Breeding Carrots for Production, Resilience, Flavor, and Fun in Organic Systems




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Breeding in a nutshell

- Establish breeding goals
- Conduct trials and select best materials
- Make crosses, allow crossing
- Select the best plants
- Allow crossing of best plants
- Select, cross, repeat



Terminology

Open Pollinated (OP)

A variety that is produced by allowing plants to openly pollinate with others in the population. Seed saved from an OP will grow out true to type.

Hybrid (F1)

A variety that is the product of a controlled cross between two genetically distinct parents. Seed saved from a hybrid carrot variety will usually be male-sterile.

Phenotype = Genotype + Environment

Phenotype: the appearance of the plant – what you see

Genotype: the genetic makeup of the plant – what you don't necessarily see

Both the environment and the production system have significant impacts on the phenotype

Not everything you see will be passed onto the progeny

Terminology

Gene: unit of inheritance that controls a given trait. At a fixed location, or locus, on a chromosome.

Allele: one of a number of possible gene variants at a given locus

F1, F2, F3: abbreviation for Filial, indicating the generation after a cross

Terminology

Heterozygous: having non-identical alleles at a locus

Homozygous: having identical alleles at a locus

Dominant Allele: allele that produces a given phenotype regardless of whether it is homozygous or heterozygous

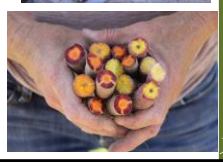
Recessive Allele: allele that produces a given phenotype only when it is homozygous

Trait inheritance – simple to complex

- **Simple**
  - Single, or few, gene(s) involved in trait expression
  - Phenotypes fit into distinct categories
  - Expression is less environmentally sensitive
- **Complex**
  - Many genes are involved in trait expression
  - Range or spectrum of phenotypes
  - Expression is more environmentally sensitive

Selection considerations

- Vigorous individuals
- Genetics and field conditions
- Disease and pest pressure
- Ideotype
- Eating quality: flavor and texture



Mass selection

Selecting individuals from a population

Tips:

- Large population
- Uniform conditions
- Select from whole field
- Good for phenotype traits

Disadvantages:

- Based only on phenotype
- Cannot realize maximum genetic potential
- Less uniformity achieved

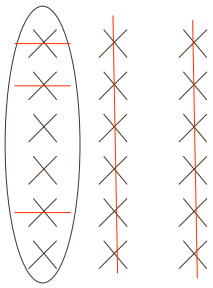
Family Selection

Selection of related individuals from a population (aka progeny selection)

Tips:
- Save seed in individual bags
- Plant individual rows next season
- Select between & within families
- Faster progress than mass selection
- Better for complex traits

Disadvantages:
- Selection more time consuming
- More field space needed
- Record keeping
- Inventory management

Family Selection



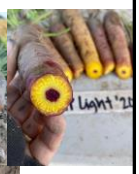
Selection Summary

Mass selection	Family selection
Slower progress	Faster progress
Easier	More difficult
Good for single gene and simple inheritance traits	Families give you insight into hidden genetics
No record keeping	Maintain separate families

Making Progress with Selection

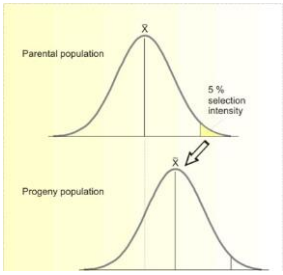
- Need variation for the trait(s) of interest within the population or cross
- Trait of interest may show high or low heritability
- Related individuals give you information about one another
- Higher potential for expression of recessive traits with family selection



How much to select?

- Selection intensity
- Seed from selected plants will grow into plants with less extreme phenotype
- Must balance against population size
  - Existing diversity



PYP Population Creation and Development



2019

2018

2021 PYP Light

2021 PYP Dark

Thank you!

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Carrot Color Genetics

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Madison, WI

Historical Developments in Carrot Improvement

- Domesticated 1100 years ago in area of Afghanistan (quite recent)
  - Yellow or purple (purple better-flavored)
  - Some possibility carrot was known in Roman Empire (100BCE)
- Spread west through Middle East, north Africa and Europe; east through India and China
- Selected for color, smooth roots, flavor, non-flowering
- Orange types selected in southern Europe in the 1400's-1500's
- Hybrid era began in 1950's

