THE ORIGIN AND TRANSMISSION OF A SERIES OF PLASTOGENE MUTANTS IN DIANTHUS AND EUPHORBIA

ROBERT N. STEWART¹

Plant Industry Station, U.S. Department of Agriculture, Beltsville, Maryland

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RECENT demonstrations of the occurrence of DNA and RNA in cytoplasmic organelles (GIBOR and GRANICK 1964) and the demonstration of segregation and recombination of particulate cytoplasmic factors (SAGER and RAMANIS 1963) have stimulated interest in extranuclear inheritance. The extensive literature on the non-Mendelian inheritance of plastid differences has not contributed substantially to the understanding of the genetics of the cytoplasm: first, because of unsuccessful attempts to fit observations to a single model when there are almost certainly several different systems; and second, because of failure to appreciate the effect of the ontogeny of various tissues of the individual plant and its organs upon the patterns of variegation displayed as a result of somatic segregation of mutant and normal plastids.

BURK, STEWART, and DERMEN (1964), on the basis of random segregation of mutant and normal plastids in meristematic cells of the shoot tip and interpretation of the ontogeny of the tissues of the shoot appendages, described many patterns of variegation in tobacco resulting from somatic segregation of a single mutant plastid type. The observation of two series of similar plastid mutants described here suggest a general occurrence of this model of plastid-based inheritance in higher plants.

MATERIALS AND METHODS

The variegated poinsettias, *Euphorbia pulcherrima* Willd. ex Klotzsch. described herein were discovered in commercial cultivars which are propagated asexually by stem cuttings and in the seedlings produced in a breeding program. The genetic base of the poinsettia material is very narrow. The breeding program has been based on commercial cultivars derived by bud sports or somatic mutation from only two original seedlings. The variegated carnations were found among seedling populations derived from very diverse parentage of several *Dianthus* species.

Plants of both genera are easily propagated by rooting cuttings of various branches produced from lateral buds formed at the nodes by the elongating apical meristem. The lateral buds from such normal origins develop from and include in their apical meristem the same hierarchy of histogenic layers as occur in the parent stem. Both plants were also propagated from seed. Both genera store enough food in the seeds that albino seedlings germinate, their cotyledons expand, and the young plants survive for several weeks.

The distribution of green and white tissues and cells within the variegated plants was determined by macroscopic examination and by observation of fresh, unstained sections with phase and

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bright field microscopy. Mature, intermediate and very young tissues were sectioned free-hand or with a cryostat-microtome and mounted in water or sucrose nutrient solution. The proplastids in the meristematic cells could not be observed with the light microscopes and their characteristics were deduced from the plastids in the cells derived from them.

The convention of using L-I, L-II, and L-III to designate the outermost, the second, and the third independent histogenic layers in the apical meristems has been followed. Letters are used in the same sequence to describe the makeup of each histogen with respect to the potential of its proplastids or content of plastid determiners. The letter "G" signifies that all the cells of a histogenic layer contain wild-type proplastids which become normal green plastids at maturity. The letter "W" signifies that cells of a histogenic layer contain only mutant proplastids which in mature cells will be white or pale. The letter "M" is used to describe a histogen from which mature cells are derived which may be green or white and form a mosaic pattern of variegation. Most cells in an "M" histogen are considered to contain a mixture of wild-type and mutant proplastids. The mutant genetic determinants of defective plastid type are designated Dpl-1, Dpl-2, etc., indicating a series of nonchromosomal, genetic factors carried in the plastids.

OBSERVATIONS AND RESULTS

Poinsettia plastome mutants: Dpl-1; In the second season's growth of a poinsettia seedling (No. 61-517-1) with normal green leaves (Figure 1) an area of mosaic-patterned variegation appeared on a single leaf (Figure 2). Other leaves on the stem, above or below, showed no variegation. The stem was cut off just above the node at which the variegated leaf appeared so that the dormant lateral bud at that node was forced into shoot growth. On leaves of the new shoot larger patches of mosaic variegation appeared (Figure 3). From the new shoot lateral shoots were obtained on which all the leaves showed a mosaic pattern of variegation (Figure 4) with small green, white, or greyish green patches scattered over the leaf. Borders between the areas of different shades of green were sharp and distinct. The mutation to Dpl-1 and its establishment in a G-M-G shoot is indicated by the arrow with the dashed shaft in Figure 16 pointing out the shift from (a) to (b).

Sections of mature leaves with the mosaic pattern of variegation showed large green plastids in the guard cells of the epidermis normally derived from L-I and also in the central, spongy parenchyma of the center of the leaf, derived from L-III. In areas derived from L-II (the palisade layer, the lower row of spongy parenchyma, and all internal tissue near the edge of the leaf) groups of cells with wild-type plastids were interspersed with groups of cells with mutant, Dpl-1 plastids. In these mature cells mutant plastids were small, colorless, and indistinct. Cells with wild-type plastids all appeared to have approximately the same number of large green plastids and no mutant plastids. In mature leaves, no cells were observed that contained both mutant and wild-type plastids. Green plastids, in cells next to groups of cells with mutant plastids, were normal whether the adjacent G and W cells were from the same or from different histogens.

Sections of young leaves from shoots producing leaves with a mosaic pattern (Figure 4) contained cells with mixtures of small light-green and smaller, colorless plastids. These heteroplastidic cells were in only part of the area derived from the L-II histogen. Cells derived from L-III contained only normal, small, light-green plastids as did the guard cells derived from L-I. In primordial leaves

FIGURES 1 to 9.—Normal leaf of poinsettia seedling No. 61-517-1 and leaves carrying Dpl-1 plastogene mutant. FIGURE 1.—Normal G-G-G leaf. FIGURE 2.—Leaf with small mosaic area in L-II (G-M-G) similar to first expression of Dpl-1. FIGURE 3.—Leaf with larger mosaic area from shoot developed at node of first variegated leaf. FIGURE 4.—Mosaic pattern of variegation from shoot with G-M-G histogenic layers. FIGURE 5.—Leaf from initial shoot which contained mostly W cells and a few M cells in L-II. FIGURE 6.—Leaf from stable G-W-G shoot derived from G-M-G by plastid segregation. FIGURE 7.—Leaf in transmitted light from G-G-W shoot derived from G-W-G shoot by replacement. FIGURE 8.—Leaf in transmitted light from G-G-M shoot, derived from G-M-G shoot by replacement. FIGURE 9.—Leaf from G-W-W shoot derived from G-G-W shoot by displacement.

no differences were observed in the small colorless inclusions considered to be proplastids. However, the second histogenic layer in the meristem forming mosaicly variegated leaves was considered to have both proplastids with wild-type genetic information and proplastids with mutant genetic information whether or not they were morphologically distinguishable.

