

Correlated morphological and genetic patterns in *Embothrium coccineum* (Proteaceae) across climate and geography: can *Embothrium* survive patagonian climate change?

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Abstract. Adaptive radiation and reproductive isolation can determine the biogeographic structure of any species. We examine patterns of biotic variation in *Embothrium coccineum*, a Proteaceae tree that spans 20° of latitude and is both morphologically and genetically highly variable. We aim to (1) explore the correspondence between these biotic patterns and current geographic and climatic gradients, and (2) determine whether and how those patterns are likely to respond to changing climate. We conducted separate principal component analyses on biotic and abiotic sets of variables for 34 populations of *Embothrium coccineum*, accounting for a large fraction of the total variation in each. We then used canonical correlation analyses to optimise the match of those gradients onto each other. Smaller, rounder leaves and particular alleles typify the colder and drier parts of the range, whereas larger, lanceolate leaves and other alleles typify warmer and moister areas. Finally, we mapped biotic profiles onto a predicted climatic landscape, on the basis of doubling of CO₂ projections. The climatic regime is predicted to shift geographically, but this lineage has successfully responded to repeated and dramatic climatic shifts since the Oligocene, and it should also be able to move and adapt quickly enough to meet the present challenge. More generally, our analytic approach can be extended to analysis of biotic and abiotic patterns in other species facing climatic challenges. Where there is enough biogeographic variation to provide adaptively relevant substrate, and where propagule dispersal is sufficiently extensive to keep up with the pace of spatial climatic shift, such taxa should be able to cope with shifting climate.

Additional keywords: climatic gradients, genetic variation, geographic metrics, multivariate analysis, phenotypic patterns.

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Introduction

Both adaptive radiation and reproductive isolation are thought to be major determinants of species biogeographic structures (Wright 1943; Epperson 1993; Hamrick and Nason 1996). Reproductive isolation represents a balance between the stochastic aspects of genetic drift and the homogenising influences of gene flow (Broughton and Harrison 2003). With growing interest in climate change, there has been a resurgence of interest in the impact of climatic adaptation on biogeographic structure. Any shift in a species habitat, of either anthropogenic or natural origin, could result in selection on fitness-relevant traits (Gienapp *et al.* 2008).

Under changing selective pressures that are driven by climate change (Houghton *et al.* 2001), populations could respond in three basic ways (Holt 1990). They could elude the change by shifting their ranges. They could stay and adapt to the new conditions, through phenotypic plasticity, entailing no basic genetic changes. They could evolve, in response to those new conditions. Evasion leads to local extinction and emergence

elsewhere, whereas plastic phenotypic change and/or genetic adaptation might well permit local persistence. Range shifts have been a common reaction to past climatic changes (Parmesan and Yohe 2003; Perry *et al.* 2005; Soto *et al.* 2010). Plastic responses have also been demonstrated (Pigliucci *et al.* 1995; DeWitt *et al.* 1998; Canale and Henry 2010). Adaptation *in situ* might also occur, providing long-term evolutionary adjustment at a local scale (Clausen *et al.* 1940; Linhart *et al.* 1996; Maddox and Antonovics 1983; Sork *et al.* 1993; Galloway 2005). A combination of responses is both possible and likely (Davis and Shaw 2001). If the organism's ability to respond is not sufficient to meet the challenge, then of course, there is always a fourth possibility, and not uncommonly the ultimate outcome of dramatic climate shift, namely extinction.

Geographically widespread species, particularly those encountering diverse environmental conditions, are instructive exemplars that have successfully coped with dramatic climatic change over extended space and time. Temperate Patagonian forests exhibit complex climatic gradients that occur over

micro- to macro-scales. *Embothrium coccineum* (J.R. et Forst.), Proteaceae, is an early successional species and one of only three widespread trees within the Patagonian forest zone. Climatic variation is considerable and well documented across its extensive geographic range (Daniels and Veblen 2000; Montecinos and Aceituno 2003). Substantial geographic variation among populations of this species, both for leaf morphology (Souto *et al.* 2009) and genetic-marker loci (Souto and Premoli 2007), has been demonstrated.

Geography and climate are strongly correlated across Patagonia, with climate exhibiting two principal axes (Souto *et al.* 2009). The major axis runs roughly along the Andes, with temperature decreasing southward; the minor axis runs roughly from coast to inland sites, with precipitation decreasing longitudinally. Elevational effects tend to be masked by stronger latitudinal and longitudinal effects; the Andes are higher in the north, where climate is relatively warmer, whereas elevation declines near the coast, wherever conditions are relatively wetter. This, broadly two-dimensional, climatic pattern has strong implications for the distributions of most Andean species, and the impacts of projected climate change on such species should be considered in light of such climatic patterns.

Many authors have combined ecological and genetic data to study correlated patterns of biotic variation with geography, typically using isolation-by-distance or autocorrelation methods (e.g. Morton 1973; Sokal and Wartenberg 1983; Smouse *et al.* 1986; Smouse and Long 1992; Britten *et al.* 1995; Hardy *et al.* 2000; Telles and Diniz-Filho 2005). Studies of the correlation of geographic separation and phenotypic differentiation in animals (Schluter and Rambaut 1996; Wilkinson and Edds 2001) and in plants (Linhart and Grant 1996; Telles and Diniz-Filho 2005) have also been ongoing and numerous (Blanchet *et al.* 2008). These studies have been extended to the relationship between spatial separation and environmental divergence (Legendre and Legendre 1998). In recent years, interest has shifted to integrating population genetics, landscape ecology and a variety of spatial statistics (Manel *et al.* 2003; Gaggiotti and Fol 2010; Sork *et al.* 2010). Here, we develop a statistical approach that takes advantage of sets of highly intercorrelated biotic and abiotic features, establishing a basis for climatologically predictive biogeography, extracting broad signals from assemblages of highly inter-dependent characters.

Our objectives for the present study were to (1) explore the correspondence between morphological and genetic patterns and current Patagonian geographic and climatic gradients in *E. coccineum* and (2) determine whether and how those patterns are likely to respond to shifting climatic zones. Previous experience with this species (Souto and Premoli 2007; Souto *et al.* 2009) suggests the following: (i) although adaptive responses to climatic divergence have undoubtedly contributed to geographic patterns in morphology, we cannot rule out some secondary geo-spatial impact of distributed Pleistocene refugia and barriers to gene flow that have not been subsequently erased by post-glacial reoccupation; (ii) although genetic divergence over such vast expanses of geography is often attributed to isolation by distance *per se*, at least some adaptive signature is plausible, particularly across a region where palaeo-environmental reconstruction suggests that

cold-tolerant species may have survived in locally favourable microsites throughout the current range (Markgraf *et al.* 1995); and (iii) *Embothrium*, as a clade, has survived repeated climatic shifts since the Eocene, including those associated with repeated Pleistocene glacial cycles, and might well survive this one. We tested those predictions by (1) elucidating the correlation between geographic and climatic features across the range of *E. coccineum*, (2) analysing the correlation structure of genetic-marker loci and morphological leaf traits, (3) determining the association of morphology and genotype with climate and geography and (4) using models of changing climate, projecting those biotic traits onto a future landscape, so as to assess the likely biogeographic structure of *E. coccineum*, as a consequence of the predicted climatic shift.

