▲ 'Pink Stripes' (Derrow, 2006)
— Photo courtesy of the hybridizer

▲ 'Peppermint Ice' (Lovell, 2004)
— Kyle Billadeau photo

The unabridged version

Daylily Genetics

Part 3 Variegated or broken flower colors: Jumping genes?

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Any characteristic can be variegated but usually we think of variegated leaves or flowers and of differences in color. However, variegation can be present in both plants and animals and in any tissue and affect any characteristic. Variegation does not need to be obvious to the unaided human eye. It can be defined as any visible differences in the appearance or phenotype of the cells in a tissue. The causes of variegation in daylilies are not known. Comparison to the causes of all types of variegation found in any species may help explain those found in daylilies.

Clonal or non-clonal

When we look at individual cells in a flower petal or leaf and we measure the amount or concentration of pigment in each cell we find considerable variability. Some of this variability follows the same patterns as those of the cell divisions. We call those patterns clonal since cells are genetically identical, except in rare cases. Other times the variability does not follow the cell division pattern and these are non-clonal patterns. Both clonal and non-clonal patterns may be genetic involving a change in DNA sequence or non-genetic. However, non-clonal patterns are somewhat more likely to be environmental or non-genetic.

Clonal sectors (see glossary) can provide some information about when during development the event occurred. Large sectors indicate the event occurred early during development of the tissue or organ. Small sectors indicate that the event occurred late during development[1]. Few sectors indicate that the event is infrequent or rare. Many sectors indicate that the event is common. The shape of a clonal sector is determined by the location of the event and the time during development at which it occurred[1]. It provides hints about how the tissue or organ develops.

For more information about clonal analysis see http://www.jstor.org/stable/2443838.

Autonomous or Non-autonomous

When the amount of pigment in a cell depends only on the conditions in that cell, it is autonomous. When the amount of pigment in a cell depends on conditions in the surrounding cells, it is non-autonomous. A non-autonomous example would be when some compound which affects pigmentation diffuses between cells

Patterns help to identify possible causes

By looking at the patterns of pigmentation in individual cells we can get general clues about the causes of the variegation. For example a pattern on the upper epidermis of a variegated flower or leaf which is repeated and very similar to a pattern on its lower epidermis is unlikely to be genetic[2].

Variegation can be genetic or non-genetic

Finding that variegation is transmitted from one generation to the next unfortunately does not necessarily indicate that the variegation is genetic. Variegation that is induced by non-genetic factors can be transmitted from parent to offspring[3]. A phenotype, such as variegation, which is caused by an environmental effect and which is identical to a known genetically caused phenotype is called a phenocopy.

Variegation can be Environmental

Variegation can be caused by environmental factors, for example, high temperatures

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▲ **'Doctor Strange Love' (Cochenour, 2007) — Photo courtesy of the hybridizer**

during growth and development. Mineral deficiencies due to environmental factors can cause symptoms which include variegation[4]. There are mutations which affect the processing of the same minerals producing deficiencies with similar symptoms. As an example of the complexities of variegation, in corn/maize (*Zea mays*) there is a yellow stripes Mendelian (see glossary) mutation; iron deficiency can cause yellow stripes; there is a yellow-stripe virus and there is a non-chromosomal (see glossary) stripe mutation.

Genetic mutations causing variegation can be located in any of three locations

Plant cells have genes in three different locations. Genes are present in the mitochondria. These are structures within cells associated with processes involving energy in the cell. Genes are present in plastids. Plastids are structures within cells associated with producing and storing the food manufactured by the cell. Genes are also present in the nucleus where they are packaged into chromosomes. The genes in the nucleus are described as Mendelian. Both the mitochondria and the plastids reproduce within plant cells. Both also are typically primarily transferred to the offspring through the seed parent (80% of angiosperm [see glossary] species)[5]. In a typical plant, such as a daylily, the plastids have about 110 to 120 genes, the mitochondria have about 50 genes and the nucleus has about 40,000 genes[8]. Both the mitochondria and plastids require several thousand genes to function; nearly all of those genes are present in the nucleus. The DNA in plastids and mitochondria has been sequenced for a number of species and the identity and function of most of the genes has been analyzed.

Variegation can be caused by mutations in the mitochondrial genes, the plastid genes or the nuclear genes. In general, the number and importance of each gene location is proportional to the number of genes present in that location. Most of the mutations which affect pigmentation, including variegation, occur in nuclear genes[6]. This is also true for the vast majority of the mutations which cause abnormally colored seedlings (e.g. white or albino)[6]. Perhaps even more interesting or confusing is that nuclear mutations are known that cause permanent non-genetic changes in plastids and these can be inherited completely independently of the original nuclear mutations and cause maternally (80% of the angiosperm species) transmitted variegation[7].

