Woody climbers show greater population genetic differentiation than trees: Insights into the link between ecological traits and diversification

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The climbing habit is a key innovation in plants: climbing taxa have higher species richness than nonclimbing sister groups. We evaluated the hypothesis that climbing plant species show greater among-population genetic differentiation than nonclimber species. We compared the among-population genetic distance in woody climbers (eight species, 30 populations) and trees (seven species, 29 populations) coexisting in nine communities in a temperate rainforest. We also compared within-population genetic diversity in co-occurring woody climbers and trees in two communities. Mean genetic distance between populations of climbers was twice that of trees. Isolation by distance (increase in genetic distance with geographic distance) was greater for climbers. Climbers and trees showed similar within-population genetic diversity. Our longevity estimate suggested that climbers had shorter generation times, while other biological features often associated with diversification (dispersal and pollination syndromes, mating system, size, and metabolic rate) did not show significant differences between groups. We hypothesize that the greater population differentiation in climbers could result from greater evolutionary responses to local selection acting on initially higher within-population genetic diversity, which could be driven by neutral processes associated with shorter generation times. Increased population genetic differentiation could be incorporated as another line of evidence when testing for key innovations.

KEY WORDS: ΨST, evolutionary innovations, genetic diversity, isolation by distance, lianas, population differentiation.
and Hauser 1995; Barraclough et al. 1998). Cases of key innovations that meet the two conditions include phytophagy in insects (Mitter et al. 1988), latex and resin canals in plants (Farrell et al. 1991), four-cusped molars in herbivorous mammals (Hunter and Jernvall 1995), floral nectar spurs in columnines (Hodges 1997), and water-saving CAM photosynthesis in bromeliads (Quezada and Gianoli 2011).

The speciation process embodies a continuum of divergence and population differentiation is one of the first stages of this continuum (Nosil 2012). Genetic differentiation among populations has been considered a suitable surrogate for speciation in comparative studies at ecological time scales (Avise 2000; Levin 2000; Coyne and Orr 2004). In the same vein, ecological divergence is consistently associated with reproductive isolation across plants and animals (Funk 1998; Filchak et al. 2000; Coyne and Orr 2004; Rundle and Nosil 2005; Funk et al. 2006; Peccoud et al. 2009; but see and Rabosky (2016) for a critical discussion of the relationship between reproductive isolation and speciation rates). Several studies have specifically addressed whether patterns of population genetic differentiation are consistent with patterns of diversification. For instance, Martin and McKay (2004) found—controlling for geographic distance—greater genetic divergence in populations at lower latitudes across 60 vertebrate species (including amphibians, birds, fishes, mammals, and reptiles), thus mirroring the reported pattern of greater diversification rates in tropical latitudes (Cardillo 1999; but see Jetz et al. 2012). Likewise, a metaanalysis comprising 409 populations from 45 plant taxa found greater genetic differentiation in populations from lower latitudes after accounting for geographic distance (Eo et al. 2008). Kisel and Barraclough (2010) found that the magnitude of within-species genetic divergence explained up to 76% of the variation in the minimum area required for speciation for a broad range of taxa. On the other hand, Kisel et al. (2012) tested whether five pairs of sister clades of orchids markedly differing in species richness concomitantly differed in the magnitude of population genetic differentiation, but did not find such a correlation; this approach followed the notion that population genetic differentiation could be an emergent species-level trait heritable within clades and influencing diversification rates (Jablonski 2008).

The pattern of genetic differentiation among populations is set at an intermediate scale between the ecological/functional argument (organismal level) and the comparative test (macroevolutionary level). Information on population genetic structure in systems where a key innovation is known may provide insights into the link between organismal traits and diversification rates mediated by population genetic processes. In other words, this approach, focusing on key traits, could enable us to identify population genetic correlates of ecological traits that might play a causal role in mediating patterns of diversification. Furthermore, if this information is supplemented with within-population genetic diversity data, inferences may be stronger because the analysis may account for intrinsic determinants of genetic variability in the group bearing the key trait that are better observed at the local scale. Surprisingly, to our knowledge, this approach has not been undertaken so far.