Materials and methods

Embothrium, its geographic range and environment

Embothrium coccineum has a natural distribution that ranges from 35°S to 55°S in Argentina and Chile. This species is hermaphroditic and mostly self-incompatible, is highly dependent on pollinators, mainly hummingbirds and bumblebees, and is predominantly outcrossed. It is an early successional coloniser, often being associated with open areas, as well as occurring in gaps and along roadsides. It has a naturally patchy distribution in mixed forests, making it a keystone element for natural recovery of formerly agro-forested areas. In the northern portion of its distribution (latitude 40°S), *E. coccineum* is found along elevation gradients from sea level to treeline, which can reach 1500 masl (Escobar *et al.* 2006). At the same latitude, near the coast in Valdivia, Chile, the mean annual temperature (MAT) is 11°C, but at Andean treeline, annual temperatures average 5°C. In austral locations (Tierra del Fuego, latitude 55°S), MAT is only 4°C, and populations descend to within a few meters of sea level. The species also has a wide longitudinal range, and exhibits a steep precipitation gradient from areas with mean annual precipitation (MAP) of >3500 mm in southern Chile to areas with MAP of <400 mm in the Patagonian steppe of Argentina (Souto *et al.* 2009). Adults are small trees or shrubs, bearing leaves of variable sizes and shapes. On the eastern slope of the Andes, where continental climates are colder and drier in winter, plants have smaller and rounder leaves. In more northerly locations, where warmer summers are accompanied by an increase in aridity, leaves are also smaller. Sites with lower annual climatic variability occur to the west, near the Pacific Ocean, where the leaves tend to be elongated (Souto *et al.* 2009).

Sampling

We used previously published data (Souto and Premoli 2007; Souto *et al.* 2009) from 34 populations, 18 from Chile and 16 from Argentina, covering the entire range of *E. coccineum* (Fig. 1). Data consist of GPS location for each population, registering latitude and longitude (UTM coordinates), as well as elevation, which varies from 1050 m in more northern Andean sites, declining southward and westward to 20 m for coastal sites. We used the available genetic data for 30 individuals from each population (Souto and Premoli 2007) and subsampled



Fig. 1. Map depicting the 34 Patagonian locations of *Embotrium coccineum* sampled in Chile and Argentina. Black dots represent sampled localities, numbers agree with population data in Table S1, available as Supplementary Material for this paper.

10 individuals for leaf morphological data (Souto *et al.* 2009); the reader is referred to both papers for more detail. Location-specific averages for all of these features are recorded in Table S1, available as Supplementary Material for this paper. Our immediate objective here was to use these datasets for an integrated analysis of concordant patterns of geography, climate, morphology and genetic markers across the Patagonian range.

Climatic profiles

Climate metrics are useful predictors for leaf morphology in *E. coccineum*, but variation among sampled sites reflects a complex combination of regional gradients of temperature and precipitation. For broad characterisation of the variable climatic conditions of the region, we extracted local climate variables from WorldClim 1.4, a set of global climate layers with a 30 s by 30 s spatial resolution (Hijmans *et al.* 2005), including monthly total and monthly mean precipitation, minimum and maximum temperatures, and 19 derived bioclimatic variables (<http://www.worldclim.org/>, verified 1 November 2013). We then used DIVA-GIS (Hijmans *et al.* 2001) to adjust climate data for the specific elevations of our sampled sites. Correlations between WorldClim raw data and their DIVA-GIS elevational translations were all strong and significant ($r \geq 0.95$, $P < 0.05$).

Souto *et al.* (2009) showed that a quartet of variables explain most of the climatic variation experienced by *E. coccineum* across its range. These variables were temperature of the coldest and warmest months and precipitation of the driest and wettest months. Minimum temperature (T_{\min}°) and maximum precipitation (PPT_{\max}) occur in winter, whereas maximum temperature (T_{\max}°) and minimum precipitation (PPT_{\min}) occur in summer.

Leaf morphology

Souto *et al.* (2009) sampled a subset of 10 adults per population, collecting five terminal and unshaded twigs with fresh foliage, and measured fully expanded leaves from the 5th spiral of each terminal branch (1750 leaves, total) to control for developmental variation in leaf shape. Nine metric traits were extracted for each leaf, but several of those traits were strongly cross-correlated, so we here represent the essential patterns of leaf morphology with the following four traits: leaf area (LA), dry mass (DM), specific leaf area ($\log(\text{SLA})$) and a leaf shape factor (SF, defined as $(4\pi \cdot \text{area})/(\text{perimeter})^2$), a unitless measure of an object's circularity; $\text{SF} = 1$ for a perfect circle and $= 0$ for a straight line. Population mean values for each variable were approximately normally distributed for the entire collection (Souto *et al.* 2009).

Genetic markers

Souto and Premoli (2007) sampled 30 individuals from each population for allozyme analysis of 16 genetic loci. They recovered a total of 59 alleles for the 16 loci, in 934 individuals, with an average effective number of alleles of $A_e = 1.34/\text{locus}$ for the entire species. In all, 15 of the 16 loci exhibited a common allele and a collection of one or more uncommon alleles, plausibly treated as an 'ensemble alternative'. To a good first approximation, each of these 15 loci exhibited a binary distribution, so we have used the frequency of the most common allele to represent each locus. The Mdh-2 locus expressed two common alleles, but also a collection of rarer alleles; pooling the rarer alleles into the 'ensemble alternative', we listed the frequencies of the two common alleles. For the analyses here, we thus reduced our 59 allelic variants to 17 informative characters.

A summary of the raw data used for the analyses described below can be found in Table S1.

Principal component analysis

To reduce our 28-trait dataset to an even more parsimonious representation, we conducted a separate principal component analysis (PCA) for each set of traits, using STATISTICA 7 software (StatSoft). To reduce the dimensionality further, we retained only those PC axes whose collective variance was a large fraction of the total. For further analysis, we retained the first two axes from each of the climatic, geographic and morphological datasets. For each set, the first two axes extracted 88–90% of the total variation, and the remaining eigenvalues were all < 1.0 . For the genetic dataset, the first six axes accounted for 67% of the total variation, all with eigenvalues > 1.0 ; each of the residual axes accounted for less than 10% of the remaining variation, and only the 7th axis had an eigenvalue of > 1.0 . Preliminary analyses with either six or seven PC axes yielded virtually identical inference, so

we retained just the first six axes for further analysis. Each of the PC analyses yielded a set of eigenvectors (loading coefficients) that described linear combinations of the original variables, each combination with its own variance (eigenvalue). In effect, we reduced a hyper-dimensional dataset to a signal-dense, 12-dimensional representation.

Canonical correlation analysis

For purposes of signal extraction, PCA uses the correlation structure among features within a dataset to extract a parsimonious subset of indices capturing as much of the variation as feasible. It does not, however, optimise the mapping of one set of those signal sets onto another set, say climate onto geography or morphology onto genetics. For optimal pattern matching across datasets, we have used the correlations between their PCA representations to conduct a canonical correlation analysis (CCA). Effectively, we correlated the PCA variates from one dataset with those from another dataset, in such a way as to optimise the match between the two patterns. Starting with the 12 PCA scores computed for each of the 34 populations, we performed CCA, again using STATISTICA 7 software, for each of the six pairwise comparisons, as follows: (1) climate vs geography, (2) morphology vs genetics, (3) morphology vs climate, (4) morphology vs geography, (5) genetics vs climate and (6) genetics vs geography.

