Species Radiation by Niche Shifts in New Zealand's Rockcresses (*Pachycladon*, Brassicaceae)

SIMON JOLY[1,2,*], PETER B. HEENAN[3], AND PETER J. LOCKHART[2]

[1]*Institut de Recherche en Biologie Végétale, Montreal Botanical Garden and Université de Montréal, 4101 Sherbrooke East, Montreal H1X 2B2, Canada;*
[2]*Institute of Fundamental Sciences, Massey University, Private Bag 11222, Palmerston North, New Zealand; and* [3]*Landcare Research, P.O. Box 40, Lincoln, New Zealand*
Correspondence to be sent to: Institut de recherche en biologie végétale, 4101 Sherbrooke East, Montreal (QC) H1X 2B2, Canada; E-mail: joly.simon@gmail.com.

Abstract.—Adaptive radiations such as the Darwin finches in the Galapagos or the cichlid fishes from the Eastern African Great Lakes have been a constant source of inspiration for biologists and a stimulus for evolutionary thinking. A central concept behind adaptive radiation is that of evolution by niche shifts, or ecological speciation. Evidence for adaptive radiations generally requires a strong correlation between phenotypic traits and the environment. But adaptive traits are often cryptic, hence making this phenotype-environment approach difficult to implement. Here we propose a procedure for detecting adaptive radiation that focuses on species' ecological niche comparisons. It evaluates whether past ecological disparity in a group fits better a neutral Brownian motion model of ecological divergence or a niche shift model. We have evaluated this approach on New Zealand rockcresses (*Pachycladon*) that recently radiated in the New Zealand Alps. We show that the pattern of ecological divergence rejects the neutral model and is consistent with that of a niche shift model. Our approach to detect adaptive radiation has the advantage over alternative approaches that it focuses on ecological niches, a key concept behind adaptive radiation. It also provides a way to evaluate the importance of ecological speciation in adaptive radiations and will have general application in evolutionary studies. In the case of *Pachycladon*, the high estimated diversification rate, the distinctive ecological niches of species, and the evidence for ecological speciation suggest a remarkable example of adaptive radiation. [Adaptive radiation; diversification rates; ecological niche modeling; ecological speciation; niche shifts.].

Adaptive radiation is the evolution of ecological diversity within a rapidly diversifying lineage (Schluter 2000). The theory at the origin of adaptive radiations dates back to Darwin (Darwin 1859; Gavrilets and Losos 2009; Glor 2010), but it was largely Simpson (1953) who developed it. Simpson believed that species radiations occur by taking benefit of ecological opportunities (niche shifts) and that unoccupied niches provide the raw material for speciation. These ideas led to the concept of ecological speciation (reviewed in Schluter 2000, 2001, 2009; Rundle and Nosil 2005), the process by which species are formed via adaptation to environmental conditions. Confirming adaptive radiation generally involves demonstrating ecological speciation in a rapidly diversifying group (Schluter 2000; but see Glor 2010), a difficult task that requires detailed gene-up or phenotype-down approaches (Schluter 2009). Another difficulty is that ecological adaptations are often cryptic and can take place at the physiological, cellular, or molecular level (e.g., Dambroski et al. 2005; Verslues and Juenger 2011). In such groups where the traits under selection are unlikely to be easily identified, hypotheses of adaptive radiation are less likely to be formulated.

Ecological speciation implies that a population adapts to a novel habitat via natural selection on traits, and that selection is responsible for reproductive isolation. An expectation for species that evolved via ecological speciation is that they should occupy distinct ecological niches, in particular among sister species or lineages. Although ecological divergence can also occur between species that did not evolve by ecological speciation, different patterns are expected for the two scenarios. By definition, ecological speciation implies that ecological divergence occurs during speciation and that the process is not independent in sister lineages. In contrast, with non-ecological speciation, ecological divergence can occur at any time as a result of evolutionary processes that could be independent in different lineages.

Recent developments in the modeling of species' ecological niches from observation data have greatly facilitated species comparisons at the ecological level by allowing researchers to quantify differences in niches (e.g., Pearman et al. 2008; Elith and Leathwick 2009). Differences in niches could be used to test hypotheses of niche shift evolution in species radiations. A common approach in evolutionary biology consists of evaluating how well some data fit the predictions of an evolutionary model. In the case of adaptive radiations or traits, the Brownian motion model (Felsenstein 1985) has been considered to be a good null model for hypothesis testing (Harvey and Rambaut 2000; Freckleton and Harvey 2006; Davies et al. 2012). Specifically, adaptive radiation models have been shown to be incompatible with a Brownian model (Price 1997; Freckleton and Harvey 2006). The key features of the Brownian model that are relevant in a context of adaptive radiation are that (i) evolution is constant; (ii) evolution is independent in different lineages (i.e., evolution in one lineage is not influenced by that in other lineages); and (iii) there is no selection. These three characteristics are clearly incompatible with the concept of adaptive radiation

(Schluter 2000), which makes the Brownian model a good null model to test adaptive radiation hypotheses. In the case of ecological speciation, an interesting alternative model is a "niche shift" model where all divergence occurs at speciation events, thus simulating ecological speciation. This model, sometimes called a punctuated or a speciation model, makes all branch lengths equal and forces all modifications to occur at speciation events (Pagel 1999). Although it is possible to have some ecological divergence outside speciation events in an adaptive radiation, this niche shift model nevertheless represents an interesting alternative to the Brownian model in a context of ecological speciation.

Building on these predictions of ecological niche evolution within adaptive radiations, we suggest a novel approach for identifying adaptive radiations based on the following criteria: (i) the group should show high species diversification rates; (ii) species should have distinct and specific ecological niches; (iii) ecological divergence among sister species/lineages should favor a niche shift model of ecological divergence over a neutral model. Although these criteria cannot provide definitive evidence of ecological speciation, a group of organisms that meet these three criteria should be seen as a very good candidate for adaptive radiation.

The Southern Alps in New Zealand, a recent mountain range of circa 5 myr (Batt et al. 2000), provides a natural laboratory for studying niche shifts hypotheses and adaptive radiation. Indeed, its recent uplift has resulted in new ecological niches that several plant and animal groups have exploited in radiation and range expansion (Chinn and Gemmell 2004; Winkworth et al. 2005; Heenan and McGlone 2013). The New Zealand rockcresses (genus *Pachycladon*; Brassicaceae), which are close relatives of the model species *Arabidopsis thaliana*, is one such group that has radiated from 1 to 11 species in the last million years following an allopolyploid event (Joly et al. 2009a; Mandakova et al. 2010). Despite the fact that the group is morphologically diverse and that species have specialized on different types of soils (Heenan and Mitchell 2003), there is no obvious phenotypic adaptation that could categorize the group as an adaptive radiation (Voelckel et al. 2008, 2012). We thus test the three criteria mentioned above to determine whether *Pachycladon* shows evidence of past speciation by niche shifts.

MATERIALS AND METHODS

Fifty-two individuals from all 11 *Pachycladon* species were sampled for molecular studies (Supplementary Table S1; supplementary and on-line only material can be found in the Dryad data repository with http://dx.doi.org/10.5061/dryad.h97pk). Individuals were sequenced for 10 single-copy nuclear genes; DNA extraction, PCR, cloning, and sequencing were performed as in Joly et al. (2009a) and the list of the genes, primers, and PCR conditions are given in Supplementary Table S2. DNA sequences (see

Supplementary Table S1 for GenBank accession numbers) were aligned with Muscle (Edgar 2004) and alignments were edited manually when necessary. There was no evidence of recombination in the data sets ($P > 0.314$ after Holm Sidak correction) according to the PHI test (Bruen et al. 2006) as implemented in SplitsTree (Huson and Bryant 2006) and so data sets were used without further modifications.

