Does genetic delineation exist between Eastern Australian Helmet orchids?

Aiden Kennedy Varan (U. Melbourne)

CSIRO **PLANT INDUSTRY**
CENTRE FOR PLANT BIODIVERSITY RESEARCH
summer student program
THE AUSTRALIAN PASTORAL RESEARCH TRUST
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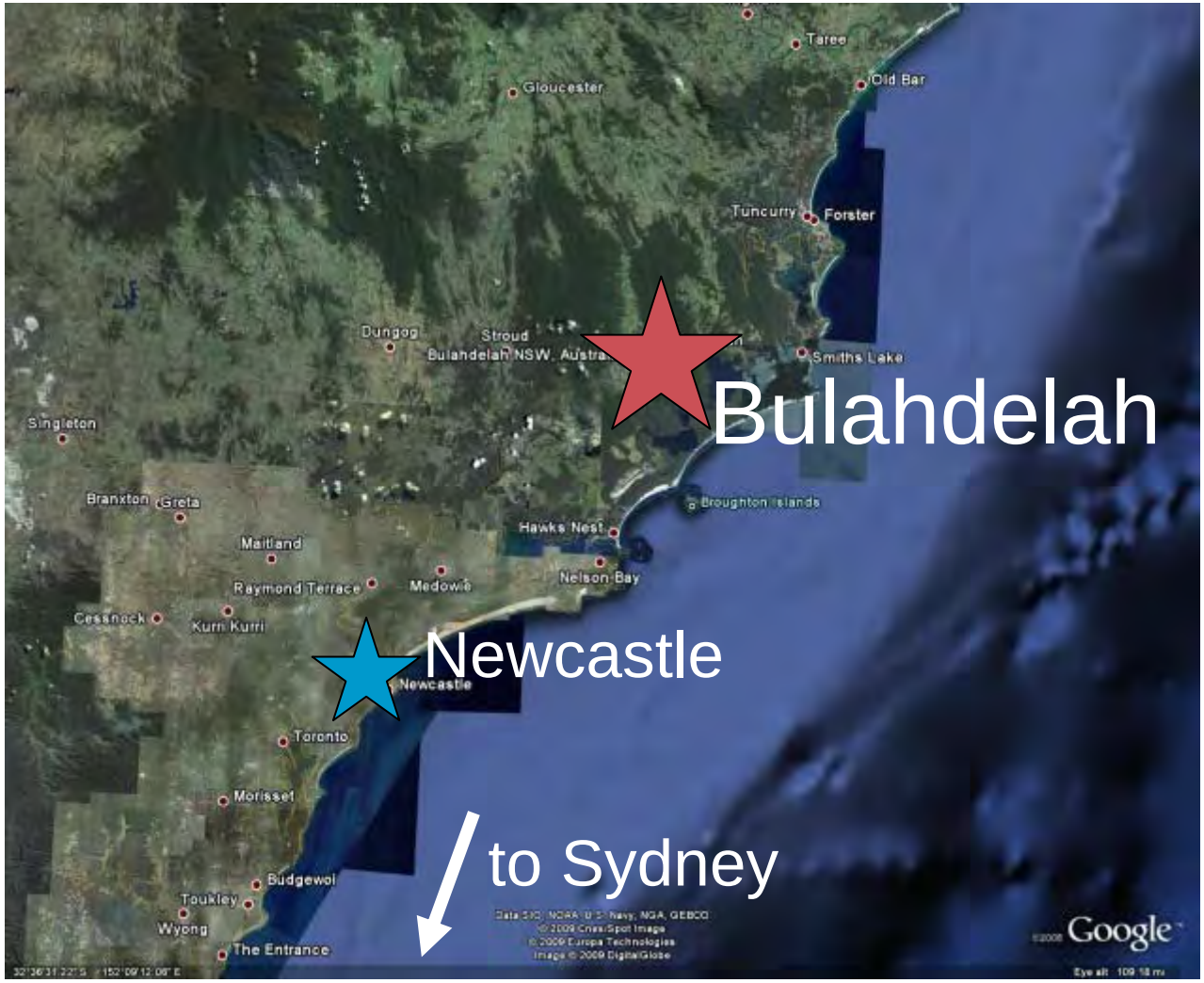
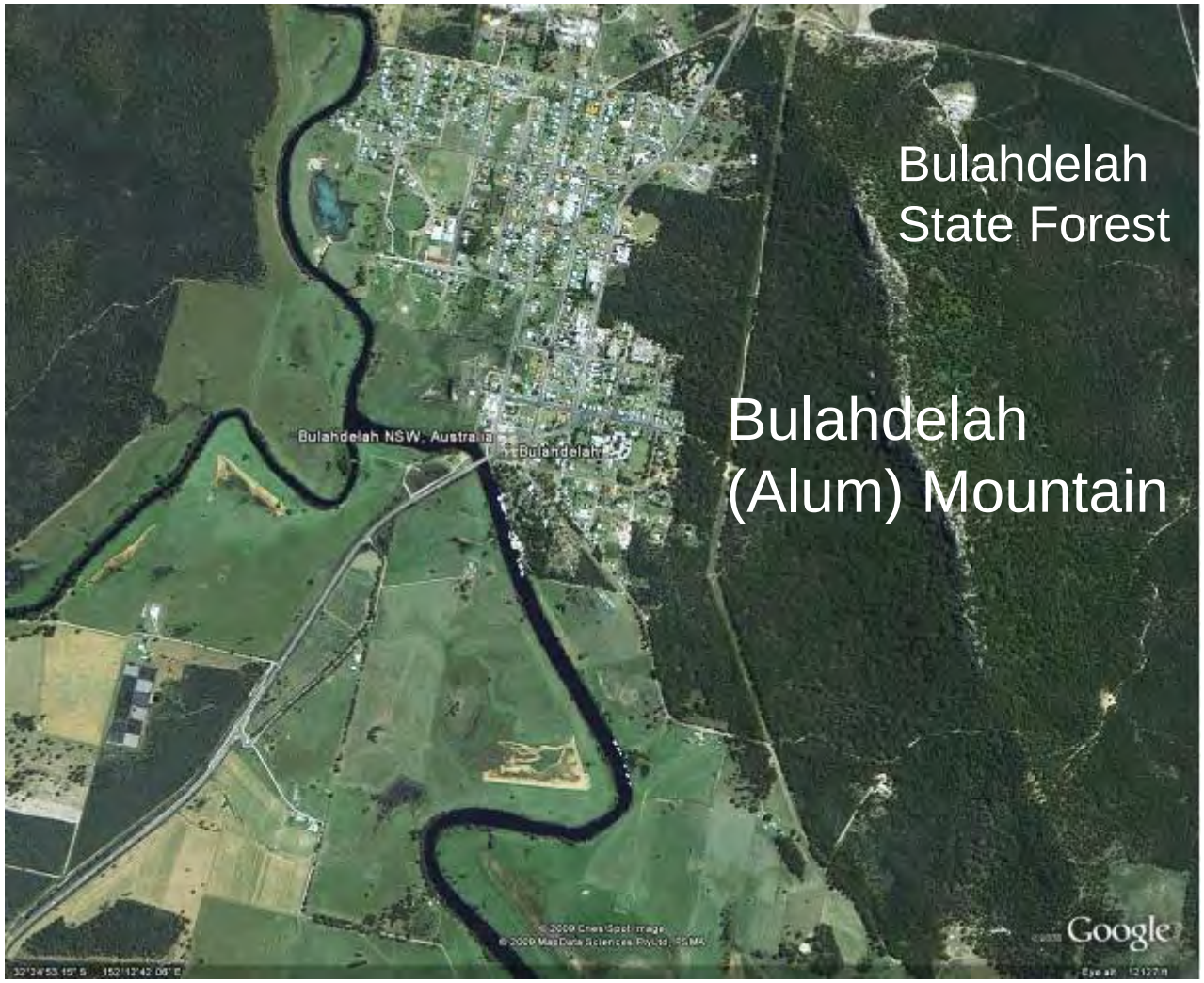
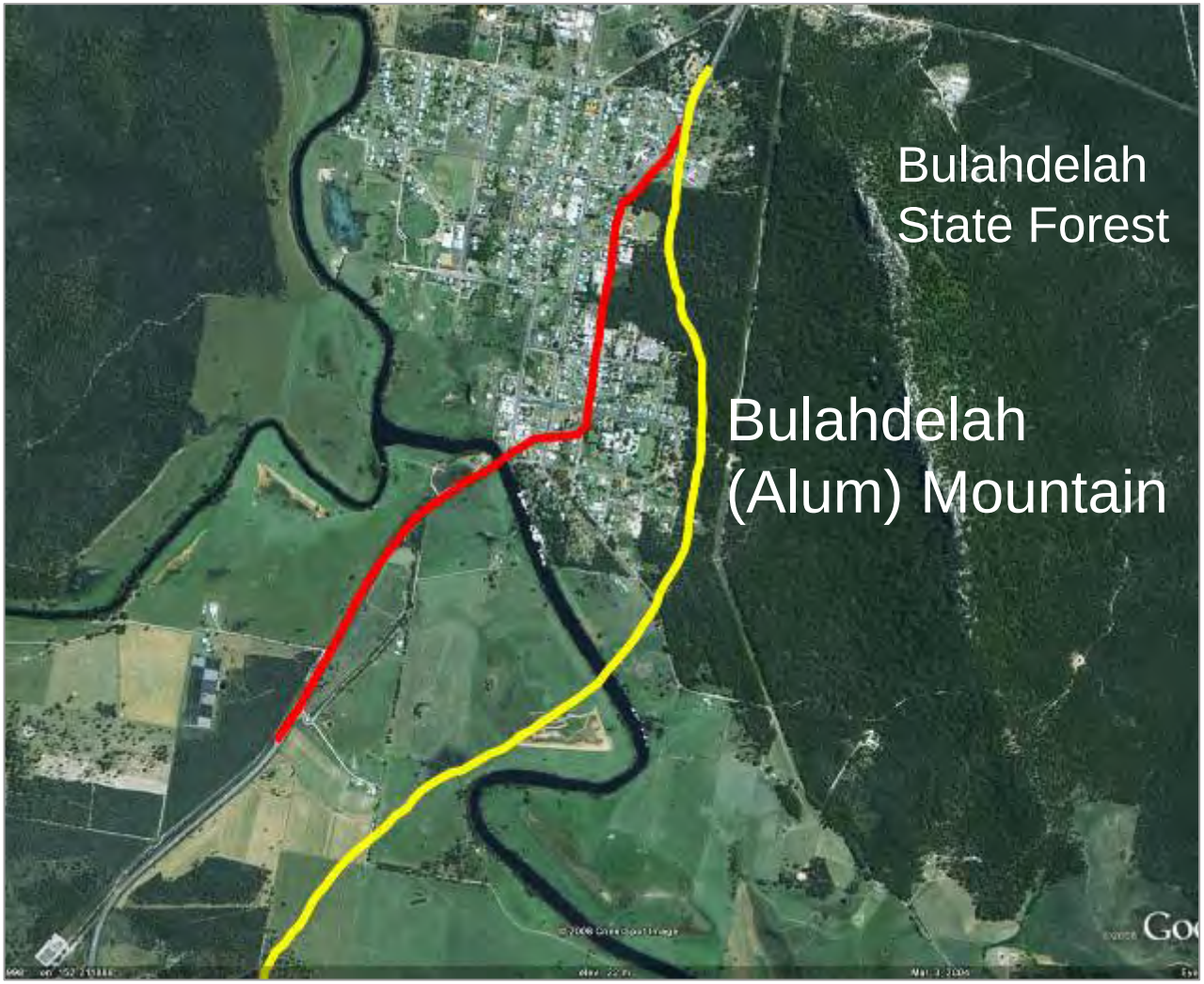
Study Site: Bulahdelah