The stems of G-M-G shoots (Figure 13C) and those of G-G-G shoots (Figure 13A) are both green and indistinguishable. Fruits formed on G-M-G shoots display a mosaic pattern similar to G-M-G leaves (Figure 14C, D), some with large white areas (Figure 14C), and others with entirely green areas (Figure 14D). Seed from fruits on G-M-G shoots produced green seedlings, white seedlings and mosaic variegated seedlings (Figure 15) regardless of the pollen parent used. Young mosaic-variegated seedlings differ from the maternal parent in that they are M-M-M, rather than G-M-G. F_2 populations showed no transmission of the mutant plastid through the male gametes.

Most of the growth from Dpl-1 G-M-G meristems had the mosaic pattern of variegation, small sectors often showed a different pattern but rarely a change in an entire shoot. The most frequent change was to a G-W-G pattern in which all cells derived from L-II were white and contained only mutant plastids (Figure 16 b to c). The shift was first observed in small areas on individual leaves or in sectors along the side of a G-M-G stem (Figure 5). Whole G-W-G shoots and leaves (Figure 6) usually arose from lateral buds. However, there were rare transitions of a Dpl-1 G-M-G apical meristem to G-W-G (Figure 10). Stems on G-W-G shoots were green (Figure 13B) but the fruits were white (Figure 14A). Seedlings from fruits on G-W-G shoots were albino (W-W-W, Figure 15B). There was no transmission through the pollen in F_2 and backcross tests.

G-M-G shoots shifted to G-G-G somewhat less frequently and green shoots were recovered (Figure 16 b to a). Recovered G-G-G shoots have been entirely stable, showing no variegation in many branches through two seasons of growth.

The Dpl-1 G-W-G shoots were also very stable with only a slight variability in the relative contribution to stem and leaf by the L-II and L-III histogens. The lighter shades of green at the edge of the central green tissue from L-III in the leaf in Figure 6 are the result of more periclinal divisions in L-II during leaf development and formation of another layer of colorless cells over green cells in that area. There was no opportunity for segregation and variability within the histogens since each histogen was homoplastidic.

One type of change from G-W-G was observed when a rare periclinal division occurred in the histogen close enough to the apical meristem that a sector of the apical bud or one of the lateral buds included the change. Branches have been derived from G-W-G stems which were G-G-W (Figure 16 c to f). Leaves on G-G-W shoots appeared normal green with incident light (Figure 11C); but when observed or photographed with transmitted light (Figure 7), the central area was lighter. Sections showed that palisade and lower spongy parenchyma cells derived from L-II contained green plastids and the central spongy parenchyma cells from L-III contained small, colorless plastids. The stems of G-G-W

FIGURES 10 to 15.—Dpl-1 plastogenes in poinsettia. FIGURE 10.—Shoot in which L-II in apical meristem has segregated from M to W. Leaves have changed from G-M-G to G-W-G pattern. FIGURE 11.—Leaves from seedling originally M-M-M on which branches arose which had segregated to W-W-W (11A), to G-W-G (11B), and to G-G-W (11C). FIGURE 12.—A, Leaf with L-II homoplasmic for Dpl-1a, a plastogene mutant from Dpl-1 shown in B. The leaf tissues from Dpl-1a greatly reduced (note narrow white edge). There is greatly increased frequency of displacement of mutant L-II by green L-I (note dark area on leaf A). FIGURE 13.—Stems on shoots with various distribution of Dpl-1 in the histogenic layers of the apex: A, G-G-G; B, G-W-G; C, G-M-G; D, G-G-W, and E, G-G-M. FIGURE 14.—Fruits from shoots with various distributions of Dpl-1 in the histogenic layers of the apex: A, G-W-G; B, G-W-W; C, G-M-G; D, G-M-G; E, G-G-G; F, G-G-M. FIGURE 15.—Seedlings from G-M-G shoot; A, G-G-G; B, W-W-W; C and D, M-M-M.

shoots were yellowish white (Figure 13D) and the fruits were normal green (Figure 14E). Seedlings from fruits on G-G-W stems were all normal green.

FIGURE 16.—Somatic inheritance of plastid mutant Dpl-1 in a poinsettia seedling and origin of sub-mutant Dpl-1a. Letters represent the plastid genotype of the three histogenic layers and the tissues derived from them. G indicates all wild-type proplastids and green mature tissue. W indicates all mutant proplastids and white mature tissue. M indicates a mixture of wild-type and mutant proplastids in the cells of a histogenic layer and a mixture of green and white cells in mature tissue. Pattern changes have occurred in the directions indicated by arrows. The dashed arrows indicate the mutations from Dpl-1⁺ to Dpl-1 and from Dpl-1 to Dpl-1a, each having occurred only once. The standard arrows represent shifts of plastid type from one histogenic layer to another by replacement or displacement and have been observed from one to five times in each case. The broad arrows indicate complete and permanent change within a histogen from heteroplastidic (M) cells to either type of homoplastidic cell (G or W) as a result of plastid segregation in mitosis. This type of change has been observed from 20 to 100 times at each point.

Another green branch which developed on a G-W-G shoot proved to be G-G-G (Figure 16 c to e). Cells from all the histogenic layers contained wild-type plastids; the stems, fruits, and seedlings were all normal green.

G-G-W shoots have shifted to G-W-W with a similar low frequency (Figure 16 f to j). The leaves of G-W-W shoots are white (Figure 9), the stems the same pale yellow as G-G-W stems, the fruits white (Figure 14B), and all seedlings albino (Figure 15B). G-G-W shoots have also shifted to G-G-G (Figure 16 f to i).

One further pattern of variegation has been observed. One green shoot developed from a lateral bud on a G-M-G shoot (Figure 16 b to d). With transmitted light, the leaves were paler in the center (Figure 8) and looked like the leaves from G-G-W shoots, except that there seemed to be more dark areas near the center of the leaf. The stems were green with pale-yellow streaks (Figure 13E). Sections of the leaves showed both cells with wild-type plastids and cells with Dpl-1 plastids in the central spongy parenchyma derived from L-III. The pattern was considered to develop from a G-G-M meristem. Fruits on G-G-M shoots were green (Figure 14F) and seedlings were all green.

