Genetic Structure and Early Effects of Inbreeding in Fragmented Temperate Forests of a Self-Incompatible Tree, *Embothrium Coccineum*

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Abstract: Deforestation of temperate forests has created landscapes of forest remnants in matrices of intense human use. We studied the genetic effects of fragmentation in southern Chile on *Embothrium coccineum* J.R. et G. Forster, an early colonizing, bird-pollinated tree. We tested the hypothesis that, because of its self-incompatibility and life-history strategy, *E. coccineum* is less strongly affected by fragmentation. We studied the effects of reduced population size and increased isolation on population genetic structure and early performance of progeny. Samples were collected from spatially isolated trees and six fragments of differing sizes (small, 1 ha; medium, 20 ha; large, >150 ha). Based on isozyme polymorphisms we estimated parameters of genetic diversity, divergence, and inbreeding for adults and greenhouse-grown progeny. We also measured germination, seedling growth, and outcrossing rates on progeny arrays. Genetic variation of adults did not correlate significantly with population size, as expected, given that fragmentation occurred relatively recently. Weak effects of fragmentation were measured on progeny. Only adults yielded significant inbreeding. Similar total genetic diversity was found in adults and progeny. Low but significant genetic differentiation existed among adult and progeny populations. Seedling growth correlated positively with the effective number of alleles, showing deleterious effects of inbreeding on progeny. Seeds from small fragments had the highest outcrossing rates and germination success, indicating that higher pollinator activity in such fragments reduced selfing, thereby buffering genetic erosion and maintaining adaptive variation. The effects of forest fragmentation were detectable in *E. coccineum*, but these effects will probably not be detrimental to the viability of remnant populations because small, fragmented populations demonstrated higher levels of gene flow and lower inbreeding than larger stands. Pioneer species that are insensitive to forest clearing may be crucial in recovery plans to facilitate the establishment of species intolerant to such disturbance.

Keywords: fragmentation, genetic diversity, outcrossing rates, population size, progeny vigor, temperate forest

Estructura Genética y Efectos Tempranos de la Endogamia en Bosques Templados Fragmentados de un Árbol Auto Incompatible. *Embothrium coccineum*

Resumen: La deforestación de bosques templados ha creado paisajes con remanentes de bosques en matrices de uso humano intenso. Estudiamos los efectos genéticos de la fragmentación en el sur de Chile sobre *Embothrium coccineum* J.R. et G. Forster, un árbol colonizador primario, polinizado por aves. Probaron la hipótesis que, debido a su auto incompatibilidad y estrategia de historia de vida, *E. coccineum* es poco afectada por la fragmentación. Estudiamos los efectos de la reducción del tamaño poblacional y del incremento del aislamiento sobre la estructura genética de la población y el vigor temprano de la progenie. Se recolectaron muestras de árboles espacialmente aislados y en seis fragmentos de diferente tamaño (pequeño, 1 ha; mediano, 20 ha; grande, >150 ha). En base a polimorfismos de isozimas estimamos parámetros de la diversidad
genética, divergencia y endogamia en adultos y progenie cultivada en invernadero. También medimos las tasas de germinación, crecimiento de plántulas y de exogamia en pruebas de progenie. La variación genética de adultos no se correlacionó significativamente con el tamaño de la población, como se esperaba, debido a que la fragmentación ocurrió relativamente recientemente. Los efectos de la fragmentación sobre la progenie no fueron severos. Sólo los adultos presentaron endogamia significativa. Encontramos similar diversidad genética total en los adultos y la progenie. Hubo baja diferenciación genética entre las poblaciones de adultos y de progenie. El crecimiento de plántulas se correlacionó positivamente con el número efectivo de alelos, lo que muestra los efectos deletéreos de la endogamia sobre la progenie. Las semillas provenientes de fragmentos pequeños tenían las mayores tasas de exogamia y de éxito de germinación, lo que indica que la mayor actividad de polinizadores en tales fragmentos redujo la autofecundación, por lo tanto amortiguó la erosión genética y mantiene la variación adaptativa. Los efectos de la fragmentación del bosque fueron detectables en Embothrium coccineum, pero estos efectos probablemente no serán perjudiciales para la viabilidad de las poblaciones remanentes, porque las poblaciones fragmentadas mostraron altos niveles de flujo de genes y menor endogamia que las poblaciones de mayor tamaño. Las especies pioneras que no son sensibles a la tala de bosques pueden ser cruciales para los planes de recuperación para facilitar el establecimiento de especies intolerantes a tales perturbaciones.