Study Site: Bulahdelah



Pacific Highway Bypass



The protagonist

Corybas dowlingii (D.L. Jones 2004)

- Endemic Australian orchid
- Exclusively found in NSW, <1000km^2 range
- Bulahdelah population: ~ 3400 plants (2004)
- <u>Endangered species</u>

Corybas in Eastern Australia

Photos: C.G. Howard, D.L. Jones

Corybas in Eastern Australia

Photos: C.G. Howard, D.L. Jones

C. aconitiflorus

Corybas in Eastern Australia

Photos: C.G. Howard, D.L. Jones

C. aconitiflorus

C. barbarae

Corybas in Eastern Australia

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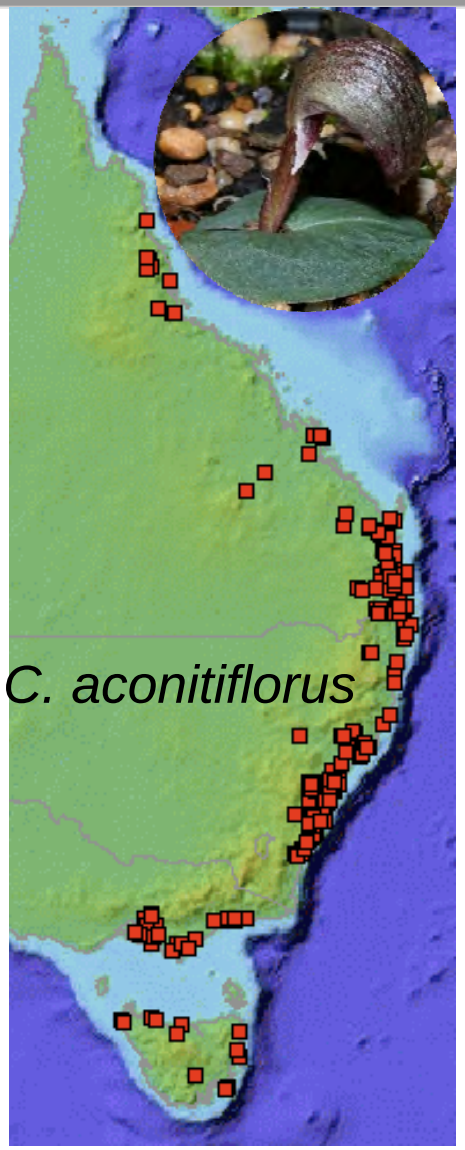
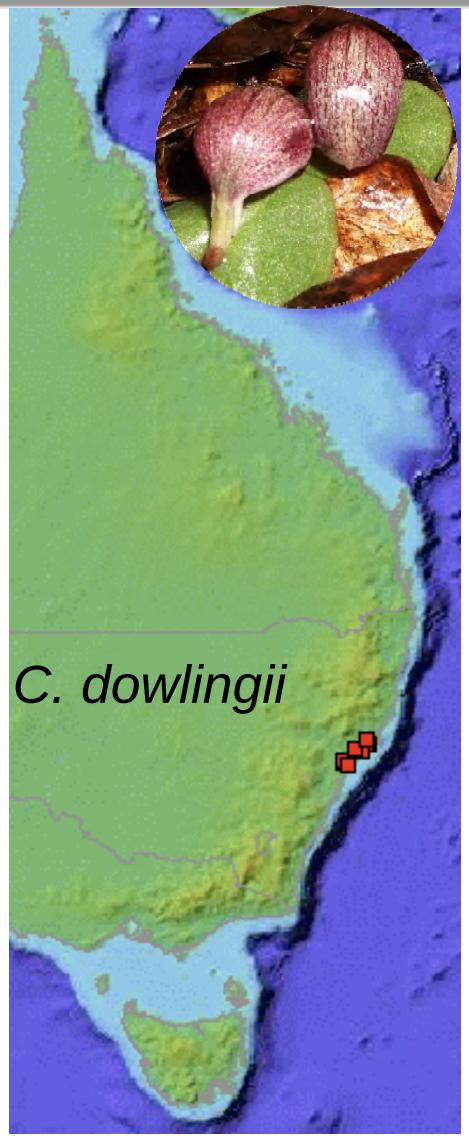
C. aconitiflorus

