Potentially species-variable DNA regions of *Cheesemania* 'Chalk Range'

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Published by
Department of Conservation
Head Office, PO Box 10-420
Wellington, New Zealand

This report was commissioned by Nelson/Marlborough Conservancy.

ISSN 1171-9834

Reference to material in this report should be cited thus:

Mitchell, A., 2000.
Potentially species-variable DNA regions of *Cheesemania* 'Chalk Range'. *Conservation Advisory Science Notes No. 317*, Department of Conservation, Wellington.

Keywords: *Cheesemania*, threatened plants, DNA sequencing, species identification.

Summary

Three of 5 potentially useful gene regions have been sequenced as part of a survey of genetic variation within the *Cheesemania* complex, with particular emphasis on the status of *C.* 'Chalk Range'. The 3 regions screened to date have shown no variation between these entities. It is recommended that the remaining potentially variable DNA regions be sequenced. If no variation is shown for any of these regions, this supports the hypothesis that these taxa in fact represent a single species. It is also recommended that this hypothesis be further tested by using population genetic methods. This will allow us to estimate gene flow between populations and to better understand species limits within this closely related group.

1. Background and rationale

Cheesemania 'Chalk Range' is a habitat-specific, range-restricted member of New Zealand's endemic Brassicaceae that is considered critically endangered (Cathy Jones; pers. comm.). According to molecular analyses of the New Zealand Brassicaceae based on ITS sequence data (Mitchell & Heenan 2000), *Cheesemania* 'Chalk Range', *C. fastigiata,* and *C. stellata* represent a very closely related species complex. Although the taxonomic status of the close relatives have been studied based on morphology (Heenan & Garnock Jones 1999), the status of *C.* 'Chalk Range' entity has not been investigated. The genetic variation and gene flow within and between populations of *C.* 'Chalk Range', *C. fastigiata,* and *C. stellata* are unknown.

Genetic data will play an important role in estimating diversity within this closely related complex. The advantage of analysing the DNA directly is that environmental and other influences are avoided (Mitchell 2000). If genetic, morphological and physiological data support specific status for *C.* 'Chalk Range', then we need to act urgently to maximise the chances of this taxon surviving.

2. Methods

A range of primers were selected and designed based on literature and sequences available from Genbank. Regions of interest have proven to be useful at species level and below and include both chloroplast and ribosomal DNA.

2.1 CHLOROPLAST DNA

Specific chloroplast DNA (cpDNA) sequences were compared for *Cheesemania* 'Chalk Range' and *C. fastigiata* and *C. stellata.* Three pairs of primers were used to amplify three non-coding regions: (1) an intergenic spacer between *trnT (UGU)* and the *trnL* (UAA) 5' exon, (2) the *trnL* (UAA) intron and (3) another intergenic spacer between the *trnL* (UAA) 3' exon and *trnF* (GAA) (Bakker et al. 1998, 1999, Fennell et al. 1998, Lanner 1998, Molvray et al. 1999, Sang et al. 1997, Small et al. 1998, Taberlet et al. 1991).

2.2 RIBOSOMAL DNA

The external transcribed spacer region (ETS) of 18S-26S rDNA has shown potential for augmenting ITS data in phylogenetic studies (Bena et al. 1998, Baldwin & Markos 1998, Tremousaygue1992). It is possible that ETS is evolving at a faster rate than ITS and therefore may allow better resolution of species relationships when divergence times have been more recent.

The primary barrier to sequencing the ETS in plants is the lack of a highly conserved region flanking the 5' end of the spacer. Although the highly conserved 18S gene offerrs various options for primer sites downstream from the 3' end of the ETS, the highly variable non-transcribed spacer (NTS) borders the 5' end of the ETS and is evolving too rapidly to provide a universal primer site. I have attempted to overcome this situation by using long-distance polymerase chain reaction for amplifying the entire intergenic spacer (IGS = NTS + ETS) and by developing a primer that may be used within the ETS for our study group.

3. Results and discussion

To date DNA has been extracted from representatives of *Cheesemania* 'Chalk Range', *C. fastigiata* and *C. stellata.* Primers have been purchased to allow sequencing of all potentially variable regions detailed above for chloroplast and ribosomal DNA. Amplifications using the polymerase chain reaction have been successful for each of these regions using all 3 taxa. DNA sequencing has been carried out for *C.* 'Chalk Range', *C. fastigiata* and *C. stellata* for nuclear ribosomal spacer regions (ITS-1 and ITS-2, Mitchell & Heenan 2000); chloroplast regions (the *trnL* (UAA) intron, using primer TabC and the intergenic spacer between *trnL* 3' exon and *trnF* using primerTabE). These regions have shown no sequence variation for well over 1000 bp in total, which suggests these taxa are a closely related complex that has undergone little molecular divergence.

Now that we have purchased the primers necessary for screening multiple potentially variable ribosomal and chloroplast regions, I recommend we use these to their full advantage by completing this survey. This would involve

sequencing 2 further regions, i.e. the remaining chloroplast region; a intergenic spacer between *trnT (UGU)* and the *trnL* (UAA) 5' exon in addition to the nuclear ribosomal ETS region. If variability were discovered for either of these regions between the taxa in question we could then proceed to evaluate relationships and better define species limits.

However, it is recommended that future work approach the question of the specific status of this taxon using DNA fingerprinting methods. AFLP have been used in our lab to generate informative DNA fingerprints for 2 separate projects, one on variation in *Arthropodium cirratum* (Heenan et al in prep.) and another on differentiation of *Magnolia* hybrid cultivars (Mitchell & Edwards in prep.). Results for these studies are supported by other data, such as those from morphology, ecology and anatomy. Application of this technique would allow us to estimate gene flow between populations of *Cheesemania* 'Chalk Range' and *C. fastigiata* and *C. stellata*, giving us a far clearer picture of relationships within this closely related group.

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