Palabras Clave: bosque templado, diversidad genética, fragmentación, tamaño poblacional, tasas de exogamia, vigor de la progenie

Introduction

Loss of continuous habitats and conversion to smaller patches reduces population size rapidly and severely and increases population isolation (Young et al. 1993). In particular populations that suffer severe bottlenecks lose genetic variation because of founder effects, genetic drift, and elevated inbreeding. Together these processes can lead to fixation of deleterious alleles and inbreeding depression, which can reduce viability and fecundity (Young et al. 1993, 1999; Couvet 2002). Isolated populations also tend to differentiate due to limited interpopulation gene flow (Templeton et al. 1990; Ledig 1992). The resulting decline in heterozygosis and allelic richness limit the species’ ability to respond to changing selective regimes in the long term (Lesica & Allendorf 1995; Hamrick & Nason 1996).

Fragmentation and degradation of continuous forests modify the habitat and thus affect the nature of plant-animal interactions during different life-history stages, such as pollination and seed dispersal (Aizen & Feinsinger 1994). Nevertheless spatially isolated trees may facilitate pollen movement (Nason & Hamrick 1997), connecting fragments genetically (Guevara & Laborde 1993; Aldrich & Hamrick 1998). Conversely small populations may attract fewer pollinators, which may reduce pollen movement (Morgan 1999). Therefore the effects of fragmentation on plant species’ responses to pollinators depend on compatibility systems and pollination mechanisms (Aizen et al. 2002). In fragmented landscapes of southern Chile, pollinators visit individual Embothrium coccineum (Proteaceae) in isolation and in small forest fragments more frequently than in medium and large fragments (Smith-Ramírez & Armesto 2003).

Temperate forests of austral South America are experiencing increasing deforestation (Armesto et al. 1998), with native forests of southern Chile having been nearly exterminated during the second half of the twentieth century (Aravena et al. 2002). Conversion of this region to mainly agricultural uses and plantations of exotic species has affected the spatial continuity of several plant and animal species and the mutualistic interactions among them (Smith-Ramírez & Armesto 2003).

We examined the impact of forest fragmentation on genetic traits of E. coccineum J.R. et G. Forster, a very common tree in fragmented landscapes of southern Chile. An analysis of satellite images for this region for 1976, 1985, and 1999 indicates that studied fragments are 20–25 years old, half of which were produced from one large ancestral population of approximately 130,000 ha (C. Echeverría, personal communication). In addition the oldest and largest E. coccineum trees are found in patches of different sizes (Mathiasen 2004), suggesting that fragmentation in southern Chile occurred relatively recently.

We tested the hypothesis that trees in small fragments and isolated trees are not genetically separated from the larger populations and thus experience normal or even enhanced gene flow and do not suffer from inbreeding depression. We predicted that genetic diversity and progeny performance would vary directly with population size. A limited effect of fragmentation on genetic structure of E. coccineum was expected because it is self-incompatible, so enforced outbreeding may counteract genetic erosion and inbreeding in small fragments. We focused on forest fragments and spatially isolated trees of E. coccineum in a matrix of intense human use, which had been the subject of prior pollination studies (Smith-Ramírez & Armesto 2003). Because fragmentation occurred recently, we
consider adult trees representative of the genetic composition of forests before fragmentation. Therefore we discuss our results in relation to population size, age class (adults vs. seedlings), time since fragmentation, dependence on pollination vectors, and vigor of early progeny.