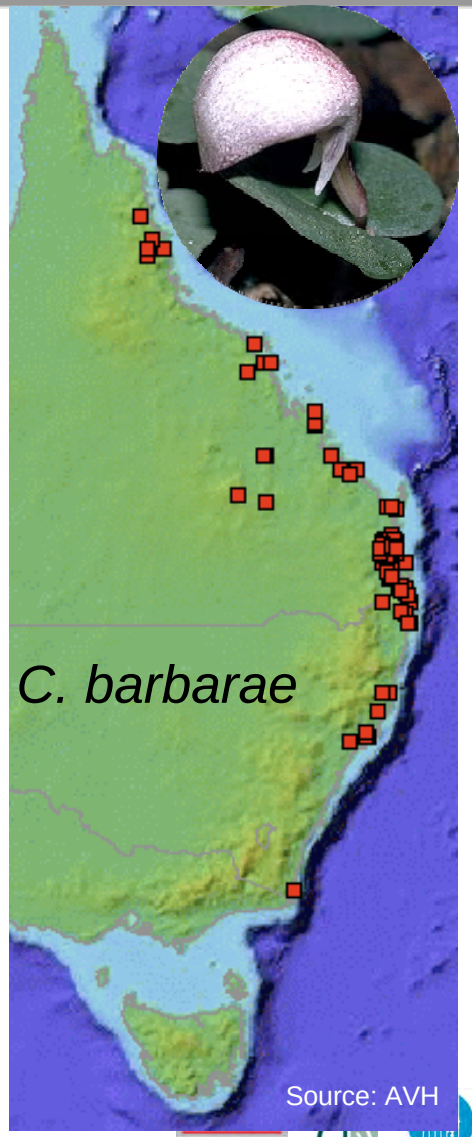
C. dowlingii

C. barbarae

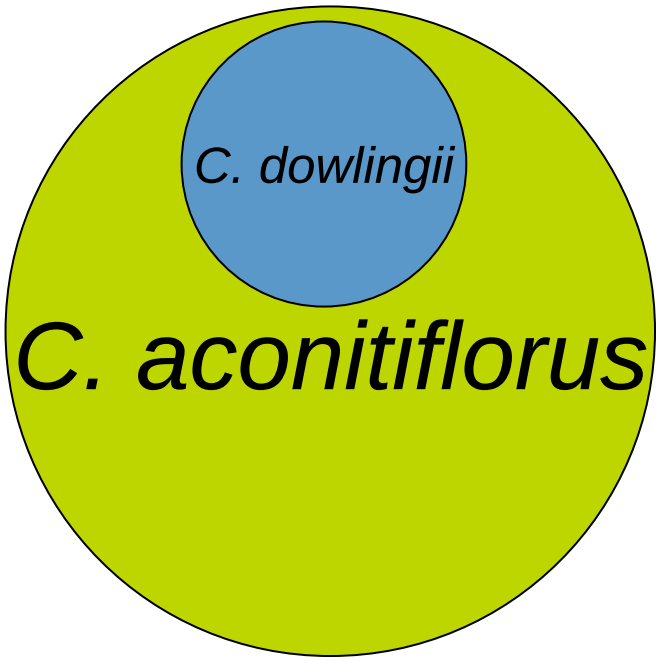
Corybas distribution

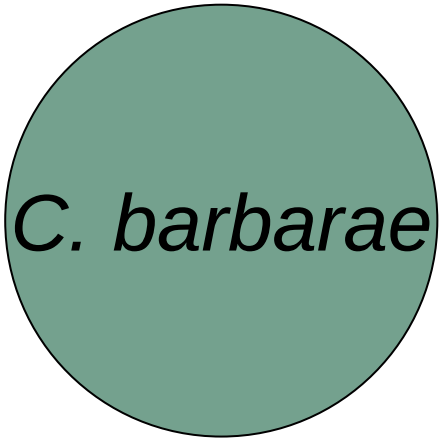






Competing hypotheses – rethinking *Corybas*





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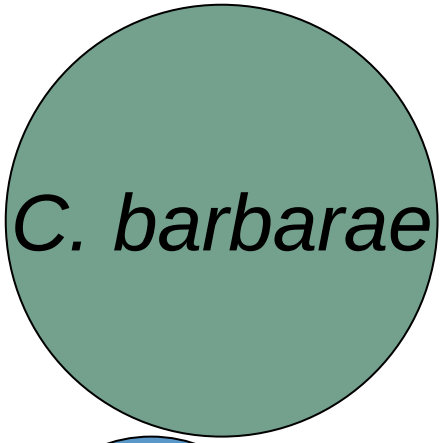
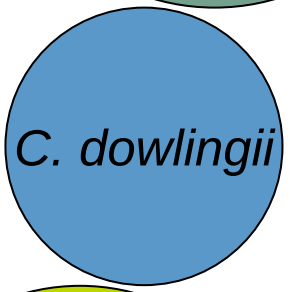
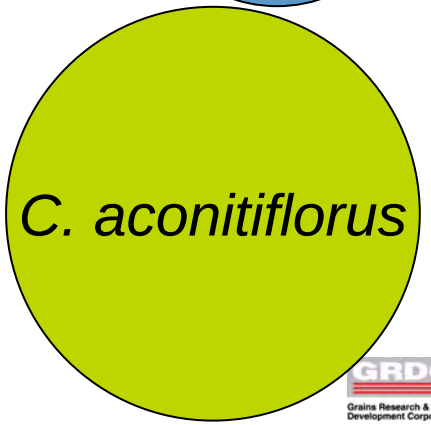