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Glossary

Allele – A variant of a gene. Alleles have one or more differences in their DNA sequences. Natural genetic variation in plant populations is present as multiple alleles for most genes. An allele of a particular gene may have a very large effect on the phenotype, causing complete loss of the function of the gene or it may have a smaller effect or no effect on the phenotype. Few alleles will have large effects on a phenotype while most will have a small or no measurable effect. In a diploid individual each gene has two alleles which may be the same or different.

Angiosperm – flowering plant species. In the gymnosperm, for example, coniferous trees, plastids are predominantly inherited through the pollen parent.

Chromoplast – A self-contained structure within a plant cell. Chromoplasts contain sufficient carotenoid pigments to color the cell.

Cytoplasmic genes – present in both the mitochondria and the plastids, small organelles in cells. Mitochondria have 3 - 67 genes and plastids have 15 - 209 genes. The genes in these structures show much less genetic variability than those in the nucleus.

Dominant – The dominance component of a phenotype is the difference between the average of the two homozygous parental phenotypes and that of the F1 offspring. When the dominance component is exactly equal to the additive component the phenotype is completely dominant. When the dominance component is less than the additive component partial dominance is present or the dominance is incomplete. When the dominance component is zero the phenotype is perfectly additive. Few phenotypes will be perfectly additive or completely dominant. At the molecular level of gene expression usually both alleles in a diploid will be expressed.

Expressivity – when individuals with the same genotype show differences in some aspects of the phenotype this is known as variable expressivity. This can also occur when a genotype affects structures in an individual that are produced in multiple copies and these may show differences, for example, single and double flowers on the same plant.

Genotype – the sequence of the DNA making up the nuclear genes of an individual.

Heterozygous – in a diploid the presence of two different alleles for one gene, e.g. W/w individuals are heterozygous. The terms for a tetraploid are different.

Homozygous – in a diploid the presence of two identical alleles for one gene, e.g. both W/W and w/w individuals are homozygous.

Mendelian – Genes and their mutations which are similar in inheritance to those studied by Gregor Mendel. These are found on the chromosomes in the nucleus of the cell. The inheritance of the phenotypes associated with these follow simple ratios, for example 3:1 for dominant versus recessive phenotypes in the F2 generation.

Mutation – A change in the sequence of the DNA of a gene. This results in a different allele. It may or may not result in a different phenotype. Mutations may be selectively neutral and have no measurable effect on a plant, or they may be deleterious and lost by natural selection or be advantageous and increase by natural selection to replace disadvantageous alleles. Some alleles may form what are called balanced polymorphisms in which case two or more alleles have advantages and disadvantages in different circumstances, none is consistently better and the alleles are main-

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Glossary
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tained in the population at intermediate frequencies. Mutations with visible effects occur very rarely, roughly once in a million to once in a hundred thousand.

Non-chromosomal – Genes and their mutations which do not follow Mendelian rules of inheritance and are not located on the chromosomes in the nucleus. They may be present in the mitochondria or in plastids. See also cytoplasmic genes.

Nuclear genes – present on the chromosomes in the nucleus. They are what Mendel studied in his peas. There are approximately 40,000 different genes in a typical diploid higher plant such as daylilies. They show a high level of genetic variability and affect all characteristics.

Phenotype – the observed characteristics of an individual, for example, measured height or flower color.

Organelle – small structures within a cell. The mitochondria and plastids are examples of organelles.

Recessive – A phenotype that is completely masked by an alternative phenotype.

Sector – Part of a tissue or organ with an unexpected or different appearance. Each stripe of white in a leaf with green and white stripes is a sector. In a flower with white petals showing splashes of blue each splash is a sector.

Somatic recombination – Cells which are genetically heterozygous, e.g. A/a should produce two daughter cells which are genetically identical. However, sometimes during cell division the two chromosomes of a pair 'break' and are rejoined incorrectly. This can result in one daughter cell which is AA and the other daughter cell which is aa and is called somatic recombination. When both daughter cells survive to produce visible clonal sectors and the phenotype of AA cells is different from that of the Aa cells, visibly obvious twin sectors are produced.

Leaf Variegation

Leaf variegation includes green versus white or yellow sectors or as in Coleus leaves sectors of other colors such as red or purple. It can have several different causes. The same causes of leaf variegation may affect flower, stem, root etc., variegation, since these plant organs may contain one or more of various types of plastids e.g. proplastids, chloroplasts, amyloplasts, elaioplasts, chromoplasts, etc at some times during their development. Variegation which affects plastids in organs other than the leaves will not necessarily involve green colored sectors. Such variegation in petals might involve yellow, or orange versus white sectors or might involve orange versus yellow sectors, etc.

