

Darwin and DNA: explaining the New Zealand flora

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Introduction

The publication of the *Origin of Species* (Darwin 1859) ranks amongst the most important scientific events of the last 200 years. In it Charles Darwin outlined his theory of natural selection — the mechanism that affects speciation over time. Natural selection is both simple and elegant. Briefly it works like this: individuals within a species vary, some of these heritable variations may confer an advantage that is ‘selected’, and over time differences accumulate that result in the formation of new species. To illustrate the idea, we can consider a famous example, the Galápagos Island finches. Traditional morphological and ecological studies suggest that the Galápagos finches are derived from a mainland South American ancestor and that diversification on the islands was driven, at least in part, by adaptation to different food sources. One can imagine a newly arrived ancestor being confronted by various open feeding niches in its new home. As the founder population expanded, variants that were able to exploit a certain resource more efficiently would have been favoured. Eventually the accumulation of behavioural and morphological changes gave rise to novel species each adapted to a specific ecological niche. This explanation of Galápagos finch diversity is certainly plausible, but since these events are historical, how can we test these ideas?

To test evolutionary hypotheses we need a ‘family tree’ (technically a ‘phylogeny’) describing who is related to whom. For example, given the traditional explanation for Galápagos finch diversity we would expect relationships among the extant species to trace back to a single point representing the mainland ancestor. By constructing a phylogeny based on the characteristics of the species involved we can directly evaluate such an expectation. Until the early 1990s evolutionary trees were commonly based on morphology. Although this approach has provided numerous insights, it is not always easily applicable or appropriate. However, around that time it became routine to characterise an organism’s DNA; this advance provided a wealth of new data and allowed researchers to address a raft of new questions. The ongoing development of ever more sophisticated methods for characterising and analysing DNA data has revolutionised our understanding of life on earth. The application of these tools allows us to address fundamental questions about

why there are so many species and why they occur where they do. For those who are interested, molecular analyses do support the traditional view of the evolutionary processes in the Galápagos finches (Sato *et al.* 1999, 2001).

The origins of the New Zealand flora have long been of interest to biologists, from the earliest visitors (e.g. Hooker 1853) until the present day. The reasons for this interest are similar to those that drove research on the Galápagos finches – where did our flora come from and what led to the diversity of the groups present? Until recently, considerable uncertainty has remained about even broad-scale evolutionary patterns. However, the application of molecular approaches has helped transform our understanding of evolutionary processes in the New Zealand flora and are likely to contribute further in the future.

The importance of dispersal

For 40 years or so, the mainstream view has been that the New Zealand flora is a Gondwanan relict, having survived virtually intact for the last 80 million years (e.g. Bellamy *et al.* 1990). The basic idea goes something like this: as tectonic activity rafted New Zealand away from the rest of Gondwana, it carried a collection of organisms that have since been protected in New Zealand by virtue of its isolation. The subdivision of ancestral distributions by the formation of a physical barrier is generally referred to as *vicariance*; this idea has dominated explanations for the origins of the New Zealand flora over the last four decades. Despite the dominance of vicariance, some biologists have clung to an alternative point of view – specifically that *dispersal*, long-distance movements between widely separated areas, explains the New Zealand flora (Raven 1973), ‘long-distance’ meaning at least 2000 km over the Tasman Sea and potentially further if the dispersal source is somewhere other than Australia. A slight digression: Charles Darwin also favoured dispersal explanations and was so intrigued by dispersal that he devoted two chapters of the *Origin of Species* to the subject.

Until recently it has been difficult to distinguish between vicariance and dispersal. To differentiate between them we need to know when the New Zealand species diverged from their closest non-New Zealand relatives. If the split occurred more than 80 million years ago (Ma), when New Zealand sepa-



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rated from Gondwana, then vicariance is the likely explanation (Figure 1A, B). However, if divergence was younger, then the distributional pattern must be the result of dispersal (Figure 1C, D). Although morphological characters and the fossil record can be used to examine relationships among species it is difficult to estimate the timing of evolutionary events from these data. In contrast, molecular data allow inferences about both pattern and time. As an example, morphological studies of *Myosotis*, the forget-me-nots, typical of many New Zealand plant groups, can be interpreted as supporting either Northern or Southern Hemisphere origins (e.g. Grau & Liens 1968; Raven 1973). On the other hand, molecular evidence strongly supports a Northern Hemisphere origin, with subsequent dispersal to New Zealand. This inference is based on genetic diversity; levels of diversity are higher amongst Northern Hemisphere representatives than those from New Zealand, implying that the New Zealand species arose more recently (Figure 2). By calibrating genetic diversity with a known time point we can take this general inference a step further and assign absolute ages. Molecular age estimates for *Myosotis* suggest that the New Zealand lineage diverged from its Northern Hemisphere relatives less than 15 Ma — consistent with dispersal (Winkworth *et al.* 1999, 2002). Similar dispersal

stories have been reported for numerous groups in the New Zealand alpine flora. Commonly these plants have originated in the Northern Hemisphere or in southern South America and have arrived in New Zealand within the last 15 million years or so (Winkworth *et al.* 2005).

