

Variegation Studies in Camellia Japonica

Variegation describes the on/off patterning of color that occurs in flower petals. As an artist I'm interested in the visual effects, as well as the molecular mechanisms which allow color synthesizing genes to turn on and off.

Color Pathways: RNAi and CRISPR Gene Knockout

Variegation results from the over expression, or the silencing, of structural gene expression in the flavonoid biosynthetic pathway. A process called RNAi, or RNA interference, is a type of cellular silencing mechanism that uses the gene's own DNA sequence to turn the gene off. This process was discovered when researchers (Jorgensen, Napoli 1990) were trying to create darker purple petunias by injecting a transgene into the flower's own genome. This gene encoded for chalcone synthase, which was responsible for the petunia's purple coloring. Instead of creating a darker flower, the results yielded variegated patterns of purple and white, as well as pure white flowers. The insertion of an extra copy of the gene had resulted in gene silencing-an unpredicted result. This discovery was termed "co-suppression" of gene expression.

In 2017, in another landmark study, the first gene knockout of the purple color in petunias using CRISPR was achieved at the University of Tsukuba, by targeting a gene synthesizing an anthocyanin enzyme.

Both of these studies are examples of the significance that variegation and the ability to control color synthesizing genes in plants have for the broader field of gene regulation and molecular biology research. Jorgensen's RNAi studies became the cornerstone for the discovery of double stranded RNA and its ability to modulate gene expression, and provided the basis for researchers to combat cancer and retro-viruses such as AIDS. The Tsukuba gene knockout experiment demonstrated the preciseness of CRISPR technology to target a single gene positioned among two similar genes, and its potential in identify gene function through loss-of-function deactivation.

Landmarks in RNAi discovery
Jorgensen & Napoli: 1990's

What happened?
->Trying to insert a transgene for chalcone synthase (purple flower gene) into petunia to make darker colored flower
->Instead, produced white and variegated flowers
->Termed this "co-suppression"

Why?
->introduced (transgenes) were silenced, as well as plant's 'purple flower' gene
->Interference RNA blocks gene functionary inserting short sequences of RNA that match target gene's sequence, thus no proteins are produced

Transgene	Gene	Phenotype
Primary transgene	35S Pro - ChsA - nos3'	ChsA nos3' Fusion zone pattern
Secondary transgene	35S Promoter duplication	ChsA nos3' Solid purple
ChsA - nos3' duplication	ChsA nos3'	ChsA nos3' Vein patterns

PETUNIA KNOCK-OUT GENE
University of Tsukuba, Yokohama City University, Japan
Sept, 2017
used CRISPR to target a single gene to change flower color
dihydroflavonol-4-reductase (F3), encoding an anthocyanin biosynthesis enzyme

25 30 35 CR cycles 25 30 35

Variegation-differential cell lines:

The following projects and artworks are my attempts to investigate the phenomenon of variegation both visually and scientifically.

Studies of Variegation in Camellia Japonica Flowers:

Camellias are a highly manipulated cultivar that has been bred extensively for variegation. By their very nature they are a genetically unstable plant, as all camellia species can and will mutate. When a camellia bud shows variegation, even a single small streak of color, it serves as an indicator. It signals that the plant will at some time in the future form a “sport,”-a mutated branch. When genetically induced, variegation indicates the turning on or silencing of a specific color gene. When virally induced, it indicates the presence of a pathogen. Variegation evidences biological and evolutionary change-a resequencing of DNA.

I. Herbarium: Sacramento Capital Grove Variegated Camellias



Materials: Cotton tag board, botanical specimen, glue. Size: approx. 24" x 14"

II. Camellia Petal Lists



A series of ~30 Photographs, Inkjet on Hahnemuhle paper, size approximation: 24" x 14"
Each photograph depicts an individual camellia flower which has been taken apart and arranged in a manner that shows the sequential development of variegation in each blossom.