The climbing habit has arisen multiple times during plant evolution and over one-thirds of all angiosperm families have climber representatives (Gianoli 2015). Using a comparative test, Gianoli (2004) showed that the climbing habit is a key innovation in angiosperms: climbing plant taxa had higher species richness than nonclimbing sister groups in 38 of 48 pairs of sister groups from 45 families. Likewise, epiphytism has been shown to promote species diversification both in orchids and other plant groups (Gravendeel et al. 2004). Ecological arguments for the evolutionary success of climbers include enhanced reproductive or ecological specializations that allow niche subdivision, leading to an increase in the number of species coexisting within a clade (Heard and Hauser 1995). The evidence of broader light niches (Gianoli et al. 2010; Valladares et al. 2011) and more—or more specialized—pollinators interacting with climbers compared to trees (Gentry 1991; Riveros and Smith-Ramírez 1995; Chazdon et al. 2003) suggests that opportunities for specialization (and local differentiation) are greater in climbers.

In the present study, we evaluated the hypothesis that climbing plant species have undergone greater among-population genetic differentiation than nonclimber species. We compared—using AFLP molecular markers—the genetic distance between populations (ΦST) in woody climbers (eight species, 30 populations) and trees (seven species, 29 populations) coexisting in nine forest communities along a ≈ 600 km latitudinal gradient in the temperate rainforest of southern Chile. We evaluated whether there was a phylogenetic bias in our results by estimating the phylogenetic signal (K, Blomberg et al. 2003) of ΦST (mean value) across the 15 species of woody climbers and trees, which are not reciprocally monophyletic. In addition, we compared the within-population genetic diversity in coexisting woody climbers and trees in two forest communities. This was done to gain insights into whether the (expected) pattern of greater population differentiation in climbers could result from a greater response to selection or from neutral processes. The former would predict lower genetic diversity in climbers’ populations, while the latter would predict the opposite, which could result from an inherently greater genetic diversity in climbers at any spatial scale. Likewise, to identify possible explanatory factors we compared the study climbers and trees with regard to biological features that have been associated with genetic differentiation and/or speciation. Specifically, we compared pollination and dispersal syndromes, mating system, height, maximum diameter (as longevity estimate), and metabolic rate (see Loveless and Hamrick 1984; Ricklefs and Renner 1994; Hamrick and Godt 1996; Heilbuth...
### Table 1. Biological features of woody climbers and trees included in the study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Dispersal vector</th>
<th>Pollination vector</th>
<th>Sexual system</th>
<th>Selfing?</th>
<th>Height (m)</th>
<th>DBH (cm)</th>
<th>$A_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Climbers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boquila trifoliolata</td>
<td>Lardizabalaceae</td>
<td>Animals</td>
<td>Insects</td>
<td>Monoecious</td>
<td>—</td>
<td>10</td>
<td>13</td>
<td>6.1</td>
</tr>
<tr>
<td>Cissus striata</td>
<td>Vitaceae</td>
<td>Animals</td>
<td>Insects</td>
<td>Monoecious</td>
<td>—</td>
<td>20</td>
<td>24</td>
<td>9.3</td>
</tr>
<tr>
<td>Elytropus chilensis</td>
<td>Apocynaceae</td>
<td>Wind</td>
<td>Insects</td>
<td>Monoecious</td>
<td>No</td>
<td>3</td>
<td>13</td>
<td>7.1</td>
</tr>
<tr>
<td>Hydrangea serratofolia</td>
<td>Hydrangeaceae</td>
<td>Animals</td>
<td>Insects</td>
<td>Dioecious</td>
<td>No</td>
<td>23</td>
<td>37</td>
<td>5.0</td>
</tr>
<tr>
<td>Lasiocarpa radicans</td>
<td>Alstroemeriaceae</td>
<td>Animals</td>
<td>Insects</td>
<td>Monoecious</td>
<td>No</td>
<td>4</td>
<td>4</td>
<td>4.4</td>
</tr>
<tr>
<td>Mitraria coccinea</td>
<td>Gesneriaceae</td>
<td>Animals</td>
<td>Birds</td>
<td>Monoecious</td>
<td>Yes</td>
<td>6</td>
<td>13</td>
<td>4.2</td>
</tr>
<tr>
<td>Muehlenbeckia hastulata</td>
<td>Polygonaceae</td>
<td>Animals</td>
<td>Insects</td>
<td>Dioecious</td>
<td>—</td>
<td>15</td>
<td>3</td>
<td>8.4</td>
</tr>
<tr>
<td>Sarmienta scandens</td>
<td>Gesneriaceae</td>
<td>Animals</td>
<td>Birds</td>
<td>Monoecious</td>
<td>—</td>
<td>23</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td><strong>Trees</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aextoxicon punctatum</td>
<td>Aextoxicaceae</td>
<td>Animals</td>
<td>Wind</td>
<td>Dioecious</td>
<td>No</td>
<td>30</td>
<td>170</td>
<td>6.5</td>
</tr>
<tr>
<td>Aristotelia chilensis</td>
<td>Elaeocarpaceae</td>
<td>Animals</td>
<td>Insects</td>
<td>Dioecious</td>
<td>No</td>
<td>10</td>
<td>35</td>
<td>11.1</td>
</tr>
<tr>
<td>Eucryphia cordifolia</td>
<td>Cunoniaceae</td>
<td>Animals</td>
<td>Insects</td>
<td>Monoecious</td>
<td>No</td>
<td>40</td>
<td>200</td>
<td>7.5</td>
</tr>
<tr>
<td>Gevuina avellana</td>
<td>Proteaceae</td>
<td>Animals</td>
<td>Insects</td>
<td>Monoecious</td>
<td>No</td>
<td>20</td>
<td>80</td>
<td>10.8</td>
</tr>
<tr>
<td>Luma apiculata</td>
<td>Myrtaceae</td>
<td>Animals</td>
<td>Insects</td>
<td>Monoecious</td>
<td>No</td>
<td>25</td>
<td>50</td>
<td>6.4</td>
</tr>
<tr>
<td>Nothofagus dombei</td>
<td>Nothofagaceae</td>
<td>Wind</td>
<td>Wind</td>
<td>Monoecious</td>
<td>No</td>
<td>50</td>
<td>400</td>
<td>6.1</td>
</tr>
<tr>
<td>Rhaphithamnus spinosus</td>
<td>Verbenaceae</td>
<td>Animals</td>
<td>Birds</td>
<td>Monoecious</td>
<td>Yes</td>
<td>6</td>
<td>25</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Both height and DBH (diameter at breast height) correspond to maximum values reported. $A_{\text{max}}$ = maximum photosynthetic rate ($\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$). —: information not available. Information sources appear in Table S1.