Variegation can be caused by Simple (Mendelian) Nuclear Mutations

In these cases, variegation appears when the plant has a specific genotype[9]. All cells in the plant have the same genotype. Whether a cell is green or not green is dependent on other non-genetic factors and some sort of threshold effect. Threshold effects are typically present whenever a characteristic is categorized as being present or absent and the assumption is that there is an underlying continuous factor. This type of variegation is not maternally transmitted. For variegation which is completely recessive it will be present in the F1 generation of seedlings only if the original variegated plant is self-pollinated and self-compatible. If the plant is cross-pollinated with pollen from a normal green plant the seedlings of the F1 generation will not show any variegation. The variegation will re-appear in the F2 generation of sibling crosses of the normal green F1 seedlings. This sort of variegation has not yet been identified in daylilies.

Variegation can be caused by Nuclear Mutations with Permanent Non-Genetic Effects in Plastids

The initial variegation which appears in these cases is due to the nuclear genotype of the initial plant[10]. However, the mutation causes a permanent non-genetic change in some of, either the mitochondria or the plastids. This permanent change cannot be repaired and is reproduced when the mitochondria or plastids divide. This type of variegation can be maternally transmitted. It is also inherited as a normal Mendelian characteristic. For recessive mutations variegation will appear in the F2 generation of sibling crosses of normal green F1 seedlings from cross-pollinations.

A typical permanent change in plastids involves changes in their ribosomes, a structure used for protein synthesis, or the loss of the ribosomes in the plastids. This type of variegation can become independent of the original nuclear genetic changes after specific types of cross-pollinations. If the original variegated plant is cross-pollinated with a normal green plant the F1 will be genetically heterozygous for the typically recessive variegation mutation and therefore not genetically variegated. However, at least some of the offspring may inherit the permanently changed abnormal mitochondria or plastids from the seed parent and be variegated. If one of these variegated individuals is again cross-pollinated with a normal green individual then half of the seedlings will be homozygous normal. However, some of those individuals may have inherited abnormal organelles from their variegated seed parent and be variegated themselves. The variegation would then be independent of the original Mendelian mutation. These sorts of variegation have been found in barley (four different genes), corn/maize (two different genes) and Pennisetum. It has not yet been found in daylilies.

Variegation can be caused by Nuclear Mutations which change the DNA in Organelles

This type of variegation follows the same pattern as when nuclear mutations cause permanent non-genetic changes in organelles[11]. It requires microscopic analysis, DNA sequencing and observations of the results of various crosses to distinguish between these two types of causes. In both cases, if the original plant showing the variegation is no longer available it may not be possible to determine the true original cause of the variegation with any certainty.

Variegation can be caused by Mutations in the Organelles

Mutations in the genes within the mitochondria or plastids can cause variegation by creating genetically and phenotypically different types of plastids[12], (e.g. green versus white or yellowish). Often these genetic changes involve the deletions of many genes from the organellesee glossary DNA. This type of variegation is inherited maternally when organelles are inherited maternally and not paternally or biparentally.

Variegation can be caused by Environmental Effects which make Permanent Changes to Plastids

Just as mutations can make permanent non-genetic changes to the plastids so can some environmental effects. For example, a plastid may lose its ribosomes because of extreme temperatures or because of the effects of various chemicals, such as antibiotics[13,3]. Plastids without ribosomes can

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▲ 'Willy Nilly' (Saxton, 1963)
— Jane M. Carson photo

▲ 'Willy Nilly' (Saxton, 1963)
— Coral Kincaid photo

▲ Note the streak at a petal's edge in both flowers.

reproduce and cannot repair the loss. One might think that such chemical factors might be unlikely in natural environments; that is not necessarily the case as some antibiotics are produced by soil microorganisms[14]. Plant cells are also capable of making antibiotic-like compounds and these are known to cause effects similar to antibiotics[15]. Plants also produce ribosome-inactivating proteins in response to various stresses and infections. One current hypothesis is that albino plants may often be due to stress causing plants to produce these sorts of compounds, for example, during micropropagation[13] which then act on their own cellular structures. Plastids without ribosomes may be very frequent in variegated plants; for example, five variegated Pelargonium cultivars were examined and all were found to lack ribosomes in their chloroplasts[40].

The inheritance of variegation can be very complex. This makes genetic analysis more than a simple case of finding a variegated plant and crossing it as pod and pollen parent with a normal plant. Even if variegation in the offspring from such crosses indicates maternal transmission, the involvement of a recessive nuclear gene in creating the original variegation is not disproved. To test for that possibility, the phenotypically normal seedlings from the normal X variegated cross need to be self-pollinated or sib-crossed to produce the F2 generation. Enough offspring need to be grown to check for an approximately 3:1 segregation of normal to variegated offspring. Nor is leaf variegation that is not nuclear/Mendelian necessarily due to chloroplasts; changes in the mitochondria can cause leaf variegation[16].