Genetic Species Delimitation

To test for genetic species cohesiveness, an individual distance matrix was estimated from allelic Hamming distances from all genes using POFAD (Joly and Bruneau 2006). Allelic distances were standardized to give equal weight to all genes and distances were corrected using a Jukes and Cantor (Jukes and Cantor 1969) model. The distance matrix of individuals was visualized using NeighborNet (Bryant and Moulton 2004) in SplitsTree4. Species boundaries were also estimated using the Bayesian BP&P software (Yang and Rannala 2010). The MCMC chain was run under the two algorithms and different priors: algorithm 0 was run with $\varepsilon = 5$ or $\varepsilon = 10$, and algorithm 1 was run with parameters $\alpha = 2$ and $m = 1$ or with $\alpha = 1$ and $m = 0.5$. A gamma (2,1000) prior was used for θ and τ, and the fine-tuning parameters GBtj, GBspr, theta, tau, mix, locusrate, seqerr were, respectively, set to 0.5, 0.00010, 0.0011, 0.00026, 0.1, 0.01, 0.01, and 0.01. The chain was sampled every 10 generations and 5000 samples were collected after discarding a burnin of 1000 samples.

Species Tree Analyses

To select the best-fitting nucleotide model for each gene, we first reconstructed a maximum-likelihood phylogeny using Garli (Zwickl 2006) with the GTR + Γ + I model. The phylogeny was then used in ModelTest 3.7 (Posada and Crandall 1998) to select the best-fitting model according to the Akaike Information Criterion (AIC). The species phylogeny was reconstructed using *BEAST (Heled and Drummond 2010), using a birth–death tree prior and the best-fitting nucleotide model for each marker. The population size parameter was given a gamma (2,0.0005) prior. A relaxed lognormal clock (gamma (2,1) prior) was favored over a strict clock according to a Bayes Factor analysis (Suchard et al. 2001) performed in Tracer (Rambaut and Drummond 2009) ($\log_{10}$ BF > 5; see Kass and Raftery (1995) for interpretation), whereas the piecewise linear population size model was rejected in favor of the simpler constant population size model ($\log_{10}$ BF < 0.5).

Because sequences for some genes were not always obtained for a given species, dummy sequences consisting of "N" had to be included in the analysis. Although this should not affect the result of the species tree search in a likelihood framework, there might nevertheless be a convergence issue when several dummy sequences are included. To address this, we

estimated the species tree with all species that had sequences for all genes as well as with different combination of species that had missing information. All tree searches resulted in congruent phylogenies, suggesting that the inclusion of dummy sequences did not affect tree inference. The xml file used for the analyses is available as supplementary material.

Three independent MCMC chains were run for 1×10^8 generations and sampled every 1×10^4 generations. Chain convergence was confirmed with Tracer (Rambaut and Drummond 2009) and by comparing the tree topologies and branch support between runs. The three independent runs were combined for downstream analyses after removing the first 2000 trees as burnin.

Evidence of hybridization in the data sets was tested by posterior predictive checking (Joly et al. 2009b) using JML (Joly 2012). A single sequence from a single locus showed evidence of introgression over all data sets ($P < 0.001$) and was removed from the analyses.

Diversification Rate Analysis

The rate-constant diversification rate per lineage was estimated by maximum likelihood (Rabosky 2013) using the laser package (Rabosky 2013) in R (R Development Core Team 2009) on 2000 species trees sampled according to their posterior probabilities, fixing the crown age for *Pachycladon* to 0.82 myr (Joly et al. 2009a). This age estimate was obtained using a synonymous substitution estimate obtained from 256 genes (Blanc et al. 2003) and calibrated using independent fossil-calibrated estimates of divergence between *Arabidopsis* and *Brassica* (Yang et al. 1999; Koch et al. 2001); see Joly et al. (2009a) for further details. Birth–death and pure birth models were compared with the AIC to select the best-fitting model. Parametric bootstrapping (Boettiger et al. 2012) was performed to evaluate if there is sufficient information in the data to estimate diversification rates.

Niche Modeling

Latitude and longitude information for niche modeling was obtained for 368 specimens coming from 6 herbaria (CHR, AK, OTA, RARE, WELT, and CANU; Supplementary Table S3; Fig. 1a). *Pachycladon radicatum*, the only species found outside New Zealand (Tasmania, Australia), was excluded because of lack of information. The geographic distribution of *Pachycladon* species has been well represented in our sampling. However, species with extremely restricted distributions (Heenan and Mitchell 2003; Heenan 2009) are only represented by a small number of accessions. For the niche modeling, we considered the 19 bioclimatic variables developed by Hijmans et al. (2005), as well as geological layers obtained from the Land Resource Information System (LRIS) from Landcare Research, New Zealand: total carbon, cation exchange capacity, maximum slope in quadrat, minimum slope in quadrat, minimum pH, phosphate retention, and toprock type.
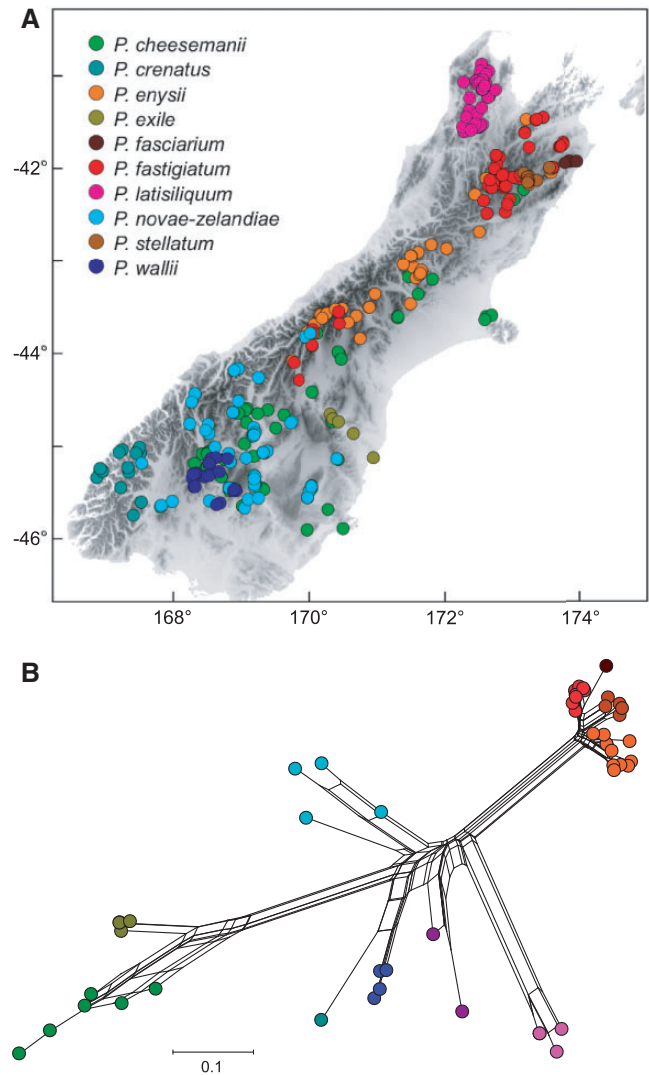

FIGURE 1.   Occurrence points used for niche modeling of all New Zealand *Pachycladon* species (a) and NeighborNet network of inter-organism distances computed from 10 single-copy nuclear genes with the POFAD algorithm (b). *Pachycladon radicatum* (Tasmania, Australia) is not shown.