2000; Allen et al. 2006; Duminil et al. 2007, 2009; Lanfear et al. 2013). Our ultimate goal was to address the link between organismal traits and diversification rates mediated by population genetic processes.

### Methods

#### STUDY SITES AND SPECIES

The 15 study species are among the most common climbers and trees in the temperate rainforest of southern Chile (Gianoli et al. 2010; Valladares et al. 2012) and have comparable geographic ranges (Donoso 2006; Marticorena et al. 2010); they are listed in Table 1 together with some biological features. The nine study sites are located in the Coastal and Andean mountain ranges and span a latitudinal gradient of nearly 600 km, with mean annual temperature and mean annual precipitation decreasing and increasing with latitude, respectively (data in Fig. S1). By sampling both climbers and trees in the same study sites we avoided a bias in the comparative analysis due to differences in geographic distance between populations.

#### SAMPLING

Plant material for molecular analysis was collected randomly along forest paths and across the forest interior. All conspecific individuals sampled were at least 10 m apart to avoid sampling the same genotype twice because of vegetative reproduction. Sample size was generally 15–20 individuals per species per population, totaling nearly 1000 individuals. We collected ca. 100 mg of young leaves (fresh weight) from each individual plant. Sample collection took place in 2010 and 2011 during continuous field trips. Samples were dried in bags with silica gel and stored at 4°C until DNA extraction.

#### DNA EXTRACTION AND AFLP ANALYSIS

Total DNA was extracted from dry leaf material following a standard protocol (Doyle 1987) and treated with RNase (30 min at 37°C). AFLP profiles were obtained following the original procedures of Vos et al. (1995) and the modifications described in Hasbún et al. (2012). The details of AFLP analysis and the list of primer combinations chosen for each species are available in the supplementary material.

