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

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

Selection Summary

<table>
<thead>
<tr>
<th>Mass selection</th>
<th>Family selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slower progress</td>
<td>Faster progress</td>
</tr>
<tr>
<td>Easier</td>
<td>More difficult</td>
</tr>
<tr>
<td>Good for single gene and simple inheritance traits</td>
<td>Families give you insight into hidden genetics</td>
</tr>
<tr>
<td>No record keeping</td>
<td>Maintain separate families</td>
</tr>
</tbody>
</table>
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

2018

2019
Thank you!

Questions?
laurenc@seedalliance.org or mcara@seedalliance.org
solveighan@ubc.ca

Stay in touch with OSA:

seedalliance.org/newsletter
Organic_Seed_Alliance
@Seed_Alliance
Organic_Seed_Alliance

Carrot Color Genetics

Phil Simon
USDA-ARS, Vegetable Crops Research & University of Wisconsin
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: Pre-900's
  - Location: Afghanistan and vicinity
  - Color: Purple and yellow

- Time: 900's
  - Location: Iran and northern Arabia
  - Color: Purple and yellow

- Time: 1000's
  - Location: Syria and North Africa
  - Color: Purple and yellow

- Time: 1100's
  - Location: Spain

- Time: 1200-1300
  - Location: Italy and China
  - Color: Orange and white

- Time: 1300's
  - Location: France, Germany, and Netherlands
  - Color: First orange carrots

- Time: 1400's
  - Location: England
  - Color: Purple and yellow

- Time: 1500's
  - Location: Italy, Spain, & Germany
  - Color: Orange and white

- Time: 1600's
  - Location: Northern Europe & North America
  - Color: Orange

- Time: 1700's
  - Location: Japan
  - Color: 'Long Orange' & 'Horn' types described

- Time: 1721
  - Location: Northern Europe
  - Color: 'Long Orange' & 'Horn' types described

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

---

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
  - 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

GGPPS
phytoene synthase, PSY1, PSY2
phytoene
phytoene desaturase, PDS
\( \zeta \)-carotene

\( \zeta \)-carotene desaturase, ZDS1, ZDS2
lycopene
LCYe, LCYe
lycopene \( \beta \)-cyclase
lycopene \( \beta \)-cyclase
lycopene \( \beta \)-cyclase
lycopene \( \beta \)-cyclase
hydroxylase
CHX
hydroxylase

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
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

**$Y_2$ Mapping population**

**Population**
- 4938 (Orange) x Wild (White)
- 213 $F_2$ individuals

**Phenotyping - HPLC**

<table>
<thead>
<tr>
<th>Phenotypic class</th>
<th>Beta-carotene ($\mu g/g$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow (161)</td>
<td>0.5 ± 1.5</td>
</tr>
<tr>
<td>Orange (52)</td>
<td>112.25 ± 59.0</td>
</tr>
</tbody>
</table>

**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
Or phenotype

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 posttranscriptionally 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)

Red is the most recessive carrot color—Most difficult to develop but easiest to maintain in a breeding program

- yyyy2y2 – orange
- yyyy2y2oror – orange
- yyyy2y2r1r1r2r2 – red
Carotenoid Color Genetics

- **Y_Y2_** - white
- **Y_y2y2** - pale orange
- **yyY2_** - yellow
- **yyy2y2** - orange
- **yyy2y2oror** - orange
- **yyy2y2r1r1r2r2** - red

~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

- P1 root phloem, B7262, eastern Turkey
- Roa1 acylation of anthocyanins, P9547, central Turkey
- P3 root (DcMYB6, 7) & petioles (DcMYB11), P9547, central Turkey, and P1652188, China
- P3 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)

Cy3XGG Cy-3-Xyl-SinapoylGlu-Gal → Acylated

Cy3XF GG 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

Anthocyanin Bioavailability

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
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

Phil Simon
USDA-ARS, Madison, WI

Laurie McKenzie
Organic Seed Alliance

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

~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 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, 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

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

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

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 2011-51300-31093 & 2016-51300-25721, Calif. Fresh Carrot Advisory Board, vegetable seed companies

philipp.simon@ars.usda.gov; psimon@wisc.edu