To avoid model over-parameterization, especially given that some species have few data points, we reduced the number of variables to a minimum. The most significant variables according to the biology of the species were retained. Correlated variables or variables that were not contributing significantly to the niche models were discarded. Six ecological variables were retained for the niche modeling: annual mean temperature, mean diurnal range, annual precipitation, precipitation seasonality, maximum slope, and toprock type (a categorical variable). Because six variables are considerable for *P. exile* and *P. fasciarium* that have only five occurrence points, the niche models for these species were also performed with two variables (the ones that contributed most in the six variables models, see Results) to evaluate whether over-parameterization affected our results and conclusions. Because of the

abundance of toprock types in the LRIS classification (28 for the North and South Islands), rock types were classified according to the New Zealand Land Resource Inventory categories for modeling purposes: surficial, sedimentary/weakly indurated, sedimentary/strongly indurated, igneous, and metamorphic. Landcare soil and topology characteristics were rasterized according to the same grid as the bioclim variables, that is using a 30 arc-second quadrat resolution.

Climatic niches were modeled from presence-only data with MaxEnt (vers. 3.3.3: Phillips et al. 2006). All occurrence points were used to build the niche models used in our analyses. Pseudo-absences were sampled from a restricted background to avoid model over-fitting (Anderson and Raza 2010). This is important in the present context as model over-fitting could potentially exacerbate niche differences between species (Anderson and Raza 2010). But the choice of an appropriate background region for sampling pseudo-absences is delicate. A too restricted background could make it difficult to compare niche models among species. Indeed, if a model is trained on a very narrow ecological range, it could become difficult to compare the model with that of other species that occur outside the ecological range of the model (Elith et al. 2011). The approach used to delimit the background region was to take the union area of circles of radius of 80 km around each occurrence (VanDerWall et al. 2009). Such a radius falls in the most optimal range observed by VanDerWall et al. (2009), but more importantly it reflects the potential of the species to disperse. For instance, the current range of *P. enysii* (400 km) and its age (60 ky; see Results) predicts a minimum dispersal rate of 33 km per 10,000 years. 1000 pseudo-absences were sampled at random within this background region to train the model. We then checked if the predictions over the whole South Island were affected by a prediction outside the ecological range of the model ("clamping"). Only the *P. fasciarium* model was affected; for that species background points were sampled from a square extent delimited by the longitudes 170 and 174.5 and the latitudes –46 and –41.5.

Model performance was evaluated by cross-validation using the area under the receiving operating characteristic curve (AUC). The AUC is expected to vary from 0.5 for a model that performs no better than random to 1.0 for perfect ability to predict presence versus absence. However, the use of AUC for testing ecological niche models obtained from presence-only data has been criticized (e.g., Raes and ter Steege 2007; Hijmans et al. 2012; Jiménez-Valverde 2012), in part because AUC values are strongly affected by the extent of the background from which pseudo-absences are drawn (VanDerWall et al. 2009; Hijmans et al. 2012). The models were thus also evaluated using the adjusted AUC (adjAUC) that corrects for the spatial sorting bias between testing presence and testing absence points (Hijmans et al. 2012). These adjAUC are then comparable between studies and null geographic models get an expected value of 0.5 (Hijmans et al. 2012).

If adjAUC values are generally much smaller than the standard AUC, at least it allows one to address whether the models perform better than a null model. For comparison purposes, the adjAUC were also estimated on a null geographic model in which the probability of occurrence is inversely proportional to the distance from the occurrence points. This model was fitted with the geoDist function in the dismo R package. Model cross-validation (75% of occurrences were used for training and 25% for testing) was performed from 10 independent random sampling of occurrence points and mean values were reported. Model testing was performed in R using the dismo package (Hijmans et al. 2012).

Niche Specificity

To test niche specificity, we estimated the logistic probability of occurrence for all specimens and evaluated the mean logistic prediction per species. We also calculated the mean logistic prediction for 1000 random background occurrences sampled from within a background region consisting in the union area of circles of radius of 80 km around each occurrence of the species corresponding to the model, which is assumed to represent the noise level. The fit of a species relative to the species for which the model was developed was estimated using the formula

$$\sigma_{ij} = \frac{\delta_{ij} - \delta_{bj}}{\delta_{jj} - \delta_{bj}}$$

where σ_{ij} is the relative fit of species i on the niche model of species j, δ_{ij} is the mean logistic fit for individuals of species i on the model of species j, and δ_{bj} is the mean logistic fit for random background points on the model of species j. Although the statistic σ_{ij} can have values below 0 (i.e., when the mean fit of the species is worse than for background points) and above 1 (when the fit of the model on a species is better than on the species for which the model was developed), it is forced to fall within the interval [0,1] by giving values below 0 and above 1 values of 0 and 1, respectively. A value of 0 means that the fit cannot be distinguished from random points and a fit of 1 means a fit as good as that of the species for which the model was developed.

Fitting Neutral and Niche Shift Models

To test whether ecological differences between species fit a neutral (Brownian) or niche shift model, we used disparity through time (dtt) plots (Harmon et al. 2003). This method compares, from the root to the leaves of the phylogeny, the amount of ecological disparity observed within clades relative to that of the whole genus for a set of characters. Ecological disparity, which could also be called ecological diversity or variation, is a variance-related measure that varies between 0 and 1

and that estimates the ecological dispersion of points in multivariate space (Ciampaglio et al. 2001; Harmon et al. 2003). Values near 0 at a specific time along the phylogeny imply that there is little disparity within clades and consequently most ecological variation is partitioned between clades. dtt plots were estimated for 5000 trees sampled from the posterior distribution of the species tree search to account for phylogenetic uncertainty. A new species tree search was performed for this analysis, which was identical to the one described above except for excluding the Tasmanian species *P. radicatum* for which we did not have reliable information on its ecological niche. Disparity was estimated from the average squared Euclidean distance between taxa using the continuous ecological variables used in the niche modeling. The value per species for each ecological variable was the median value of all occurrence points for a given species. Each variable was standardized to give all variables the same weight in the disparity estimates.

Observed dtt plots were compared with expected distributions under a Brownian motion model and a niche shift model. Distributions for these models were obtained by simulating a new ecological data set of five continuous variables using the covariance matrix of the original data for each of the 5000 trees from the posterior distribution. A single data set was simulated per tree because we were interested in evaluating the whole range of possible values and not just the mean expectation (note that this is different from the original description of dtt plots; see also Slater et al. 2010; Burbrink et al. 2012). Loess regression curves were used to represent the mean expectations and standard deviations for the observed and simulated data. To further calculate if the observed disparity was significantly greater than the disparity expected under the models, we divided the time along the phylogeny into 50 equal bins and tested significance for each bin using a paired *t*-test with a *P*-value threshold of 0.001. dtt calculations and Brownian and niche shift simulations were performed using the geiger package (Harmon et al. 2008) in R.

Correlation between Ecological Distance and Geographical Overlap

We tested the hypothesis that species with overlapping distributions had more divergent ecological niches. To obtain the geographical overlap between species, we estimated the geographical distribution of each species using a convex Hull approach using the R package dismo (Hijmans et al. 2012). For *P. cheesemanii*, *P. fastigiatum*, and *P. enysii*, the geographic distribution was better estimated using three convex Hull areas, whereas one area was appropriate for the other species (Supplementary Fig. S1). Intersect polygons between all pairs of species and areas of species distributions and of overlapping regions were estimated using the R package PBSmapping (Schnute et al. 2012). The proportion of overlap between species was obtained by dividing the

area of the intersect polygon by the area of the species with the smallest distribution. Consequently, two species have an overlap of 1 if a species range is completely enclosed in the other (Nakazato et al. 2010).

Niche overlap between species was estimated using Warren's I statistic (Warren et al. 2008) using the R package phyloclim (Heibl 2013) from probability surfaces of the niche models as obtained from MaxEnt. Because our modeling approach aimed at minimizing over-fitting, we do not think that our approach could result in an underestimated niche overlap (Broennimann et al. 2012). Moreover, such tendencies should not affect the presence of a correlation, but only the absolute value of niche overlap for a given pair of species. The correlation between the ecological niche distance and geographical overlap was tested by a Mantel test (999 permutations) with the vegan R package (Oksanen et al. 2012).