#### STATISTICAL ANALYSES

AFLP data were analyzed using AMOVA (Excoffier et al. 1992) to estimate the proportion of total variance explained by within- and between-population variation for each group (climbers and trees). To assess pairwise genetic distances between populations we focused on $\Phi_{ST}$, a parameter analogous to $F_{ST}$ (Excoffier et al. 1992) whose significance was determined against the null hypothesis of no structure using GENALEX (version 6.1, Peakall and Smouse 2006). To test whether there was higher genetic differentiation among populations of climbing plant species compared to tree species we used a t-test. To evaluate whether the relationship between genetic distance (among-population...
**Table 2.** Mean genetic distance ($\Phi_{ST}$ = proportion of total genetic variance attributable to differences between populations) and mean geographic distance (km) between populations of woody climbers and trees.

<table>
<thead>
<tr>
<th>Species</th>
<th>Genetic distance</th>
<th>Geographic distance</th>
<th>Number of populations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Climbers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boquila trifoliolata</td>
<td>0.230</td>
<td>300</td>
<td>3</td>
</tr>
<tr>
<td>Cissus striata</td>
<td>0.102</td>
<td>338</td>
<td>4</td>
</tr>
<tr>
<td>Elytropus chilensis</td>
<td>0.095</td>
<td>425</td>
<td>3</td>
</tr>
<tr>
<td>Hydrangea serratifolia</td>
<td>0.084</td>
<td>279</td>
<td>5</td>
</tr>
<tr>
<td>Luzuriaga radicans</td>
<td>0.085</td>
<td>234</td>
<td>4</td>
</tr>
<tr>
<td>Mitraria coccinea</td>
<td>0.132</td>
<td>123</td>
<td>3</td>
</tr>
<tr>
<td>Muehlenbeckia hastulata</td>
<td>0.050</td>
<td>300</td>
<td>3</td>
</tr>
<tr>
<td>Sarmienta scandens</td>
<td>0.144</td>
<td>377</td>
<td>5</td>
</tr>
<tr>
<td>Mean ± se</td>
<td>0.115 ± 0.019</td>
<td>297.0 ± 32.5</td>
<td></td>
</tr>
<tr>
<td><strong>Trees</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aextoxicon punctatum</td>
<td>0.065</td>
<td>315</td>
<td>5</td>
</tr>
<tr>
<td>Aristotelia chilensis</td>
<td>0.037</td>
<td>343</td>
<td>5</td>
</tr>
<tr>
<td>Eucryphia cordifolia</td>
<td>0.120</td>
<td>257</td>
<td>5</td>
</tr>
<tr>
<td>Gevuina avellana</td>
<td>0.051</td>
<td>319</td>
<td>4</td>
</tr>
<tr>
<td>Luma apiculata</td>
<td>0.050</td>
<td>300</td>
<td>3</td>
</tr>
<tr>
<td>Nothofagus dombeyi</td>
<td>0.053</td>
<td>616</td>
<td>2</td>
</tr>
<tr>
<td>Raphithamnus spinosus</td>
<td>0.027</td>
<td>211</td>
<td>5</td>
</tr>
<tr>
<td>Mean ± se</td>
<td>0.058 ± 0.011</td>
<td>337.3 ± 49.4</td>
<td></td>
</tr>
</tbody>
</table>

Regarding within-population genetic diversity, we calculated the average unbiased expected heterozygosity ($H_E$), Shannon information index ($I$), percentage of polymorphic loci ($PPL$), and the effective number of alleles ($N_E$) for each species in each of two communities, Antigua (seven climbers vs. seven trees) and San Martin (seven climbers vs. six trees). We used GENALEX and assumed Hardy–Weinberg equilibrium. We compared genetic diversity in woody climbers and trees using $t$-tests and computed the statistical power of these tests. Finally, depending on the nature of the data, we compared biological features of woody climbers and trees (listed in Table 1) using $t$-tests and proportions tests. We also explored whether any of these features were associated with $\Phi_{ST}$ across species.

**Results**

Primer combinations produced enough polymorphism to quantify genetic diversity for all study species. The number of scored bands ranged from 92 to 157 in climbing plant species, and from 119 to 166 in tree species (Table S2). The percentage of polymorphic loci was nearly identical in woody climbers (58.6%) and trees (58.7%), and the effective number of alleles per locus was also very similar (climbers: 1.25; trees: 1.19) (Table S2).
Mean genetic distance between populations (ΦST) of woody climbers was nearly twice that of trees (0.115 vs. 0.058) and this difference was statistically significant (t13 = 2.471, P = 0.028) (Table 2). Mean geographic distance (km) between pairs of populations was similar for climbers (297.0) and trees (337.2) (t13 = −0.698, P = 0.497) (Table 2). Isolation by distance that is a significant relationship between geographic distance (ln) and linearized pair-wise genetic differentiation, was verified for both woody climbers (r = 0.349, P = 0.002) and trees (r = 0.389, P = 0.001). However, climbing plants showed a steeper slope for this relationship (F1,13 = 8.003, P = 0.006, ANCOVA) (Fig. 1). There was no phylogenetic signal for ΦST across the study species (K = 0.656, P = 0.459; Fig. 2).