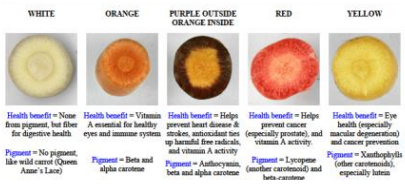
Records of cultivated carrots

Time	Location	Color
Pre-900's	Afghanistan and vicinity	Purple and yellow
900's	Iran and northern Arabia	
1000's	Syria and North Africa	
1100's	Spain	
1200-1300	Italy and China	
1300's	France, Germany, and Netherlands	
1400's	England	
1500's	Italy, Spain & Germany	First orange carrots
1600's	Japan	Purple and yellow
1600's	Northern Europe & North America	Orange and white
1700's	Japan	Orange
1721	Northern Europe	'Long Orange' & 'Horn' types described

- **Reviewed by O. Banga, 1950's and 60's;**
- **Stolarczyk and Janick, 2011**

Boy Holding a Carrot
1738 by F. Boucher (French)

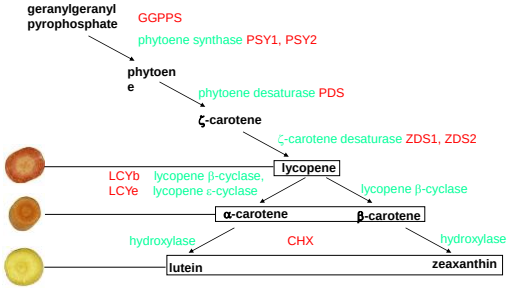
Carrot Color and Human Health

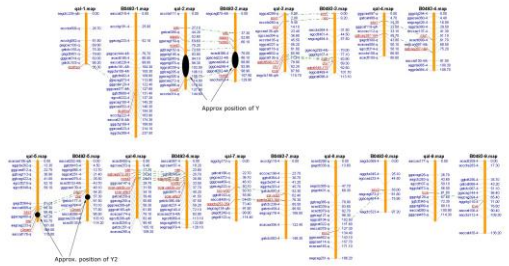


- Orange carrots are high in vitamin A precursors
  - alpha- and beta- carotene
- Vitamin A is an essential nutrient
  - Rarely a deficiency disease in more developed countries
- A significant problem in the developing world
  - Most frequent nutritional disease after protein-energy deficiency
  - 250M subclinical deficient children/year
  - Estimated 300-500,000 die annually from vitamin A deficiency
- Nutritional value of U.S. carrots has increased with classical breeding

Carrot carotenoids

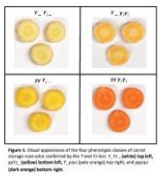
Carotene Biosynthesis





Y and *Y2* mapped by Carlos Santos and Brian Just

The *Y* and *Y2* genes control white vs. yellow and yellow vs. orange color

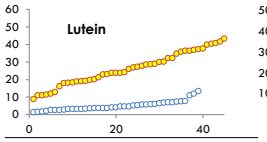


*Y_Y2_* - white
Y_y2y2 – pale orange
*yyY2_* - yellow
yyy2y2 - orange

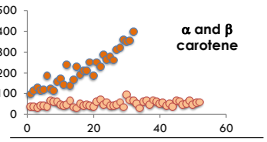
The *Y* gene

Pop 5249
Y2Y2

Pop 466
y2y2



Phenotypic class	No. roots	p-value
1) White (3)	262	0.75
2) Yellow (1)	78	



Phenotypic class	No. roots	P-value
1) Pale orange (3)	148	0.65
2) Orange (1)	53	

Ellison *et al.* 2017, *G3*

Y_2 Mapping population

Population

- 493B (Orange) x Wild (White)
- 213 F_4 individuals

Phenotyping - HPLC

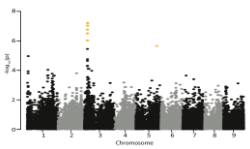
Phenotypic class	Beta-carotene (µg/g)
Yellow (161)	0.5 ± 1.5
Orange (52)	112.25 ± 59.0

Genotyping - GBS

- 33,712 high quality SNPs; one GBS marker every 11.3 kb

Or was recently identified via a genome-wide association study (GWAS)

- 154 wild and 520 domesticated carrots were used in this study
- *Or* is within a 143 kb region on Chromosome 3 flanked by the most significant SNPs
- Metrics to detect selective sweeps (pi, F_{st}, and XP-CLR) identified a region on chromosome 3



Ellison et al, 2018 (Under Review)

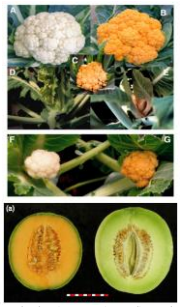
Or phenotype

yy y_2y_2 *oror* *yy* y_2y_2 *OrOr*

yy y_2y_2 *Oror*

What is the function of *Or* in plants?