RESULTS

Species Delimitation

Prior to investigating the rate of diversification and ecological niche separation, it is important to have sound species boundaries. Although *Pachycladon* has been extensively studied at the morphological level (reviewed in Yogeeswaran et al. 2011), it is still unclear whether morpho-species form genetically cohesive units (Templeton 1989). To investigate species boundaries, we used a visual network representation of genetic distances as well as a Bayesian approach. The NeighborNet network of the genetic distances between individuals showed that individuals cluster by species and that in most cases species are supported by strong splits (Fig. 1b). Further, the network did not show important intra-specific genetic structure suggestive of cryptic species. We thus used the taxonomic species as input in the Bayesian program BP&P, which calculates the posterior probability of different species delimitations using the multi-species coalescent (Yang and Rannala 2010). Multiple independent runs based on different models and priors all supported the 11 species model with posterior probability of 1.0; the lumping of species did not receive any statistical support. Consequently, we considered these 11 species to be independent evolutionary lineages and to represent distinct species.

Diversification Rates

To test whether *Pachycladon* meets our criteria for an adaptive radiation, we first estimated the diversification rate (criterion a) for the genus. The diversification rates were estimated on the *BEAST phylogeny (Supplementary Fig. S2) using a birth and death model, which had a smaller AIC score than a pure birth model on the highest clade credibility tree. The highest density value (Fig. 2) was 1.99 species per myr for the lineage diversification rate (95% CI = 0.35–2.19)
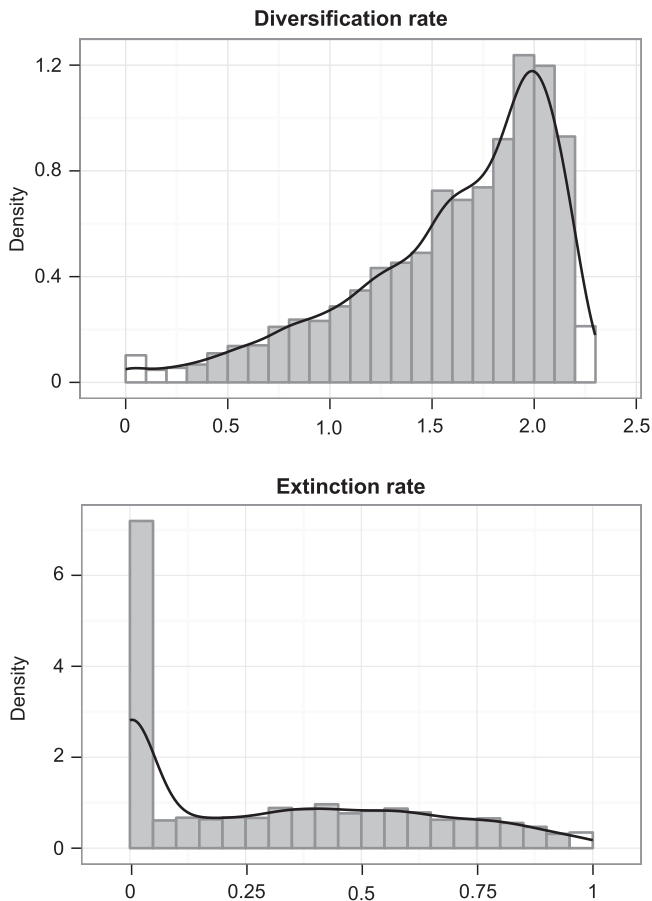
FIGURE 2.   Estimation of the lineage diversification and extinction rates (species per myr) according to a birth and death model on the posterior distribution of species trees.

and 0.005 for the lineage extinction rate (95% CI = 0–0.92). Not surprisingly, confidence intervals were quite wide, which is expected for a data set with so few species. Parametric bootstrapping confirmed this and further showed that the uncertainty in parameter estimation is caused by the joint estimation of diversification and extinction rates (Supplementary Fig. S3). Indeed, when the extinction rate was fixed at 0, there was enough information to precisely estimate the diversification rates (Supplementary Fig. S3).

Ecological Niche Modeling

The ecological niches of most species are relatively narrow and are confined to the South Island of New Zealand (Fig. 3). The exceptions are *P. cheesemanii*, generally considered to be a generalist (Heenan and Mitchell 2003), and *P. fastigiatum* and *P. crenatus*, which have relatively large predicted niches in the Southern Alps of the South Island (Fig. 3). Species models showed a good performance overall with adjAUC demonstrating a better performance than the null geographic models (Table 1). The variable contributions in the models suggest that all included variables were relatively

important in the niche model of at least one species, but the most important variables overall were annual mean temperature, mean diurnal range, annual precipitation, and toprock composition (Table 1). The niche models of *P. exile* and *P. fasciarium* trained with only two ecological variables were very similar to the models with six variables (Schoener's D of 0.72 and 0.98 for *P. fasciarium* and *P. exile* models, respectively) and substituting these models in the downstream analyses had no effect on the results and conclusions (data not shown). Only the data obtained with the six variables models are thus presented to facilitate the comparison among species.

We used the niche models to estimate niche specificity (criterion b) by evaluating the fit of the occurrence of each species on the models of the other species. Niche models were specific, with very few exceptions (Fig. 4). One exception is the *P. cheesemanii* model for which several other species had a relatively good fit, again supporting previous observations that this species is a generalist (Heenan and Mitchell 2003). Interestingly, the niche fitting heatmap is generally not symmetric. For instance, if several species showed a relatively good fit for the *P. cheesemanii* model (first column), the opposite is not true and *P. cheesemanii* had a very bad fit for all species niche models (first row). In some cases, the observed patterns may be informative of speciation processes such as between *P. fastigiatum* and *P. stellatum*, where *P. stellatum* might be considered to be derived from *P. fastigiatum* since its narrower—and distinct—niche seem to be included in that of *P. fastigiatum*. Despite the overall niche specificity, we observe that closely related species tend to have more similar niches than distantly related species (Fig. 4).

Ecological Disparity

The last criterion we tested is whether the ecological differences, or disparity, show a pattern that would support evolution by niche shifts (criterion c). To do this, we compared the observed ecological disparity to that expected under Brownian and niche shift models using dtt plots (Harmon et al. 2003). We found that the observed disparity was greater than that expected under a neutral model throughout the phylogeny and that it was significantly greater ($P < 0.001$) for 98% of the evolutionary history of the genus (Fig. 5a). In contrast, the observed disparity clearly fell within one standard deviation of the mean expected for a niche shift model of evolution (Fig. 5b), and it was significant over a much smaller fraction of the history of the group (20%). Together, these results reject a neutral model of ecological niche differentiation and support an evolution by niche shifts for *Pachycladon*.