Mapping current and future climates

For future projections of climate in this region, we used the CCM3 scenario, based on a doubling of CO₂ (Govindasamy *et al.* 2003), downscaled to 2.5-min resolution and downloaded from <http://www.diva-gis.org/climate> (verified 1 November 2013). Raw data used for the analyses described below can be found in Table S1. We included the same four climatic features in our future climate projections as we used to describe current climate, extreme temperatures of the warmest and coldest months (FT_{min}^o and FT_{max}^o, respectively) and precipitation extremes for the driest and wettest months (FPPT_{min} and FPPT_{max}, respectively).

To project the currently occupied climatic habitat of *E. coccineum* onto the map, under future (CCM3) conditions, we used our current climatic PCA loadings (*a* coefficients, Table S2, available as Supplementary Material for this paper, Table 2) and CCM3 climatic features to compute future climate PCA scores,

$$\begin{aligned} \text{PCA} - \text{FC1} &= (a_{11} \times \text{FPPT}_{\min} + a_{12} \times \text{FPPT}_{\max} \\ &\quad + a_{13} \times \text{FT}_{\min}^{\circ} + a_{14} \times \text{FT}_{\max}^{\circ}), \text{ and} \\ \text{PCA} - \text{FC2} &= (a_{21} \times \text{FPPT}_{\min} + a_{22} \times \text{FPPT}_{\max} \\ &\quad + a_{23} \times \text{FT}_{\min}^{\circ} + a_{24} \times \text{FT}_{\max}^{\circ}), \end{aligned} \quad (1)$$

for each of the 34 sampled locations. Using inverse distance weighted interpolation (IDW) in ArcGis 9.3 (ESRI 2008), we interpolated those future climate PCA scores smoothly across the map. To illustrate how the climatic map would shift over time, we plotted the PCA-1 and PCA-2 scores for both current

and future climates on the Patagonian map, and compared the two.

Projecting morphology and genetics onto a CCM3 map of Patagonia

So as to map morphology onto the CCM3-predicted climatic landscape, we correlated future climate and biotic factors via CCA translation. We used the future climate PCA scores for the 34 locations to calculate future climate CCA scores, using the current CCA loadings (*b* coefficients, Table S6) for climate, in association with morphology, as follows:

$$\text{CCA} - \text{FC1} = (b_{11} \times \text{PCA} - \text{FC1} + b_{12} \times \text{PCA} - \text{FC2}), \quad (2)$$

and used those future climate CCA scores to compute morphological translations, via

$$\text{CCA} - \text{FM1} = (\rho_{\mu\chi-1}) \times \text{CCA} - \text{FC1}. \quad (3)$$

The CCA correlation value ($\rho_{\mu\chi-1}$) can be found in Table 3. We then used the 34 pairs of CCA–FM1, along with IDW interpolation, to provide continuous morphological maps across the CCM3 landscape of Patagonia, comparing those with their present time CCA morphological pattern (CCA–PM1), treated in the same fashion.

To project our genetic scores onto a similar CCM3 map, we followed a similar strategy, although with corresponding coefficients for climate, in association with genetics (*c* coefficients, Table S7) for translation, as follows:

$$\text{CCA} - \text{FC1} = (c_{11} \times \text{PCA} - \text{FC1} + c_{12} \times \text{PCA} - \text{FC2}), \quad (4)$$

and used those future climate CCA scores to compute their genetic translations, via

$$\text{CCA} - \text{FG1} = (\rho_{\gamma\chi-1}) \times \text{CCA} - \text{FC1}. \quad (5)$$

The correlation value ($\rho_{\gamma\chi-1}$) can again be found in Table 3. We then used the 34 pairs of CCA–FG1, along with IDW interpolation, to provide continuous maps across the CCM3 landscape of Patagonia, comparing those with their present-time CCA analogues (CCA–PG1).

Results

Eliciting the principal axes of variation

Geography and climate

PCA yielded an essentially two-dimensional representation of geographic location (Table 1; Table S2). The first geographic axis (L₁) declined towards the south and west, consistent with the geographic orientation of the Andes, and the second axis (L₂) declined with increasing elevation. Together, these two axes accounted for 90% of the geo-locational variation among our 34 sampled populations. PCA of the four variables used to describe present climate also yielded a two-dimensional representation that explained 88% of the total variation (Table 1). The first climate axis (C₁) increased as winter temperature and both precipitation measures declined. The second axis (C₂) decreased with increasing summer temperature.

Morphology and genetics

Souto *et al.* (2009) showed that all four leaf traits were strongly intercorrelated, and PCA reduced the quartet to a two-dimensional representation that accounted for 89% of the total variation (Table 1). The first axis (M_1) decreased with increasing leaf size and weight, but increased with increasing specific leaf area. The second axis (M_2) increased as the leaves became rounder, and decreased as they became more lanceolate. The variation pattern for the genetic analysis was more complex, given that the 16 loci are thought to segregate independently, and variation should be more than two-dimensional. After extensive preliminary analyses (not shown), we chose to retain six genetic PC axes (G_1 – G_6), each with an eigenvalue exceeding 1.0, each accounting for more than 10% of the variation, and collectively yielding 67% of the total. The remaining 11 axes accounted for <10% of the variation each, and did not change our conclusions (results not shown); they have not been pursued further. The loci

Table 1. Principal component analysis principal axes retained for further analysis, with eigenvalues and cumulative percentages of total variation accounted for, within each of four datasets, in 34 populations L1 and L2, geo-location axes; C1 and C2, climatic axes; M1 and M2, morphology axes; and G1–G6, genetic axes

Principal axis	Eigenvalue (variance)	Cumulative percentage
Geo-location		
L1	1.911	63.69
L2	0.788	89.96
Climate		
C1	2.250	56.27
C2	1.284	88.36
Genetic		
G1	2.780	17.38
G2	2.034	30.09
G3	1.728	40.89
G4	1.537	50.49
G5	1.454	59.58
G6	1.124	66.60
Morphology		
M1	2.369	59.22
M2	1.205	89.34

with the heaviest loadings (≤ -0.55 or $\geq +0.55$; Table S2) are those that showed the most inter-population divergence (Table 1); so, removing the others was of no inferential consequence.

Constructing correlated pattern gradients with CCA

The cross-set correlations in Table 2 collectively imply strong biotic–abiotic associations across the range of *E. coccineum*, the targets of interest here. To optimise those cross-set mappings, we conducted a CCA for each pair of feature sets, reducing each cross-set comparison to a pair of canonical (orthogonal) contrasts, enumerated in Table 3.

Abiotic associations

The correlation between the respective 1st canonical axes for geographic location (λ_1) and climate (χ_1) was large and significant ($\rho_{\lambda\chi-1} = 0.88$; $P < 0.001$). The respective 2nd CC axes (λ_2 and χ_2) were minimally and non-significantly correlated ($\rho_{\lambda\chi-2} = 0.05$; $P > 0.81$). Over the Patagonian range of *E. coccineum*, the mapping of climate onto geography was approximately linear and one-dimensional, reflecting the dominant climatic pattern of the region, a decrease in temperature and an increase in precipitation as one moves southward.

Biotic associations

Biotic divergence among populations of *E. coccineum*, measured in terms of leaf morphology and genotype, also exhibited substantial association. The respective 1st canonical axes for leaf morphology (μ_1) and genotype (γ_1) were strongly correlated and statistically significant ($\rho_{\mu\gamma-1} = 0.70$; $P < 0.005$). The respective 2nd canonical axes (μ_2 and γ_2) were modestly correlated, although not quite significant ($\rho_{\mu\gamma-2} = 0.55$; $P < 0.065$); the morpho-genetic results exhibited some cross-population ‘synchronised pattern’; however, leaf morphology and genotype are not rigidly coupled across geo-climatic space.

