Molecular Systematics & Ethnobotany Case Study: Breadfruit

• Hawaii, California, Bering Straight
• “Bounty-hunting”
• “Pandora’s Box”
• Breadfruit
• Molecular systematics

I. Circumstances Leading to the Mutiny on the Bounty

- James Cook’s sailing master (2nd Voyage; 1776)
- Captain, HMS Bounty (1787-1789)
George III (reign: 1760-1820) portrait by Allan Ramsay, 1762

Mutiny on the Bounty, 1789

HMS Bounty

Mutiny on the Bounty, 1789

The voyage of the HMS Bounty, 1787-1789

-23 Dec 1787, departed for Tahiti

Mission: To collect breadfruit for West Indies.

Crew:
Bligh (Captain)
Fletcher Christian (Master’s Mate)
2 Civilian botanists
42 other a

…the truth behind the legend.
The voyage of the HMS Bounty, 1787-1789

-28 Oct 1788, reached Tahiti
  5 mos. collecting & preparing 1015 breadfruit trees.
-4 Apr 1789; set sail for West Indies

-29 Apr 1789, mutiny. Led by Fletcher Christian

After the mutiny, 1789

Bligh setting out (ultimately) for Timor and the Malay Archipelago (3618 nautical miles or 6701 km over 47 days with only a sextant, pocket watch, and no compass)
After the mutiny, 1789

Mutineers: attempts to settle Tubuai (3 mo.; cannibals), then 14 back to Tahiti, 17 others went along with 9 Tahitians, 7 Tahitian women, and 1 baby.

Why the mutiny?
After the mutiny

HMS *Pandora*

- 14 mutineers in Tahiti caught, held in “Pandora’s Box”

*Pandora (G.) =*

- Ca. 8th Century BCE
- First woman, a gift from Zeus & other gods
- She comes bearing another gift – a mysterious jar.
- She gives into temptation & The rest is history (or myth).

Return (1791): Great Barrier Reef, 4 prisoners and 31 crew lost, Timor refuge.

-Britain (1792): 10 surviving “mutineers” tried.

— *Pandora* by John W. Waterhouse, 1896

Pitcairn Islands

- 1789: 8 mutineers, 6 Tahitian men, 11 women and 1 baby.
- 1783: 4 mutineers & 10 women plus their children remaining, Cristian not among them.
- By 1808: “Discovered” by British; 1 surviving mutineer plus original women and descendants.
- Today: ca. 50 inhabitants.
II. Breadfruit Origins
A. Artocarpus & Artocarpus altilis

- 60 spp of trees & shrubs
- SE Asia & Pacific
- Moraceae (mulberry & fig family)
II. Breadfruit Origins

A. Artocarpus & Artocarpus altilis

- Produce large, multiple fruits
- Source of breadfruit & jackfruit
Breadfruit, Artocarpus altilis

- Starch staple of Pacific islands, millennia.
- Humans selected for sterile (seedless) cultivars.
- Sterile throughout much of range.
- Classic example of crop species that evolved & spread with humans.

- Where, how, and when did breadfruit originate?
- What is the wild progenitor species?
- Why & how have sterile varieties persisted?
- What role have humans had in breadfruit origins and dispersal?
II. Breadfruit Origins
B. 3 Prior Hypotheses
1. Morphological Evidence:
   - similarity to two other species

II. Breadfruit Origins
B. 3 Prior Hypotheses
1. Morphological Evidence:
   - Fruit shape & texture is variable in A. altillis and intermediate between the other two.

II. Breadfruit Origins
B. 3 Prior Hypotheses
1. Morphological Evidence:
   - Leaves are variable, but suggest contributions from both
II. Breadfruit Origins

B. 3 Prior Hypotheses

2. Biogeographical Evidence:
   - Overlapping distributions

C. Hypothesis Testing (Zerega et al. 2005)

1. Methods Used
   - a. Phylogeny reconstruction within Artocarpus
      1) Phylogenetic (cladistic) inference can be done with either molecular or morphological characters.
II. Breadfruit Origins
C. Hypothesis Testing (Zerega et al. 2005)

1. Methods Used
   a. Phylogeny reconstruction within Artocarpus

   2) Plants have 3 genomic sources of molecular data

   3) Zerega et al. used two genes: ITS and trnLF

   4) PCR & DNA sequencing allows selective amplification and determining
      the nucleotide sequence of a small region of DNA

   5.8S 18S 26S

   nuclear ribosomal DNA locus
II. Breadfruit Origins

C. Hypothesis Testing (Zerega et al. 2005)

1. Methods Used
   a. Phylogeny reconstruction within Artocarpus
      4) PCR & DNA sequencing
         allows selective amplification and determining
         the nucleotide sequence of a small region of DNA

         \[
         \begin{array}{ccc}
         \text{trnL intron} & \text{trnL-F spacer} \\
         \text{trnL 5' exon} & \text{trnL 3' exon} & \text{trnF exon}
         \end{array}
         \]

         trnL – trnF region

   b. Conclusions:
      A. camansi & A. mariannsis
      are BF's closest relatives. BF may
      be derived from A. mariannensis.

   c. But Hybrid Hyp. can't be
      ruled out cladistically.