DISCUSSION

Although the theory behind the concept of adaptive radiations is sound and well developed (Schluter 2000), categorizing species radiations as such is often less obvious and sometimes relatively arbitrary (Glor 2010).
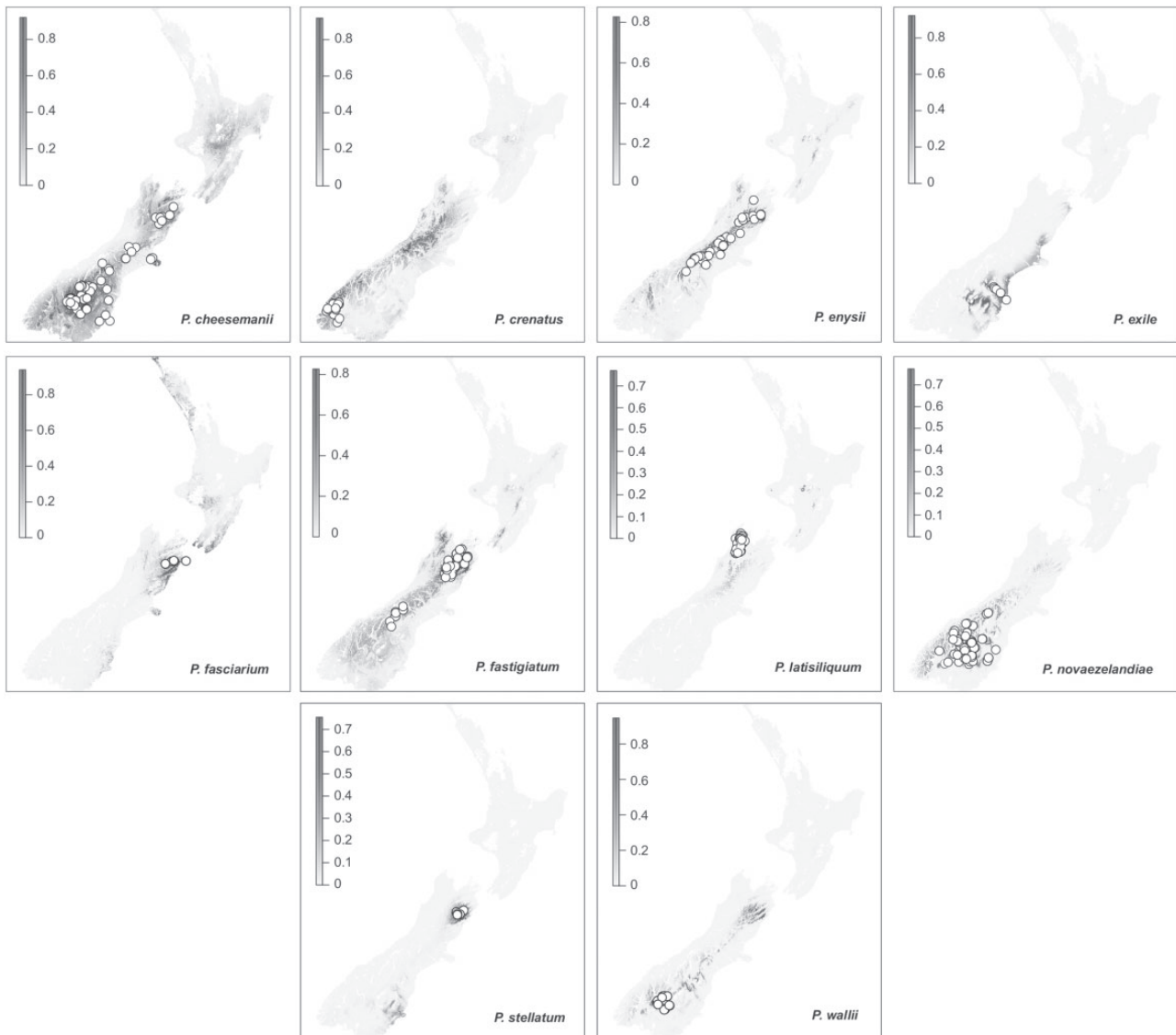
FIGURE 3.   Probability of presence (logistic output) predicted from the MaxEnt ecological niche models for all *Pachycladon* species. White dots represent occurrence points.

TABLE 1.   Ecological niche model evaluation and variable contributions in the models.

Species	AUC	adjAUC	Null adjAUC[a]	Variable contribution					
				Annual temperature (%)	Diurnal range (%)	Annual precipitation (%)	Precipitation seasonality (%)	Maximum slope (%)	Toprock type (%)
P. cheesemanii	0.85	0.70	0.51	7	39	23	1	17	13
P. crenatus	0.79	0.64	0.47	21	4	39	7	7	23
P. enysii	0.94	0.80	0.50	63	0	6	1	2	27
P. exile	0.88[b]	0.90[b]	0.55	14	0	67	13	5	1
P. fasciarium	0.93[b]	na[c]	na[c]	3	24	28	4	33	9
P. fastigiatum	0.88	0.65	0.51	58	24	2	5	1	10
P. latisiliquum	0.98	0.89	0.54	57	0	36	6	0	1
P. novae-zelandiae	0.95	0.85	0.53	80	2	1	10	1	7
P. stellatum	0.99[b]	0.64[b]	0.44	38	3	51	2	0	6
P. wallii	0.98	0.81	0.49	61	2	10	4	8	14

Notes: [a]adjAUC value obtained on a null geographic model; [b]50% of the samples were used for testing for these species; [c]impossible to obtain an adjAUC value due to the clumping of the samples.
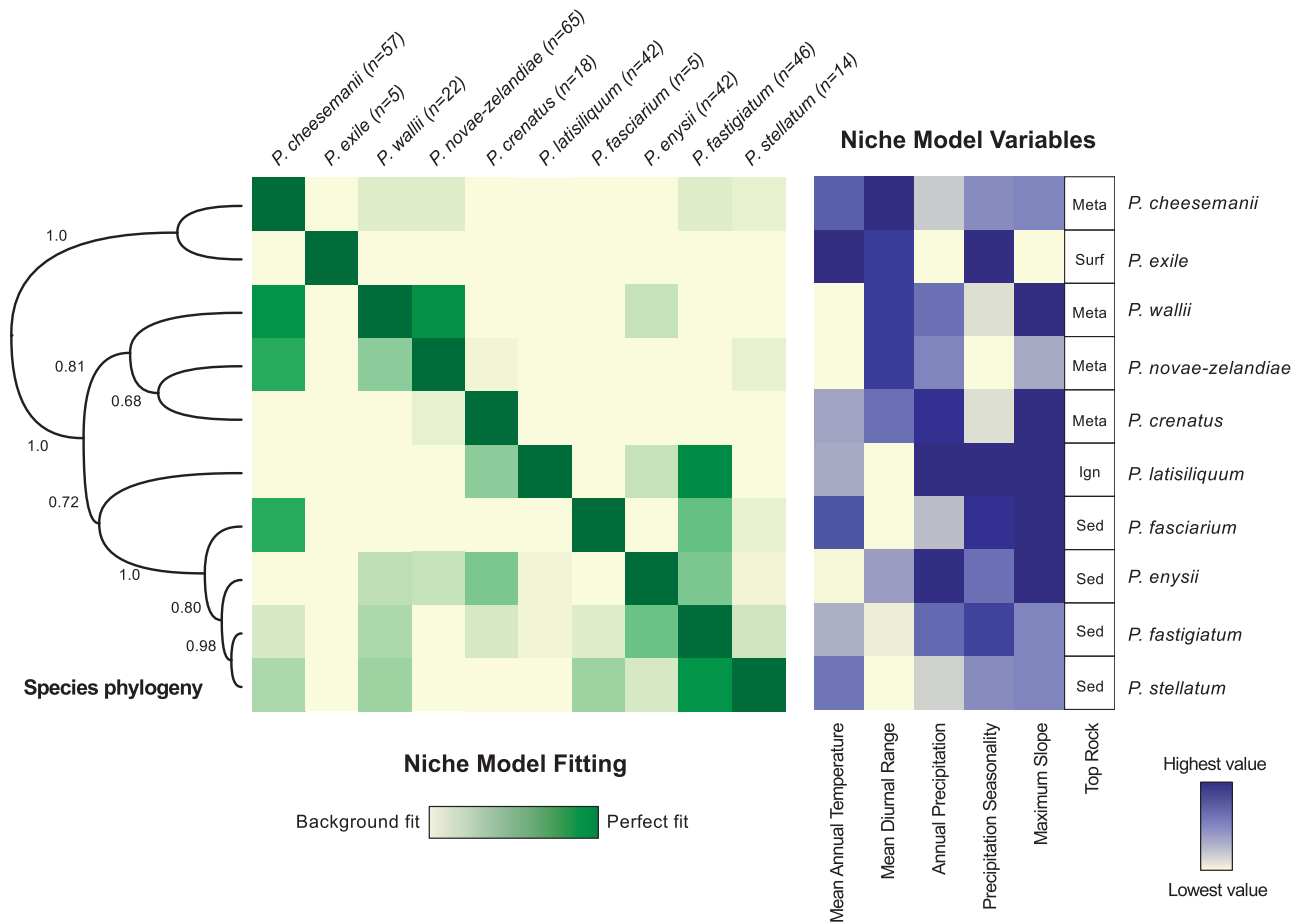
FIGURE 4.   Niche model fitting (left) and standardized median values of the variables for the different species (right). The green heatmap on the left shows the standardized fit of the species ecological niche models. The columns indicate the species niche model (with number of samples used to train the models) and the rows the species on which each model was fitted. A dark green color indicates a fit equivalent to the species that was used to train the model (hence the dark green diagonal), whereas a light color indicates a fit that is not different from random points. The blue heatmap on the right shows the standardized median values per species for the different variables that were used to build the ecological niche models. Species are sorted according to the *BEAST species phylogeny (to the left), where numbers represent clade posterior probabilities. Toprock abbreviations: Meta = metamorphic, Surf = surficial, Ign = igneous, Sed = sedimentary/strongly indurated.