**Methods**

**Study Species and Area**

*Embothrium* is a monotypic genus in the Proteaceae, a family of Gondwanic origin. It is endemic to temperate forests of southern Argentina and Chile and ranges in the north from Linares (35° S) in Chile and Lago Ñorquinco (39° S) in Argentina to Tierra del Fuego (55° S). *Embothrium coccineum* grows as a tree or shrub, usually associated with forest edges, although it never occurs as dominant element or in pure stands. It is the only red-flowered tree within austral forests. The bright red, tubular flowers are hermaphrodite and open for 4 days, although the dense inflorescences flower for up to 16 weeks (Smith-Ramírez & Armesto 1994). Birds and a few insect species visit *E. coccineum* flowers for nectar. On Chiloé Island, Chile, *E. coccineum* blooms during spring (September-January) and is pollinated primarily by two bird species: *Elaenia albiceps* (flycatcher) and *Sephanoides sephanoides* (hummingbird; Smith-Ramírez & Armesto 2003). *E. coccineum* is self-incompatible in mountain areas at 41° S (Riveros 1991) and farther south at 50° S (Arroyo & Squeo 1990), although it was recorded as self-compatible at low elevations at 39° S (Riveros et al. 1996).

We studied *E. coccineum* on northern Chiloé Island, between 41° 53′–41° 59′ S and 73° 32′–73° 41′ W. The study area consisted of forest fragments ranging from 1 to more than 150 ha separated by a maximum of 15 km and isolated trees in a matrix of intense human land use. Fragments in the study area were generated by forest clearing during the past 100 years, and most were remnants of continuous forests that covered Chiloé Island in the mid 1800s (Willson & Armesto 1996). Remnant vegetation was dominated by evergreen broadleaf species, including an emergent tree layer of endemic Myrtaceae, *Drynis winteri* J.R. et Foster (Winteraceae); two other Proteaceae *Gevuina avellana* Mol. and *Lomatia bursuta* (Lam.) Diels ex J.F. Macbr.; southern beech (*Nothofagus nitida* (Phil.) Krasser), and conifers such as *Pilgerodendron uviferum* (D. Don) Florin (Cupressaceae) and *Podocarpus nubigena* Lindl. (Podocarpaceae).

**Adult Sampling and Experiments on Seedling Performance**

We studied adult trees in six forest fragments and isolated trees at two locations. The number of adult trees in each fragment was estimated from the product of the average density in four 25-m² plots and the area occupied by *E. coccineum*. This area was estimated as the fragment perimeter (measured with GIS satellite imagery) multiplied by an approximately 2-m-wide band along fragment edges. Based on this approach, the six fragments included 47, 82, 97, 172, 934, and 990 individuals. These were identified as small fragments S-KS and S-ST, medium-sized fragments M-GB and M-WF, and large-sized fragments L-KC and L-KG, respectively. Isolated individuals were trees separated by at least 10 m and surrounded by pasture, identified as I-MD and I-CP. Isolated trees were as old as trees in forest fragments (Mathiassen 2004), indicating that these remnants were part of the original continuous forest prior to fragmentation. We excluded isolated trees from statistical analysis of the effects of population size because they were widely dispersed across the landscape and could not be considered populations.

We measured germination and progeny vigor to assess whether inbreeding effects increased with population size. We collected fruits produced by five inflorescences from each of the canopies of 8–10 maternal trees of similar age in each fragment and from each isolated tree during the austral summer of 2002. Each inflorescence produces approximately five fruits, which resulted in a total sample of 20–25 fruits per tree. Ten fruits from each maternal tree were randomly selected to assess fruit mass, the number of seeds per fruit, and average seed mass. The latter was based on 10 batches of 10 randomly selected seeds from the total of the pooled fruits produced by a single maternal tree. Out of those total seeds produced per tree, we randomly selected seeds that were maintained in progeny arrays to measure germination ability and progeny vigor. Seeds were germinated following Figueroa et al. (1996) in a greenhouse under natural light at Laboratorio Ecotono, Bariloche, Argentina (41° 08′ S, 71° 19′ W).