Variable expressivity

Variegation can be produced by developmental errors or problems. For example, in a plant genetically capable of producing anthocyanins, developmental processes control when those genes are active and where they are active. Genes must be switched on and switched off at the correct times during development in the flower bud before it opens. Since all cells in a plant are usually genetically identical but phenotypically may differ, the ultimate causes of the differences in gene expression are non-genetic. In modern terms developmental processes are considered epigenetics. Epigenetic effects have an element of randomness in their occurrence which can result in variegation[38]. Some developmental changes can be transmitted through somatic cell divisions (mitosis) and others can be transmitted through gamete production (meiosis). Such 'inheritance' does not necessarily follow Mendelian rules/ratios and may be inconsistent[39].

Somatic mutations

Mutations or sports can occur in any cell within a plant. When mutations occur at any time in cells which are part of the germ line (the cell lineage which will produce the male and female gametes) they can be inherited. When mutations occur in cells which are not part of the germ line (the somatic lineage) they can be inherited if they occur early enough in an appropriate tissue layer in a meristem or growing point[18]. Plants, unlike animals do not separate their germ line from their somatic line until very late in development.

In daylilies there are vegetative meristems which later become inflorescence (or scape) meristems, inflorescence branch meristems and floral meristems. In plants, somatic mutations in mature individuals can be inherited, depending on when and where the mutation occurred. This is the basis for the inheritance of mutations produced when seeds are treated with mutagenic chemicals or radiation. This is also the basis for the use of pollen from converted diploid cultivars with tetraploid cultivars as pod parents. Treatment of the vegetative growing point (shoot apical meristem or SAM) with various chemicals (e.g. colchicine) produces somatic changes in ploidy in the vegetative meristem that can be inherited when the meristem becomes a reproductive meristem and eventually produces gametes.

For more information about meristems see http://www.public.iastate.edu/~bot.512/lectures/SAM.htm

Daylily meristems have three tissue layers. Cells derived from layer 2 ultimately form approximately 75-90% of the gametes on average; layer 1 is responsible for approximately 10-25% of the gametes[17]. Layer 1 also forms the epidermal layer in the petals and sepals. The epidermis contains the anthocyanin pigments. Somatic mutations visible as sectors of changed cyanic color in petals and sepals can be inherited if they occurred early enough in the floral meristem. We can identify such mutations by a change in the pigments which appears to start at the base of petals or sepals. The larger such sectors are, the earlier during flower development the mutation occurred and the more likely the mutation has affected some of the gametes.

A somatic mutation, visible as a colored sector, that affects only a small fraction of one petal is less likely to have affected tissues in the stamens or the pistil and less likely to be present in any gametes. On the other hand, a somatic mutation which affects at least part of one sepal and of one petal is more likely to also affect the associated stamens and part of the pistil. Somatic mutations in plants can be inherited[18]. If the phenotypes of appropriately early and large sectors are found to be not inheritable, when tested in genetic crosses, this suggests that the variegation was non-genetic.

Somatic Recombination

Sectors of a changed color have sometimes been used to predict whether a plant is heterozygous for a mutation in a pigment determining gene. The assumption is that a somatic mutation in the remaining normal allele will produce a sector with the appropriate visibly altered color. However, phenotypically different sectors may not be genetically different from the rest of the plant. Even if the sector is genetically different from the rest of the plant the phenotype of the sector does not necessarily provide information which helps correctly determine the genotype of the plant.

Let's look at an example. A plant has a red-colored flower. On one petal there is a pink sector on the normal red background. If we know or assume that pink is recessive to red then the appearance of the pink sector suggests that the sector is homozygous for the pink mutation. That conclusion is the basis for assuming that the plant is heterozygous for the pink mutation. Unfortunately, although this may sometimes be the case it is by no means necessary or likely. Mutations occur more or less at random. There is a low probability that a new mutation, even in the appropriate gene will be the same and pro-

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duce an identical mutant phenotype as the previous mutation. In the above example, the plant could be heterozygous for an allele which would cause a recessive white-colored flower (null or knock-out mutation). The new mutation, even if it is at the same gene, could have produced a partially active product and a pink appearance when associated with the white allele. The plant would be heterozygous for the 'white' allele and heterozygous for the 'pink' allele in the sector but actually heterozygous for the 'white' allele elsewhere not heterozygous for a 'pink' allele elsewhere as would be assumed because the sector was pink.

