Introduction

The Asplenium ferns of New Zealand appear to have had diverse biogeographic origins, but most of the species belong to a comparatively closely related ‘Austral’ group (Brownsey 1977a, Perrie & Brownsey 2004). There are no diploid Asplenium species in New Zealand (Dawson et al. 2000), but amongst the tetraploid representatives of the Austral group, three groups can be recognised by both morphological (Brownsey 1977a) and molecular (Perrie & Brownsey 2004) evidence: (i) the Bulbiferum Group of A. bulbiferum G.Forst. sensu stricto and A. hookerianum Colenso; (ii) the Flaccidum Group of A. flaccidum G.Forst. subsp. flaccidum, A. flaccidum subsp. bauhuiense Brownsey, A. chathamense Brownsey, and A. lamprophyllum Carse; and (iii) the Obtusatum Group of A. obtusatum G.Forst. subsp. obtusatum and A. oblongifolium Colenso.

In addition to these tetraploid taxa, there are a similar number of octoploid taxa, and these appear to include examples of both auto- and allopolyploid evolution (Brownsey 1977b). The tetraploid chloroplast parents of the putative allo-octoploids Asplenium hyallii (Hook.f.) T.Moore and A. scleroprium Hombr. were recently determined to be either A. oblongifolium or A. obtusatum subsp. obtusatum (Perrie & Brownsey 2004). That study investigated variation in the DNA sequence of the trnL-trnF intergenic spacer of the chloroplast, which is maternally inherited in at least some European Asplenium (Vogel et al. 1998). However, which of the two tetraploids from the Obtusatum Group were involved in the respective polyploid events could not be distinguished as all four taxa had identical trnL-trnF sequences. Further, A. oblongifolium and A. obtusatum subsp. obtusatum were found to have identical sequences for the rbcL gene and the psbC-trnS intergenic spacer (Perrie & Brownsey 2004).

Asplenium oblongifolium is endemic to New Zealand, and occurs in coastal to lower montane areas of the North Island and northern South Island. In contrast, A. obtusatum subsp. obtusatum is considered to be restricted to coastal habitats, and occurs from Wellington to the subantarctic islands south of New Zealand. It is also found outside New Zealand, and probably has a circumantarctic distribution. However, while Tristan de Cunha plants have been demonstrated to be tetraploid, the majority of
putative populations outside New Zealand have not been investigated cytologically (Brownsey 1977a, Brownsey & Smith-Dodsworth 2000).

Asplenium oblongifolium and A. obtusatum subsp. obtusatum are morphologically very similar, but can usually be distinguished by the shape of their frond pinnae, the form of their stipe scales, and the ornamentation of their spores (Brownsey 1977a, Brownsey & Smith-Dodsworth 2000). The distinction is, however, less clear in the north-west of the South Island, where plants with A. oblongifolium-like frond morphology but A. obtusatum-like spore ornamentation and stipe scales occur inland, in some instances several kilometres from the coast (e.g. Pororari River, c. 1 km from the coast, Perrie et al., 26 February 2002, WELT P020548; Karamea Bluff, c. 6 km from the coast, Perrie et al., 28 February 2002, WELT P020549).

Asplenium lyallii occurs principally on calcareous substrates from coastal to subalpine areas, and is distributed from the Waikato to Stewart Island (Brownsey 1977a, Brownsey & Smith-Dodsworth 2000). Based on its morphology, Brownsey (1977b) suggested A. lyallii was an allopolyploid with the parentage of either A. bulbiferum x obtusatum subsp. obtusatum or A. bulbiferum x oblongifolium, although the involvement of A. hookerianum instead of A. bulbiferum should also be considered. A putative collection of A. hookerianum x oblongifolium (Masters Shelter, northeastern Ruahine Ranges, Shepherd et al., 20 October 2002, WELT P020550) bears a striking resemblance to some of the morphological forms of A. lyallii.

Asplenium scleroprium occurs from Invercargill southwards, where it is mostly confined to coastal habitats, and is at its most prevalent on the Auckland Islands (Brownsey 1977a, Brownsey & Smith-Dodsworth 2000). Brownsey (1977b) indicated that A. flaccidum subsp. flaccidum x A. obtusatum subsp. obtusatum was the most likely allopolyploid parentage for A. scleroprium. Asplenium obtusatum subsp. obtusatum occurs sympatrically with A. scleroprium, but A. oblongifolium does not (Brownsey 1977a).

Here we report data from the trnL intron, a DNA sequence marker that has only recently been investigated in ferns, and which neighbours the trnL-trnF intergenic spacer (Trewick et al. 2002). We hoped these data would
allow us to distinguish genetically *Asplenium oblongifolium* and *A. obtusatum* subsp. *obtusatum*, and to identify which was the chloroplast parent in the allopolyploid events producing *A. lyallii* and *A. scleroprium*.

**Methods**

DNA was extracted from the samples listed in Table 1 as described by Perrie & Brownsey (2004). DNA sequence for the *trnL* intron was obtained using the PCR reagents of Perrie & Brownsey (2004), and the primers and thermocycling conditions of Trewick *et al.* (2002), except a primer annealing temperature of 65°C rather than 48°C was used.

PCR products were purified by incubation at 37°C for 30 minutes with 2 units Shrimp Alkaline Phosphatase and 10 units Exonuclease I (both USB Corporation), followed by heat inactivation of the enzymes at 80°C for 15 minutes. The purified PCR products were sequenced in both directions using an Applied Biosystems ABI3730 Genetic Analyser.