Mapping morphological pattern onto geography and climate

For morphology and geography, the respective 1st CC axes (μ_1 and λ_1) were strongly correlated ($\rho_{\mu\lambda-1} = 0.88$; $P < 0.001$;

Table 2. Cross-variable contrasts of principal component axes of *Embothrium coccineum*

Covariances are below the diagonal, variances along the diagonal and correlations above the diagonal. C1 and C2, the 1st and 2nd climatic axes; L1 and L2, the 1st and 2nd geographic axes; M1 and M2, the 1st and 2nd morphological axes; and G1–G6, the 1st–6th genetic axes

	C1	C2	L1	L2	M1	M2	G1	G2	G3	G4	G5	G6
C1	2.319	0	0.227	−0.323	0.509	0.409	0.135	0.000	0.370	−0.283	−0.258	0.163
C2	0	1.322	−0.532	0.582	−0.731	0.475	0.332	0.371	−0.389	−0.091	0.099	−0.023
L1	0.486	−0.858	1.968	0	0.560	−0.364	−0.125	−0.313	0.277	−0.222	−0.369	−0.115
L2	−0.443	0.603	0	0.812	−0.572	0.130	0.296	0.044	−0.485	−0.164	−0.126	−0.044
M1	1.212	−1.314	1.228	−0.805	2.441	0	−0.111	−0.241	0.475	0.058	−0.345	0.246
M2	0.694	0.609	−0.569	0.131	0	1.241	0.495	0.127	0.059	0.079	−0.109	0.169
G1	0.347	0.647	−0.297	0.451	−0.293	0.933	2.865	0	0	0	0	0
G2	−0.001	0.618	−0.636	0.058	−0.546	0.205	0	2.095	0	0	0	0
G3	0.752	−0.596	0.519	−0.583	0.991	0.088	0	0	1.780	0	0	0
G4	−0.543	−0.132	−0.391	−0.186	0.114	0.111	0	0	0	1.583	0	0
G5	−0.480	0.139	−0.634	−0.139	−0.660	−0.148	0	0	0	0	1.498	0
G6	0.267	−0.028	−0.173	−0.043	0.413	0.202	0	0	0	0	0	1.158

Fig. 2a), whereas the respective 2nd axes (μ_2 and λ_2) showed almost no association ($\rho_{\mu\lambda-2}=0.15$; $P=0.39$; Table 3). Morphology was also associated with climate; the respective 1st CC axes (μ_1 and χ_1) were again highly correlated ($\rho_{\mu\chi-1}=0.92$; $P<0.001$; Fig. 2b), whereas the respective 2nd axes (μ_2 and χ_2) were more modestly correlated ($\rho_{\mu\chi-2}=0.59$; $P<0.001$; Table 3), suggesting that both precipitation and temperature have an impact on leaf size and shape. Together, these results suggest that leaf morphology is slightly more associated with climate than with geography, but that those mappings are tightly associated. Basically, climatic divergence

among the 34 locations was highly correlated with their geographic separation, and leaf morphology varies accordingly.

Mapping genotypes onto geography and climate

When paired with geographic location (λ_1 and λ_2), genotypes were substantially and convincingly correlated for their first axes ($\rho_{\gamma\lambda-1}=0.69$; $P=0.006$; Fig. 2c) and less so for their second axes ($\rho_{\gamma\lambda-2}=0.53$; $P=0.094$; Table 3). The respective 1st CC axes for genotypes and climate (γ_1 and χ_1) showed virtually the same pattern, ($\rho_{\gamma\chi-1}=0.69$; $P=0.007$; Fig. 2d), and again their respective 2nd CC axes (γ_2 and χ_2) were modestly and non-significantly correlated ($\rho_{\gamma\chi-2}=0.51$; $P=0.125$; Table 3). The association of genotypes with geography and climate are entirely credible, essentially equivalent and largely confounded.

Table 3. Canonical axis correlation values for pairs of feature sets

λ_1 and λ_2 , geographic axes; χ_1 and χ_2 , climatic axes; μ_1 and μ_2 , morphology axes; γ_1 and γ_2 , genetic axes. CCA, canonical correlation analysis. n.s. = not statistically significant; ** $P<0.05$; *** $P<0.001$

CCA axis	Geography climate morphology genetic							
	λ_1	λ_2	χ_1	χ_2	μ_1	μ_2	γ_1	γ_2
λ_1	–							
λ_2	0	–						
χ_1	0.88***	0	–					
χ_2	0	0.05 ^{n.s.}	0	–				
μ_1	0.88***	0	0.92***	0	–			
μ_2	0	0.15 ^{n.s.}	0	0.59***	0	–		
γ_1	0.69***	0	0.69***	0	0.70**	0	–	
γ_2	0	0.53 ^{n.s.}	0	0.51 ^{n.s.}	0	0.55 ^{n.s.}	0	–

Assembling the future association puzzle

We mapped present PCA–PC1 and PCA–PC2, as well as future PCA–FC1 and PCA–FC2 (Fig. 3), to illustrate the projected shift in climate under CCM3 projections. In the southern portion of *E. coccineum* range, our results suggested that 1st axis climatic scores would increase under CCM3 conditions, suggesting decreasing winter temperature and both winter and summer precipitation measures. This is represented by an expansion of the pink zone in this portion of the range (Fig. 3b), relative to that at present (Fig. 3a). The climate in the central portion of Patagonia is not projected to change much under CCM3 conditions, but in the north-western part of the range, the 1st

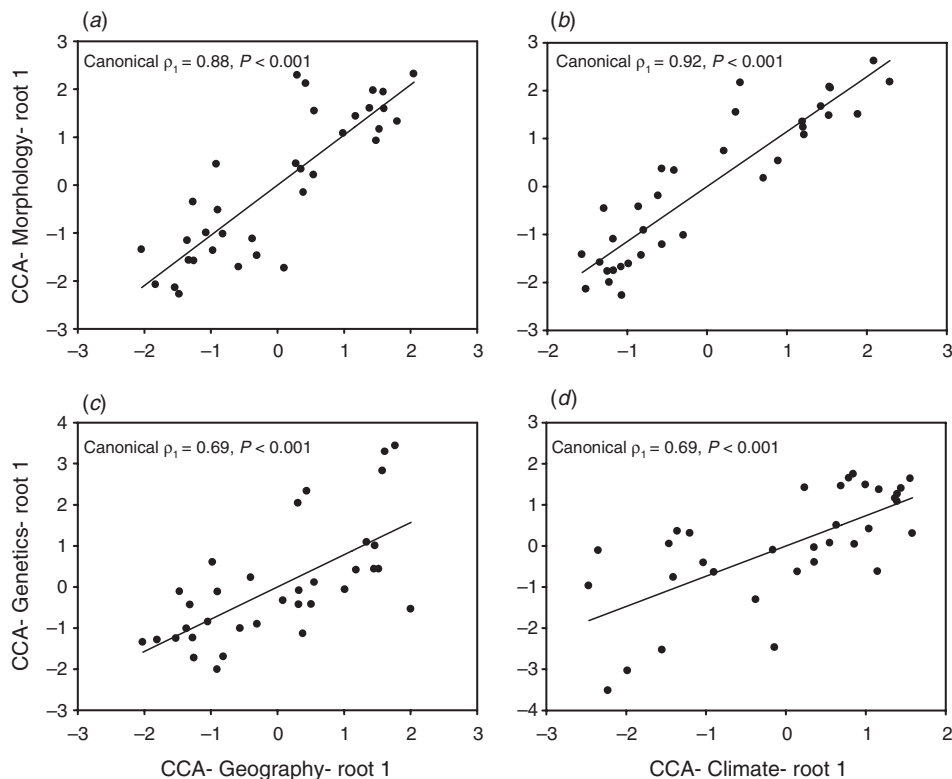


Fig. 2. Canonical correlations, 1st axis plots: (a) morphology vs geography, (b) morphology vs climate; (c) genetics vs geography and (d) genetics vs climate, for 34 locations of *Embothrium coccineum*.