Currently our understanding is biased towards the alpine flora, and we have a much poorer understanding of other elements. However, three recent molecular studies provide important clues. The first involves a textbook example of the ‘Gondwanan distribution’. In contrast to the traditional view, molecular data indicate that the split between Australian and New Zealand *Nothofagus* species occurred around 30 Ma — again too young to be explained by vicariance (Knapp *et al.* 2005). When taken together with fossil evidence for a much longer presence in New Zealand, these results suggest a more complex history. Specifically, we must assume that recent colonists from Australia have replaced the lineages present when New Zealand separated from Gondwana. Dispersal also appears to have played an important role in various groups of ferns and their allies (Perrie & Brownsey 2007). These authors examined 31 New Zealand/non-New Zealand species pairs, finding average divergence times of between 1 and 53 million years — a much

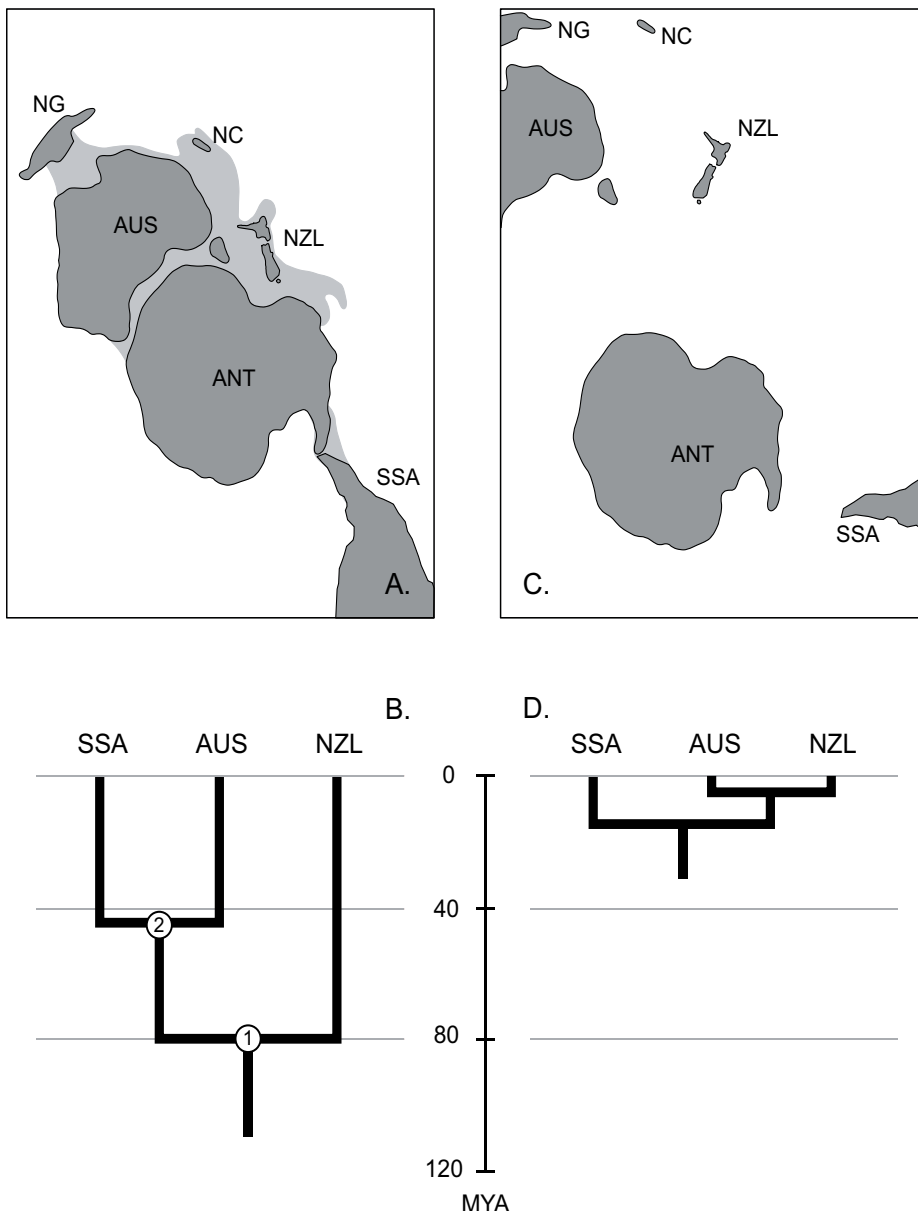
Figure 1. Distinguishing hypotheses about the origins of the New Zealand flora based on the timing of events.

