II. Breadfruit Origins
A. *Artocarpus* & *Artocarpus altilis*

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

A. *Artocarpus & Artocarpus altilis*

Produce large, multiple fruits
Source of breadfruit & jackfruit
Jackfruit, *A. heterophyllus*

Breadfruit, *Artocarpus altilis*
Breadfruit, *Artocarpus altilis*

Starch staple of Pacific islands, millennia.

Sterile throughout much of range.
Classic example of species (crop plant) 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 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.

![Fruit images](image)

*A. mariannensis*  *A. altilis, breadfruit*  *A. camansi*

Leaf variation suggests contributions from both

![Leaf images](image)

*A. mariannensis*  *A. altilis, breadfruit*  *A. camansi*
Distribution of three species.

A. mariannensis (dugdug)

A. camansi (breadnut)

Breadfruit, Artocarpus altilis

Seed & Sterile

Seeded & Sterile

Sterile

Sterile

Seeded & Sterile

Sterile
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.
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

- cpDNA maternally inherited
- mtDNA maternally inherited
- nDNA biparentally inherited

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

II. Breadfruit Origins

4) PCR & DNA sequencing

a.) PCR

1.) Design 2 primers that match conserved flanking regions
II. Breadfruit Origins

a.) PCR

1.) Design 2 primers that match conserved flanking regions

2.) PCR Mix: target DNA, primers, nucleotides, DNA polymerase from *Thermus aquaticus*

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**nuclear ribosomal DNA locus**

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3.) DNA Amplification Using Polymerase Chain Reaction

- Denaturation then primer annealing
- Copying (extension)
- Denaturation then primer annealing
- Copying (extension)
A typical PCR program

1. **Denaturing**: 95 °C for 1 min
2. **Annealing**: 60 °C for 1 min
3. **Extension** (polymerization): 72 °C for 1 min per 1000 bp

Repeat for 29 times for total of 30 cycles.
II. Breadfruit Origins

4) PCR & DNA sequencing
a.) PCR
b.) Sequence

Typical sequence output for 1 sample

A. altilis, Melanesia
A. altilis, Micronesia
A. altilis, E. Polynesia
A. altilis, W. Polynesia
A. blancoi
A. camansi (breadnut)
A. camansi (breadnut)
A. kemando
A. maingayi
A. sericicarpus
A. taman
A. elasticus
A. scortecheni
A. mariannensis (dugdug)
A. M. M. (dugdug)
A. altillis, Micronesia
A. altillis, E. Polynesia
A. altillis, Melanesia

Conclusions:
A. camansi & A. mariannsis 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

X

↓

Arto. *altilis*  
AaBb

Hybrids are expected to have genetic markers of both species (“additivity”) for nuclear genes.

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

A. *mariannensis*  
dugdug  
AABB

A. *camansi*  
breadnut  
aabb

X

↓

Arto. *altilis*  
AaBb

Sex in hybrid species would thoroughly mix alleles.

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

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

Then we expect additivity of AFLP markers

If breadfruit is a hybrid

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

Proportion of breadfruit population with dugdug markers (black = dugdug-specific markers)

Proportion of breadfruit population with breadnut markers (grey = breadnut-specific markers)

Does archeological evidence help explain this pattern?

Lapita: 4000-3000 ybp

Lapita reach Hawaii 1700 ybp

Lapita reach Easter Island 1700 ybp
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 Hyp 2:

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

Hypothesis 2
Lapita: 4000-3000 ybp
Lapita reach Easter Island 1700 ybp
Lapita reach Hawaii 1700 ybp

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

Does archeological evidence help explain this pattern?

A bit of the hybrid hypothesis (original Hyp 3):

Hypothesis 2

Hypothesis 3
3. Hybrid breadfruits dispersed/traded out of Micronesia.

Does archeological evidence help explain this pattern?

Lapita reach Easter Island 1700 ybp

Unseeded, asexual cultivars derived. Today primarily unseeded exist here.

Lapita reach Hawaii 1700 ybp

Seeded, sexual cultivars initially. Today both seeded and unseeded exist.

Lapita: 4000-3000 ybp

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