- *Or*, first identified in Cauliflower, stimulates the biogenesis of chromoplasts in non-green tissues (Lu et al, 2006)
- In Arabidopsis, *Or* was shown to posttranstriptionally regulate PHYTOENE SYNTHASE (PSY), the rate-limiting enzyme in the carotenoid pathway (Zhou et al, 2015)
- Mutants of *Or* in melon were shown to prevent metabolism of β-carotene (Chayut et al, 2017)



Lu et al, 2006 (Top) and Tzuri, et al, 2015 (bottom)

Red is the most recessive carrot color-

Most difficult to develop but easiest to maintain in a breeding program

yyy2y2 – orange

yyy2y2oror – orange

yyy2y2r1r1r2r2 – red

Carotenoid Color Genetics

*Y_Y2_* - white

Y_y2y2 – pale orange

*yyY2_* - yellow

yyy2y2 – orange

yyy2y2oror – orange

yyy2y2r1r1r2r2 – red

Beta III progenitor HCM
270 ppm 140 ppm 450 ppm

~8 QTL across the genome control the range of orange color (alpha- and beta- carotene) in orange carrots
Ranges from 50ppm to 400ppm(Santos & Simon, 2002)
Several high carotene inbreds and populations released (Simon et al., 1989 to present)

Carrot anthocyanins

Water soluble pigments from a different biochemical pathway than carotenoids

Segregation of *Y2* and *P1* loci

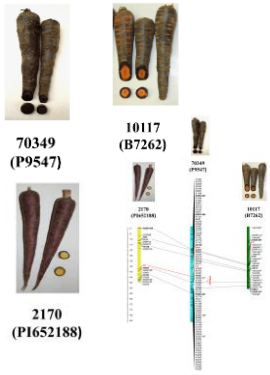
All on chromosome 3

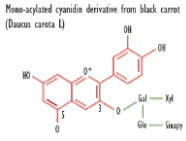
P_1 root phloem, B7262, eastern Turkey

Raa1 acylation of anthocyanins, P9547, central Turkey

P_3 root (*DcMYB6, 7*) & petioles (*DcMYB11*), P9547, central Turkey, and PI652188, China

P_2 nodes, B7262, eastern Turkey, not mapped





Cyanidin - Cy

Cy3XG Cy-3-Xyl-Gal -- **Non-acylated** Cyanidin-3-(2″-xylose-galactoside)

Cy3XGG Cy-3-Xyl-Glu-Gal -- **Non-acylated** Cyanidin-3-(2″-xylose-glucose-galactoside)

Cy3XSGG Cy-3-Xyl-SinapoylGlu-Gal -- **Acylated**

Cy3XFGG Cy-3-Xyl-FeruloylGlu-Gal -- **Acylated**

Cy3XCGG Cy-3-Xyl-(4-Coumaroyl)Glu-Gal **Acylated** Cyanidin-3-(2″-xylose-6″-feruloyl-glucose-galactoside)

Carrot Anthocyanins

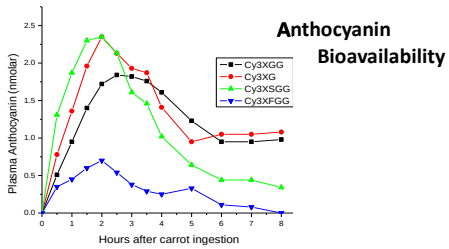
Cy3XG Cy-3-Xyl-Gal -- **absorbed readily**

Cy3XGG Cy-3-Xyl-Glu-Gal -- **absorbed readily**

Cy3XSGG Cy-3-Xyl-SinapoylGlu-Gal -- **absorbed somewhat**

Cy3XFGG Cy-3-Xyl-FeruloylGlu-Gal -- **absorbed somewhat**

Cy3XCGG Cy-3-Xyl-(4-Coumaroyl)Glu-Gal -- **not absorbed**



Anthocyanins Mask All Carotenoids

*P1_Y_Y2_* - white

P1_ Y_y2y2 – pale orange

*P1_ yyY2_* - yellow

P1_ yyy2y2 – orange

P1_ yyy2y2oror – orange

P1_ yyy2y2r1r1r2r2 – red

Thank you

This material is based upon work that is in part supported by USDA, ARS, the National Institute of Food & Agriculture, U.S. Department of Agriculture, under award numbers 2011- 51300-30903 & 2016-51300-25721 ; Calif. Fresh Carrot Advisory Board; vegetable seed companies

Challenging Traits to Breed Away From and Discovering New traits to Improve Carrots

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USDA-ARS, Madison, WI
Laurie McKenzie
Organic Seed Alliance

USDA Agricultural Research Service

THE UNIVERSITY of WISCONSIN MADISON

Variation in the carrot genome has provided the raw material that early farmers and modern breeders used to drive the domestication of wild carrot to the crop we have today
and will drive improvements of the crop to meet future needs and challenges