We determined germination success for 20 randomly selected seeds from each of the maternal families sampled per site and counted the number of emerging seedlings every 7–10 days for 3 months. Seedling mortality was recorded after 80 days as the proportion of germinated seeds that died. Eight to fifteen of the remaining seedlings per maternal family (*n* = 432) were grown in the common garden for 3 months and then measured to quantify progeny vigor. We measured plant height, root length, and mass after drying seedlings at 40°C for 3 days. We also used these seedlings, maintained in maternal families (i.e., progeny arrays), for genetic analysis of progeny and to analyze the relationship between vigor parameters with population and genetic traits.

**Isozyme Electrophoresis**

We analyzed genetic characteristics of adult trees and their progeny from each fragment and the isolated trees. We considered that seedlings were representative of the current mating system interacting with the genetic structure of the adult population(s). Fresh leaf tissue was collected from approximately 50 adult trees per site and
seedlings grown in a common garden. Adults included the maternal trees from which we collected seeds and other randomly selected individuals. A total of 406 adult trees and 432 seedlings were analyzed by protein-electrophoresis with 12% starch gels (Starch Art Corporation, Smithville, Texas). Nine enzymes coding for 22 putative isozyme loci were resolved in two buffer systems. We ran MDH (malate dehydrogenase, EC 1.1.1.37), PERc (cathodic peroxidase, EC 1.11.1.7), PGI (phosphoglucomutase, EC 5.3.1.9), and SKDH (shikimate dehydrogenase, EC 1.1.1.25) in O’Malley et al.’s (1979) buffer system. We used Poulik’s (1957) system to resolve IDH (isocitric dehydrogenase, EC 1.1.1.42), ME (malic enzyme, EC 1.1.1.40), MNR (menadione reductase, EC 1.6.99.2), PERa (anodic peroxidase, EC 1.11.1.7), and PGM (phosphoglucomutase, EC 5.4.22). Eleven of the 22 analyzed loci were polymorphic in at least one site, including MDH-2, MNR-4, PERa-1, PERc-1, PERc-2, PERc-3, PGI-2, PGM-1, PGM-2, and SKDH.

Genetic Parameters

We quantified genetic parameters separately for adult trees and seedlings from each site because we considered the two generations representative of pre- and postfragmentation mating environments. This analysis considered standard within-population parameters of genetic variation and inbreeding estimated with POPGENE (version 1.32; Yeh et al. 1999), including mean number of alleles (A); effective number of alleles (Ae); number of rare alleles (Ar); percent of polymorphic loci under the sensu stricto criterion (Ps); observed heterozygosity (Ho); and gene diversity (H). We estimated the number of rare alleles following Premoli et al. (2000), as Ar = A - [A/A], where A is the number of low-frequency (<0.10) alleles corrected by the number of alleles in a population (A) relative to the total number of alleles (A) observed in all populations. In addition we calculated the individual heterozygosity for seedlings (Hi) as the number of heterozygous loci to the total number of analyzed loci per individual.

We used the maximum-likelihood model of Ritland and Jain (1981) and the MLTR (version 2.4, Ritland 2002) computer program to estimate the following mating system parameters: average single-locus (ts) and multilocus (tm) outcrossing rates, coefficient of biparental inbreeding (tm-ts: Brown 1990), and the correlation of outcrossed paternity (rp) for progeny arrays from fragments and isolated trees. We performed a hierarchical analysis of inbreeding by Fis as a measure of the average within-population inbreeding and FST as an indicator of among-population divergence, which—in case of a genetic equilibrium—is also an indirect estimator of gene flow (Wright 1965, but see Whitlock & McCauley 1999). Finally we calculated total genetic diversity (H) for adult trees and seedlings based on polymorphic loci with FSTAT (version 2.9.3, Goudet 2002).

Several aspects of our analyses considered statistical hypotheses about means, which we tested with various t tests. We used simple linear regression to investigate the effects of log-transformed population size on the genetic variation parameters determined on adult trees and seedlings, germination rate, and mating-system parameters. We used stepwise multiple linear regression to analyze the relationships among average seedling vigor characteristics per family (plant height, root length, shoot weight, and total weight) and population characters (seedling mortality, germination rate, seed weight, fruit weight, and number of seeds per fruit) and genetic variation parameters (individual heterozygosity, polymorphism, effective number of alleles, number of rare alleles, and mean number of alleles per locus).