The edited DNA sequences for the *trnL* intron were combined with those from the *trnL-trnF* intergenic spacer DNA sequences (Perrie & Brownsey 2004) and aligned using ClustalX 1.8 (Thompson *et al.* 1997). PAUP 4b10 (Swofford 2002) was used to conduct a phylogenetic analysis using a maximum parsimony criterion with a branch and bound search and sites encompassing insertion-deletion events treated as missing data. *Asplenium bulbiferum, A. flaccidum* subsp. *flaccidum, A. lamprophyllum, A. hookerianum,* and *A. obtusatum* subsp. *northlandicum* were designated as the outgroup (Perrie & Brownsey 2004).

**Results**

A single tree of 60 steps was recovered from the maximum parsimony analysis (see Fig. 1), with a Consistency Index of 0.9833, a Retention Index of 0.9848, and a Rescaled
Consistency Index of 0.9684. The trnL intron sequence recovered from the two samples of Asplenium oblongifolium differs from those of the two samples of A. obtusatum subsp. obtusatum at two base-pair positions. The A. scleroprium sample and one of the A. lyallii samples have identical trnL intron sequences to those of A. obtusatum subsp. obtusatum, while the other A. lyallii sample exhibits a one base-pair difference. The trnL intron sequence of the octoploid A. obtusatum subsp. northlandicum Brownsey is identical to that of A. obtusatum subsp. obtusatum, but as noted by Perrie & Brownsey (2004), the former exhibits two autapomorphies in the trnL-trnF intergenic spacer. The sequence polymorphisms within the trnL intron and trnL-trnF intergenic spacer for the Obtusatum Chloroplast Group taxa are listed in Table 2.

Discussion

The two DNA sequence base-pair differences in the trnL intron reported here are the first detected between Asplenium oblongifolium and A. obtusatum subsp. obtusatum, and follow the investigation of three other chloroplast DNA sequence data sets (Perrie & Brownsey 2004). Indeed, the two taxa (or, at least, their chloroplasts) appear very closely related, with only two differences found across 2443 base-pairs. Investigating this trnL intron genetic polymorphism in conjunction with variation in spore ornamentation and stipe scale size may help to clarify the boundary between A. oblongifolium and A. obtusatum subsp. obtusatum in the northwestern part of the South Island, where their usual morphological and ecological distinctiveness appears to breakdown.

The two base-pair differences between the Asplenium oblongifolium and A. obtusatum subsp. obtusatum samples are reconstructed as autapomorphic in the former (see Fig. 1). These base-pair differences occur in two geographically distant samples, which suggests that they may be widespread in A. oblongifolium rather than representing only geographically local variation. One of the changes, an A to C transversion at alignment position 56, has been independently acquired by A. flaccidum subsp. flaccidum (see Outgroup, Table 2).

Unfortunately, the trnL intron data are unable to resolve which of Asplenium oblongifolium and A. obtusatum subsp. obtusatum were the chloroplast parents in the allopolyploid events producing A. lyallii and A. scleroprium. The chloroplast trnL intron sequences of A. lyallii and A. scleroprium are identical to those of A. obtusatum subsp. obtusatum, except for an autapomorphy exhibited by one of the A. lyallii specimens, but the resemblance is symplesiomorphic, being based on shared, ancestral character states. The data are therefore unable to discriminate between A. obtusatum subsp. obtusatum, A. oblongifolium (e.g. ancestral A. oblongifolium before the base-pair

<table>
<thead>
<tr>
<th>Locus</th>
<th>trnL intron</th>
<th>trnL-trnF spacer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alignment position</td>
<td>56</td>
<td>196</td>
</tr>
<tr>
<td>A. oblongifolium</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>A. oblongifolium</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>A. obtusatum subsp. obtusatum</td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td>A. obtusatum subsp. obtusatum</td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td>A. lyallii</td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td>A. lyallii</td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td>A. scleroprium</td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td>A. obtusatum subsp. northlandicum</td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td>Outgroup</td>
<td>A/C</td>
<td>G</td>
</tr>
</tbody>
</table>
autapomorphies evolved, or, if these differences are not fixed in *A. oblongifolium*, individuals that retain the pleiomorphic states), or their common ancestor, being the chloroplast parent in the allopolyploid events producing *A. lyallii* and *A. scleroprium*.

Given the seemingly very high similarity of the chloroplast DNA of *Asplenium oblongifolium* and *A. obtusatum* subsp. *obtusatum*, how best to identify the chloroplast parents of *Asplenium lyallii* and *A. scleroprium* is uncertain. Already four chloroplast DNA loci, encompassing 2443 base-pairs, have been investigated. Possibly, sequencing of additional chloroplast DNA loci may find synapomorphies between the octoploids and one or other of the tetraploids, thereby identifying which of *A. oblongifolium* and *A. obtusatum* subsp. *obtusatum* was involved in each instance. Alternatively, methods that assess variation in biparentally inherited genetic markers from the nuclear genome (e.g. DNA fingerprinting, Perrie *et al.* 2003; DNA sequencing of the nuclear ITS locus, Van den Heede *et al.* 2003) may provide sufficient resolution to address this question.

**Acknowledgements**

We would like to thank the Palmerston North campus of the Allan Wilson Centre for Molecular Ecology and Evolution for the use of their laboratory facilities, and Mike Bayly and two reviewers for comments that greatly improved this manuscript. This work was funded by the Foundation for Research, Science and Technology (contract MNZX0201).

**References**