No statistical difference in within-population genetic diversity between woody climbers and trees was detected for any of the four parameters evaluated (HE, I, PPL, Nef) in the two forest communities, yet there was a consistent trend for a higher genetic diversity in climber populations (Table 3). The statistical power of the tests was rather low (range: 0.17–0.52) (Table 3), so these results should be taken with caution.

Climbers and trees did not differ in the proportion of species with animals as dispersal vectors (χ² = 0.02, P = 0.876), the prevalence of insect pollination (χ² = 0.54, P = 0.464), or the proportion of monoecious species (χ² = 0.02, P = 0.876) (Table 1). Concerning self-compatibility for the study climbers (Table 1), but we compared the proportion of selfers in woody climbers and trees/shrubs from the temperate rainforest of southern Chile (data from Riveros et al. 1996; Smith-Ramírez et al. 2005) and it was not different (χ² = 0.95, P = 0.33). The difference in maximum height attained (Table 1) (Mean ± SE; climbers = 13.00 ± 2.96 m, trees = 25.86 ± 5.94 m) was marginally nonsignificant (t13 = −2.02, P = 0.196). Maximum diameter at breast height (DBH, Table 1) was greater (t13 = −2.60, P = 0.022) in trees (137.14 ± 50.71 cm) than in climbers (13.75 ± 4.17 cm). Maximum photosynthetic rate (Amax, Table 1) was similar (t13 = −1.37, P = 0.196) in climbers (6.36 ± 0.75 μmol CO₂ m⁻² s⁻¹) and trees (7.87 ± 0.81 μmol CO₂ m⁻² s⁻¹).

ANOVA did not find differences in genetic distance in species with animal-mediated dispersal vs. species with other dispersal vectors (F1,13 < 0.001, P = 0.989), insect-pollinated species versus noninsect-pollinated species (F1,13 = 0.043, P = 0.839), or monoecious versus dioecious species (F1,13 = 0.043, P = 0.839). Likewise, regression analyses showed that ΦST was not associated with maximum height (R² = 0.02, F1,13 = 0.196, P = 0.665), DBH (R² = 0.05, F1,13 = 0.633, P = 0.440) or Amax (R² = 0.12, F1,12 = 1.604, P = 0.229) across species.

**Discussion**

We empirically verified the hypothesis that woody climbers have undergone greater among-population genetic differentiation than trees in the temperate rainforest of southern Chile. Both trees and woody climbers showed isolation by distance, but the increase in among-population genetic distance with geographic distance was greater for climbers. A recent metaanalysis compared published values of genetic differentiation among populations in forests and shrublands of southern South America and also found higher genetic distance in populations from climbing plant species compared to nonclimbing taxa (Gianoli 2015). Given that a macroevolutionary comparative test had previously shown that the climbing habit is linked to increased diversification in angiosperms (Gianoli 2004), our study provides further support to the positive association between genetic differentiation and diversification (Funk 1998; Filchak et al. 2000; Coyne and Orr 2004; Martin and McKay 2004; Rundle and Nosil 2005; Funk et al. 2006; Eo et al. 2008; Peccoud et al. 2009). The next step would be to identify the link between ecological traits (presumably overrepresented in climbers) and diversification rates and/or population genetic correlates. We address this issue below, discussing evidence related to the traits listed in Table 1.