Sacramento Capital City Historic Pioneer Camellia Grove, Sacramento, California

III. Photographic and video documentation Of Capital City Pioneer Camellia Grove:



Reverse variegation in one individual bush



Single streak sector in a camellia flower



Splotchy variegation pattern -caused by a virus

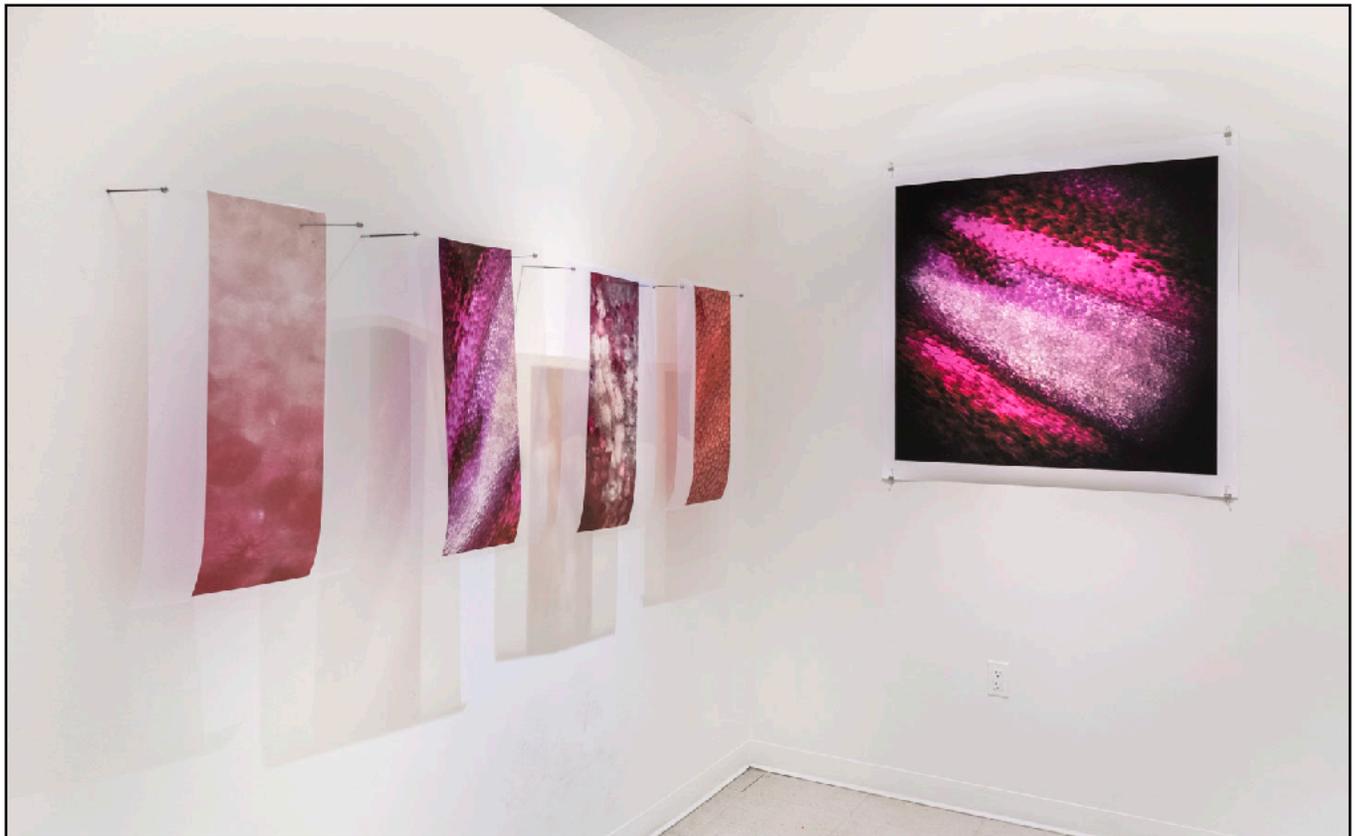


Sharply defined sectoring pattern
-created through genetic inheritance

IV. **Variegated Cell Lines, Micrographs**

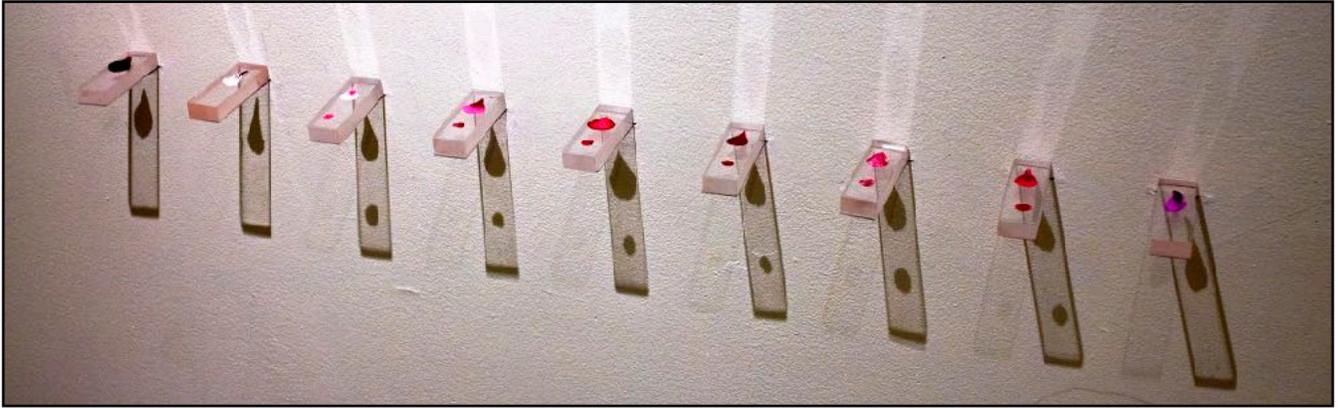