      1) Cladograms only resolve
         phylogeny, and not
         taxogony.
      2) Although ITS or trnLF may
         be from A. mariannensis, other
         genes may be from A. camansi.
      3) >200 cultivars exist for A.
         altilis breadnut

Problems with 1- to few-gene approach when dealing with hybrid hypothesis (for nuclear genes):

A. mariannensis

\[\begin{array}{c}
\text{duplug} \\
AABB
\end{array}\]  

A. camansi

\[\begin{array}{c}
\text{breadnut} \\
AaBb
\end{array}\]  

X

A. altilis

\[\begin{array}{c}
\text{Nelimgie} \\
AaBb
\end{array}\]  

\[\begin{array}{c}
\text{Polynesia} \\
AaBb
\end{array}\]  

Hybrids are expected to have genetic markers of both species ("additivity") for nuclear genes.
Problems with 1- to few-gene approach when dealing with hybrid hypothesis (for nuclear genes):

A. mariannensis

dugdug

AABB

A. camansi

breadnut

aabb

Arto. altilis

AaBb

Arto. altilis

AaBb

Problems with cpDNA or mtDNA approach when dealing with hybrid hypothesis:

A. mariannensis

dugdug

cpHaplotype M

A. camansi

breadnut

cpHaplotype C

Arto. altilis

cpHaplotype M or C, depending who the mother was
Problems with cpDNA or mtDNA approach when dealing with hybrid hypothesis:

\[ A. \text{marianensis} \quad \times \quad A. \text{camansi} \]

- **A. mariannensis**: dugdug (cpHaplotype M)
- **A. camansi**: breadnut (cpHaplotype C)
- **Artocarpus altillis**: cpHaplotype M or C, depending who the mother was

II. Breadfruit Origins

C. Hypothesis Testing (Zerega et al. 2005)

1. Methods Used
   b. AFLP Analysis in *Artocarpus marianensis*, *A. altillis* and *A. camansi*.

1) Key points to AFLP approach for testing hybrid hypothesis
   - Sampling of many individuals within species and cultivars.
   - Rapid, genome-wide rather than 1 or 2 genes.
   - Look for additivity of *A. marianensis* and *A. camansi* markers in *A. altillis*.

2) Method Summary
   - Extract DNA from many different trees for each species
   - Cut-up into tiny pieces using restriction enzymes that cut at precise sequence motifs
   - PCR-amplify all fragments
   - Electrophoresis to visualize fragments

Amplified Fragment Length Polymorphisms (AFLPs)
(DNA fingerprinting)
II. Breadfruit Origins

C. Hypothesis Testing (Zerega et al. 2005)

2. Results

Map depicting the presence and abundance of *Artocarpus mariannensis* (dugdug, in black) and *A. camansi* (breadnut, in grey) AFLP genetic markers in breadfruit cultivars throughout Oceania (incl. Micronesia, Melanesia, and Polynesia).

- breadnut markers found in all breadfruit, dugdug in some.
- breadfruit in some regions are clearly exclusively of breadnut heritage.

If breadfruit is a hybrid

- *A. camansi* (breadnut)
- *A. altilis* (breadfruit)
- *A. mariannensis* (dugdug)

Then we expect additivity of AFLP markers

- *A. camansi* (breadnut)
- *A. altilis* (breadfruit)
- *A. mariannensis* (dugdug)
BreadfruitOrigins
C. Hypothesis Testing (Zerega et al. 2005)
3. Does archeology help explain this pattern?

Lapita: 4000-3000 ybp
Lapita reach Easter Island 1700 ybp
Lapita reach Hawaii 1700 ybp

Support for
breadrub origin
dugout origin
hybrid origin
II. Breadfruit Origins

- Later trade with people of Micronesia
- Breeding with Dugdug introduces dugdug genes

Breadfruit Genomics in the Pacific

Lapita reach Hawai'i 1700 ybp
Lapita reach Easter Island 1700 ybp
Lapita 4000-5000 ybp

Support for 1) the A. camansii-origin hypothesis & 2) the hybrid hypothesis

A. mariannensis (dugdug)
A. camansii (breadnut)
A. altilis (breadfruit)

Breadnut origin followed by introgression with dugdug