Unique Challenges in Breeding Specialty and Perennial Crops

Presentation for Advanced Plant Breeding Symposium
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Carl Jones PhD  Plant Sciences Director
Mars Advanced Research Institute
A hard crop,

A really hard crop,

Why a single trait can be hard even in an easy crop.
Cocoa is essential to the livelihoods of 40-50 million people worldwide. For most of them, cocoa is their main source of income.
Cocoa is produced in 13 countries

1. Côte d'Ivoire 39%
2. Ghana 17%
3. Indonesia 13%
4. Nigeria 6%
5. Cameroon 6%
6. Brazil 5%
7. Ecuador 4%
8. Peru 2%
9. Dominican Republic 2%
10. Colombia 1%
11. Papua New Guinea 1%
12. Uganda 1%
13. Mexico 1%

Côte d'Ivoire produces nearly 40% of the world’s cocoa.

Four West African countries produce 70% of the world’s cocoa.

The top four producers: Côte d'Ivoire, Ghana, Indonesia, Nigeria.

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The top four producers: Côte d'Ivoire, Ghana, Indonesia, Nigeria.

Are in the bottom half of nations GDP per capita.
Challenges in Cacao Production

30-40% of production is lost to 5 major pest and diseases each year

- Black Pod (Phytophthora spp.)
- Frosty Pod (Moniliophthora rorieri)
- Cacao Swollen Shoot Virus
- Vascular Streak Dieback
- Witches Broom Disease
Increased production as a result of expansion - not efficiency

1990: 443 kg/ha
2017: 442 kg/ha

1990: 8 T/ha
2017: 16.8 T/ha

Source: FAOSTAT (May 11, 2019)
THE GENETIC TOOLKIT
Historically, Three Groups Recognized

Two subspecies based on morphological traits and geographic origins, and a third that arose from their hybridization (Cheeseman 1944; Cuatrecasas 1964)

“Forastero” (*Theobroma cacao* subsp. *sphaerocarpum*)
- Amazonian
- Broader genetic diversity base

“Criollo” (*Theobroma cacao* subsp. *cacao*)
- Mesoamerican domestication
- Highly homozygous
- Fine flavor characteristics but highly susceptible to diseases

“Trinitario”
- Spontaneous hybrid when other types were brought to plantations in Trinidad
Figure 1. Localization of the origin of individuals analyzed; colors indicate the inferred genetic cluster to which they belong.

Figure 2. Neighbor joining tree from Cavalli-Sforza and Edwards genetic distance [16] matrix among the 36 subclusters identified using Structure (559 clones).


https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0003311

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Structure analysis of 700 cacao germplasm accessions
Implications for Breeding

Understand ancestry sources of production and resistance traits

Exploit heterosis, avoid inbreeding depression

Better estimations of heritability

Population structure to correct for GWAS
### Using molecular tools for detection of Off-types

#### Prevalence of off-types in Cacao collections

<table>
<thead>
<tr>
<th>COLLECTION/TRIAL</th>
<th>% OFFTYPES</th>
<th>REFERENCE</th>
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<tbody>
<tr>
<td>HYBRID</td>
<td>5.9%-8.6%</td>
<td>Dadzie et al., 2013</td>
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<tr>
<td>HYBRID</td>
<td>11.8%</td>
<td>Cervantes-Martinez et al., 2006</td>
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<tr>
<td>HYBRID</td>
<td>30%</td>
<td>Schnell et al., 2013</td>
</tr>
<tr>
<td>HYBRID</td>
<td>54.5%</td>
<td>Padi et al., 2015</td>
</tr>
<tr>
<td>CLONAL</td>
<td>0-100%</td>
<td>Padi et al., 2015</td>
</tr>
<tr>
<td>CLONAL</td>
<td>35%</td>
<td>Olasupo et al., 2017</td>
</tr>
<tr>
<td>CLONAL</td>
<td>6.9%</td>
<td>Romero Navarro et al., 2017</td>
</tr>
<tr>
<td>CLONAL</td>
<td>15-44%</td>
<td>Sounigou et al., 2001</td>
</tr>
<tr>
<td>CLONAL</td>
<td>20-100%</td>
<td>Padi et al., 2015</td>
</tr>
<tr>
<td>CLONAL</td>
<td>46.4%</td>
<td>Aikpokpodion et al., 2009</td>
</tr>
<tr>
<td>CLONAL</td>
<td>57.4-78%</td>
<td>Olasupo et al., 2017</td>
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LD in cacao genetic groups

Linkage Disequilibrium is effectively a measure of historical recombination.