Photographs: Size and format vary



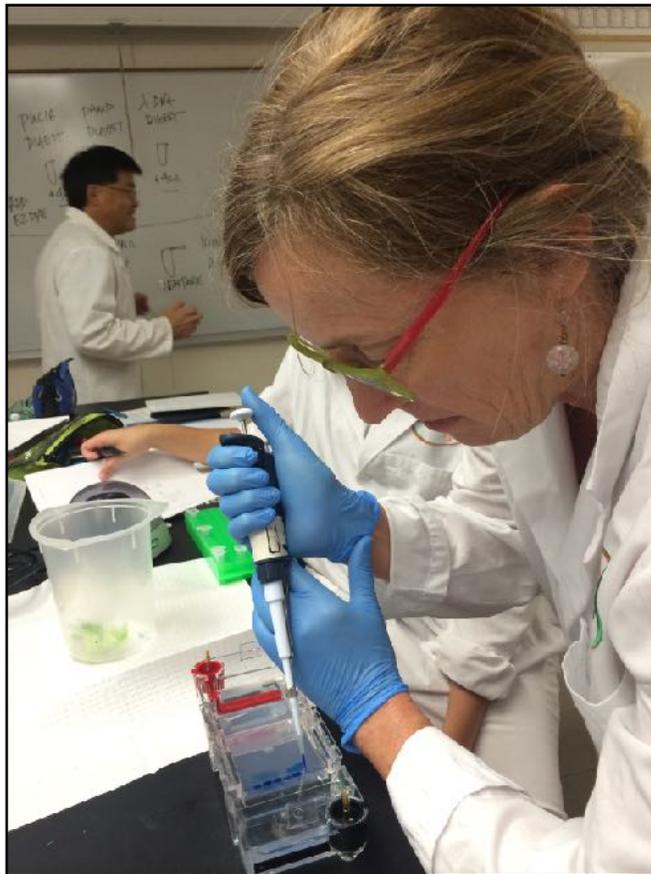
“Wall Slides, Geranium Tissue Specimens”, BioTransmutations show, SVA NYC, 2017
BioTransmutations Show, School of Visual Arts, NYC, 2017

V. Variegated Geranium Study



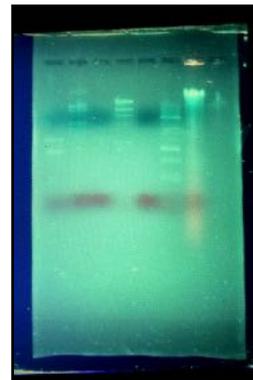
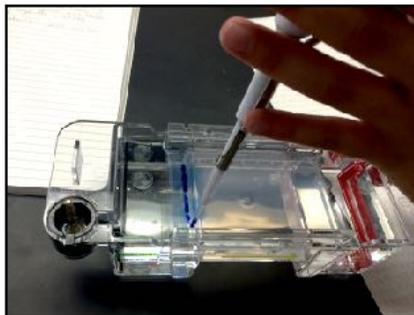
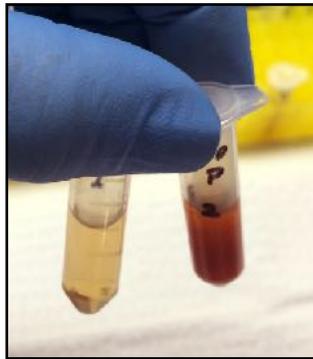
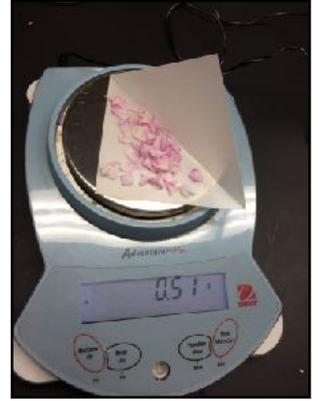
Materials: Glass, Plexiglass, geranium tissue; Size: each wall unit is 3" x 1"