G-G-M shoots frequently give rise to G-G-W shoots (Figure 16 d to g) and less frequently to G-G-G shoots (Figure 16 d to h).

Variegated seedlings have been obtained only from shoots with L-II in the M state, where the pattern of leaf variegation was mosaic and both mutant and

normal plastids were found in single cells in young leaves. All variegated seedlings had a mosaic pattern in the cotyledons indicating L-II was M. The stems were green, streaked with yellow, indicating L-III was also M. L-I was not observed. In one seedling, a sample of whose leaves are shown in Figure 11, the hypocotyl was green and streaked with yellow; the cotyledons showed a mosaic pattern of green and white; and the primary shoot became completely white at the node producing the first true leaf (Figure 11A). The terminal shoot died and two branches were formed, one from the cotyledonary node and one from the next node. The cotyledonary branch was G-W-G (Figure 11B) with the typical white-edged leaves and green stem. The other branch was G-G-W (Figure 11C photographed with incident light) with green leaves and yellow stem. In general, the behavior of Dpl-1 in seedlings was the same as in the original plant. The relative contribution of L-II and L-III to leaves on G-W-G shoots was similar (Figure 6, parent, and Figure 11B seedling). Stability of the several patterns formed in the new variegated seedlings was apparently the same as in the original seedling when allowance was made for the presence of two rather than one heteroplastidic histogen.

Dpl-1a: One branch has developed on a Dpl-1 G-W-G shoot with a modified pattern of variegation (Figure 16 c to k). The new pattern had only a very narrow white edge (Figure 12A) instead of the usual broad area of white tissue which develops around the edge of Dpl-1 G-W-G leaves (Figure 12B). The leaves with the modified G-W-G pattern were generally smaller, and there was a greatly increased frequency of dark green streaks and patches on both the upper and lower epidermis of the leaves (Figure 12A). Fresh sections of the new pattern showed no wild-type plastids in small, abnormal palisade cells or in the lowest layer of spongy parenchyma cells. In sections through the dark-green patches on either surface of the leaf, the layer of cells next to the epidermis contained wild-type plastids and the palisade cells were normal size and shape. These areas arise from displacement of the cells of the mutant layer by the genetically green cells of L-I or L-III following periclinal division in cells of those histogens relatively late in the development of the leaf primordium. In their new position descendants of these Dpl-1a⁺ cells developed into normal palisade tissue with normal green plastids.

Dpl-2: In the primary shoot of poinsettia seedling number 61-76-1 a sector consisting of about one third of the circumference of the shoot displayed a typical G-W-G pattern of variegation (Figures 17, 18). The histological constitution was the same as in the G-W-G shoots of Dpl-1. The small, colorless, mutant plastids (Dpl-2) were found in the palisade layer, the lower layer of spongy parenchyma and in all the internal tissue near the leaf edges. No heteroplastidic cells of Dpl-2 and Dpl-2⁺ have been found. The leaf margin was whiter and more narrow than on Dpl-1 G-W-G shoots. The contribution of W L-II to a leaf is less than that of a G L-II, as indicated by the reduced size of the G-W-G half of leaves at the edge of the sector shown in Figure 17. Streaks of dark-green like the one shown in Figure 30 appeared on occasional leaves. Growth of separate plants with Dpl-2 G-W-G shoots was relatively less vigorous than Dpl-1 G-W-G or wild-type plants.

FIGURES 17 to 25.—Plastogene mutants of independent origin in different seedling poinsettias. FIGURE 17.—Original mutant sector of Dpl-2 (G-W-G). FIGURE 18.—Leaf from Dpl-2 G-W-G shoot. FIGURE 19.—Leaf with original type of mosaic sector of Dpl-3 (G-M-G). A G-W-G shoot of Dpl-3 had leaves very similar to Dpl-2 in Figure 18. FIGURE 20.—Original, mosaic G-M-G pattern of Dpl-4. The mutant areas are only slightly paler green. FIGURE 21.—A leaf from G-W-G Dpl-4 shoot. FIGURE 22.—Original mosaic G-M-G pattern of Dpl-5. FIGURE 23.—A leaf from G-W-G Dpl-5 shoot. FIGURE 24.—Original mosaic G-M-G pattern of Dpl-6. FIGURE 25.—A leaf from G-W-G Dpl-6 shoot. Mutants areas are nearly normal green.

The stems of G-W-G shoots were green, the fruits nearly white. From crosses with wild-type male parent, 60 albino seedlings and one green seedling have been obtained. The G-W-G pattern has been very stable with just one observed shift to G-G-G.

Dpl-3: In the second season's growth of poinsettia seedling No. 62-133-3 a mosaic-patterned area (Figure 19) appeared on a leaf and G-M-G shoots were obtained by selective pruning and propagation. The Dpl-3 G-M-G shoot has been more stable than the Dpl-1 G-M-G and only two branches have been selected with a changed pattern, both to G-W-G. Mature tissue containing the mutant plastid is very white and the white leaf margin is about the same width as that on Dpl-2 (Figure 18) and more narrow than on Dpl-1 (Figure 6).

Dpl-4: In the sixth season's growth of poinsettia seedling No. 58-492-1 a mosaic-patterned variegation appeared from which G-M-G (Figure 20) shoots were selected. The G-M-G shoots were very unstable. The apical meristems changed to G-W-G (Figure 21) by the time eight to ten nodes were formed and most shoots from the lateral buds were G-W-G. The W areas carrying Dpl-4 were only a slightly paler green than Dpl-4⁺. The mutant plastids were the same size but slightly paler-green than wild-type. Leaf shape and size on G-W-G shoots were normal and the contribution of L-II to the leaf equal to that of green L-II.

Dpl-5: In the first season's growth of poinsettia seedling No. 63-186-5 a sector of mosaic-patterned variegation appeared and G-M-G shoots were isolated (Figure 22). Segregation to G-W-G (Figures 22, 23) occurred with about the same frequency as with Dpl-1. The mutant plastids are very small and pale-yellow in color. Mature leaf tissue from homoplastidic Dpl-5 meristem is a pale yellow-green. Leaf shape and size on G-W-G shoots are normal, and the contribution of L-II to the leaf is about equal to that of green L-II.