We care about this because we need to “observe” recombination to find where important traits are in the genome.

Recombination only occurs each reproductive cycle (7 years)
Genome Wide Association Study

Uses diverse individuals to look for regions contributing to trait expression

TcMYB113 gene on chromosome 4 associates strongly with pod color (Motamayor et al., 2013)

Transcription factor with homologues regulating fruit color in Rosaceae, Solanaceae
Breeding for Multiple Traits

Yield  Pod Index  Fat Content  Self-Compatibility

Ceratocystis  Witches’ Broom  Black Pod  Frosty Pod

Resistance  Resistance  Resistance  Resistance

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Cacao Breeding Cycle Timeline

Traditional Cacao Breeding Cycle
- **0 yrs.** Crosses
- **4 yrs.** Progeny (hybrid) trial
- **7 yrs.** Initial clonal trial
- **7 yrs.** Advanced clonal trial
- **7 yrs.** Multi-location large scale trials
- **1 yr.** Prepare for commercialization, formal release
- **1 yr.** Deployment on farm
- **30.5 yrs.**

Genomic Selection Breeding Cycle - Accelerated
- **0.5 yr.** Crosses
- **2 yrs.** Progeny (hybrid) trial - Budwood Production Only
- **2 yrs.** Initial clonal trial
- **7 yrs.** Multi-location large scale trials
- **1 yr.** Prepare for commercialization, formal release
- **1 yr.** Deployment on farm
- **18.5 yrs.**

Multi-location large scale trials
- **7 yrs.** Advanced selection multi-location large scale trials
- **1 yr.** Prepare for commercialization, formal release
- **1 yr.** Deployment on farm
- **28 yrs.**
Breeding is cyclical

<table>
<thead>
<tr>
<th>Stage</th>
<th>Duration</th>
<th>Notes</th>
</tr>
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<tbody>
<tr>
<td><strong>Genomic Selection</strong></td>
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<td><strong>CacaoBreeding Cycle</strong></td>
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<td><strong>Accelerated</strong></td>
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<td><strong>Crosses</strong></td>
<td>0.5 yr.</td>
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<td><strong>Progeny (hybrid) trial</strong></td>
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<td><strong>Parallel Path</strong></td>
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<tr>
<td><strong>Advanced selection multi-location trials</strong></td>
<td>7 yrs.</td>
<td>Prepare for commercialization, formal release Deployment on farm 1 yr.</td>
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<td><strong>Multi-location large scale trials</strong></td>
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<td>1 yr.</td>
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<tr>
<td><strong>Deployment on farm</strong></td>
<td>1 yr.</td>
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<tr>
<td><strong>After initial cycle:</strong></td>
<td></td>
<td>new releases every 2-3 yrs.</td>
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<tr>
<td><strong>Deploy on Farm</strong></td>
<td>1 yr.</td>
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Mint

Mint oil is a key source of natural flavors for gum, candy, perfumes, cosmetics and health care products.

Industry based on a few number of cultivars

**Peppermint** [Mentha x piperita L.]
Native **Spearmint** [Mentha x spicata L.]
Scotch **Spearmint** [M. x gracillis Sole]
Corn Mint [Mentha Arvensis]

Susceptible to pests
Asexual plant reproduction
Limited genetic information

Black Mitcham [ ploidy: 6X ]
Origins of cultivated mints

1. Spearmint
   - (-)-Carvone 60-70%
   - (-)-Limonene 13-20%
   - (-)-Dihydrocarvone 3-5%

2. Peppermint
   - (-)-Menthol 37.47%
   - (-)-Menthone 15-21%
   - (+)-Menthofuran 5-8%
   - 1,8-Cineole 4-6%
   - (-)-Menthyl acetate 4-6%

M. suaveolens × M. longifolia

M. aquatica

Sterile

= 12 chromosomes
Diploid progenies flavor composition analysis (GC-FID)
Mint diploid relatives are highly diverse

Oil composition does not correlate with genetic similarities

Spearmint type-like profiles are present in diploid species
Pepper Phytophthora Resistance – A 50 year journey from discovery to product

1965
Resistance discovered in Landrace pepper CM334

Breeders and scientists struggle for years to combine meaningful resistance with acceptable commercial qualities

Public and private QTL mapping identify a strong QTL on chromosome 5 2010-2014

Work at Seminis discovers key modifying loci

Years of additional work finally create key recombination events and Identify AIG1 gene 2018

Products start hitting markets 2020

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Thank you

linkedin.com/in/carl-m-jones
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