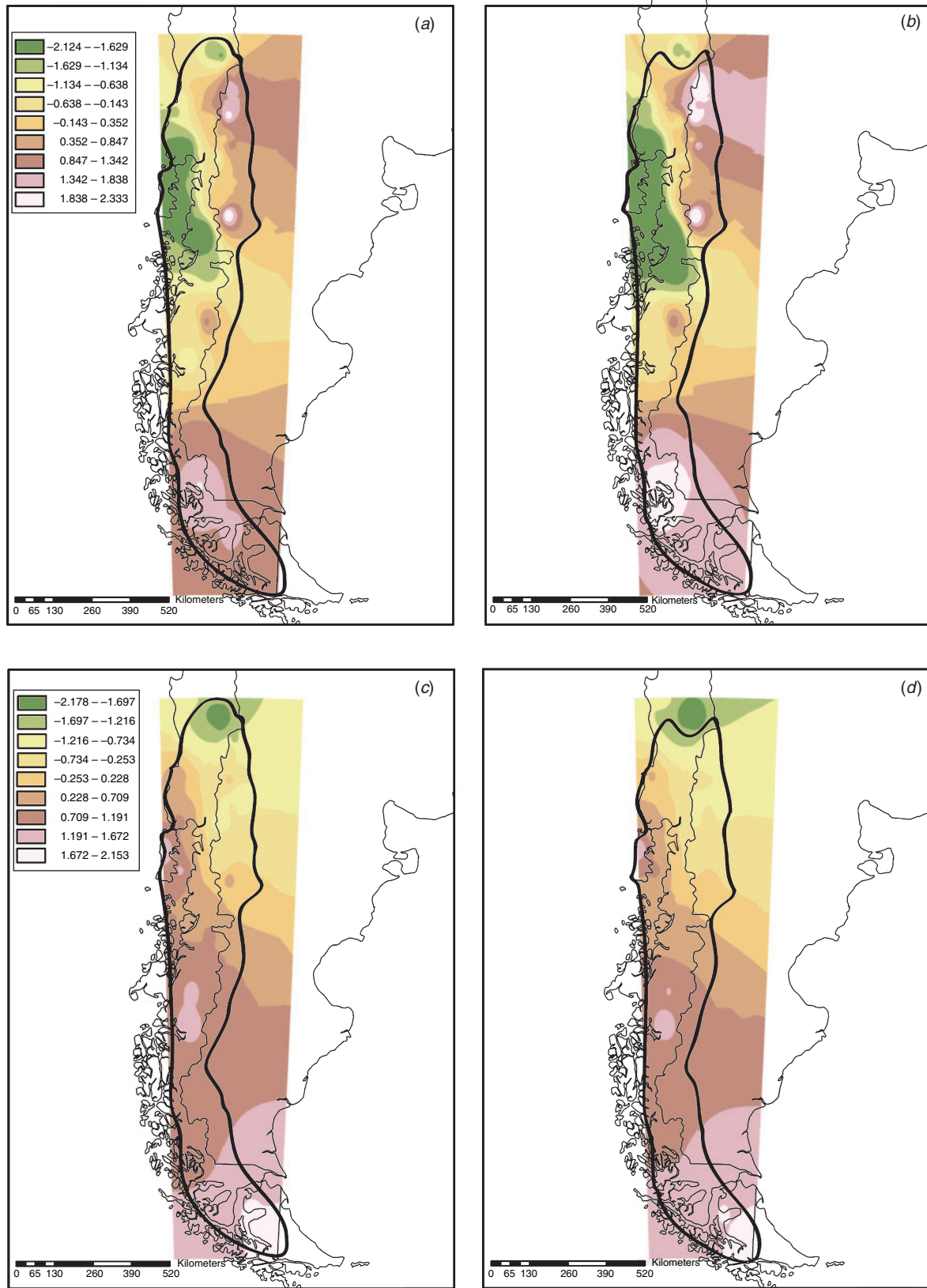


Fig. 3. Mapped 1st and 2nd principal component analysis (PCA) axes, using weighted inverse distance interpolation. (a) Present climate (PCA-PC1), (b) future climate (PCA-FC1), (c) present climate (PCA-PC2) and (d) future climate (PCA-FC2). Lines in (a) and (c) represent the current range of *Embothrium coccineum* (modified from Escobar *et al.* 2006) and those in (b) and (d) represent the projected range change, considering climate shift.