Dpl-6: In the first season's growth of poinsettia seedling No. 63-303-8 a sector of mosaic-patterned variegation appeared and G-M-G shoots were isolated (Figure 24). There were more frequent changes to G-W-G (Figure 25) than with Dpl-5. The mutant plastids were the same size as wild-type and only very slightly paler. Mature leaf tissue was almost normal and the patterns of variegation could be seen only with favorable lighting. Leaf shape and size were wild type and G-W-G shoots were as vigorous as G-G-G shoots.

Dpl-7: In the first season's growth a sector of the shoot of poinsettia seedling No. 63-305-5 showed a mosaic-patterned variegation and a G-M-G branch was isolated. One G-W-G branch has developed from the G-M-G shoot. Tissue from the homoplastidic mutant histogen is almost normal green in young leaves but becomes yellow as the leaf matures.

Dpl-8: Poinsettia seedling No. 64-46-1 apparently contained both wild-type and mutant plastids as a zygote although its parents showed no variegation. The cotyledons displayed a mosaic pattern of variegation and the stem above and below the cotyledonary node was streaked with green and yellow. Wild-type plastids were found in the guard-cells of the stomata [L-I] in some mature areas, while in other areas the guard cells contained Dpl-8 plastids. One branch which developed was M-M-M (Figure 26), another M-W-G (Figure 27), and another

FIGURES 26 to 31.—Plastogene mutants of independent origin in poinsettia. FIGURE 26.—Original mosaic M-M-M pattern of Dpl-8 which was discovered in seedling cotyledons. The mutant tissue is sensitive to high light intensity. FIGURE 27.—A leaf from M-W-G shoot of Dpl-8. FIGURE 28.—Leaves from G-W-G shoot of Dpl-13. The very narrow white margin indicates the restrictive effect of the mutant. Displacement of L-II by normal L-I is earlier and more frequent during vigorous growth (leaf A) than during slow growth (leaf B). FIGURE 29.—A leaf from G-G-W shoot of Dpl-13. The leaf shape is the same as on G-G-G shoots and again indicates the restriction of growth of L-II and its effect upon leaf morphology shown in Figure 28. FIGURE 30.—Leaf from G-W-G shoot of Dpl-14. This restrictive mutant limited development of L-II histogens carrying it, resulting in a narrow white margin and frequent displacement. FIGURE 31.—A G-G-G shoot arising from a G-W-G shoot of Dpl-14. This shoot displays the color and vigor of the parent variety.

G-W-W. The mutant plastids were about one-half the size of wild-type and were pale, greenish-yellow. Mature leaf tissue carrying Dpl-8 was yellow. Homoplastidic mutant L-II contributed a normal amount of tissue to the developing leaf but when mature the yellow tissue turned brown and dried up (Figure 26). The M-M-M shoots were not stable and the shift to all mutant plastids was more frequent in L-II than in L-III. No shifts from M to G occurred.

Dpl-9: Poinsettia seedling No. 64-135-1 developed from a heteroplastidic zygote. The cotyledons showed a mosaic pattern and the hypocotyl and epicotyl were streaked. One branch became M-W-M and another M-G-M. This mutant resembled Dpl-1 but was of separate origin.

Dpl-10: Poinsettia seedling 64-23-1 was a normal green color but later a sector of mosaic-patterned variegation appeared on the primary shoot, with slightly pale-green areas appearing in portions of the leaf derived from L-II. G-M-G shoots have developed and from one a G-W-G shoot has arisen. The Dpl-10 G-W-G shoot was normal in size, shape and vigor.

Dpl-11: Poinsettia seedling No. 64-266-1 was M-M-M from earliest observation. The hypocotyl, epicotyl, cotyledons and first true leaves all showed a mosaic pattern of variegation. The terminal growing point became M-W-M and several M-W-M branches have shown sectors of M-W-W tissue. The mature tissue from homoplastidic W histogens is a very pale yellowish white. The shift from M has been only to W, not to G, and the change occurred more quickly in L-II than in L-III.

Dpl-12: Poinsettia seedling No. 64-172-1 was normal green until a small sector developed with a mosaic pattern of variegation in tissue from L-II. A G-M-G shoot was isolated but has grown very little, although a G-G-G branch developed. No G-W-G shoots or leaves have appeared.

Dpl-13: A variegated poinsettia obtained from Paul Ecke Company, Inc., of Encinitas, California, was reported to have arisen by somatic mutation from the commercial cultivar "Ruth Ecke", a diploid form. The sport proved to be a G-W-G periclinal chimera. The mature cells from L-II contained very small colorless plastids and the white margin on leaves of the G-W-G shoots was very narrow (Figure 28). The leaves were a lighter and grayish green, and smaller and quite different in shape from those typical of the cultivar "Ruth Ecke." The origin from "Ruth Ecke" seemed doubtful until a G-G-W branch developed. On this branch the leaves (Figure 29), bracts and flowers were typical of "Ruth Ecke" but the stem was yellow rather than green.

Leaves on G-W-G shoots showed frequent dark streaks on their surface (Figure 28B). When G-W-G shoots were grown in uncrowded conditions and at a high level of nutrition, their growth was more vigorous, with larger leaves and earlier and more frequent displacement of the mutant L-II tissue by green L-I or L-III (Figure 28A).

Dpl-14: A second variegated poinsettia from Paul Ecke Company, arose as a somatic mutation of the commercial cultivar "Barbara Ecke," a tetraploid form. It proved to be a G-W-G chimera (Figure 30). The grayish-green leaves had a narrow white margin, showed frequent displacement by the Dpl-14⁺ L-I or L-III

(Figure 30), and had reduced lobes. The homoplastidic mutant cells contained small, colorless plastids. The G-G-G shoot (Figure 31, left) which arose from a G-W-G plant showed the typical leaf shape and vigor of the cultivar "Barbara Ecke."