A. Paleogeographic reconstruction of Gondwana approximately 90 Ma.

B. A vicariance explanation holds if the pattern and timing of branching events in an organismal phylogeny matches those of the geological break up scenario. Numbers on nodes denote geological events: 1, New Zealand splitting away from Gondwana approximately 80 Ma; 2, isolation of Australia and South America 45 Ma — note that South America and Antarctica remain in contact for another 15 million years.

C. Contemporary distribution of Southern Hemisphere landmasses, focusing on the southern Pacific.

D. An alternative to vicariance is needed if relationships and timing of branching events do not match those predicted by the geological scenario. In this case New Zealand and Australia are most closely related and all the divergence events post-date the geological break-up (a pattern commonly seen in empirical datasets). Abbreviations: ANT, Antarctica; AUS, Australia; SSA, southern South America; NC, New Caledonia; NG, New Guinea; NZL, New Zealand.



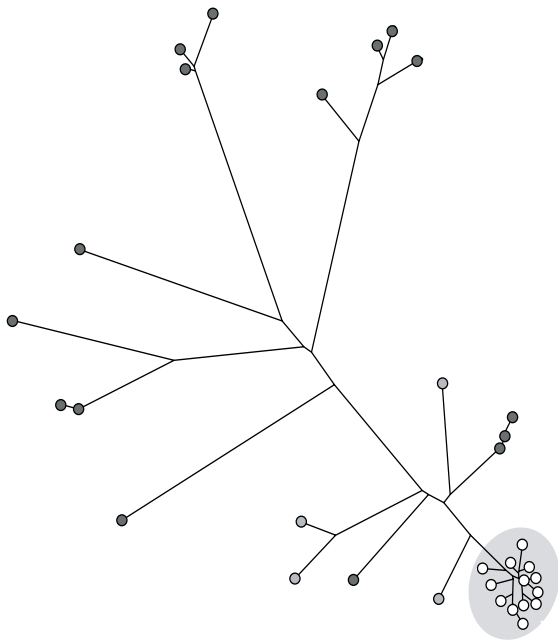


Figure 2. Myosotis phylogeny based on the nuclear internal transcribed spacer region of the ribosomal DNA repeat (see Winkworth *et al.* 2002). Dots represent species (names removed for clarity), with different shadings indicating taxonomic sections within the genus. Species with grey dot (light or dark) belong to the Northern Hemisphere sections, white indicates the predominantly New Zealand group – outliers occur in Australia, New Guinea, South America, and on several subantarctic islands (these are included in the tree). The level of genetic diversity, proportional to the lengths of the branches in the tree, is much greater for the Northern Hemisphere groups than those from the New Zealand group.

wider range than for the alpine flora but still more consistent with dispersal explanations than vicariance. For several fern groups the combination of fossil and molecular data also suggests replacement of older lineages (e.g. the tree ferns). The last study provides a possible example of Gondwanan vicariance. Molecular analyses of Araucariaceae suggest that *Agathis australis* may have been present in New Zealand for the last 80 million years. There is still some uncertainty but confidence intervals on the age estimates include 80 million years and are therefore consistent with a vicariance explanation (Knapp *et al.* 2007). Although still limited, these data suggest that a broad array of histories are represented in the lowland flora, with the modern assemblage reflecting the accumulation of lineages over a relatively long period. This finding makes sense, given evidence for the predominance of lowland habitats for most of New Zealand's history. However, a better understanding of the exact contribution of dispersed lineages to the lowland flora will require further analyses.

At present we understand much of the broad picture, but we have yet to fill in many of the details. There are numerous groups that still need to be evaluated if we are to complete our understanding of dispersal patterns. However, moving beyond documenting patterns, molecular analyses are now beginning to help us understand the underlying processes. One important set of questions relates to how some kinds of plants made it to geographically isolated New Zealand, given that they seem to lack obvious long-distance dispersal mechanisms. A possible solution involves the establishment of 'stepping-stone' populations along the route of dispersal. Perhaps the most obvious

stepping-stone is Antarctica, which until recently may have provided an overland route for movements between southern South America and New Zealand (e.g. Sanmartin *et al.* 2007). Other important questions involve dispersal direction and whether the establishment of dispersed lineages has been favoured at particular times in the past. Understanding these issues will involve a better integration of fossil and molecular data.