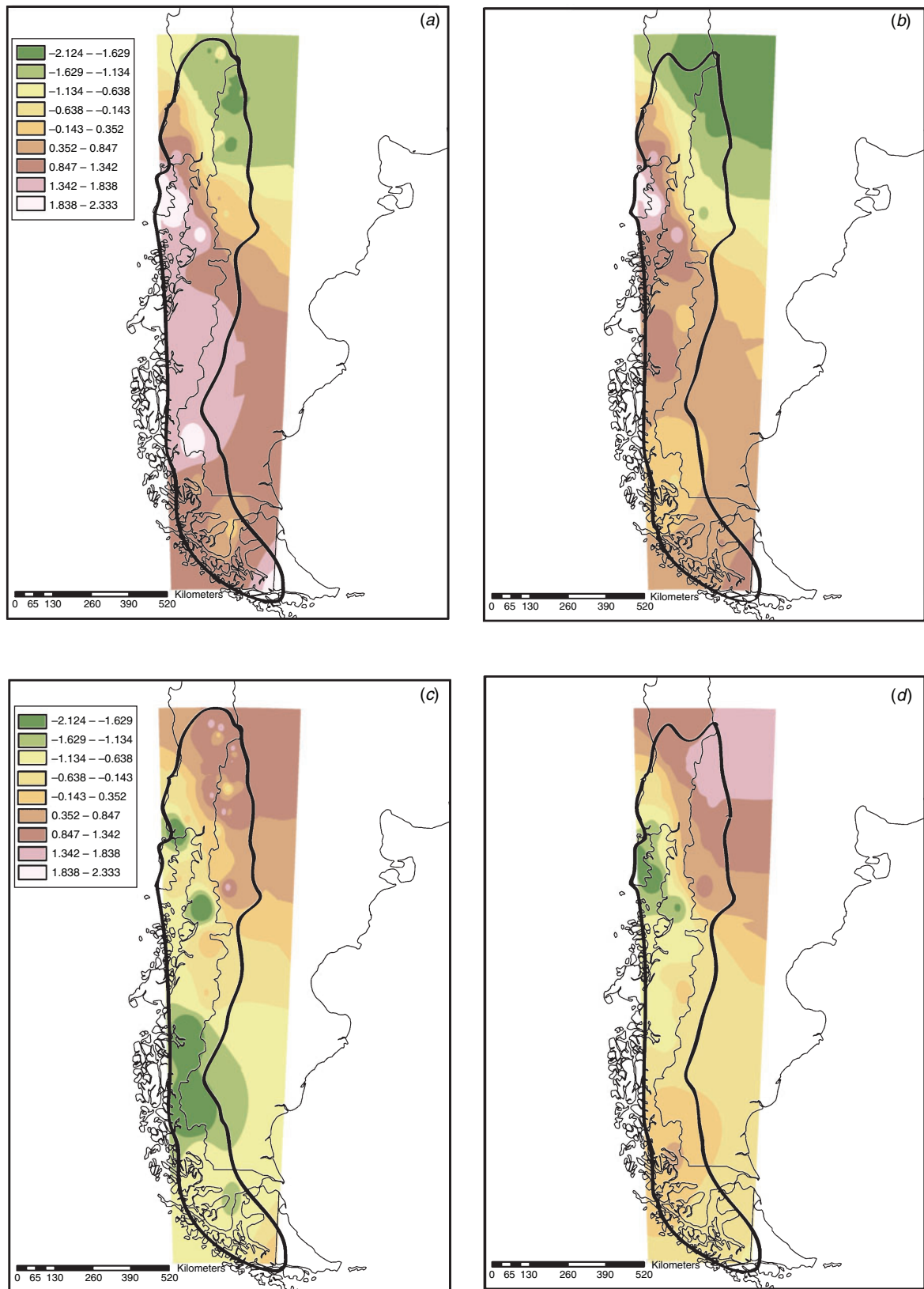


Fig. 4. Mapped 1st canonical axes, using weighted inverse distance interpolation. (a) Present climate vs morphology; (b) future climate vs morphology; (c) present climate vs genetic and (d) future climate vs genetic. Lines in (a) and (c) represent the current range of *Embothrium coccineum* (modified from Escobar *et al.* 2006) and those in (b) and (d) represent the projected range change, considering climate shift.

axis climatic scores would decrease, suggesting increasing winter temperature and both summer and winter precipitation, favouring expansion of more suitable climatic conditions for *E. coccineum*. This is shown by an increase in the area of the green zone in north-western Patagonia under CCM3 conditions (Fig. 3*b*). In north-eastern Patagonia, on the other side of the Andes, the 1st axis climatic scores would increase under CCM3 conditions, suggesting decreasing winter temperature and both winter and summer precipitation measures, represented by an expansion of the pink zone in this portion of the range. The 2nd axis, PCA-PC2 (Fig. 3*c*) is mainly determined by summer temperature, decreasing with increasing T_{\max}° ($r = -0.96$). When compared with CCM3 projected scenario, the only evident change is at the northern tip of the range, reflected in an enlargement of the green area, probably excluding *E. coccineum* from this portion of its present range because of the seriously elevated summer temperatures (average $FT_{\max}^{\circ} = 25.6^{\circ}\text{C}$, for this portion of the range) that are beyond the tolerance range of *Embothrium* ($T_{\max}^{\circ} = 13.5\text{--}25.1^{\circ}\text{C}$; Fig. 3*d*).

We have projected morphological and genetic patterns onto that same Patagonian map, under CCM3 conditions. Assuming that the mappings of morphology and genotype onto climate do not change much in the near future, the geographic patterns of both should shift slightly across the map under CCM3 conditions. For morphology, the projected CCM3 map (Fig. 4*b*) exhibits an increase in the green area, relative to the current map (Fig. 4*a*), reflecting an increase in summer temperatures, translating as leaves with reduced dry mass (DM) and leaf area, but with higher specific leaf area (logSLA) under CCM3 conditions. In the central and southern parts of the range, the pink areas in (Fig. 4*a*) will turn to brown, and brown will turn into orange under CCM3 conditions (Fig. 4*b*), translating as slightly smaller, lighter and thicker leaves.

For genotypes, the brown area in the north-eastern part of the range (Fig. 4*c*) is projected to turn to pink (Fig. 4*d*), reflecting increasing summer temperature under CCM3 conditions, and translating as a corresponding increase in the frequencies of Mnr-12 and Skdh-2 and a decrease in Idh-2. In the north-western portion of the range, the green area becomes larger (Fig. 4*d*) than at the present (Fig. 4*c*), reflecting a small reduction in FT_{\max}° , and translating into a probable decrease in Mnr-12 and Skdh-2 and an increase in Skdh-2. Toward the southern end of the range, present green areas (Fig. 4*c*) should turn to orange (Fig. 4*d*), reflecting an increase in summer temperature, and translating into probable increases in the frequencies of Mnr-12 and Skdh-2 and a decrease in Idh-2.

Discussion

Geographic separation and climate profiles separately predict morphological and genetic structure among *E. coccineum* populations today, but given the strong association between geography and climate in this region (Table 2), separating geographic from climatic causal inference remains difficult. The reality is that geography determines climate in causal fashion; they are not just statistically confounded. Moreover, palaeoenvironmental reconstruction suggests that temporal heterogeneity of Patagonian climate has been of minor

amplitude for the past 21 000 years (Markgraf *et al.* 1995). The climatic setting has been relatively more stable than that for the northern hemisphere, and could easily have allowed at least local persistence of cold-tolerant taxa, buffering them from extreme climatic shifts over that extended time span.

Climatically associated morphological pattern is usually interpreted in terms of adaptive radiation within the species, drawing on accumulated experience with common-garden work on many plant species (Clausen *et al.* 1940; and many later publications). Leaf traits, in particular, can affect productivity and competitive ability (Reich *et al.* 1998; Westoby 1998). Conversely, geographically associated genetic pattern is usually ascribed to sheer geographic separation or 'isolation by distance' (Wright 1943), particularly for markers (such as allozymes), often viewed as adaptively neutral until proven otherwise (Watterson 1978). Beyond reproductive isolation *per se*, however, the relationship between genetic traits and environmental variables has also been recognised as responsive to natural selection (Endler 1986; Mitton 1995; Manel *et al.* 2010).

Partitioning the impact of geographic and climatic predictors on the biotic structure will have to await further studies, including extensive common-garden trials. It seems clear that *E. coccineum* exhibits a coordinated biotic (morphological and genetic) pattern across its range, which is highly predictable from and also highly coordinated with abiotic (geographic and climatic) patterns. This biotic-abiotic correspondence is a common pattern for many species whose ranges span similar geographic and climatic gradients (Webb 1968; Dilcher 1973; Givnish 1979; Dolph and Dilcher 1980; Wilf *et al.* 1998), and pending further resolution into separate adaptive and plastic responses, our statistical approach allows joint analysis of the association between highly intercorrelated biotic and abiotic patterns.

Climatologically predictive biogeography

Geography will determine future climate, just as it has determined the current climate, but the geographic locations of any particular climatic combination will change. The ordination (PCA) results showed that climate in Patagonia can be represented along two axes, depicting temperature and precipitation ranges for *E. coccineum*. This result helps elucidate future climatic range for the species. Particularly in the northern portion of the range, temperature is expected to exceed the species tolerance range, probably excluding it from this region. Our climate projections indicated that the regime that occurs now near the biological centre of the species range in the Valdivian rainforest may expand slightly. This result is in agreement with the projected climatic conditions of the region (CONAMA 2007). Such climate conditions are currently associated with larger, heavier, elongated and relatively thinner leaves, along with high frequencies of the common alleles at the Mnr-12 and Percat-12 loci, but low frequencies of the common allele Mdh-22. These characteristics might become more common and more widespread, to the extent that climate predicts both morphological and genetic patterns, indicating a slightly broader area over which *E. coccineum* should thrive. In the other portions of the species range, winter temperature and both summer and winter

precipitation would decrease, relative to current conditions, but would remain within the species tolerance range. Our CCA results indicated that a single axis of variation describes most of the biotic–abiotic associations for *E. coccineum*. So, that increasing summer temperatures will drive future range shifts of the species, meanwhile resulting in smaller, lighter and thinner leaves and an increase in particular allele frequencies.