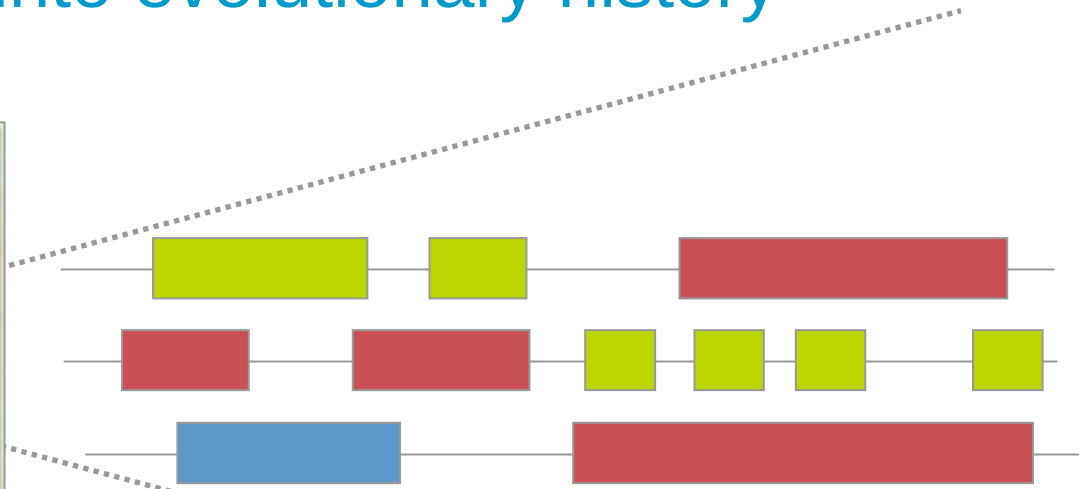




Genetic approach to systematics – Why?

- Avoid reliance on ambiguous markers
  - eg. flower colour
- Ability to develop PCR-based diagnostics
- Possible insight into evolutionary history

Photo: M.A. Clements



Genetic approach to systematics

Probe non-coding regions

- Useful for closely related organisms
- Rapid mutation & evolution capacity
- Nuclear + chloroplast regions considered

Photo: M.A. Clements

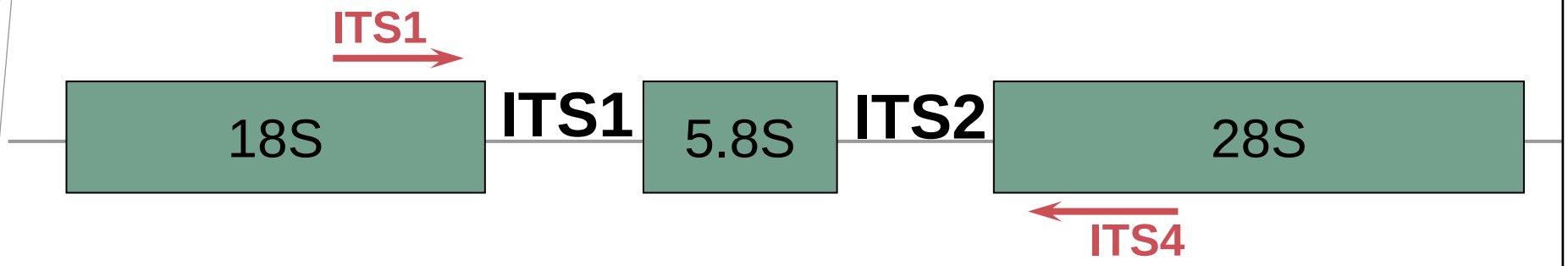
Available specimens

- Collections made prior to investigation
- Fresh material used
- DNA extracted from subset of collection

- *C. dowlingii*:
  good collection from Bulahdelah site
- *C. aconitiflorous, C. barbarae*:
  specimens from Bulahdelah and beyond

