Molecular Systematics & Ethnobotany Case Study: Breadfruit

Thanks to Tim Motley & Nyree Zerega for pictures and information.

- Hawaii, California, Bering Straight
- “Bounty-hunting”
- “Pandora’s Box”
- Breadfruit
- Molecular systematics
I. Circumstances Leading to the Mutiny on the Bounty

William Bligh

- James Cook’s sailing master (3rd Voyage; 1776)

- Captain, HMS *Bounty* (1787-1789)

George III (reign: 1760-1820)
portrait by Allan Ramsay, 1762

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 sailors
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
Bligh setting out (ultimately) for Timor and the Malay Archipelago (3618 nautical miles; 6701 km).

Painting by Robert Dodd.

-29 Apr 1789, mutiny.
Led by Fletcher Christian

After the mutiny, 1789
-Bligh went to Timor and the Malay Archipelago.
(3618 nautical mile 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, 8 to Pitcairn Islands along with 6 Tahitian men, 11 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.) =
• first woman, a gift from Zeuss & other gods
• She comes bearing another gift – a mysterious jar.
• 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.
After the mutiny

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

Jackfruit, *Artocarpus heterophyllus*
Breadfruit, *Artocarpus altilis*

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

Seeded wild species >
- 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 Based on Biogeography & Morphology
-Breadfruit is morphologically similar to two other species

A. mariannensis (dugdug)  A. Altilis (breadfruit)  A. camansi (breadnut)

Fruit shape and texture is variable in A. altilis and intermediate between the other two.

A. mariannensis (dugdug)  A. altilis (breadfruit)  A. camansi (breadnut)
Leaf variation suggests contributions from both

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

Distribution of three species.
Breadfruit, *Artocarpus altilis*

Seeded & Sterile

Sterile

Seeded & Sterile

Sterile

Dugdug Origin

Breadnut Origin

Hybrid Origin

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

A. *camansi* (breadnut)
A. *altilis* (breadfruit)
A. *mariannensis* (dugdug)
II. Breadfruit Origins

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.

   2) Plants have 3 genomic sources of molecular data
      - mtDNA maternally inherited
      - cpDNA maternally inherited
      - nDNA biparentally inherited
II. Breadfruit Origins

C. Hypothesis Testing (Zerega et al. 2005)

1. Methods Used
   a. Phylogeny reconstruction within Artocarpus

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

nuclear ribosomal DNA locus
Portion of most parsimonious cladogram

Conclusions:
- *A. camansi* & *A. mariannensis* are BF's closest relatives. BF may be derived from *A. mariannensis*.
- But Hybrid Hyp. can't be ruled out cladistically.

1) Cladograms only resolve phylogeny, and not tokogeny.
2) Although ITS or trnLF may be from *A. mariannensis*, other genes may be from *A. camansi*.

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

- *A. mariannensis* (dugdug) *AABB*
- *A. camansi* (breadnut) *aabb*
- *Arto. altillis* *AaBb*

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

Sex in hybrid species would thoroughly mix alleles.

Arto. *altilis*
- AaBb

Cladogram derived from gene A using this plant as an *A. altilis* representative would show *breadnut* as sister.

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Cladogram derived from gene A using this plant as an *A. altilis* representative would show *digdug* as sister.

Finer data needed (ca. 200+ cultivars of *A. altilis*)

A. *blancoi*
- A. *treculianus*
- A. *excelsus*
- A. *louii*
  - A. *kemando*
  - A. *maingayi*
  - A. *sericicarpus*
  - A. *tamaran*
  - A. *elasticus*
  - A. *scortechenii*
  - A. *camansi* (breadnut)
  - A. *camansi* (breadnut)
  - A. *mariannensis* (dugdug)
  - A. *Mariannensis* (dugdug)
  - A. *altilis*, Micronesia
    - A. *altilis*, E. Polynesia
    - A. *altilis*, Melanesia
    - A. *altilis*, W. Polynesia
II. Breadfruit Origins
C. Hypothesis Testing (Zerega et al. 2005)

1. Methods Used
   b. AFLP Analysis in Artocarpus marianensis, A. altilis 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 two genes.
         • Look for additivity of A. marianensis and A. camansi markers in A. altilis.
      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
If breadfruit is a hybrid

A. camansi (breadnut)

A. altillis (breadfruit)

A. mariannensis (dugdug)

Then we expect additivity of AFLP markers

A. camansi (breadnut)  A. altillis (breadfruit)  A. mariannensis (dugdug)

Much of breadfruit is breadnut, some islands (esp. Micronesia) with dugdug genes too.

Diane Ragone (NTBG), N. Zerega
Does archeological evidence help explain this pattern?

1. Breadfruit initially derived from breadnut (not dugdug). Unseeded cultivars derived later from seeded. Vegetative propagation required for long voyages b/c seeds are short lived.
Support for

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

Breadnut Origin

Does archeological evidence help explain this pattern?

2. Lapita carry/trade breadfruit into Micronesia. Hybridization of sexual forms with dugdug.

Lapita reach Hawaii 1700 ybp
Lapita reach Easter Island 1700 ybp
Dugdug range
Breadnut range

Seed, sexual cultivars initially. Today both seeded and unseeded exist. Unseeded, asexual cultivars derived. Today primarily unseeded here.
A bit of the hybrid hypothesis (original Hyp 3):

Breadnut origin

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

Hybrid Origin

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

Does archeological evidence help explain this pattern?

Lapita reach Hawaii 1700 ybp
Lapita reach Easter Island 1700 ybp

Dugdug range
Breadnut range

Seeded, sexual cultivars introd. Today both seeded and unseeded exist.
Unseeded, asexual cultivars derived. Today primarily unseeded.

3. Hybrid breadfruits dispersed/traded out of Micronesia.
Lapita: 4000-3000 ybp

Lapita reach Easter Island 1700 ybp

Lapita reach Hawaii 1700 ybp

4. Limited trade/dispersal of hybrid breadfruits south into original breadfruit range, followed by backcrossing with pure breadnut-contributed breadfruits.

Seeded, sexual cultivars initially. Today both seeded and unseeded exist. Unseeded, asexual cultivars derived. Today primarily unseeded exist here.

Does archeological evidence help explain this pattern?