Although our results suggest that sufficient variation exists among populations of *E. coccineum* to accommodate CCM3 conditions, this does not imply that processes such as plasticity and local adaptation will not be important for climatically relevant traits. It does suggest that the species will have a future climatic niche to exploit, and over a broad geographic range. Because the species is highly variable, widespread and ecologically versatile, it should be able to cope well with the predicted changes in climate. It is also outcross-pollinated and an early successional coloniser, and its propagules can and do move. Its range will probably shift a bit, perhaps shrinking in the north, but it should persist.

Our results also suggest that if sufficient variation exists among populations of any widespread, ecologically versatile species, it should be able to cope well with climatic shifts. Attention then shifts to the question of whether the species can move fast enough to occupy its appropriate niche, via normal propagule dispersal. *Embothrium*, a widespread early successional coloniser, does not have to move far to accommodate CCM3 conditions, and its propagules should be able to move fast enough to accommodate the expected pace of spatial climate shift. Its range will shift with climate change, but the species should persist.

In more general terms, our analytical approach both allows and profits from joint analysis of the association between highly intercorrelated biotic and abiotic patterns, advancing the cause of climatologically predictive biogeography. The novelty of this approach is to combine multiple datasets for an integrated geo-climatic analysis of biotic patterns across an extensive region, yielding an interpretable two-dimensional geographic map. It would be enlightening to see similar approaches applied to species whose probable responses to climate shift are less clear or rather different. Other key elements of the southern hemisphere forests, for example, members of the Nothofagaceae, Mirthaceae, and Podocarpaceae, would be worth pursuing in this same vein.

Conclusions

The lineage that has led to *E. coccineum* is thought to have evolved during the Eocene, 35.4–44.5 million years ago (Sauquet *et al.* 2009). The Proteaceae, along with other dominant and widespread woody lineages in the southern hemisphere, such as the Podocarpaceae and Nothofagaceae, have survived more than 20 climate shifts and palaeogeographical modifications of the landscape since the Oligocene (Hill 2004; Mathiasen and Premoli 2010). *Embothrium* has had a long history of adaptation, and the available morphological and genetic variation within the species would seem to be compatible with future climatic scenarios for the region. Although geographically shifted somewhat, the species should remain a keystone element of this region of South America.

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References

- Blanchet FG, Legendre P, Borcard D (2008) Forward selection of explanatory variables. *Ecology* **89**, 2623–2632. doi:10.1890/07-0986.1
- Britten HB, Brussard PF, Murphy DD, Ehrlich PR (1995) A test for isolation-by-distance in central Rocky Mountain and Great Basin populations of Edith's checkerspot butterfly (*Euphydryas editha*). *The Journal of Heredity* **86**, 204–210.
- Broughton RE, Harrison RG (2003) Nuclear gene genealogies reveal historical, demographic and selective factors associated with speciation in field crickets. *Genetics* **163**, 1389–1401.
- Canale CI, Henry PI (2010) Adaptive phenotypic plasticity and resilience of vertebrates to increasing climatic unpredictability. *Climate Research* **43**, 135–147. doi:10.3354/cr00897
- Clausen J, Keck DD, Hiesey WM (1940) 'Experimental studies on the nature of species. I. The effect of varied environments on western North American plants.' Publication no. 520. (Carnegie Institution: Washington, DC)
- CONAMA (2007) Estudio de la variabilidad climática en Chile para el siglo XXI. Available at http://www.dgf.uchile.cl/PRECIS/articles-39442_pdf_Informe_figuras.pdf [Verified 1 November 2013]
- Daniels LD, Veblen TT (2000) ENSO effects on temperature and precipitation of the Patagonian-Andean region: implications for biogeography. *Physical Geography* **21**, 223–243.
- Davis MB, Shaw RG (2001) Range shifts and adaptive responses to quaternary climate change. *Science* **292**, 673–679. doi:10.1126/science.292.5517.673
- DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution* **13**, 77–81. doi:10.1016/S0169-5347(97)01274-3
- Dilcher DL (1973) A paleoclimatic interpretation of the Eocene floras of southeastern North America. In 'Vegetation and vegetational history of northern Latin America'. (Ed. A. Graham) pp. 39–59. (Elsevier Scientific Publishing Company: Amsterdam)
- Dolph GE, Dilcher DL (1980) Variation in leaf size with respect to climate in the tropics of the western hemisphere. *Bulletin of the Torrey Botanical Club* **107**, 154–162. doi:10.2307/2484220
- Endler JA (1986) 'Natural selection in the wild.' (Princeton University Press: Princeton, NJ)
- Epperson BK (1993) Recent advances in correlation studies of spatial patterns of genetic variation. *Evolutionary Biology* **27**, 95–155. doi:10.1007/978-1-4615-2878-4_4
- Escobar B, Donoso C, Souto C, Alberdi M, Zúñiga A (2006) *Embothrium coccineum*. In 'Las especies arbóreas de los bosques templados de Chile y Argentina: autoecología'. (Ed. C. Donoso) pp. 233–245. (Marisa Cuneo Ediciones: Valdivia, Chile)
- ESRI (2008) ArcGIS Version 9.3. Environmental Systems Research Institute, Redlands, CA.
- Gaggiotti OE, Foll M (2010) Quantifying population structure with the F-model. *Molecular Ecology Resources* **10**, 821–830. doi:10.1111/j.1755-0998.2010.02873.x
- Galloway LF (2005) Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytologist* **166**, 93–100. doi:10.1111/j.1469-8137.2004.01314.x

