

Climatic refugia and the significance of range disjunctions in South Australian eucalypts.

Progress report on the grant:

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Background

Funding was awarded to explore the evolutionary significance of geographic range disjunctions in *Eucalyptus* species occurring widely in eastern Australia and disjunctly in the Flinders-Mount Lofty Ranges (South Australia). We used range wide sampling and molecular (DNA sequence) data to determine the evolutionary relationships and divergence times among samples.

Progress

Population sampling

Population samples have been collected for three species: *E. albens*, *E. dalrympleana* and *E. macrorhyncha*, including populations from South Australia, Victoria and NSW, covering much of the mainland distribution of each species. Where possible, we also collected material from closely related and co-occurring species in order to test species boundaries.

DNA sequencing and data analyses

We used hybrid-capture and high-throughput DNA sequencing (e.g. Weitemier et al. 2014) to generate data for this study using a custom bait set designed in-house. We developed two data sets, one comprising chloroplast DNA sequence data including at least 17 genes and associated introns (in many samples, whole chloroplast genomes were recovered); and a separate data set comprising DNA sequences from c. 20 nuclear genes. These data are treated separately given the different modes of inheritance (chloroplast is maternally inherited while nuclear DNA is bi-parental), which shapes the evolutionary trajectories of the genes and therefore the inferences that are drawn.

Key findings

The chloroplast data, in general, produced well-resolved phylogenies that are incongruent with morphological species boundaries, instead reflecting the geographic origin of the samples. For instance, samples assigned to *E. dalrympleana*, *E. viminalis* and *E. bicostata* collected in South Australia fall together in a well-supported clade, while samples of the above, collected from eastern Australia form a clade that is sister to the 'South Australian' lineage. Molecular dating analyses suggest the divergence of these two groups may be in the vicinity of 2 million years, which likely predates the age of the included species (Thornhill et al. 2019). The strong geographic pattern in these data may be explained by localised inter-specific hybridisation and introgression, leading to chloroplast capture (i.e. transfer of one species chloroplast into another species), a process widely reported among

Eucalyptus (e.g. Flores-Rentieria et al. 2017; Alwadani et al. 2019). The relatively deep divergence between the South Australia-eastern Australia lineages could be explained if the chloroplast type in a given region has been inherited over millions of years among species (including presumably extinct ones) that are capable of hybridising.

In each of our study species, the nuclear data supports the distinctiveness of the South Australian samples. Our analyses suggest that the South Australian and eastern Australian lineages separated approximately 800 - 1400 generations before present. While noting there are difficulties in estimating generation time (i.e. the number of years from seed to seed), applying reasonable values (c. 50-100 years/generation) yields divergence time estimates pre-dating the last glacial maximum (c. 20,000 years before present) and potentially pre-dating the onset of the last glacial period (c. 100-120,000 years before present) (Harle et al. 2002). These estimates support the *in situ* survival of our study species in the Flinders-Mount Lofty Ranges through periods of highly unfavourable regional climate consistent with a refugial status for the region.

Ongoing work

The ongoing focus of our study is the finalisation of all data analyses and the preparation of a manuscript for submission to a peer reviewed journal. It is anticipated that this work will be completed prior to July 2020.

References

Alwadani et al. 2019. *Mol. Phylogenet. Evol.* 136: 76-86; Flores-Rentieria et al. 2017. *Mol. Phylogenet. Evol.* 108: 70-87; Harle et al. 2002. *Quaternary Res.* 17: 707-720; Thornhill et al. 2019. *Aust. Syst. Bot.* 32: 29-48; Weitemier et al. 2014. *Appl. Plant Sci.* 2: 1400042.