### Results

#### Genetic Variation

Adult trees and seedlings from the six population fragments differed somewhat in their genetic variation (Table 1). Across the range of populations, seedlings were significantly more polymorphic (Ps) than adult trees (t = -2.77, p = 0.03, paired t test) and had fewer alleles (Ar) than adults (t = 3.58, p = 0.01, paired t test). Adults and progeny did not differ significantly for the other genetic parameters that we estimated (p > 0.05 in all cases, paired t test). On average inbreeding coefficients within adult populations were significantly different from zero (Fis ± SE = 0.324 ± 0.072; 95% confidence level [CL] = 0.171–0.545), whereas those of seedlings were not (0.095 ± 0.099; 95% CL = -0.045 to 0.535). Nevertheless both adults and progeny demonstrated a low, but significant, differentiation among populations (FST ± SE = 0.025 ± 0.011; 95% CL = 0.006–0.059 and 0.055 ± 0.034; 95%

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**Table 1.** Mean (SE) population estimates of genetic variation parameters for adult trees and seedlings of *E. coccineum* from six population fragments on Chiloé Island, southern Chile.*

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>A</th>
<th>Ae</th>
<th>A</th>
<th>At</th>
<th>Ps</th>
<th>Ho</th>
<th>He</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>50.8</td>
<td>1.66 (0.03)</td>
<td>1.40 (0.01)</td>
<td>3.26 (0.36)</td>
<td>37.1 (0.5)</td>
<td>42.6 (1.2)</td>
<td>0.13 (0.01)</td>
<td>0.196 (0.004)</td>
</tr>
<tr>
<td>Seedlings</td>
<td>50.8</td>
<td>1.76 (0.04)</td>
<td>1.38 (0.01)</td>
<td>4.36 (0.68)</td>
<td>33.4 (0.8)</td>
<td>48.7 (1.6)</td>
<td>0.14 (0.01)</td>
<td>0.197 (0.004)</td>
</tr>
</tbody>
</table>

*Mean number of individuals sampled; A, mean number of alleles per locus; Ae, effective number of alleles; Ar, number of rare alleles; At, total number of alleles; Ps, polymorphism sensu stricto; Ho, observed heterozygosity; and He, gene diversity.*

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lower multilocus outcrossing rates in one large fragment (tm = 0.78) and one location with isolated trees (tm = 0.53), suggesting that some selfing can occur. Multilocus and single-locus (ts) estimates of outcrossing were very similar (tm ± SE = 0.898 ± 0.060 and ts ± SE = 0.831 ± 0.039, respectively; t14 = 0.93, p = 0.37, t test) (Table 2), so the coefficient of biparental inbreeding (tm-ts) did not differ significantly from zero (t7 = 1.50, p = 0.18, unpaired t test), indicating little mating between relatives. The average correlation of outcrossed paternity (rp) among progeny within families was low (rp ± SE = 0.081 ± 0.028) but significantly greater than zero (t7 = 2.96, p = 0.02, unpaired t test), suggesting that randomly chosen pairs of outcrossed progeny from a maternal family tend to be full sibs (i.e., have the same pollen parent). Multilocus outcrossing rate (tm) decreased significantly with log-transformed population size (F1,4 = 13.02, p = 0.02: Fig. 2), whereas the other mating-system parameters (ts, tm-ts, and rp) did not correlate significantly with this variable (p > 0.1, linear regression).

Progeny Performance

Germination rate was negatively correlated with log-transformed population size (F1,4 = 17.5, p = 0.01: Fig. 3). Seeds collected from intermediate fragments had higher germination capacity than those from large fragments and isolated trees (Fig. 3), which in turn showed comparable germination rates (percent germination attained was 35.6 [L-KC] and 20.0 [L-KG] in large fragments and 34.8 [I-MD] and 25.0 [I-CP] in isolated trees).

Average measures of seedling size after 3 months did not correlate significantly with log-transformed population size (p > 0.1 in all cases, simple linear regression) but were significantly related to the effective number of alleles and, in some cases, seedling mortality (Table 3). Seedlings from families with a large effective number of alleles were taller, had longer roots and weighted more than those with fewer alleles. In addition the surviving seedlings from families with poor seedling survival tended to be shorter and lighter than those from families with high survival.