Dpl-15: A third aberrant type of poinsettia from Paul Ecke Company, also appeared as a somatic mutation of the cultivar "Barbara Ecke." The leaves were a uniform yellowish-green color with no white or pale margin. The leaf shape was similar to that of G-W-G of Dpl-14 (Figure 30) and the vigor was similarly reduced relative to "Barbara Ecke." The yellowish-green leaves showed streaks and patches of normal green color similar to those in Figures 28B and 30. Sections through such green areas and adjacent yellow-green areas out to the leaf margin showed that this was a periclinal chimera of a G-W-G constitution. The sub-epidermal layer of cells in the yellow-green areas contained small, yellow-green plastids. There was only a single layer of these cells out to the leaf margin and not the usual formation of all the chlorenchyma cells by cells from L-II. The restrictive nature of Dpl-15 was also indicated by the fact that the cells below the upper epidermis were not differentiated into a palisade layer but were essentially isodiametric and similar to the spongy parenchyma. The cross section through the green streaks showed a typical palisade layer with wild-type plastids apparently from a normal L-I or L-III by displacement. Neither these normal palisade cells next to the mutant subepidermal layer nor the normal green spongy parenchyma from L-III just internal to the mutant layer showed any effect of the neighboring Dpl-15 cells.

Dpl-16: A third somatic mutant of the cultivar "Barbara Ecke" was discovered in the greenhouses of "J. Haggarty the Florist" in Elizabeth, New Jersey, and propagations were brought to Beltsville for evaluation 3 years later. This mutant was a periclinal chimera of a G-M-G constitution showing a mosaic pattern of variegation. The leaves varied from some with as little white tissue as the one shown in Figure 2, to others with as much white area as in the leaf of Figure 5. The most vigorous plants had less white and more green tissue but this did not appear to be a result of displacement. This mosaic shoot has been remarkably stable in that during the growth and increase to approximately 300 plants with many shoots in a 3-year period, the grower has been unable to select a stable G-W-G shoot.

Additional mutants: Three other variegated seedlings were found in the 1964-65 season. A mosaic pattern was the first expression in each case but so little growth has occurred that the mutant's nature or behavior has not been determined. One seedling was from an M zygote; the other two developed mericlinal mosaic patterns.

Mutation rate: The mutation rate of the poinsettia plastome is difficult to estimate. Approximately 10,000 seedlings have been examined during two seasons of careful search for variegations and four were entirely M-M-M, i.e. they came from zygotes which contained both mutant and wild-type proplastids although neither parent was variegated. Twelve mericlinal mutant areas were found during the same two seasons in the approximately 20,000 shoots which were under

observation. The mean shoot height attained was about 40 centimeters with about 30 leaves. The growth of the poinsettia shoot from its shoot apex is similar to that in cranberry which has been described in detail (DERMEN 1945). Tissue derived from each of the three independent histogenic layers arises from the 1, 2, or 3 cells of each layer which occupy the very central part of the apical dome. Mutation in a plastid in one of these apical cells establishes the potential distribution of the mutant within the histogenic layer to a sector of $\frac{1}{3}$ or $\frac{1}{2}$ or all of the subsequent growth of the shoot. The mutant Dpl-2 occurred in one of three cells in L-II occupying the center of the apical dome. It first appeared in the sector occupying $\frac{1}{3}$ of the circumference of the stem. As the shoot grew there was an apparent shift from three to two cells occupying the central point of the apical dome and only $\frac{1}{2}$ of the upper part of the seedling showed the mutant phenotype (Figure 17). Dpl-2 is the only mutant described which occurred in one of the central apical cells.

Meristematic activity is not confined to the central cells or to the apical dome but extends into the primary nodal and internodal regions. However, cells farther away from the apex give rise to smaller areas of mature tissue and chances of observing and recovering a mutant are less. Survival of a mutant in a cell below the central apex depends upon its being included in the organization of a lateral bud. In several instances variegated areas in leaf blades, apparently the result of a mutant plastogene, were not repeated in the shoot that subsequently developed from the lateral bud at the same node. This indicated the mutation occurred below the central cells of the bud initial.

The population of dividing cells below the central cells of the apical dome is of course infinitely larger than the population of central cells. Most mutants would be expected to occur there and to appear in limited sectors. Eleven of the 12 poinsettia mutations detected in shoots occurred in subapical cells which contributed to the L-II tissue in at least one lateral bud and the leaf subtending it.

While all of the mutants in the shoots of the material under observation were found in L-II, there is no conclusive evidence that mutation was more frequent in that histogenic layer. Abnormal plastids in L-I can be detected only with microscopic observation of large areas of epidermis. A search of this nature was not undertaken. Mutant plastids in leaf tissue derived from L-II are readily visible and those that occur close to the apical zone become very conspicuous. Plastid mutations occurring in L-III are difficult to see in leaves except with transmitted light. The poinsettia stem color is principally determined by L-III and no pale or streaked stems were observed in the 20,000 shoots. However, conspicuous markings on the stem would occur only after mutation in cells at or near the apical dome.

Carnation plastome mutants. Dpl-1: In the first season's growth of carnation seedling No. 62-12-1 a sector of one branch developed an irregular streaking on the leaves (Figure 32C, D). From this sector shoots were selected on which all the leaves were streaked. The stems were marked in a similar fashion (Figure 33B, C) and the calyces of the flowers were also variegated (Figure 34). Seedlings from flowers with variegated calyces produced three kinds of seedlings

FIGURES 32 to 35.—Plastogene mutant Dpl-1 in carnations. FIGURE 32.—Leaf from shoots with various distributions of Dpl-1: A, G-G-G; B, G-W-G; C and D, G-M-G. FIGURE 33.—A, stem of G-G-G shoot, B and C, stems of G-M-G shoots. FIGURE 34.—Calyx on G-M-G shoot. FIGURE 35.—M-M-M seedling from flower like that in Figure 34.

irrespective of the source of the pollen; green, albino and variegated (Figure 35). The variegation in the nearly round cotyledons of the carnation seedlings was a mosaic pattern similar to that of M-M-M poinsettia leaves.

We have selected shoots with a stable pattern of a narrow green central stripe in an otherwise white leaf (Figure 32B) from the shoot with the irregularly

striped leaves and stem. These shoots had white stems, calyces which were white except for a narrow green stripe in the midrib. All seedlings from the flowers were albino. Therefore, the original striped sector was G-M-G, the shoot with a narrow strip of green in the white leaves was G-W-G, and the variegated seedlings were M-M-M. Tests through F_2 's showed no transmission of the mutant plastid through the male gametophyte.

Sections through young leaves on G-M-G shoots showed cells with two kinds of young plastids, one larger and greener than the other. In cells of younger leaves and in cells of older leaves all the plastids within a single cell looked alike. In mature cells there were either small, pale-green bodies which were interpreted as being mutant plastids or an apparently full complement of large, green, wild-type plastids. As in poinsettia there was no effect of mutant cells upon adjacent wild-type cells.