Plant reproductive features that influence population genetic differentiation and/or diversification, such as seed dispersal syndrome, pollination mode, and mating system (Loveless and Hamrick 1984; Hamrick and Godt 1996; Duminil et al. 2007, 2009) were similarly represented in the study climbers and trees. It has been found that woody climbers tend to be more predominantly wind-dispersed than trees or shrubs (Gentry 1991), which
Climbing plants have lower levels of population genetic differentiation compared to trees (Hamrick and Godt 1996), but such a tendency for dispersal mode was not verified in our study species. Plant height has been negatively associated with the magnitude of genetic population differentiation (Duminil et al. 2009) and evolutionary rates (Lanfear et al. 2013). Considering maximum height, we found that trees were taller than climbers, which would be consistent with the general pattern reported by Duminil et al. (2009) since we found lower values of $\Phi_{ST}$ in trees. This difference in height, however, was (marginally) not statistically significant. Moreover, the reported influence of plant stature on genetic structure lost significance once the most explicative variables—related to reproductive features—were incorporated into the general model (Duminil et al. 2009). A model supported by global datasets showed that individual metabolic rates are positively associated with rates of genetic divergence and speciation (Allen et al. 2006). This would lead to expect higher photosynthetic rates in woody climbers as compared to trees; however, we found similar values of $A_{\text{max}}$ in the two groups, a result consistent with earlier studies (Lusk and Del Pozo 2002; Gianoli et al. 2012; Wyka et al. 2013; van der Sande et al. 2016).

**Figure 2.** Phylogenetic relationships among the study species, eight woody climbers (in bold) and seven trees. The relative magnitude of species’ mean genetic distance between populations ($\Phi_{ST}$) is represented by dot size. Timescale is in millions of years before present.

The magnitude of $\Phi_{ST}$ was not associated with particular clades, that is, there was no phylogenetic signal for $\Phi_{ST}$.

**Table 3.** Compared within-population genetic diversity in woody climbers and trees from two temperate rainforest communities (Anticura and San Martin).

<table>
<thead>
<tr>
<th></th>
<th>Climbers</th>
<th>Trees</th>
<th>$t$-value</th>
<th>$P$</th>
<th>$1 - \beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anticura</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N_E$</td>
<td>1.256 ± 0.031</td>
<td>1.207 ± 0.037</td>
<td>-1.02</td>
<td>0.33</td>
<td>0.25</td>
</tr>
<tr>
<td>$H_E$</td>
<td>0.172 ± 0.019</td>
<td>0.141 ± 0.025</td>
<td>-0.97</td>
<td>0.35</td>
<td>0.23</td>
</tr>
<tr>
<td>$I$</td>
<td>0.252 ± 0.027</td>
<td>0.213 ± 0.036</td>
<td>-0.88</td>
<td>0.40</td>
<td>0.22</td>
</tr>
<tr>
<td>$PPL$</td>
<td>59.32 ± 5.985</td>
<td>52.41 ± 7.675</td>
<td>-0.71</td>
<td>0.49</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>San Martin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N_E$</td>
<td>1.255 ± 0.033</td>
<td>1.197 ± 0.018</td>
<td>-1.48</td>
<td>0.17</td>
<td>0.40</td>
</tr>
<tr>
<td>$H_E$</td>
<td>0.175 ± 0.019</td>
<td>0.135 ± 0.011</td>
<td>-1.72</td>
<td>0.11</td>
<td>0.52</td>
</tr>
<tr>
<td>$I$</td>
<td>0.255 ± 0.025</td>
<td>0.209 ± 0.016</td>
<td>-1.50</td>
<td>0.16</td>
<td>0.36</td>
</tr>
<tr>
<td>$PPL$</td>
<td>61.17 ± 4.647</td>
<td>56.66 ± 3.516</td>
<td>-0.74</td>
<td>0.47</td>
<td>0.17</td>
</tr>
</tbody>
</table>

$N_E$, effective number of alleles per locus; $H_E$, unbiased expected heterozygosity; $I$, Shannon information index; $PPL$, percentage of polymorphic loci. $1 - \beta = \text{power of the test (for } \alpha = 0.05; H_0: \mu_{[\text{climbers}]} \leq \mu_{[\text{trees}]}).$ Means ± standard errors are shown.
Consequently, the above traits are hardly linked to the increased genetic differentiation found in climbing plants. Other factors known to affect the extent of population differentiation, such as perenniality or woodiness (Duminil et al. 2009), latitudinal distribution (Eno et al. 2008), geographic range (Hamrick and Godt 1996), and geographic distance between populations (Slatkin 1993) were shared by the two groups due to our sampling strategy. Finally, none of these biological features showed a significant association with $\Phi_{ST}$ across species, which further suggests that these features do not play a role in the observed patterns of differentiation.