Figure 1. Effects of log-transformed population size on gene diversity (H_e) of E. coccineum progeny in southern Chile.

Table 2. Population size and mean (SE) estimates of mating system parameters for isolated E. coccineum (sites I-MD and I-CP) and six population fragments in Chiloé Island, southern Chile.*

<table>
<thead>
<tr>
<th>Site</th>
<th>Size</th>
<th>tm</th>
<th>ts</th>
<th>tm-ts</th>
<th>rp</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-MD</td>
<td>NA</td>
<td>0.534 (0.272)</td>
<td>0.711 (0.176)</td>
<td>−0.177 (0.199)</td>
<td>0.240 (0.399)</td>
</tr>
<tr>
<td>I-CP</td>
<td>NA</td>
<td>0.921 (0.262)</td>
<td>0.917 (0.218)</td>
<td>0.004 (0.139)</td>
<td>0.126 (0.137)</td>
</tr>
<tr>
<td>S-KS</td>
<td>47</td>
<td>1.063 (0.121)</td>
<td>0.963 (0.067)</td>
<td>0.100 (0.131)</td>
<td>0.008 (0.071)</td>
</tr>
<tr>
<td>S-ST</td>
<td>82</td>
<td>0.983 (0.152)</td>
<td>0.710 (0.093)</td>
<td>0.273 (0.082)</td>
<td>0.053 (0.068)</td>
</tr>
<tr>
<td>M-GB</td>
<td>172</td>
<td>1.007 (0.240)</td>
<td>0.945 (0.149)</td>
<td>0.063 (0.137)</td>
<td>0.008 (0.031)</td>
</tr>
<tr>
<td>M-WF</td>
<td>97</td>
<td>0.993 (0.190)</td>
<td>0.884 (0.153)</td>
<td>0.109 (0.119)</td>
<td>0.075 (0.135)</td>
</tr>
<tr>
<td>L-KC</td>
<td>990</td>
<td>0.905 (0.099)</td>
<td>0.806 (0.048)</td>
<td>0.099 (0.080)</td>
<td>0.111 (0.218)</td>
</tr>
<tr>
<td>L-KG</td>
<td>794</td>
<td>0.775 (0.377)</td>
<td>0.714 (0.160)</td>
<td>0.061 (0.335)</td>
<td>0.031 (0.024)</td>
</tr>
</tbody>
</table>

*Abbreviations: tm, multilocus outcrossing rate; ts, single-locus outcrossing rate; tm-ts, biparental inbreeding coefficient; and rp, correlation of outcrossed paternity; NA, not applicable.
Discussion

Fragmentation is probably affecting the genetic variability of *E. coccineum* populations in southern Chile. Reduced allelic richness in progeny, as measured by the total number of alleles and the tendency for a higher gene diversity of progeny with population size, most likely reflects local mate availability and mating relationships within and among fragments. Whether these have changed as a consequence of fragmentation is still a bit uncertain. Genetic theory predicts that small and isolated populations have lowered genetic variation (Nei et al. 1975), as documented in remnant habitats of other species containing few reproductive individuals after fragmentation (Young et al. 1999; Tomimatsu & Ohara 2003). In contrast to shade-tolerant species that depend on facilitation mechanisms including the availability of secure sites for establishment, early colonizers such as *E. coccineum* vigorously regenerated in cleared areas and therefore population size may even increase under fragmentation. Hence the genetic and demographic consequences of fragmentation are closely linked to autoecological traits.

Isozyme variation of adults was moderate and not related to population size. We speculate that the genetic variation of adult *E. coccineum* trees may reflect a structure that was probably established prior to habitat fragmentation. Given that fragmentation in the study area occurred recently (C. Echeverría, personal communication), too little time has elapsed for fragmentation to produce noticeable genetic effects on adults and only weak effects on progeny. In addition current genetic structure may be a reflection of the fact that the species occurred naturally in small patches within the closed canopy and was rarely found as continuous forest.