Dpl-2: In the first season's growth of carnation seedling No. 62-18-2 a sector appeared with a mosaic pattern similar to that of *Dpl-1* and G-M-G shoots were isolated from it. From the G-M-G shoots G-W-G branches were selected. Sections of mature leaves showed white cells with smaller and paler plastids than *Dpl-1*. It was also apparent that in heteroplastidic *Dpl-2* material shifts to homoplastidic mutant were not as frequent as in *Dpl-1* material.

DISCUSSION

Site of genetic control: Critical evidence for the location of the genetic control of chlorophyll variegations in the plastids themselves has been available in very few cases; and many authors have questioned the widespread occurrence of plastogene control of chloroplast development and function. However, the recent demonstration of a characteristic chloroplast DNA has led to the proposal of a model of a plastid-based, DNA-coded genetic mechanism (GIBOR and GRANICK 1964). With the conclusive evidence of the presence of DNA in plastids and other organelles in the cytoplasm, the available indirect evidence of the existence of plastogenes becomes more convincing.

The particulate, cytoplasmic nature of the control of chloroplast development is evident in cases where extensive somatic segregation occurs within the tissues derived from a single histogenic layer. Such somatic segregation is shown in the unstable mosaic-patterned variegations where the patches and sectors are variable in size and distribution as shown on poinsettia leaves in Figures 3, 4, and 5. The mosaic patterns on leaf sectors represent somatic segregation within a close lineage of cells to a degree never encountered with nuclear factors. The shifts between green and white cells, which when mature contain only normal or mutant plastids, are the result of random assortment of two kinds of proplastids in mitosis. MICHAELIS (1958) and others have questioned whether there are enough mitoses involved in leaf formation to allow complete segregation of a large number of two plastid types in the frequency indicated by the patterns of variegation. However, there are reports that meristematic cells contain few proplastids (MICHAELIS 1958; GRANICK 1963) so that complete segregation could occur more

frequently. In poinsettia and carnation it seems probable that random segregation of mutant plastogenes above a relatively low threshold number results in suppression of all normal plastid development from wild-type proplastids. Below the threshold number of mutant proplastids the normal proplastids divide as the cell differentiates and produce a green cell with a normal number of plastids. The threshold concept, suggested to explain the mosaic patterns of variegation in tobacco leaves (BURK, STEWART, and DERMEN 1964), was similar to the suggestion of RHOADES (1946) that a cell with colorless plastids might have needed only a preponderance of abnormal plasmagenes.

In several reports the segregation patterns of green and white tissues were apparently due to a somatic segregation of determiners of plastid types in the cells giving rise to the ovules. ANDERSON (1923) and RHOADES (1943) reported in corn, and KAPER (1934) in sorghum, a clustering of chlorophyll types in seeds and seedlings from various parts of the ear. Areas which produced striped plants were between areas which produced green plants and areas which produced albino plants. In tobacco (BURK *et al.* 1964), and in carnation and poinsettia, the pattern of variegation in the calyx and in the ovary wall showed a high correlation with the plastid content of the nearest seed. The pattern indicated the same kind of somatic segregation of discrete particles was taking place in both areas.

Inheritance of the chlorophyll variegations in carnation and poinsettia has been entirely maternal. Only green seedlings have been obtained from seed parents with green L-II, mutant seedlings from seed parents with mutant L-II, and green, mutant, and variegated from seed parents with variegated L-II. The statement, often encountered in texts, that nonchromosomal determinants are identified primarily by their nonsegregation at meiosis applies only to stable variegations with a homoplastidic L-II histogenic layer. When L-II is heteroplastidic there is segregation in meiosis as well as in mitosis. The only unique effect of sexual reproduction for nonchromosomal determiners is to move the determiners found in L-II of the female parent into all three histogenic layers of the seedlings.

JINKS (1964), RHOADES (1946, 1955) and others have stated that critical evidence for plastogenes is the observation of cells with mixed plastid types. Mixed plastid types in single cells have been observed by BURK *et al.* (1964), GREGORY (1915) MICHAELIS (1958), WOODS and DuBUY (1951), and others, as well as in the chlorophyll mutants of carnation and poinsettia. Each of these authors found mixed cells in relatively young tissue only, and found none in mature leaves or in cells in meristematic regions. BURK *et al.* (1964) pointed out that not all cells derived from heteroplastidic histogens would contain two types of plastids. The series of mutants in poinsettia differ in the stage of plastid development at which their effect becomes apparent and thus further limit the period during which observations of mixed plastid types are possible. VON WETTSTEIN (1961) suggests that if the determiners are in the plastids themselves then different plastid types in one cell require proplastids in the meristem to differ morphologically. However, if the mutant plastogene represents a change in the base sequence of DNA which results in failure of one of the later stages of differentiation into a chloroplast, no difference in appearance of the proplastid need be evident. While obser-

vation of mixed cells is conclusive evidence of genetic differences in the plastids themselves, the lack of such observations is not critical negative evidence.

Complexity of the plastid genetic system: Consideration of the 16 spontaneous plastogene mutants of poinsettia, described in Table 1, indicates the plastids have a complex genetic system. The varied phenotypes suggest that there are at least 16 different mutons. The mutants affect many different phases of the development and differentiation of proplastids, suggesting extensive control systems based on the genetic material in the plastid itself. Among the characteristics affected are the degree of proplastid development in terms of size and color of the mature plastid, the rate of proplastid division, the sensitivity of the chlorophyll to light, the growth of cells and tissues carrying the mutant, early necrosis of cells and tissues carrying the mutant, and stability of the histogenic layers. These divergent characteristics under plastogenic control seem to require a genetic system complex enough to include a number of cistrons. GRANICK (1963) in *Euglena* and Woods and DuBUY (1951) in *Nepeta* have described similar series of plastid mutants controlling many different kinds of characters and indicating complex, multigenic, genetic systems.

The stepwise change from Dpl-1⁺ to Dpl-1 and from Dpl-1 to Dpl-1a presents limited evidence of a different kind on the complexity of the genetic material in the plastid. The stepwise mutations could be the result of successive loss of two bits of information, of successive alteration in two units, or of stepwise change in a single unit. Backmutation of mutant plastogenes is practically impossible to establish because of the possible persistence of suppressed wild-type loci in L-I tissue. When these move into subepidermal regions of a leaf through a rare periclinally oriented mitosis, plastid development is no longer suppressed. The occurrence of very small subepidermal green islands on all white M-W-W leaves in tobacco (BURK *et al.* 1964) was attributed to replacement or displacement by L-I.