There are other circumstances, in which a plant heterozygous for a mutation at one gene is apparently normal in appearance and a sector of a different color appears on a petal. It might be assumed that the plant was heterozygous for a particular flower color. However an apparently recessive mutation may have occurred in a completely different gene. Although the plant is now heterozygous for mutations at two different genes and is expected to be normal in appearance it can actually be mutant. This can occur when the phenotypes of plants heterozygous for one mutation are only apparently dominant (for example, they produce 80% of the normal amount of pigment and we cannot distinguish 80% from 100% with the unaided eye). A heterozygous individual at one gene with 80% of the normal pigment which suffers a new mutation (heterozygous) at a different gene that results in say 85% of the normal amount of pigment, will have only 68% (80% X 85%) of the normal pigment and may now be visibly mutant[19]. The plant may have a mutant colored sector on a normal background but not be heterozygous at any specific gene for the mutant flower color. As an example a plant may be heterozygous for a mutation 'a' which causes white flowers when homozygous but red flowers when heterozygous. Plants which are Aa are red flowered and apparently identical to plants which are AA to the unaided eye but actually produce only 70% of the normal amount of pigment. A somatic mutation occurs at another gene 'r' which also causes white flowers when homozygous. Plants which are Rr are apparently identical to the unaided eye to plants which are RR and are red-flowered but produce only 80% of the normal pigment. The cells in the sector with the somatic mutation are Aa Rr. Those cells only produce 56% of the normal amount of pigment (70% X 80%) and are light red. The observer sees a light red sector on a red background and assumes that the plant is heterozygous for the light red mutation when in fact it is heterozygous for a white mutation at a different gene.

There are situations in which a sector of a changed color occurs in a plant which is heterozygous for a pigment mutation and the same mutation is homozygous in the sector. In these cases recombination may have occurred in the somatic tissues and an originally heterozygous cell has produced one daughter cell which is homozygous normal and one daughter cell which is homozygous mutant[20]. If the homozygous normal sector is phenotypically different from the heterozygous petal background then there will be two visibly different twin sectors. Twin sectors may not be visible as such if the mutation is completely recessive, in which case only the homozygous mutant sector is visibly obvious. Twin sectors will not occur when one of the daughter cells dies for any reason before reproducing and thus does not create a clonal sector. Single sectors may also be produced[20] when the recombination occurred through somatic gene conversion rather than somatic crossing over.

Chimeras

Variegation can be produced in individuals which are mixtures of two or more different cell types, whether those cell types are initially caused by genetic or non-genetic factors, as long as the different types are reproduced when the cells divide. These sorts of individuals are called mosaics or chimeras.

See reference 21 for a more detailed discussion of chimeras.

Developmental instability and canalization

When a new mutation occurs its effects on the phenotype are often highly variable. For example, to the unaided eye the amount of pigment in each cell of an apparently uniformly colored petal may vary widely when viewed with a microscope. The amount of variability or the range in the amount of pigment in each cell, is a measure of the developmental stability of pigment production in each individual. Selection, both artificial by the hybridizer and natural can act to make the phenotypic effects of the mutation more uniform and also more resistant to environmental disturbances. That is, selection will act to reduce the variability within each individual or to make the amounts of pigment in each cell more consistent, for example, by reducing the range. Those changes result in what is called canalization and developmental stability. They affect the expression of the mutation.

When individuals with a history of such selection for a characteristic, for example from a red-flowered species, population or line – selection for stable uniform cyanic pigmentation) are crossed with those without such a history for example from a yellow-flowered species, population or line, the stability will break down. Each individual offspring of the crosses will be more variable in color; the amount of pigment in each cell will vary more widely, or the range in the amount of pigment will be larger than in the parent. They will also, on average, have a lower concentration of pigment per cell than the parental populations since they are heterozygous. The combination of a lower average amount of pigment in each cell with a wider range in the amount of pigment in each cell may result in patchiness or variegation in color, for example, there may be more cells with no pigment.

Figure 1 provides an example of the mean and variability in the amount of pigment per cell for one parent and one offspring individual from a cross of a yellow line with a red line.