For instance, although we expect ecological speciation to be preponderant in adaptive radiation, it is unlikely to underlie all speciation events. Consequently, the relative importance of ecological speciation in an adaptive radiation remains unclear and has been subject to debate (Glor 2010). Furthermore, species adaptation could be cryptic and this complicates traditional approaches for identifying instances of adaptive radiations that are based on phenotype-environment associations (Schluter 2009). In order to offer an alternative to the traditional criteria for identifying adaptive radiations, we propose an approach that focuses on the ecological niche of species. The main idea is that the observed pattern of ecological divergence through time should have a better fit to a niche shift model than to a neutral (Brownian) model. Clearly, an approach based on the ecological niche comparison can hardly confirm the presence of ecological speciation, but if the observed pattern better fits a niche shift than a Brownian model, it is likely that ecological speciation has taken place in the group.

The New Zealand rockcresses (*Pachycladon*) represented a good candidate to test the approach because although it superficially looks like an adaptive radiation with species living in very different habitats, no clear key adaptations were known (but see Voelckel et al. 2008, 2012 for potential cryptic adaptations). *Pachycladon* also consists of a modest number of species, a common feature of several adaptive radiations (Freckleton and Harvey 2006) that, because of sample size, poses a challenge in terms of power for detecting non-neutral evolution.

Diversification rate estimation is a challenge for groups with few species (Freckleton and Harvey 2006; O'Meara et al. 2006; Boettiger et al. 2012) and *Pachycladon* is no exception. Nevertheless, the most likely estimate points toward high diversification rates for *Pachycladon*. These rates compare to that of the highest rates observed among flowering plants (< 0.35 for angiosperm orders: Magallon and Sanderson 2001; 1.9–3.7 for the *Lupinus* radiation in the Andes: Hughes
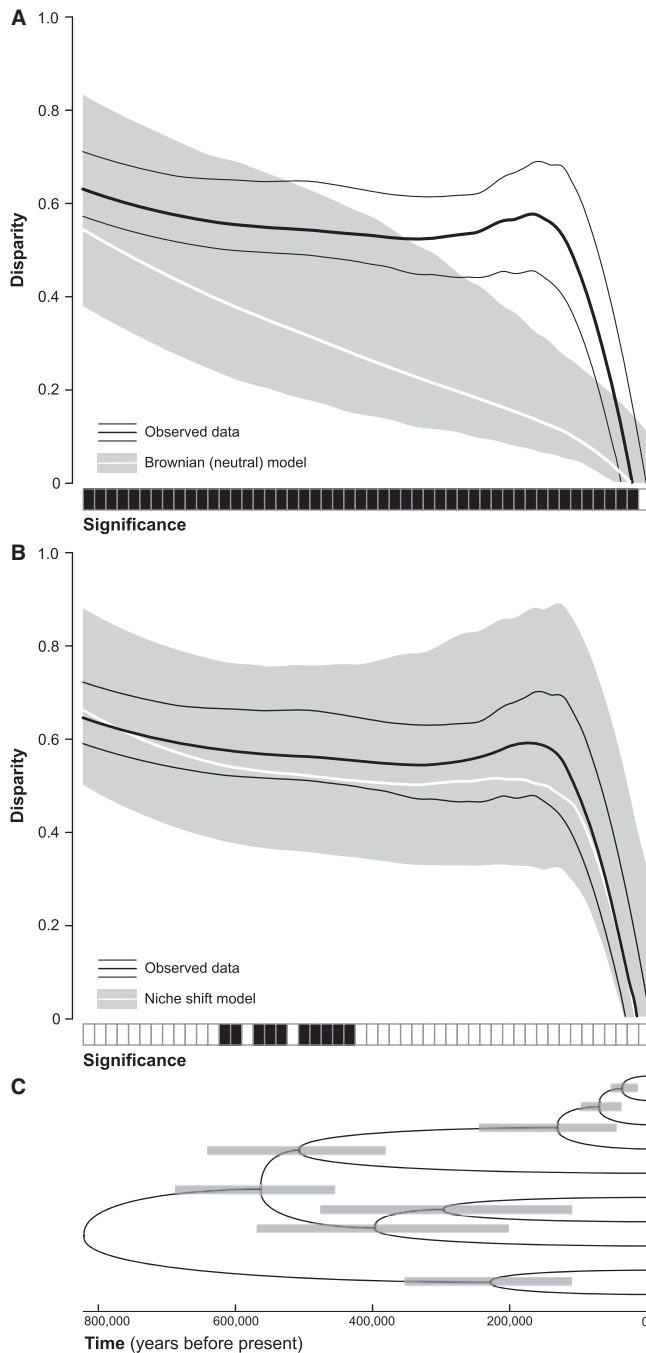
FIGURE 5.   dtt plots for the *Pachycladon* data (black lines) as well for data simulated under (a) a Brownian (neutral) model and (b) a niche shift model (simulated data shown as white line and gray shadow). The thick lines represent the loess fitted regression model and the shadow (for simulated data) and thin lines (for the *Pachycladon* data) show the fitted standard deviation. The significance bar indicates, for equally spaced bins along the time axis, if the observed disparity for the *Pachycladon* data was significantly greater than that for the simulated data (paired *t*-test; black indicates that $P < 0.001$). The phylogeny (c) of the genus is shown at the bottom as a reference, with the boxes at the nodes representing the 95% Bayesian credible intervals for the estimated node ages.

and Eastwood 2006), which is certainly consistent with a species radiation hypothesis. We then showed that apart from *P. cheesemanii*, which has a wide ecological niche and is considered to be a generalist (Heenan and Mitchell 2003), all other species have distinct and relatively narrow ecological niches. Such levels of niche specificity within a monophyletic group are remarkable. But more importantly, results showed that the ecological disparity within lineages in the history of the genus is significantly greater than expected according to a neutral model of evolution. In other words, closely related species are more divergent ecologically than would be expected if ecological divergence were occurring constantly through time as result of an evolutionary process that is independent among lineages. In contrast, the observed pattern of ecological disparity is much more similar to that expected with a niche shift model in which all ecological divergence occurs at speciation events. Together, these results provide good evidence that *Pachycladon* represents an adaptive radiation.