Stability of the plastid genetic system: The spontaneous mutation rate of the plastogenes in poinsettias and carnations is very low. The apparently large number of mutants observed is the result of the ease of their detection and propagation in the descendants of an enormous population of meristematic cells. For any mutant plastogene to survive, it must occur in a proplastid in a meristematic cell which will give rise to cells and tissues involved in the initiation of leaves, lateral buds, or flowers. This zone of meristematic activity includes not only the central cells of the apical dome but also large numbers of cells below the dome in the areas of initiation and differentiation of lateral structures. As these areas mature, new ones are formed from the cells in the apical dome so that there is a large and constantly renewed population of dividing cells in which plastid mutants could survive and be detected. Sixteen mutations were detected in 20,000 shoots, but each shoot provided a constantly renewed population of meristematic cells over a period of about 4 months. The three independent histogenic layers in the apical meristem all contribute to the formation of the shoot and its appendages but only L-II gives rise to tissue in which mutant plastids are readily detected macroscopically. L-I gives rise to the epidermis of the leaf in which plastid development

TABLE 1
Characteristics of a series of plastogene mutants in poinsettia shoots

Mutant No.	First expression	Mutant plastid size	Mutant plastid color	Mutant tissue color	Width of L-II at leaf edge	Balance off-tissue W:G	G-W-G leaf shape	G-W-G leaf size	G-W-G shoot vigor
1	M*	Very small	Colorless	White	Wide	60:40	Normal	Normal	Reduced
1a	W	Very small	Colorless	White	Narrow	100:0	Lobes reduced	Normal	Reduced
2	W	Very small	Colorless	White	Medium	100:0	Normal	Reduced	Reduced
3	M	Very small	Colorless	White	Medium	60:40	Normal	Reduced	Reduced
4	M	Normal	Light green	Light green	Wide	80:20	Normal	Normal	Normal
5	M	Very small	Pale yellow	Pale green	Wide	60:40	Normal	Normal	Reduced
6	M	Normal	Green	Green	Wide	70:30	Normal	Normal	Normal
7	M	Small	Yellow	Yellow	Wide	60:40	Normal	Normal	Reduced
8	M	Small	Yellow	Yellow	Wide	70:30	Normal	Normal	Reduced
9	M	Small	Colorless	Pale yellow	Wide	50:50	Normal	Normal	Reduced
10	M	Normal	Green	Green	Wide	60:40	Normal	Normal	Normal
11	M	Small	Pale yellow	Pale yellow	Medium	70:30	Normal	Normal	Reduced
12	M	Small	Pale yellow	Yellow	..	40:60			
13	..	Very small	Colorless	White	Narrow		Lobes reduced	Reduced	Reduced
14	..	Small	Pale yellow	White	Narrow		Lobes reduced	Reduced	Reduced
15	..	Very small	Colorless	None	None		Lobes reduced	Reduced	Reduced
16	M	..	..	White	Wide	50:50	Normal	Normal	Reduced

* M indicates heteroplasmic cells in meristem which give rise to both green and mutant-colored cells, W indicates homoplasmic mutant cells which give rise to mutant-colored cells.
 † A measure of the relative frequency of shift from M to W or G.

is suppressed except for small chloroplasts in the guard cells so that mutant plastids could not be observed. L-II gives rise to the layer of subepidermal cells over the entire leaf and to deeper cells near the leaf edge. L-II tissues are not masked by the colorless epidermis and small groups of mutant cells are readily detected. L-II is also the source of the female gametes. Thus plastogene mutants in L-II of differentiating flowers can be included in the zygote and observed in the seedling cotyledons. L-III cells give rise to internal tissue, and mutant areas are masked by normal green cells of L-II. Of the poinsettia plastogene mutants which occurred in our material, 12 were discovered as mericlinal L-II chimeras and four were discovered as M-M-M seedlings. It seems probable that mutation in L-I and L-III occurs at rates comparable to the rate in L-II but that the mutants escape detection.

As indicated in Table 1, the several plastogene mutants in poinsettia differ in the frequency with which the heteroplastidic cells in M histogens segregate to homoplastidic mutant or wild-type cells. More frequent segregation to homoplastidic mutant cells indicates that the proplastids carrying the mutation divide more rapidly than wild-type. Mutants which resulted in slower division probably occur but would usually be left behind in nondividing cells before increasing to numbers which could be observed. In two of the poinsettia mutants, Dpl-9 and Dpl-16, the mutant and wild-type proplastids appeared to be dividing at equal rates and no homoplastidic histogens were found. One mutant, Dpl-12, was very difficult to maintain because most of the growth segregated to wild-type plastids indicating a lower rate of mutant proplastid division. There has been some indication of differential nutritional effect on rate of proplastid divisions and at the time the mutation to Dpl-12 occurred, its rate of division must have been favored by the current level of nutrition. Under standard conditions, it does not compete successfully.

With a low spontaneous mutation rate, one would expect mutation to occur in a single plastid, in a single cell, and in a single histogenic layer. With all but the most aggressive or rapidly dividing mutants the first expression would be mixed cells in a mericlinal chimera. If the mutation occurred in L-II of a differentiating inflorescence, mosaic seedlings would be expected. As indicated in Column 2 of Table 1, all but two of the poinsettia mutants first appeared as heteroplastidic areas. Dpl-1a and Dpl-2 first appeared as stable homoplastidic L-II mericlinal chimeras. Dpl-1a and Dpl-2 appeared either to suppress division of normal proplastids or to divide at a much more rapid rate than normal so that the areas of the histogen carrying the mutants became homoplastidic by the time the first cells matured and revealed the presence of the mutation.

After considering the number of chloroplasts per cell, the possibility of replicates of plastid genes within chloroplastid, and the possibility that the mutated plastid (if it arose in a meristematic cell) might divide more slowly than the normal proplastids and be lost in subsequent cell generations, GRANICK (1955, 1961) concluded: "From such considerations it would appear that in order to bring about an inheritable observable plastid change, it is necessary to change at the same time all replicates of a particular plastid gene in all the plastids of a

cell." The "bleaching" of *Euglena gracilis* by a variety of agents occurs at a very high frequency and on a "mass mutation" basis (GRANICK 1963; GIBOR and GRANICK 1964; McCALLA 1965). Bleaching is clearly different from the spontaneous plastogene mutation in tobacco (BURK, STEWART, and DERMEN 1964), in carnation and in poinsettia. In all higher plants, plastogene mutations must occur in a meristematic cell in order to survive. Early observations of mixed cells indicate that only one of the plastogenes mutated.