Figure 1

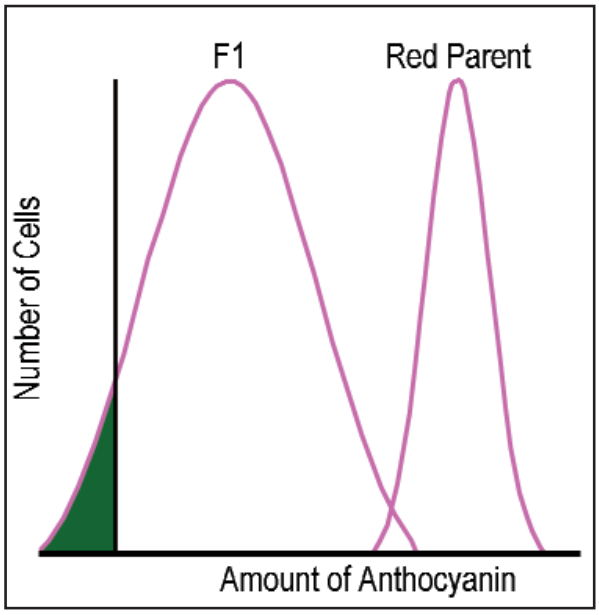

***Figure 1**. The frequency distributions of the amount of pigment per cell within individual plants of a cross involving a developmentally stable red line with a developmentally stable yellow line. The area under the curve colored green indicates the total number of cells with no apparent pigment. The F1 will show variegated color.*

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Virus Infection can cause Variegation

The story of broken flower colors caused by virus infections in tulips is well known. Cases of virus infections causing variegated flower colors are known from camellia[22], abutilon[23], and other plant species[24]. Viruses are known to infect daylilies and any new cases of broken flower color must be considered as potentially caused by a virus infection. Although, it should not be assumed that broken flower color necessarily means a virus infection is present, plant pathologists do consider flower color breaking as a good diagnostic feature of virus infection[25].

The patterns created by virus infection can be very similar to those created by several different genetic causes. The symptoms of infection can vary from no visibly obvious abnormal effects ('symptomless', although potentially with effects on growth, height, yield, etc.) to plant death and will depend on the both the genotype of the virus and of the cultivar[25]. For example, all Easter lilies are probably virus infected[26]. Viruses can become integrated into chromosomes and be inherited[27]. Some viruses can be transmitted through seed or pollen and passed to offspring[25]. It is not simple to determine whether a new case of broken flower color is caused by a virus infection and it requires laboratory testing to be confident about whether any viruses are present or not.

Different types of Nuclear (Mendelian) Mutations can cause Variegation

Variegation can be caused by mutations, inherited equally from both the pod and pollen parent[9]. There are many different types of mutations which change the DNA sequence of the genes. Simple English sentences can be used as an analogy to illustrate these mutation types. In the following examples, sentence 1 is the equivalent of one normal gene and each letter is part of the sequence of instructions coded in the DNA of the gene. Sentences 2 to 8 are examples of particular types of mutations.

1) To create a new flower first select the appropriate parents. Normal or wild-type

2a) To creyte a new flower first select the appropriate parents. Point mutation

2b) To cremte a new flower first select the appropriate parents. Point mutation

2c) To preate a new flower first select the appropriate parents. Point mutation

2d) To create a new flower lirst select the appropriate parents. Point mutation

3a) To create a new flwer first select the appropriate parents. Deletion

3b) To create select the appropriate parents. Deletion

4a) To create a new xflower first select the appropriate parents. Insertion

4b) To create a new flower first select the zopg appropriate parents. Insertion

5a) To create a neww flower first select the appropriate parents. Duplication

5b) To create a new flower first select select the appropriate parents. Duplication

6a) To create a new flower first tceles the appropriate parents. Inversion

6b) To create a new flower etairporppa eht tceles tsrif parents. Inversion

7) To create a new [cut this & paste elsewhere] flower first select the appropriate parents. Transposon

8) To create a new [copy this & paste elsewhere] flower first select the appropriate parents. Retrotransposon

A mutation may have no effect on the function of the gene, or the effect may vary from slight to completely destroying the entire gene's function (e.g. a knock-out mutation). The same gene may have some recessive mutations and some dominant mutations, although the majority of new mutations are more or less recessive. Most new mutations cause a loss of function; a few cause a gain in function. The average gene[28] has approximately the equivalent of 1350 'letters' which code for 450 'words' using the sentence analogy from above. Mutations can occur at any of the 'letters' approximately in a random manner. The effect a mutation has on the function of the gene is determined by where in the gene the mutation occurs and the type and characteristics of the mutation.

Active Transposons (Jumping Genes) can cause Variegation

One particular type of mutation, an insertion, occurs when a piece of extraneous DNA is inserted into a gene. Sometimes the piece of inserted DNA is large and contains complete gene sequences. When a large piece of DNA is inserted into a gene the usual result is a knock-out mutation and the gene no longer functions at all.

One specific type of insertion is called a transposable element popularly known as a 'jumping gene'. Transposable elements are able to move or transpose about the DNA in a cell. Transposable elements can be cut out from their current location and pasted into a new location (a transposon) or they can be simply copied from their current location while the original remains in place and pasted into a new location[29] (a retrotransposon).