Daucus.carota ssp. *carota* (wild carrot), *D. carota* ssp. *sativus* (cultivated carrot)

A colorful history of carrot consumer value

- Domesticated 1100 years ago in area of Afghanistan (quite recent)
  - Yellow or purple (purple better-flavored)
  - Some possibility carrot was known in Roman Empire (100BCE)
- Spread west through Middle East, north Africa and Europe; east through India and China
- Selected for color, smooth, large roots, flavor, non-flowering
- Orange types selected in southern Europe in the 1400's-1500's
- Hybrid era began in 1950's

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Carrot Selection

Yellow core & green shoulders difficult to breed away

Flavor is an important trait for consumers of horticultural crops

Extensive genetic variation for flavor in diverse carrots

Flavor gene expression and mapping underway for several crops

"Sorry kid. That's the only flavor carrots come in."

Improving Carrot Farm Value

Diseases and Pests

- What U.S. growers want
- Hybrid cultivars for uniformity
- Disease and pest resistance
  - Alternaria leaf blight
    - QTL analysis
  - Root-knot nematodes
    - Some QTL analysis
  - Cavity spot, mildew, carrot fly, etc.
    - No published genetic mapping
- Bolting resistance
    - Some gene analysis
- Early seedling & plant vigor
    - No published genetic analysis
- Lower pesticide, fertilizer, energy input
  - U.S. organic production 11-14% of total crop
  - Larger top size suppresses weed competition
    - Some QTL analysis
- Heat/drought/stress tolerance

Root-knot nematode resistance

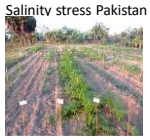
2014-17 Carrot Crop Wild Relative Project valuating diverse germplasm for abiotic stress

Heat stress Bangladesh

- Heat, drought, and saline field trials in Bangladesh by Md. A. Rahim and in Pakistan by A. Ali
  - **Heat & Drought Stress**
    - Stress tolerance in wild carrot
      - **Salinity Stress**
        - Stress tolerance in cultivated carrot

Drought stress Bangladesh

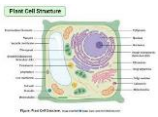
Salinity stress Pakistan



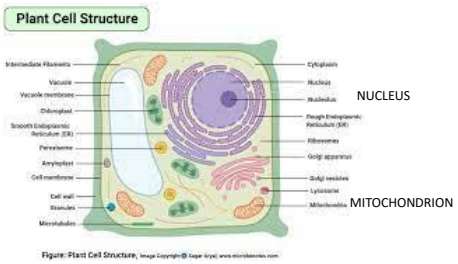
Funded by the Global Crop diversity Trust

CMS types in carrot

- Brown anther type (Sa)
  - OP varieties and wild carrot
  - Alloplasmic lines
- Petaloid type (Sp)
  - Wild carrot
    - Massachusetts, USA ('Cornell', SpC)
    - Wisconsin, USA ('Wisconsin Wild', SpW)
    - Guelph, Canada
    - Sölvesborg, Sweden (SpS)
    - Hiddensee, Germany (SpH)
  - Alloplasmic line, *D.c. maritimus*



DNA is in 3 parts of a plant cell



CHLOROPLAST

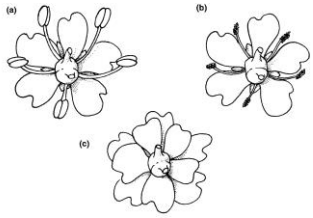
NUCLEUS

MITOCHONDRION

Figure: Plant Cell Structure, Image Copyright© Sagar Aryal, www.microbenotes.com

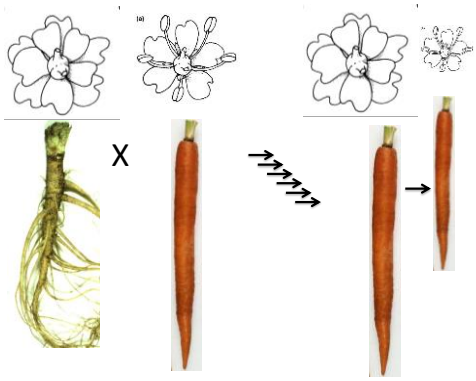
From microbenotes.com

Flower phenotypes in carrot



a) Normal (N-cytoplasm, restored CMS plants)
b) Brown anther CMS (Sa)
c) Petaloid CMS (Sp)

Petaloid Carrot Male Sterility



A-Male sterile cytoplasm B-Male fertile cyto. No fert. restorer (nuc.) Fertility restorer

Using Cytoplasmic Male Sterility

- Takes additional generations to produce seed
- Requires more record keeping and see production facilities
- Used for most large-scale production because of greater uniformity

Thank you

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