- Gienapp P, Teplitsky C, Alho JS, Mills JA, Merilä J (2008) Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology* **17**, 167–178. doi:10.1111/j.1365-294X.2007.03413.x
- Givnish TJ (1979) On the adaptive significance of leaf form. In 'Topics in plant population biology'. (Eds OT Solbrig, S Jain, GB Johnsonand, PH Raven) pp. 375–407. (Columbia University Press: New York)
- Govindasamy B, Caldeira K, Duffy PB (2003) Geoengineering Earth's radiation balance to mitigate climate change from a quadrupling of CO₂. *Global and Planetary Change* **37**, 157–168. doi:10.1016/S0921-8181(02)00195-9
- Hamrick JL, Nason JD (1996) Consequences of dispersal in plants. In 'Population dynamics in ecological space and time'. (Eds OE Rhodes, RK Chesser, MH Smith) pp. 203–236. (University of Chicago: Chicago, IL)
- Hardy J, Vanderhoeven S, Meerts P, Vekemans X (2000) Spatial autocorrelation of allozyme and quantitative markers within a natural population of *Centaurea jacea* (Asteraceae). *Journal of Evolutionary Biology* **13**, 656–667. doi:10.1046/j.1420-9101.2000.00199.x
- Hijmans RJ, Guarino L, Cruz M, Rojas E (2001) Computer tools for spatial analysis of plant genetic resources data: 1. DIVA-GIS. *Plant Genetic Resources Newsletter* **127**, 15–19.
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**, 1965–1978. doi:10.1002/joc.1276
- Hill RS (2004) Origins of the southeastern Australian vegetation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **359**, 1537–1549.
- Holt RD (1990) The microevolutionary consequences of climate change. *Trends in Ecology & Evolution* **5**, 311–315. doi:10.1016/0169-5347(90)90088-U
- Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K, Johnson CA (2001) 'Climate change 2001: the scientific basis.' Contribution of Working Group I to the third assessment report of the intergovernmental panel on climate change. (Cambridge University Press: Cambridge, UK)
- Legendre P, Legendre L. (1998) 'Numerical ecology.' Elsevier.
- Linhart YB, Grant MRC (1996) Evolutionary significance of local genetic differentiation. *Annual Review of Ecology and Systematics* **27**, 237–277. doi:10.1146/annurev.ecolsys.27.1.237
- Maddox GD, Antonovics J (1983) Experimental ecological genetics in *Plantago*: a structural equation approach to fitness components in *P. aristata* and *P. patagonica*. *Ecology* **64**, 1092–1099. doi:10.2307/1937819
- Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution* **15**, 290–295.
- Manel S, Joost S, Epperson BK (2010) Perspectives on the use of landscape genetics to detect genetic adaptive variation in the field. *Molecular Ecology* **19**, 3760–3772. doi:10.1111/j.1365-294X.2010.04717.x
- Markgraf V, McGlone M, Hope G (1995) Neogene paleoenvironmental and paleoclimatic change in southern temperate ecosystems – a southern perspective. *Trends in Ecology & Evolution* **10**, 143–147. doi:10.1016/S0169-5347(00)89023-0
- Mathiasen P, Premoli AC (2010) Out in the cold: genetic variation of *Nothofagus pumilio* (Nothofagaceae) provides evidence for latitudinally distinct evolutionary histories in austral South America. *Molecular Ecology* **19**, 371–385. doi:10.1111/j.1365-294X.2009.04456.x
- Mitton JB (1995) Genetics and the physiological ecology of conifers. In 'Ecophysiology of coniferous forests'. (Ed. TM Hinckley) pp. 1–36. (Academic Press: New York)
- Montecinos A, Aceituno P (2003) Seasonality of the ENSO related rainfall variability in central Chile and associated circulation anomalies. *Journal of Climate* **16**, 281–296. doi:10.1175/1520-0442(2003)016<0281:SOTERR>2.0.CO;2
- Morton NE (1973) Isolation by distance. In 'Genetic structure of populations'. (Ed. NE Morton) pp. 76–79. (University Press of Hawaii: Honolulu, HI)
- Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37–42. doi:10.1038/nature01286
- Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate change and distribution shifts in marine fishes. *Science* **308**, 1912–1915. doi:10.1126/science.1111322
- Pigliucci M, Whitton J, Schlichting CD (1995) Reaction norms of *Arabidopsis*. I. Plasticity of characters and correlations across water, nutrient and light gradients. *Journal of Evolutionary Biology* **8**, 421–438. doi:10.1046/j.1420-9101.1995.8040421.x
- Reich PB, Walters MB, Tjoelker MG, Vanderklein D, Buschena C (1998) Photosynthesis and respiration rates depend on leaf and root morphology and nitrogen concentration in nine boreal tree species differing in relative growth rate. *Functional Ecology* **12**, 395–405. doi:10.1046/j.1365-2435.1998.00209.x
- Sauquet H, Weston PH, Anderson CA, Barker NP, Cantrill DJ, Mast AR, Savolainen V (2009) Contrasted patterns of hyperdiversification in Mediterranean hotspots. *Proceedings of the National Academy of Sciences, USA* **106**, 221–225. doi:10.1073/pnas.0805607106
- Schluter D, Rambaut A (1996) Ecological speciation in postglacial fishes. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **351**, 807–814. [and discussion]
- Smouse PE, Long JC (1992) Matrix correlation analysis in anthropology and genetics. *Yearbook of Physical Anthropology* **35**, 187–213. doi:10.1002/ajpa.1330350608
- Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology* **35**, 627–632. doi:10.2307/2413122
- Sokal RR, Wartenberg DE (1983) A test of spatial autocorrelation analysis using an isolation-by-distance model. *Genetics* **105**, 219–237.
- Sork VL, Stowe KA, Hochwender C (1993) Evidence for local adaptation in closely adjacent subpopulations of northern red oak (*Quercus rubra* L.) expressed as resistance for leaf herbivores. *American Naturalist* **142**, 928–936. doi:10.1086/285581
- Sork VL, Davis FW, Westfall R, Flint A, Ikegami M, Wang H, Grivet D (2010) Gene movement and genetic association with regional climate gradients in California valley oak (*Quercus lobata* Née) in the face of climate change. *Molecular Ecology* **19**, 3806–3823. doi:10.1111/j.1365-294X.2010.04726.x
- Soto A, Robledo-Arnuncio JJ, González-Martínez SC, Smouse PE, Alía R (2010) Climatic niche and neutral genetic diversity of the six Iberian pine species: a retrospective and prospective view. *Molecular Ecology* **19**, 1396–1409. doi:10.1111/j.1365-294X.2010.04571.x
- Souto CP, Premoli AC (2007) Genetic variation in the widespread *Embothrium coccineum* (Proteaceae) endemic to Patagonia: effects of phylogeny and historical events. *Australian Journal of Botany* **55**, 809–817. doi:10.1071/BT06183
- Souto CP, Premoli AC, Reich PB (2009) Complex bioclimatic and soil gradients shape leaf trait variation in *Embothrium coccineum* (Proteaceae) among austral forests in Patagonia. *Revista Chilena de Historia Natural* **82**, 209–222. doi:10.4067/S0716-078X2009000200004
- Telles MPC, Diniz-Filho JAF (2005) Multiple Mantel tests and isolation-by-distance, taking into account long-term historical divergence. *Genetics and Molecular Research* **4**, 742–748.
- Watterson R (1978) The homozygosity test of neutrality. *Genetics* **88**, 405–417.

- Webb LJ (1968) Environment relationships of the structural types of Australian rainforest vegetation. *Ecology* **49**, 296–311. doi:[10.2307/1934459](https://doi.org/10.2307/1934459)
- Westoby M (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil* **199**, 213–227. doi:[10.1023/A:1004327224729](https://doi.org/10.1023/A:1004327224729)
- Wilf P, Wing SL, Greenwood DR, Greenwood CL (1998) Using fossil leaves as paleoprecipitation indicators: an Eocene example. *Geology* **26**, 203–206. doi:[10.1130/0091-7613\(1998\)026<0203:UFLAPI>2.3.CO;2](https://doi.org/10.1130/0091-7613(1998)026<0203:UFLAPI>2.3.CO;2)
- Wilkinson C, Edds D (2001) Spatial pattern and environmental correlates of a midwestern stream fish assemblage: including spatial autocorrelation as a factor in community analyses. *American Midland Naturalist* **146**, 271–289. doi:[10.1674/0003-0031\(2001\)146\[0271:SPAECO\]2.0.CO;2](https://doi.org/10.1674/0003-0031(2001)146[0271:SPAECO]2.0.CO;2)
- Wright S (1943) Isolation by distance. *Genetics* **28**, 114–138.