Retrotransposons differ from one type of virus by only one gene[30] and are thought to have evolved from that type of virus or vice versa. Both transposons and retrotransposons are apparently able to move laterally between species[31] somewhat like infections.

Insertions, duplications and other mutations can cause variegation due to gene silencing[32]. Transposons can cause variegation due to genetic changes (clonal sectors), for example, when they excise from a gene. Transposons, retrotransposons, viruses and some mutations are similar enough that plants react to them with similar mechanisms. This can make it next to impossible to distinguish between the causes of variegation outside of a molecular genetics laboratory.

The important difference between a transposon and other insertion mutations is that a transposon can be removed from the gene in which it is inserted. A mutation which has been created by the insertion of a transposon is not necessarily a permanent mutation. Such mutations are described as unstable or mutable. This creates a pattern in the expected changes in phenotype (variegation) when an active transposon is involved. The pattern is a distinguishing and a somewhat diagnostic feature of transposon insertions and their excision.

Variegation Patterns caused by Transposons

We begin with a normal gene functioning to produce its normal product – the wild-type phenotype. The transposon, which typically is a large piece of DNA, becomes inserted into the gene. It disrupts the gene's function causing a mutation. The mutation may become homozygous by appropriate crosses. This causes a change in phenotype from wild-type to mutant as most mutations are recessive[21]. When, occasionally, the transposon is cut out of the gene in a particular cell then there is a chance that the gene returns to its normal function, for example, when the entire transposon is removed. The phenotype will no longer be mutant in all the descendents of that original cell. Their phenotype will be normal or near-normal. This is called a reversion. A clonal sector will be created with the normal phenotype. Since the transposon is not excised from all cells, some of the cells will have the original mutant phenotype while others will have the reverted phenotype. Once the transposon has been cut from the gene it may be lost or it may be inserted into any gene. This causes a new somatic

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mutation in some characteristic although it will not be obviously visible since it will be heterozygous.

The expected pattern that is present in the vast majority of transposon-based variegation, is for the basic characteristic of the plant to be mutant with sectors of reverted normal or near normal appearance. Since most mutations are recessive this can also be described as sectors of the dominant phenotype on a recessive phenotypic background. For those studying variegation without molecular genetic techniques this is one of the basic rules to help identify potential transposon-based variegation[33]. These sorts of variegation are genetic, and clonal. Typically the phenotype of the sectors is autonomous but this depends on the normal characteristics of the gene in which the insertion occurred.

Transposons are one of many factors which can cause mutations. In homozygous mutant individuals with active transposons the occasional excision of the transposon causes somatic mutations which may be visible as variegation. Some of the somatic mutations are complete reversions back to the normal wild-type but others may only be partial reversions and have phenotypes which differ from both the normal and the mutant. For example, if the normal is red-flowered and the transposon based mutation is to white-flowered, a homozygous mutant individual would have white flowers with red sectors. Sometimes a sector might be light red or pink, etc. Red sectors would be complete reversions; light red or pink sectors would be examples of partial reversions. In both cases of reversions the affected gene would no longer contain the transposon and the phenotype would be stable. Somatic mutations in plants can be inherited[18]. It is possible to produce individuals, by appropriate crosses, with the stable partially reverted phenotypes and corresponding genotypes (in the example, individuals with stable completely light red or pink flowers).

The mutations caused by transposons can be inherited in two different ways. Transposons may excise in tissues which permit visibly obvious phenotypes to appear, such as variegation in anthocyanin pigments in the epidermis of flower petals. Those somatic mutations which occur early enough to be present in a meristem of any sort can be inherited in the same manner as any chimeric 'sport' can be inherited. Transposons may also independently excise in any tissue without visibly obvious phenotypes appearing. For example, an active transposon might excise in pollen mother cells directly in germ line tissues which do not produce anthocyanin but do produce the pollen.

Distinguishing Types of Sectoring Patterns

For tentative identification of transposon based variegation one would look for sectors of a dominant phenotype on a recessive phenotype background or sectors of near normal phenotype (wild-type) on mutant backgrounds. Usually this is fairly easy to do, for example, in daylilies a pattern of reddish sectors on a whitish or yellowish background fits the expected pattern. However, the frequency and size of sectors differs among different types of transposons. Some may excise very early in development and create large sectors. Some may excise very frequently and create many sectors. Sometimes, there may be so many and such large sectors that they may overlap and create the appearance of sectors of a recessive phenotype on a dominant phenotype background. Such sectoring is most likely produced by non-transposon based causes. However, to help distinguish between the causes one would need to know the parentage of the phenotypically mutable or variegated individual and one might have to carefully examine many sectored tissues looking for the rare occurrence of few and small sectors.