The mutant form a poinsettia plastogene is apparently as stable as wild-type; only one case of further mutation has been observed. The appearance of a shoot developing from a G-M-G apex is one of constant change difficult to reconcile with stable genetic control. Numerous authors have been unable to see any relationship between the patterns of variegation and the developmental morphology or ontogeny of the different tissues in the leaves. The extensive description of Dpl-1 in the text, and the numerous photographs (Figures 1 through 16) of Dpl-1 variegations, attempt to describe the somatic segregation of a single mutant within a histogenic layer by replication and segregation in mitosis, and the extension of the mutant into the other histogens by replacement and displacement (DERMEN 1960). Replacement results from periclinal division of a cell in L-I or L-II. Daughter cells from one histogenic layer now form two layers and L-III is left behind in growth of the affected sector of the shoot apex. Displacement results from periclinal division of a cell in a histogenic layer followed by intrusion of one of the daughter cells between cells of the adjacent histogen, displacing them so that they are left behind. The third histogenic layer is retained. Displacement usually proceeds from an inner layer into one external to it. All of the patterns illustrated result from different distributions of Dpl-1.

The interpretation of the patterns of variegations in terms of ontogeny and histogenic layers depends first upon a paper by SATINA, BLAKESLEE and AVERY (1940) who described three germ layers in the shoot apex of *Datura* differentiated by induced periclinal polyploidy. SATINA and BLAKESLEE (1941) presented an interpretation of absolute independence of the germ layers and development of specific tissues from specific histogens. DERMEN (1945), using induced chromosomal periclinal chimeras of cranberry, suggested that the independent layers in the apical meristem be referred to as primary histogenic layers. He pointed out that cytological differences within and between different tissues in the cytochimeras had their origin in the primary histogenic layers making it possible to trace the ontogeny of various tissues in all parts of the plants using differential polyploidy as the marker. DERMEN (1947a) described the ontogeny of the various fundamental tissues in cranberry, again using chromosomal chimeras as markers. He pointed out that the contribution of the three histogens to various tissues could be quite variable and that an anatomically defined single tissue was often made up of cells derived from two different histogenic layers. DERMEN's chromosomal chimeras were genetically entirely stable and the variation in contribution to tissues resulted from changes in plane of division in mitosis whose cause was not determined. DERMEN (1947b, 1960) has described the ontogenetic significance of the patterns of variegation in a whole series of stable, periclinal chimeras in

which one or more of the histogenic layers was homoplastidic for a plastogene mutant. BURK *et al.* (1964) extended the same type of interpretation to include a plastogene mutant in tobacco which was discovered in a heteroplastidic condition. In tobacco, segregation of two kinds of proplastids in mitosis led to the mosaic pattern of variegation. Segregation to homoplastidic condition in the central cells of the apical dome led to the stable, homoplastidic patterns of variegation previously described. The poinsettia and carnation material described here show two series of plastogene mutants whose patterns of variegation can all be interpreted on the same basis; i.e., mutation in a single plastid resulting in a heteroplastidic cell and subsequent segregation of the two kinds of proplastids in both mitosis and meiosis. We suggest the general occurrence of this model of plastid-based hereditary factors whose somatic segregation reveals, and is controlled by the ontogenetically independent areas of the shoots in which they appear.

Relation of variegation patterns to morphological development: The variable linear striping in the carnation leaves shown in Figure 32C and D is the result of a heteroplastidic L-II histogenic layer of exactly the same type as gives rise to the mosaic-pattern of variegation in the broad poinsettia leaf shown in Figure 4. The different patterns reflect the different developmental morphology of these structures.

The stems of carnation derive more of their stem from L-II than does poinsettia. The stem of a G-W-G poinsettia is normal green (Figure 13B) while the stem of a G-W-G carnation is white. The stem of a G-M-G carnation is striped (Figure 33B, C) while the stem of a G-M-G poinsettia is green (Figure 13C). The stem of a G-G-W poinsettia is white (Figure 13D) and a G-G-M poinsettia stem is striped (Figure 13E). However, the stems of both G-G-W and G-G-M carnations are green. These examples illustrate the usefulness of plastogene mutations in ontogenetic studies. The mutant and wild-type cells are clearly different and their distribution is much easier to trace than the nuclear and cell-size differences in the chromosomal chimeras.

SUMMARY

Critical evidence is presented that a series of independently occurring chlorophyll variegations in *Dianthus* and *Euphorbia* are due to change in genetic material carried in the plastids themselves (plastogenes). Individual cells with mutant and wild-type plastids were observed. The two types of plastids segregated within histogenic layers at mitosis. Inheritance was entirely maternal, and—when the tissue forming the female gametophyte was heteroplastidic—green, white, and variegated seedlings were obtained. The data established that the plastogenes of the two species undergo extensive spontaneous mutation including stepwise change, indicating that there are a number of sites capable of change and that at least some of the mutants are the result of change in and not loss of genetic material. Among the characters controlled by plastogene are the size and color of chloroplasts, the sensitivity of chlorophyll to light, the rate of division of the mutant proplastid, and the growth and differentiation of tissue derived from the histogenic layer carrying the mutant. The spontaneous mutation rate of

plastogenes in the two species is very low when the size of the cell population within which a mutant could be detected is considered. The meristematic cells in the apical dome and in the zone of differentiation of lateral appendages just below the apex constitute a very large and constantly renewed population within which any mutant whose rate of replication is not impaired has a high probability of survival and detection. Because of their low frequency, plastogene mutants occur in a single plastid, in a single cell, and in a single histogenic layer. When first observed, they are in heteroplastidic cells in a histogenic layer which produces a mosaic pattern of variegation as a result of the segregation of wild-type and mutant plastids in mitosis. The further distribution of the mutant plastid in vegetative growth both reveals, and is controlled by, the ontogeny of the tissues of the shoot in which they appear. The mutant plastogene is thus a powerful tool for the study of developmental morphology and the ontogeny of plant tissues.

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