Comparable results have been found in several other species. In *Vincetoxicum hirundinaria*, a perennial herb, genetic characteristics are not directly related to population size but rather to the demographic history and mating system of the species (Leimu & Mutikainen 2005). Similarly genetic diversity did not vary with current population size in long-lived *Grevillea iaspicula* and *G. caleyi* (Proteaceae), which had suffered recent fragmentation.

Table 3. Summary statistics for stepwise multiple regression analyses (*p* < 0.05) of seedling size after 3 months against life-history characters and genetic traits (independent variables) of *E. coccineum*.

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>$R^2$</th>
<th>F</th>
<th>df</th>
<th>p</th>
<th>Independent variables</th>
<th>$\beta$</th>
<th>$R^2_{\text{partial}}$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height</td>
<td>0.295</td>
<td>2.72</td>
<td>4.26</td>
<td>0.05$^b$</td>
<td>$A_e$</td>
<td>0.589</td>
<td>0.176</td>
<td>0.02</td>
</tr>
<tr>
<td>Root length</td>
<td>0.343</td>
<td>3.39</td>
<td>4.26</td>
<td>0.02$^b$</td>
<td>$A_e$</td>
<td>0.799</td>
<td>0.132</td>
<td>0.01$^b$</td>
</tr>
<tr>
<td>Shoot weight</td>
<td>0.477</td>
<td>4.03</td>
<td>5.25</td>
<td>0.01$^b$</td>
<td>$M$</td>
<td>-0.430</td>
<td>0.184</td>
<td>0.01$^b$</td>
</tr>
<tr>
<td>Total weight</td>
<td>0.327</td>
<td>4.36</td>
<td>3.27</td>
<td>0.01$^b$</td>
<td>$A_e$</td>
<td>0.359</td>
<td>0.101</td>
<td>0.03</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$M$</td>
<td>-0.370</td>
<td>0.160</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$A_e$</td>
<td>0.327</td>
<td>0.098</td>
<td>0.05</td>
</tr>
</tbody>
</table>

$^a$Key: $A_e$, effective number of alleles; $M$, percentage of seedling mortality after 80 days.

$^b$Significant after Bonferroni correction (Rice 1989).
(Hoebee & Young 2001; Llorens et al. 2004). In these species self-incompatibility and the production of seed banks may buffer the loss of diversity, even following severe reductions in population size (Levin 1990). In contrast predominantly outcrossed, self-compatible species may be more susceptible to the genetic effects of fragmentation. This is due to higher inbreeding in small populations and the expression of lethal recessive alleles when homozygosity increases (Charlesworth & Charlesworth 1987).

Similar genetic diversity in adults and progeny is maintained by the predominant self-incompatibility of *E. coccineum*, which creates a dependence on pollinators to facilitate mating. Self-incompatibility may favor pollen importation from other fragments, particularly where few reproductive trees are available for successful pollination. Previous data revealed that the main pollinator of *E. coccineum* in the study area, the bird *E. albiceps* (Tyrannidae, Passeriformes), frequently visits spatially isolated individuals and those at forest edges within small fragments (Smith-Ramirez & Armesto 2003). In contrast pollinators are more territorial in larger fragments, where they tend to defend three to five adjacent flowering trees on which they forage for nectar. Moreover in such large fragments, nearby *E. coccineum* individuals tend to be genetically similar because seeds primarily disperse within 20 m of the maternal tree (Rovere & Premoli 2005). Hence trees in larger fragments may commonly inbreed if pollinator behavior favors mating between related neighboring plants in such structured populations. This biparental inbreeding may explain the lower percentage of fruit production in medium-sized and large fragments (5% and 6%, respectively) compared with that in small fragments and isolated trees (11% and 13%, respectively) within the study area (A.E.R., unpublished data). In addition, larger fragments are probably composed of several small demes that may suffer higher drift than isolated trees and individuals at forest margins and in small fragments, where genes are more freely exchanged (Aldrich & Hamrick 1998).