Transposons are not always active

Although mutations due to transposons are not initially stable when they first appear, they can become permanently stable when the transposon is inactivated. Transposons are inactivated by mutations which occur within the transposon sequence or by epigenetic processes. Mutations can also become permanently stable if the transposon leaves a small portion of DNA in the gene when it is excised.

Most species do not have currently active transposons. Most individuals of those species with active transposons do not have active transposons. In plants, when geneticists do not have active transposons in a particular species they transfer active transposons from other species. Thus plant species are not thoroughly surveyed for active transposons. Much of genetics research in one species is directly applicable to other species – that is one reason why particular species have been chosen for specific types of genetics research. One of the best genetically researched group of species are mammals, including humans. Geneticists have searched very hard to find active transposons in mammals but have been unable to find any currently active[37] transposons. As has been typically found in other species, there is evidence that active transposons were once present but they were inactivated in the distant prehistoric past. Variegation in mammals cannot be caused by active transposons. Just as active transposons are absent from some species, such as mammals, one cannot assume that active transposons are present in daylilies. We cannot simply assume that variegation in daylilies is caused by active transposons.

Figure 2

The flowers of morning glory showing broken colors due to different causes.
A) Variegation due to the excision of a transposon. The colored sectors show where the transposon has been cut out of the gene in which it was located. The cells in the colored sectors are genetically different from those in the uncolored sectors.
B), C) and D) Non-clonal variegation due to three different types of gene silencing. The cells in the darker colored sectors are genetically identical to those in the lighter colored sectors.

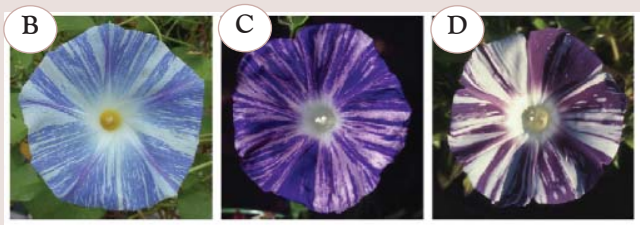

Figure 1 A, B, C and D reproduced with permission from *The Science of Flavonoids* edited by E. Grotewold, 2006, Springer, New York.

Gene Silencing

Variegation can be caused by gene silencing[38]. Gene silencing occurs when the expression of a gene is interrupted or suppressed and is an epigenetic effect. Some viruses affect gene expression, for example, producing broken flower color. Geneticists

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use deliberate virus infections to learn how differences in gene expression may affect particular phenotypes.

Infections caused by certain viruses can induce gene silencing where none was present before. Infections caused by other viruses can turn-off gene silencing when it was previously present. These sorts of epigenetic effects may show random differences between genetically identical (clones) individuals or petals in a flower or flowers on a plant, etc. and may result in variegation.

There are several phenotypes in ornamental flowers which have an epigenetic component. Some dominant white mutations are due to gene duplications and/or inversions which activate certain types of gene silencing. If an individual heterozygous for such a dominant white mutation is infected with a virus capable of switching-off gene silencing the result is variegation with white and pigmented sectoring[34].

Another phenotype with an epigenetic component is the star pigmentation pattern in petunia flowers. If an individual with a star-type pattern is infected with a virus which is capable of switching-off gene silencing the star phenotype is altered and the resulting effect is variegation with sectoring[35].

Active transposons in daylilies do not seem to be common. Daylilies seem particularly good at keeping transposons inactive or inactivating them quickly. Cultivars which show broken flower color which suggest the excision of transposons are rare. The cultivars 'Doctor Strange Love' (Cochenour, 2007) and 'Willy Nilly' (Saxton, 1963) may have active transposons (compare with Figure 2 A). The cultivars 'Pink Stripes' (Derrow, 2006) and 'Peppermint Ice' (Lovell, 2004) may be examples of variegation due to gene silencing (compare with Figures 2 B, C and D).

The presence of an active transposon in a species is very useful to geneticists because transposons are as efficient as radiation and mutagenic chemicals in creating new mutations. However, apart from producing broken colored flowers, transposable elements are not likely to be of practical importance to daylily hybridizers. Using an active transposon to generate inheritable genetic mutations requires thousands to tens of thousands of offspring from appropriate crosses. As there are several elements of randomness in the size, number, shape and location of sectors associated with the excision of transposons, it is unlikely that transposons would be of practical importance in producing daylilies with consistent spotted or similar type patterns. Transposons are not implicated in the causes of spots or dots, etc. in plant species in which all or almost all individuals are spotted. For example, the genetic basis for patterns such as the dots or spots in Lilium has not been completely defined but current research indicates that they are not due to transposons[36].

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