We reiterate that we do not provide definitive evidence of ecological speciation—more detailed studies are required for this. Yet, the data are in agreement with such a scenario. Ecological divergence could certainly occur following reproductive isolation through gradual selection and genetic drift due to geographic isolation and environmental variation (Drummond et al. 2012). However, in such cases ecological differences are expected to accumulate randomly in different lineages and at a relatively constant rate, a scenario rejected by our analyses. Divergence could be promoted and accelerated if species came into secondary contact with each other, as might be expected during Pleistocene climate changes. However, because relatively few species of *Pachycladon* overlap in their current distributions (Fig. 1a and Supplementary Fig. S1) and because several species have very restricted distributions, it would be difficult to attribute all niche shifts to secondary contacts. Moreover, there is no significant positive correlation between the distance in ecological niche and the geographic overlap of species (Mantel statistic $r_M = -0.366$; $P = 0.975$). Rare interspecific hybrids (Yogeeswaran et al. 2011) and evidence for introgression of adaptively significant gene loci (Becker et al. 2013) have been found among some *Pachycladon* accessions, but our analyses of neutral independent gene loci reported here suggests that the extent of introgression is not sufficient to break down the genetic cohesiveness of species. Nevertheless, it is impossible to completely discard a potential influence of secondary contacts. Further studies are needed to investigate in more detail what traits could have been involved in speciation events and what role these might have played in reproductive isolation. There are certainly several good candidates for adaptive traits in *Pachycladon*, including genes involved in glucosinolate biosynthesis that are differentially expressed between the high alpine *P. enysii* and the mid-alpine *P. fastigiatum* (Voelckel et al. 2008).

The method proposed here that tests for evidence of evolution by niche shifts have several advantages.

It focuses on ecological niche comparisons instead of phenotype-environment correlations and as such it is useful for testing adaptive radiation in groups that do not have apparent key innovations. Another advantage is that it is directly aligned with Simpson's view of adaptive radiations. Moreover, by relying on evolutionary models, it provides a statistical framework to test adaptive radiation hypotheses and to investigate the power of the data to test the hypothesis, a key component in phylogenetic comparative studies (Boettiger et al. 2012). Perhaps more importantly, this approach has the potential to help understand the role of ecological speciation in adaptive radiations, a debated issue (Glor 2010), but also in evolution in general. Indeed, the increasing popularity for the concept of niche conservatism (Ackerly 2003; Wiens 2004), which is the tendency of niche-related ecological traits to remain similar over time (Wiens et al. 2010), tend to minimize the importance of ecological speciation in nature. The present approach thus provides a framework to test these ideas and reveal further organismal groups where ecological evolution has been particularly exceptional.

REFERENCES

Ackerly D.D. 2003. Community assembly, niche conservatism, and adaptive evolution in changing environments. Int. J. Plant Sci. 164:S165–S184.

Anderson R.P., Raza A. 2010. The effect of the extent of the study region on GIS models of species geographic distributions and estimates of niche evolution: preliminary tests with montane rodents (genus Nephelomys) in Venezuela. J. Biogeogr. 37:1378–1393.

Batt G.E., Braun J., Kohn B.P., McDougall I. 2000. Thermochronological analysis of the dynamics of the Southern Alps, New Zealand. Geol. Soc. Am. Bull. 112:250–266.

Becker M., Gruenheit N., Steel M., Voelckel C., Deusch O., Heenan P.B., McLenachan P.A., Kardailsky O., Leigh J.W., Lockhart P.J. 2013. Hybridization may facilitate in situ survival of endemic species through periods of climate change. Nature Clim. Change 3:1039–1043.

Blanc G., Hokamp K., Wolfe K.H. 2003. A recent polyploidy superimposed on older large-scale duplications in the Arabidopsis genome. Genome Res. 13:137–144.

Boettiger C., Coop G., Ralph P. 2012. Is your phylogeny informative? Measuring the power of comparative methods. Evolution 66:2240–2251.

Broennimann O., Fitzpatrick M.C., Pearman P.B., Petitpierre B., Pellissier L., Yoccoz N.G., Thuiller W., Fortin M.-J., Randin C., Zimmermann N.E., Graham C.H., Guisan A. 2012. Measuring ecological niche overlap from occurrence and spatial environmental data. Global Ecol. Biogeogr. 21:481–497.

Bruen T.C., Philippe H., Bryant D. 2006. A simple robust statistical test for detecting the presence of recombination. Genetics 172:2665–2681.

Bryant D., Moulton V. 2004. Neighbor-Net: an agglomerative method for the construction of phylogenetic networks. Mol. Biol. Evol. 21:255–265.

Burbrink F.T., Ruane S., Pyron R.A. 2012. When are adaptive radiations replicated in areas? Ecological opportunity and unexceptional diversification in West Indian dipsadine snakes (Colubridae: Alsophiini). J. Biogeogr. 39:465–475.

Chinn W.G., Gemmell N.J. 2004. Adaptive radiation within New Zealand endemic species of the cockroach genus *Celatoblatta* Johns (Blattidae): a response to Plio-Pleistocene mountain building and climate change. Mol. Ecol. 13:1507–1518.

Ciampaglio C.N., Kemp M., McShea D.W. 2001. Detecting changes in morphospace occupation patterns in the fossil record: characterization and analysis of measures of disparity. Paleobiology 27:695–715.

Dambroski H.R., Linn C., Berlocher S.H., Forbes A.A., Roelofs W., Feder J.L. 2005. The genetic basis for fruit odor discrimination in Rhagoletis flies and its significance for sympatric host shifts. Evolution 59:1953–1964.

Darwin C. 1859. On the origin of species by means of natural selection, or, the preservation of favoured races in the struggle for life. London: John Murray.

Davies T.J., Cooper N., Diniz-Filho J.A.F., Thomas G.H., Meiri S. 2012. Using phylogenetic trees to test for character displacement: a model and an example from a desert mammal community. Ecology 93:S44–S51.

Drummond C.S., Eastwood R.J., Miotto S.T.S., Hughes C.E. 2012. Multiple continental radiations and correlates of diversification in Lupinus (Leguminosae): Testing for key innovation with incomplete taxon sampling. Syst. Biol. 61:443–460.

Edgar R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797.

Elith J., Leathwick J.R. 2009. Species distribution models: ecological explanation and prediction across space and time. Ann. Rev. Ecol. Evol. Syst. 40:677–697.

Elith J., Phillips S.J., Hastie T., Dudík M., Chee Y.E., Yates C.J. 2011. A statistical explanation of MaxEnt for ecologists. Divers. Distrib. 17:43–57.

Felsenstein J. 1985. Phylogenies and the comparative method. Am. Nat. 125:1–15.

Freckleton R.P., Harvey P.H. 2006. Detecting non-Brownian trait evolution in adaptive radiations. PLoS Biol. 4:e373.

Gavrilets S., Losos J.B. 2009. Adaptive radiation: contrasting theory with data. Science 323:732–737.

Glor R.E. 2010. Phylogenetic insights on adaptive radiation. Ann. Rev. Ecol. Evol. Syst. 41:251–270.

Harmon L.J., Schulte J.A., Larson A., Losos J.B. 2003. Tempo and mode of evolutionary radiation in iguanian lizards. Science 301:961–964.

Harmon L.J., Weir J.T., Brock C.D., Glor R.E., Challenger W. 2008. GEIGER: investigating evolutionary radiations. Bioinformatics 24:129–131.

Harvey P.H., Rambaut A. 2000. Comparative analyses for adaptive radiations. Philos. Trans. R. Soc. Lond. B Biol. Sci. 355:1599–1605.

Heenan P.B. 2009. A new species of Pachycladon (Brassicaceae) from limestone in eastern Marlborough, New Zealand. New Zeal. J. Bot. 47:155–161.

Heenan P.B., McGlone M.S. 2013. Evolution of New Zealand alpine and open-habitat plant species during the late Cenozoic. New Zeal. J. Ecol. 37: 105–113.

Heenan P.B., Mitchell A.D. 2003. Phylogeny, biogeography and adaptive radiation of Pachycladon (Brassicaceae) in the mountains of South Island, New Zealand. J. Biogeogr. 30:1737–1749.

Heibl C., Calenge C. 2013. phyloclim: integrating phylogenetics and climatic niche modelling, version 0.9-4. http://CRAN.R-project.org/package=phyloclim (last accessed December 20, 2013).