Initial Studies: ITS

- ITS (Internal Transcribed Spacer)
  - Nuclear DNA, widely used in phylogenetic analysis
  - 2 introns (non-coding) between ribosomal genes
  - Primers: **ITS1, ITS4**
  - **Variation between species:**
    - **Indels** (insertions/deletions)
    - **SNPs** (Single Nucleotide Polymorphisms)



ITS Results

- Successful amplification, sequencing
  - 2 specimens from each species used
- Contig constructed
  - Forward, reverse sequences for each specimen
  - Contig ~800bp across species

```
dowlingii           TGTCGAGACCCGAAAAGATCGAGCAATTTTGACAACCTGTGAACAATCAATAAGGTGGCG
aconitiflorous      TGTCGAGACCCGAAAAGATCGAGCAATTTTGACAACCTGTGAACAATCAATAAGGTGGCG
barbarae            TGTCGAGACCCGAAAAGATCGAGCAATTTTGACAACCTGTGAACAATCAATAAGGTGGCG
                    ************************************************************

dowlingii           ATGCGTGTCGTTGCCATACACCCTTTCTTTTGTCTTGTTTTCTTGAATGAAATATCTTGG
aconitiflorous      ATGCGTGTCGTTGCCATACACCCTTTCTTTTGTCTTGTTTTCTTGAATGAAATATCTTGG
barbarae            ATGCGTGTCGTTGCCATACACCCTTTCTTTTGTCTTGTTTTCTTGAATGAAATATCTTGG
                    ************************************************************

dowlingii           CATAAAGACTGGGTGGAAAACAAAATCGGCACAGCTTGGTGCCAAGGAATATATATAATG
aconitiflorous      CATAAAGACTGGGTGGAAAACAAAATCGGCACAGCTTGGTGCCAAGGAATATATATAATG
barbarae            CATAAAGACTGGGTGGAAAACAAAATCGGCACAGCTTGGTGCCAAGGAATATATATAATG
                    ************************************************************

dowlingii           CGAGTGCGCACGATACATCCTTTAAGCTGGATCTGTTTGAGGGACTATCGTTGTTGCTTC
aconitiflorous      CGAGTGCGCACGATACATCCTTTAAGCTGGATCTGTTTGAGGGACTATCGTTGTTGCTTC
barbarae            CGAGTGCGCACGATACATCCTTTAAGCTGGATCTGTTTGAGGGACTATCGTTGTTGCTTC
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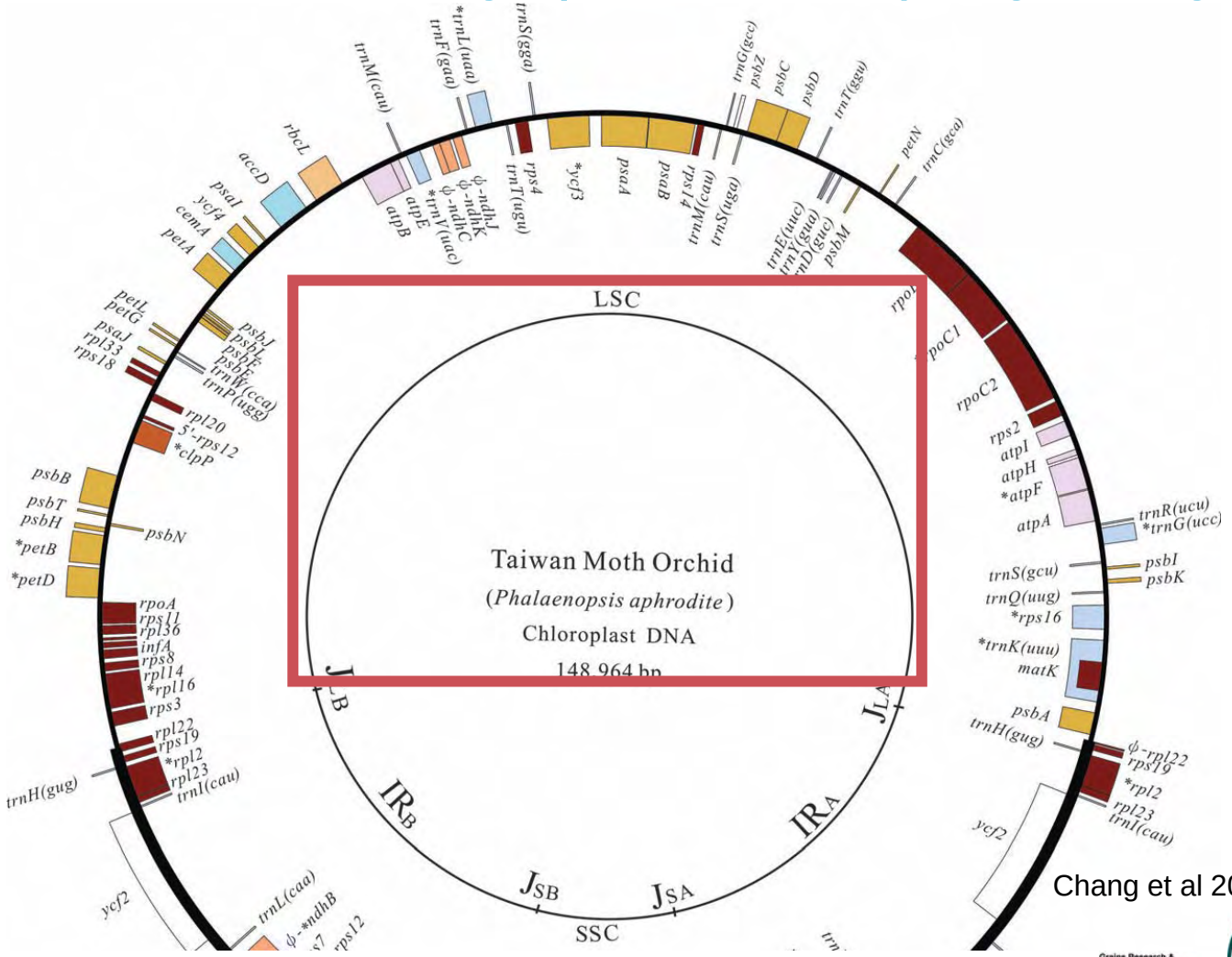
Main Studies: cpDNA

Focus on non-coding cpDNA LSC (Large Single Copy)



Chang et al 2006

Main Studies: cpDNA

- Adopting method of Ebert & Peakall (in press)