Lineage diversification

Like many island archipelagos, New Zealand has its own cast of spectacular plant radiations. In particular, the New Zealand mountains are home to a number of large, morphologically and ecologically diverse plant groups (Wilton & Breitweiser 2000). The juxtaposition of high diversity and geological evidence that mountain habitats arose only recently has led to controversy about the origins of this diversity. Essentially the question is: How long does it take to generate biological diversity? If the process of species formation is assumed to be slow and steady, these groups are likely to be older than the current mountains. On the other hand, if speciation is a punctuated, potentially rapid process, the diversity could have arisen since mountain uplift began around 5 Ma. Again, since the issue involves time, earlier morphological studies have not been sufficient to differentiate between alternative hypotheses. Given molecular evidence for recent arrival, by necessity diversification is going to be more recent. In other words, the striking differences between species have arisen in less than 15 million years. For example, the hebes are a well-known group of New Zealand plants consisting of approximately 120 predominantly montane species. The group ranges from alpine cushion plants just a couple of centimetres high to lowland woody shrubs a metre or more in height, and have a corresponding diversity of vegetative and reproductive morphologies. Recent molecular analyses indicate that this diversity arose within the last 10 million years (Wagstaff *et al.* 2002).

Having established that alpine plant diversity is young, the question becomes: What factors have driven the rapid diversification of the New Zealand alpine flora? Perhaps most simply we might assume that the expansion of mountain habitats promoted diversification of mountain plant groups (Wardle 1963; Raven 1973). Given the close correspondence between the timing of mountain building and lineage diversification, this does not seem an unreasonable suggestion. However, climatic instability during this period is also likely to have been an important influence. Changing environments would have provided opportunities for speciation via population subdivision and divergence in isolation. This follows the classic divergent model of speciation. However, recently there has been renewed interest in the evolutionary importance of hybridisation as a means of generating diversity. Since climates were changing cyclically it seems likely that there would have been opportunities for diverging populations to have interacted with closely related forms and in the process produced additional variants. The possibility that reticulate evolution has played an important role in the diversification of New Zealand alpine plant groups is consistent with observations from molecular analyses and the occurrence of natural hybrids (Winkworth *et al.* 2005).

Again, while we appear to have established the general pattern, we now need to fill in the details. It will be especially important to establish the mechanisms that underlie diversifica-

tion. One important issue relates to whether diversification is a result of adaptive changes – modifications that better fit plants to specific habitat types. For example, each of the two alpine lineages of *Pachycladon* is restricted to a different substrate, either schist or greywacke. Heenan & Mitchell (2003) suggested that adaptation to these substrates may have been important in the initial diversification of *Pachycladon*. Evaluating this possibility is not straightforward, requiring reciprocal transplantation studies to evaluate fitness as well as studies of the physiological and genetic basis of adaptation. Until recently such analyses have been hampered because the available methods were applicable only to model plant systems (e.g. *Arabidopsis*). However, the development of high-throughput sequencing technologies now allows us to study almost any plant group. Using these technologies it is possible to examine at a genetic level whether there are functional differences between species or populations that occur in different habitats (Hoffman & Willi 2008). Potentially such studies can help identify adaptations and the genes that underlie differences in a single step. High-throughput sequencing technologies will also help us to understand better the importance of hybridisation. Current studies are limited because they rely on just a few loci, but these new approaches have the potential to provide detailed genome-wide comparisons. As a result we can expect a more complete assessment of genome complexity and therefore a better understanding of both the extent and evolutionary significance of hybridisation in particular groups. In particular, it will be fascinating to examine whether hybridisation may act to promote adaptive responses (Seehausen 2004).

Conclusions and outlook

In the 150 years since the publication of the *Origin of Species*, evolutionary studies have revolutionised biology; as the title of Theodosius Dobzhansky's (1973) famous essay states 'Nothing in biology makes sense except in the light of evolution.' Over the last 20 years our understanding of the evolutionary processes that have shaped the New Zealand flora have been transformed by the application of molecular phylogenetic tools. While we have clarified many of the broad patterns we are still far from a complete picture. Addressing these outstanding questions promises general insights into the pattern and process of plant evolution.

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