Heled J., Drummond A.J. 2010. Bayesian inference of species trees from multilocus data. Mol. Biol. Evol. 27:570–580.

Hijmans R.J. 2012. Cross-validation of species distribution models: removing spatial sorting bias and calibration with a null model. Ecology 93:679–688.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25:1965–1978.

Hijmans R.J., Phillips S.J., Leathwick J.R., Elith J. 2012. dismo: species distribution modeling, version 0.9-3. http://CRAN.R-project.org/package=dismo (last accessed December 20, 2013).

Hughes C., Eastwood R. 2006. Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. PNAS 103:10334–10339.

Huson D.H., Bryant D. 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23:254–267.

Jiménez-Valverde A. 2012. Insights into the area under the receiver operating characteristic curve (AUC) as a discrimination measure in species distribution modelling. Global Ecol. Biogeogr. 21:498–507.

Joly S. 2012. JML: testing hybridization from species trees. Mol. Ecol. Resour. 12:179–184.

Joly S., Bruneau A. 2006. Incorporating allelic variation for reconstructing the evolutionary history of organisms from multiple genes: an example from Rosa in North America. Syst. Biol. 55:623–636.

Joly S., Heenan P.B., Lockhart P.J. 2009a. A Pleistocene inter-tribal allopolyploidization event precedes the species radiation of Pachycladon (Brassicaceae) in New Zealand. Mol. Phylogenet. Evol. 51:365–372.

Joly S., McLenachan P.A., Lockhart P.J. 2009b. A statistical approach for distinguishing hybridization and incomplete lineage sorting. Am. Nat. 174:e54–e70.

Jukes T.H., Cantor C.R. 1969. Evolution of protein molecules. Mamalian protein metabolism. New York: Academic Press. p. 21–123.

Kass R.E., Raftery A.E. 1995. Bayes factor. J. Am. Stat. Assoc. 90:773–795.

Koch M., Haubold B., Mitchell-Olds T. 2001. Molecular systematics of the Brassicaceae: evidence from coding plastidic matK and nuclear Chs sequences. Am. J. Bot. 88:534–544.

Magallon S., Sanderson M.J. 2001. Absolute diversification rates in angiosperm clades. Evolution 55:1762–1780.

Mandakova T., Heenan P., Lysak M. 2010. Island species radiation and karyotypic stasis in Pachycladon allopolyploids. BMC Evol. Biol. 10:367.

Nakazato T., Warren D.L., Moyle L.C. 2010. Ecological and geographic modes of species divergence in wild tomatoes. Am. J. Bot. 97:680–693.

O'Meara B.C., Ané C., Sanderson M.J., Wainwright P.C. 2006. Testing for different rates of continuous trait evolution using likelihood. Evolution 60:922.

Oksanen J., Blanchet F.G., Kindt R., Legendre P., Minchin P.R., O'Hara R., Simpson G.L., Solymos P., Stevens M.H.H., Wagner H. 2012. vegan: community ecology package, 2.0-10. http://CRAN.R-project.org/package=vegan.(last accessed December 20, 2013)

Pagel M. 1999. Inferring the historical patterns of biological evolution. Nature 401:877–884.

Pearman P.B., Guisan A., Broennimann O., Randin C.F. 2008. Niche dynamics in space and time. Trends Ecol. Evol. 23:149–158.

Phillips S.J., Anderson R.P., Schapire R.E. 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model. 190:231–259.

Posada D., Crandall K.A. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.

Price T. 1997. Correlated evolution and independent contrasts. Philos. T. Biol. Sci. 352:519–529.

R Development Core Team 2009. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Rabosky D.L., Schliep K. 2013. Laser: likelihood analysis of speciation/Extinction rates from phylogenies, 2.4-1. http://CRAN.R-project.org/package=laser (last accessed December 20, 2013).

Raes N., ter Steege H. 2007. A null-model for significance testing of presence-only species distribution models. Ecography 30: 727–736.

Rambaut A., Drummond A.J. 2009. Tracer, version 1.5. Distributed by the authors.

Rundle H.D., Nosil P. 2005. Ecological speciation. Ecol. Lett. 8:336–352.

Schluter D. 2000. The ecology of adaptive radiation. New York, USA: Oxford Univeristy Press.

Schluter D. 2001. Ecology and the origin of species. Trends Ecol. Evol. 16:372–380.

Schluter D. 2009. Evidence for ecological speciation and its alternative. Science 323:737–741.

Schnute J.T., Boers N., Haigh R., Grandin C., Johnson A., Wessel P., Antonio F. 2013. PBSmapping: mapping fisheries data and spatial analysis tools, version 2.66.53. http://CRAN.R-project.org/package=PBSmapping (last accessed December 20, 2013).

Simpson G.G. 1953. The major features of evolution. New York: Columbia University Press.

Slater G.J., Price S.A., Santini F., Alfaro M.E. 2010. Diversity versus disparity and the radiation of modern cetaceans. Proc. R. Soc. B 277:3097–3104.

Suchard M.A., Weiss R.E., Sinsheimer J.S. 2001. Bayesian selection of continuous-time Markov chain evolutionary models. Mol. Biol. Evol. 18:1001–1013.

Templeton A.R. 1989. The meaning of species and speciation: a genetic perspective. Speciation and its consequences. Sunderland, MA: Sinauer Associates. p. 3–27.

VanDerWall J., Shoo L.P., Graham C., Williams S.E. 2009. Selecting pseudo-absence data for presence-only distribution modeling: How far should you stray from what you know? Ecol. Model. 220: 589–594.

Verslues P.E., Juenger T.E. 2011. Drought, metabolites, and Arabidopsis natural variation: a promising combination for understanding adaptation to water-limited environments. Curr. Opin. Plant Biol. 14:240–245.

Voelckel C., Gruenheit N., Biggs P., Deusch O.D., Lockhart P.J. 2012. Chips and tags suggest plant-environment interactions differ for two alpine Pachycladon species. BMC Genomics 13:322.

Voelckel C., Heenan P.B., Janssen B., Reichelt M., Ford K., Hofmann R., Lockhart P.J. 2008. Transcriptional and biochemical signatures of divergence in natural populations of two species of New Zealand alpine Pachycladon. Mol. Ecol. 17:4740–4753.

Warren D.L., Glor R.E., Turelli M. 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evolution 62:2868–2883.

Wiens J.J. 2004. Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. Evolution 58: 193–197.

Wiens J.J., Ackerly D.D., Allen A.P., Anacker B.L., Buckley L.B., Cornell H.V., Damschen E.I., Jonathan Davies T., Grytnes J., Harrison S.P., Hawkins B.A., Holt R.D., McCain C.M., Stephens P.R. 2010. Niche conservatism as an emerging principle in ecology and conservation biology. Ecol. Lett. 13:1310–1324.

Winkworth R.C., Wagstaff S.J., Glenny D., Lockhart P.J. 2005. Evolution of the New Zealand alpine flora: origins, diversification and dispersal. Org. Divers. Evol. 5:237–247.

Yang Y.-W., Lai K.-N., Tai P.-Y., Wen-Hsiung L. 1999. Rates of nucleotide substitution in angiosperm mitochondrial DNA sequences and dates of divergence between Brassica and other angiosperm lineages. J. Mol. Evol. 48:597–604.

Yang Z., Rannala B. 2010. Bayesian species delimitation using multilocus sequence data. Proc. Natl Acad. Sci. 107:9264–9269.

Yogeeswaran K., Voelckel C., Joly S., Heenan P.B. 2011. Pachycladon. In: Kole C., editor. Wild crop relatives: genomic and breeding resources. Berlin, Heidelberg: Springer. p. 227–249.

Zwickl D.J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion [Ph.D. dissertation]. Austin: The University of Texas.