A plant trait that could be involved in the observed patterns is generation time, which has been negatively associated with evolutionary rates (Verdú 2002; Smith and Donoghue 2008) and population differentiation (Nyborg 2004; Duminil et al. 2009). Generation time has been invoked to explain the contrasting magnitude of genetic differentiation of seeder and resprouter populations of a fynbos shrub (Segarra-Moragues and Ojeda 2010). Accordingly, if climbing plant species have shorter generation times—and thus faster population turnover—than tree species, they would produce new alleles at a higher rate and then should show higher levels of genetic variation than trees, both at the among-population and within-population scales (Segarra-Moragues and Ojeda 2010). We lack information on the generation time of the study species, but it seems to vary significantly within each group. Thus, there are from shrub-like trees (Rhaphithammus spinosus and Aristotelia chilensis; Donoso 2006) to long-lived canopy trees (Nothofagus dombeyi and Eucryphia cordifolia; Lusk and Del Pozo 2002); and whereas most climbers are rather thin woody vines, some may develop into thick (Boquila trifoliolata) and even massive lianas (Hydrangea serratifolia may attain diameters over 35 cm; Gianoli et al., unpubl. data). Nevertheless, using DBH as an estimate of longevity (Laurance et al. 2004), which in turn is inversely associated with generation time, we found that—not surprisingly—trees were tenfold thicker than climbers. This would suggest that the greater genetic differentiation of climbers could be explained by their shorter generation times. However, if this were true then we would expect higher genetic variation in climbers at any spatial scale, which means that within-population data should show the same pattern: greater genetic diversity in climbers (see Ægisdóttir et al. 2009; Segarra-Moragues and Ojeda 2010; Wagner et al. 2011). In contrast, we found (seemingly) similar levels of genetic diversity in trees and climbers in the two forest sites where this was tested, and the same result was found earlier in another site included in this study (Torres-Díaz et al. 2013).

This would suggest that the shorter generation time of climbing plants was not involved in the among-population patterns observed. However, we could propose a hypothesis, including both selection and neutral processes, to explain why current levels of within-population genetic diversity are similar in trees and climbers but the latter show greater among-population genetic differentiation. We suggest that (i) climber populations initially had higher levels of genetic diversity due to their shorter generation times (i.e., neutral processes determining patterns of genetic variation), (ii) this higher population genetic variability together with a greater exposure to local selection agents (see below) resulted in greater within-population evolutionary responses in climbers that led to increased differentiation among populations, and (iii) this past selection probably reduced the initially higher within-population genetic diversity of climbers to levels similar to those of trees (note that there was a nonsignificant trend for higher genetic diversity in climbers). It has been shown earlier that high within-population genetic diversity may provide the raw material for local selection to operate, leading to genetic differentiation between plant populations (Kärkkäinen et al. 2004). Other non-selective explanations for the observed patterns cannot be ruled out. Differential effects of geographic barriers or differential long-distance dispersal in climbers and trees could lead to contrasting levels of gene flow and thus account for the results, at least in part. However, we lack evidence on such differential long-distance dispersal. Furthermore, the influence of dispersal ability on diversification can be positive or negative (Weeks and Claramunt 2014). Genetic drift in supposedly smaller climber populations could also explain their greater level of population differentiation, but genetic diversity data together with field observations of comparable population size in trees and climbers make this hypothesis unlikely.

We found a posteriori some support for the hypothesis that climber populations could show a greater response to local selection pressures than trees. Considering that the study species are mainly temperate rainforest species, we focused on a study site at the northern edge of this formation (36°47’S, 73°09’W, site 2 in Fig. S1), where summer drought is likely (Luebert and Pliscoff 2006), and compared climbers and trees with regard to their water-use efficiency (WUE) during summer months. WUE (ratio of carbon assimilation rate to water loss rate) is a functional trait that plays a key role in plant adaptation to reduced soil moisture and has shown adaptive population differentiation along aridity gradients (Heschel et al. 2002, Lázaro-Nogal et al. 2015). The rationale was that if climbers are more responsive than trees with regard to phenotypic adjustment to the environment (a relatively dry climate), then WUE should be higher in the climbing plant species compared to co-occurring tree species. Accordingly, the mean WUE of climbers was twice that of trees and this difference was statistically significant despite the small number of replicates (five species per group) (Table S3). Importantly, overall, WUE of climbing plants does not differ from that of trees (Santiago and Wright 2007; Van der Sande et al. 2013), so our results do not reflect intrinsic differences of the two growth forms. Actually, when we compared WUE in a “core”
southern forest community, climbers, and trees showed similar values (Table S4).