Low genetic divergence among adults (*F_{ST} = 0.025*) and seedlings (*F_{ST} = 0.055*) suggests that there is little restriction of gene flow (*N_{m} > 1*) among populations inhabiting fragmented landscapes. Nevertheless, within-population inbreeding seems to follow distinct patterns in adults and progeny. On one hand adults exhibit higher mean inbreeding (0.324) than seedlings (0.095), perhaps reflecting prefragmentation Wahlund effects and thus the existence of a former population structure (Young et al. 1996). In contrast reduced inbreeding of progeny correlates with the observed high outcrossing rates. Hand-pollination experiments in the study area indicate that outcross and open pollination yielded significantly higher fruit set (37.3% and 17.3%, respectively) than spontaneous autogamy or self-pollination (0% and 1.3%, respectively; Rovere et al. 2006). These results are consistent with a predominantly outcrossing mating system. However selfing may occur, as indicated by relatively low outcrossing rates in one large fragment (tm = 0.775) and one location with isolated trees (tm = 0.53). The large fragment (L-KG) was the only study site immersed in a matrix dominated by *Baccharis* (Asteraceae), an insect-pollinated shrub (Aizen & Ezcurra 1998). The isolated trees at site I-MD occurred on private property with beehives, where bees visit *E. coccineum* frequently (C. Smith-Ramirez, personal communication). Although open-pollinated families have paternity with low average correlation (rp < 0.13), isolated trees at I-MD had the highest (rp = 0.24). Among these isolated trees, it is likely that drift has led to fewer compatible mates and only a few fathers contributed to each seedling family, which resulted in a higher rp. Hence in addition to fragment size, the mating system and the genetic structure of *E. coccineum* were affected by pollinator assemblages and the vegetation associated with the surrounding matrix.

Seed germination was negatively correlated with population size (Fig. 3), which may reflect higher inbreeding in large fragments. Deleterious effects of inbreeding are also evident in the positive relationship of seedling performance with effective number of alleles (Table 3). Such inbreeding presumably results from selfing and/or mating between relatives caused by short interplant movements of pollinators as measured in large fragments. Open areas were characterized by abundant regeneration of *E. coccineum* from only few reproductive individuals, which may pose demographic problems. The loss of adaptive genetic variation and inbreeding depression may threaten population viability in this fragmented landscape (Oostermeijer et al. 1995; Reed & Frankham 2003) because they may alter vital characteristics of the population (Sherwin & Mortiz 2000) and reduce the population’s ability to respond to selective pressures posed by the changing environment (Reed & Frankham 2003).

To maintain genetic diversity and fitness, forest patches of pollinator-dependant species such as *E. coccineum* must be preserved. These fragments will balance genetic erosion by drift, reduce inbreeding, and retain sufficient heterozygosity through a continuous influx of alleles from locally distinct gene pools mediated by pollinators. Isolated trees may act as connectors, maintaining gene flow among distant populations (Aldrich & Hamrick 1998; Cascante et al. 2002; White et al. 2002). Our results indicate that spatially isolated trees and forest remnants are sufficient for the maintenance of viable populations undergoing fragmentation. *Embothrium coccineum* can then be considered to have low sensitivity to fragmentation, which mitigates the effects of genetic erosion. This together with its natural ability to colonize open areas facilitate the establishment of other shade-tolerant plants that in turn are highly sensitive to fragmentation.

Fragments are all that remain of native forests in southern Chile because of intense land use, including plantations of exotic species. We studied an area within

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administrative Region X, which supports 7.5% of Chile’s remaining lowland and coastal mountain forests (Neira et al. 2002). These forests are in turn the least represented in the protected-area system. Austral forests of South America have an endemic biota at high taxonomic levels. Their flora also exhibits one of the highest incidences of biotic pollination (25%) of woody plants, particularly ornithophilous, which is comparable to incidences in tropical areas (Aizen et al. 2002). Nevertheless, in contrast to the tropics, these ornithophilous plants depend strongly on a few bird species. For example, approximately 20% of red-flowered woody plants are visited by one hummingbird species (S. sepaniades; Smith-Ramirez 1993). Areas in temperate latitudes with elevated endemism and highly dependant mutualistic interactions could be as fragile as those in the tropics, so conservation practices should emphasize the role of remnant forest fragments.

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