  - Universal cpDNA sequencing primers to probe LSC
  - >85% coverage of noncoding regions
  - Primer sites located within genes in LSC (conserved)


Photo: C.G. Howard

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- Adopting method of Ebert & Peakall (in press)
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Photo: C.G. Howard

- 2 types of variation considered
  1. **Indels** (insertions / deletions)
  2. **Mononucleotide simple sequence repeats** (SSRs), min. 8 repeats

cpDNA Results

Of the **53** regions accessible with universal cpDNA primer sets,


Photo: Chris Howard

47 successfully amplified, min. 1 specimen from each species

25 were sequenced for select specimens

>17kb of cpDNA covered in sequencing

cpDNA Results

Of the **53** regions accessible with universal cpDNA primer sets,

47 successfully amplified, min. 1 specimen from each species

25 were sequenced for select specimens

>17kb of cpDNA covered in sequencing

No indels

24 SSRs identified

Variable SSR

- cpDNA noncoding region “49” (*petBex2 - petDex2*)
  - T mononucleotide SSR – between 9 and 11 repeats
  - Variation not fixed within species

C. aconitiflorous												
Sample ID	A1	A2	A3	A4	A5	A6	A7	A8	A9	A11	A12	A13	A14
# Repeats	9	9	9	11	10	10	9	9	9	9	9	9	9

C. dowlingii										
Sample ID	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
# Repeats	9	10	9	9	9	10	9	9	9	9

C. barbarae								
Sample ID	B1	B2	B3	B4	B5	B6	B7	B8
# Repeats	9	9	9	11	10	10	9	9

Future directions

1. Expand sequenced coverage of cpDNA regions
   - Identify more SSRs for possible variability
   - Increase number of individuals sequenced
2. Widen search to include coding regions
   - Alleles as definitive indication of variation

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- If little substantial variation identified, reconsider structure of *Corybas* genus
  - Review of endangered listing of *C. dowlingii*
  - Implications for conservation at Bulahdelah site

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