Apart from the differential response to water availability in a functional trait described above, there are other pathways through which these climbing plant species might undergo increased ecological specialization. Climbing plants in this forest have broader light niches (Lusk et al. 2006; Gianoli et al. 2010) and longer flowering periods (Riveros and Smith-Ramírez 1995) than co-occurring tree species. This may allow more opportunities for specialization and ensuing local differentiation in climbers. For instance, it has been reported that canopy, subcanopy, and understory trees, which flower at rather distinct heights, differ in the diversity of their associated pollinators (Bawa et al. 1985); woody climbers, being able to show flowers at a broad range of heights, may exploit the whole guild of pollinators across forest strata and eventually develop more associations with specialist pollinators, which promote plant diversification (Pellmyr 2002). In fact, there is evidence that climbers tend to be more specialist-pollinated than trees (Gentry 1991).

Phylogenetic signal indicates to what extent the focal trait is explained by phylogenetic relatedness (Blomberg et al. 2003). It has been stressed that studies addressing the link between genetic diversity parameters and species features have often not accounted for the phylogenetic influence on the results, thus weakening the explanatory power of the relationships found (Duminil et al. 2009). We did evaluate whether observed values of among-population genetic differentiation could be explained by phylogenetic relatedness and found that there was no phylogenetic signal, that is high and low $\Phi_{ST}$ values were found interspersed across the phylogenetic tree of the study species (Fig. 2). Likewise, a recent study comparing 63 liana species and 71 tree species coexisting in tropical and temperate forests (Ríos et al. 2015) found that both lianas and trees showed patterns of functional trait variation among species that were independent of phylogenetic relatedness. Climbing plants are a polyphyletic group (Gianoli 2015) and the same was observed in our sample of species: there was no monophyletic group of climbers (Fig. 2, Table 1). In sum, the greater population differentiation found for climbers in this temperate rainforest most likely resulted from independent evolutionary events.

The present study, set at the intraspecific level and at an ecological time scale, provides further support to the climbing habit as a promoter of diversification in plants. To our knowledge, this is the first report of concordance between a putative key innovation and population differentiation patterns. The potential of this population genetic approach to address macroevolutionary questions was also stressed by Kisel et al. (2012) despite not finding a correlation between population genetic differentiation (also using AFLP) and total diversification in five pairs of sister clades of orchids. From a broader perspective, we suggest incorporating these data, greater among-population genetic differentiation, as a third line of evidence when testing for key innovations, besides a comparative test at the macroevolutionary level and an ecological-functional argument (Heard and Hauser 1995). Such comparisons of genetic differentiation should be phylogeny-wise and take into account geographic distance. Moreover, among-population data should be complemented with estimates of within-population genetic diversity, inasmuch as comparing the results from both scales can help elucidate whether particular ecological traits are involved or not in the evolutionary patterns observed.

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DATA ARCHIVING

Data are archived in Dryad. The doi is 10.5061/dryad.4s6m3.

LITERATURE CITED


Donoso, C., ed. 2006. Las especies arbóreas de los bosques templados de Chile y Argentina. Marisa Cuneo, Valdivia, Chile.


Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s website:

**Table S1.** References for information shown on Table 1.

**Table S2.** Primer combinations used to quantify genetic diversity and genetic structure, total number of scored bands, and within-population genetic diversity statistics (AE = effective number of alleles per locus, HE = unbiased expected heterozygosity, PLP = percentage of polymorphic loci).

**Table S3.** Instantaneous water-use efficiency (WUE; ratio of photosynthesis to transpiration [μmol CO2 /mol H2O]) of woody climbers and trees in a forest site at the northern (drier) edge of the temperate rainforest (site 2 in Figure S1).

**Table S4.** Instantaneous water-use efficiency (WUE; see Table S3) of woody climbers and trees in a southern forest site of the temperate rainforest (site 8 in Figure S1).

**Figure S1.** Study sites (# spp climbers / # spp trees; latitude S, longitude W; MAT, MAP; altitude)