting to determine the cause of variegation in a plant certain facts must be remembered. The apical organization of a species determines whether or not a periclinal chimera can exist. Not all plants have tunica corpus meristems. Dicots that do have tunica corpus meristems generally have a two-layered tunica while monocots tend to have a one-layered tunica. There are, however, many exceptions that do not necessarily follow close taxonomic relationships. In addition, adventitious shoots can be multicellular in origin in some species (Bergann and Bergann, 1982; Marcotrigiano, 1986b), unicellular (Arisumi and Frazier, 1968; Broertjes and Van Harten, 1985), or unihistogen in others (e.g., Pohlheim,

1981). A fraction of chimeral adventitious shoots can be recovered from cultures of chimeral tissue (Marcotrigiano, 1986b), although the probability that all of the regenerated plants would have the same periclinal arrangement is extremely low.

Although the terms "variegated" and "chimeral" can be related, all plant chimeras are not variegated, as any genetic difference between apical cell layers can result in a chimera. Conversely, all variegated plants are not chimeras, since in many variegated plants the color pattern is controlled by differences in gene expression that are positionally dependent rather than genotypically dependent. Tests can and should be performed in order to determine whether or not a variegated plant is a chimera. A single test may not yield a definitive answer. The strongest evidence comes from the observation of seedlings following controlled self- and cross-pollinations.

Evidence of nonchimeral variegation includes: 1) the maintenance of variegation pattern in plants generated via adventitious shoots; 2) the transmission of the variegated phenotype to any future generation following sexual reproduction; 3) a variegation pattern that does not appear to follow cell lineage (there are exceptions); and 4) the pattern is typical for members of the uncultivated species.

In contrast, periclinal chimeras are generally characterized by 1) most adventitious shoots sorting into component genotypes; 2) seed progeny being genetically homogeneous and generally representing only one genotype of the chimera (an exception is a heteroplastidic female); 3) variegation patterns follow the cell division patterns (lineage) of the leaf, with tunica corpus meristems yielding leaves with generally consistent and predictable variegation patterns; 4) the occasional appearance of nonvariegated shoots or mericlinal shoots appearing as "bud sports" or as adventitious sprouts from the roots or wounded stems; 5) the lack of the relevant variegated phenotype in wild populations.

Given the diverse cellular organization in apices and leaves in different plant families and the numerous causes of variegation, many beautiful variegation patterns can exist. The causes of variegation clearly are complex and diverse. The utilization of variegated plants to study plant development, therefore, should be performed only on characterized plants.

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