Molecular work w ARC-BAC at American River College

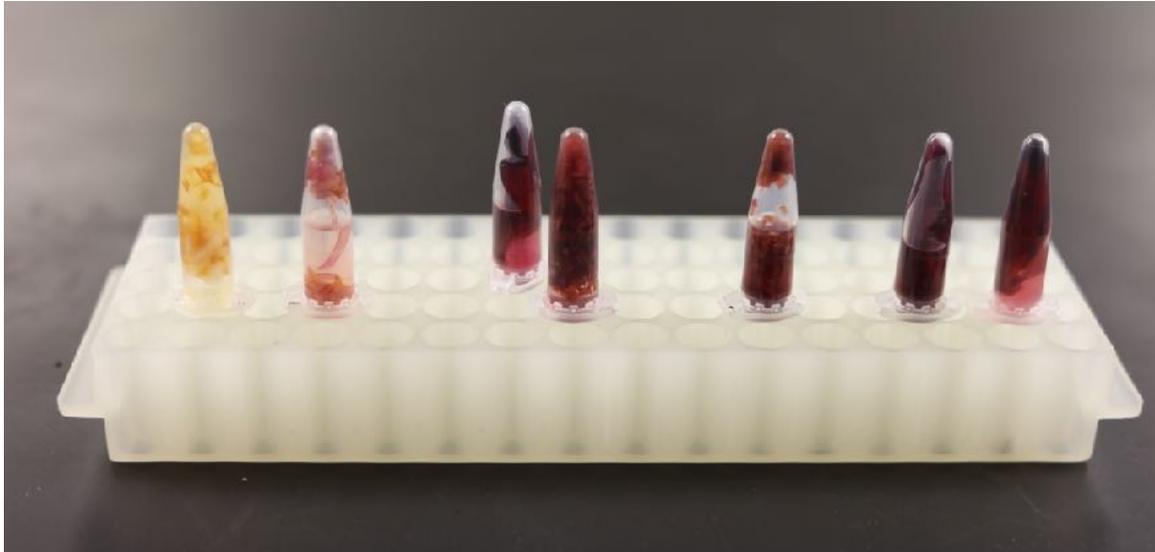


VI. Protocol: Red/White Dahlia, Plant DNA Extraction

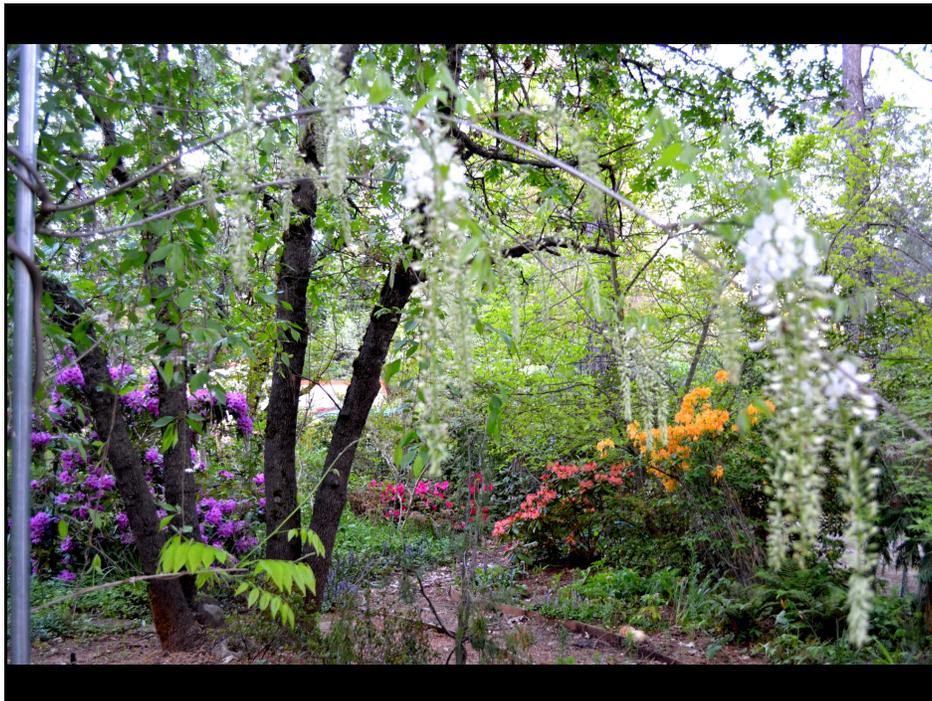
This art-based research project used a bicolored dahlia to do a plant DNA digestion.
Produced at American River College Biotechnology Department



VII: Protocol: Variegated Pigments, Paper Chromatography



VIII. Field Planting; My home gardens, experimental variegation studies



IX. **Community Biohacking Activity:** Counter Culture Plant Bio Group, Oakland, CA



X. **Educational/Biohacking Conferences:** MIT Biosummit, Cambridge, Massachusetts

