High gene flow in epiphytic ferns despite habitat loss and fragmentation

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Abstract Tropical montane forests suffer from increasing fragmentation and replacement by other types of land-use such as coffee plantations. These processes are known to affect gene flow and genetic structure of plant populations. Epiphytes are particularly vulnerable because they depend on their supporting trees for their entire life-cycle. We compared population genetic structure and genetic diversity derived from AFLP markers of two epiphytic fern species differing in their ability to colonize secondary habitats. One species, *Pleopeltis crassinervata*, is a successful colonizer of shade trees and isolated trees whereas the other species, *Polypodium rhodopleuron*, is restricted to forests with anthropogenic separation leading to significant isolation between populations. By far most genetic variation was distributed within rather than among populations in both species, and a genetic admixture analysis did not reveal any clustering. Gene flow exceeded by far the benchmark of one migrant per generation to prevent genetic divergence between populations in both species. Though populations are threatened by habitat loss, long-distance dispersal is likely to support gene flow even between distant populations, which efficiently delays genetic isolation. Consequently, populations may rather be threatened by ecological consequences of habitat loss and fragmentation.

Keywords Colonization · Epiphyte · Mexico · *Pleopeltis crassinervata* · *Polypodium rhodopleuron* · Secondary habitats · Tropical montane forest

Introduction

Even if not leading to extinction, habitat loss and fragmentation can affect gene flow and the genetic structure of plant populations (Picó and Van Groenendael 2007). Both lead to reduced population size and increased spatial isolation usually resulting in an erosion of genetic variability (González-Astorga et al. 2004; Young et al. 1996). Possible causes are genetic bottlenecks and subsequently increased inbreeding in small populations, enhanced genetic drift and reduced gene flow (Templeton et al. 1990; Young et al. 1996). Several studies found that remnant population size was positively correlated with polymorphism and allelic richness (Cardoso et al. 2005; Morden and Loeffler 1999; Prober and Brown 1994; Van Treuren et al. 1991).

Whether and to which degree genetic consequences of fragmentation are detectable depends on the one hand on the time elapsed since the onset of fragmentation, and on the other hand on life-history traits of the concerned species. In recently fragmented populations often no effects on population genetic structure were found (reviewed in Aguilar et al. 2008; González-Astorga and Núñez-Farfán 2001; Murren 2003; Neel and Ellstrand 2001). Long-lived species and/or species with far-ranging pollination or seed dispersal syndromes like neotropical tree species (reviewed in Nason 2002) and epiphytic orchids (Ackerman and Ward 1999; Avila-Díaz and Oyama 2007; Bush et al. 1999; Trapnell and Hamrick 2004; Trapnell et al. 2004) exhibit high levels of genetic diversity and low genetic differentiation among populations even when they are separated by long distances.
One of the terrestrial ecosystems most threatened by
deforestation and fragmentation are tropical montane for-
est (Bruijnzeel et al. 2010). Despite their limited spatial
extension they harbour a very diverse flora, e.g., Mexican
tropical montane forests cover <1% of the country’s ter-
ritory but contain between 10 and 12 percent of all Mexi-
can vascular plant species with epiphytes representing a
third of the plant species diversity in these forests (Wil-
liams-Linera 2007). In Mexico, less than one third of the
area originally covered by tropical montane forests
remained as closed forest by 1990 (Price and Butt 2003).
Among the most extensive forms of land-use replacing
tropical montane forests are coffee plantations. In Mexico,
770,000 hectares were dedicated to the cultivation of coffee
in 2007 (http://faostat.fao.org/site/567/default.aspx#ancor),
with coffee generally grown under shade trees (Moguel
and Toledo 1999). Epiphytes depend on host trees for their
entire life-cycle and are thus highly affected by defores-
tation and forest fragmentation (e.g., Padmawathe et al.
2004; Turner et al. 1994). Coffee plantations with large
shade trees offer a refuge for many epiphytes in areas
where few forests remain (García-Franco and Toledo-
Aceves 2008; Mehltreter 2008), but not all species are able
to colonize trees in secondary habitats (Hietz 2005).

In the present study we compare population genetic
structure and genetic diversity of two epiphytic fern species
differing in their ability to colonize secondary habitats
(Mehltreter 2008). Pleopeltis crassnervata is a successful
colonizer of isolated trees and shade trees in coffee plantations
(Hooper and Huafler 1997). In contrast, Polypodium rhodo-
pleuron is a shade-loving species, with sporophytes occurring
preferentially in the lower parts of large tree trunks with dense
bryophyte mats, in riparian forests or in the most humid parts
of montane forests which are reduced to fragments with sig-
nificant man-made isolation between populations today. Thus,
the level of anthropogenic habitat loss and fragmentation is
much more pronounced in Polypodium. Using highly repro-
ducible and variable AFLP markers (Vos et al. 1995), we aim
to discern between two scenarios: (1) High gene flow among
populations prevents genetic consequences of fragmentation.
In this case we expect high levels of genetic diversity and low
levels of genetic divergence among populations. (2) The
epiphytic ferns are sensitive to fragmentation with low levels
of gene flow and genetic diversity and with most genetic
variability located among populations.

Materials and methods

Study species

Polypodium rhodopleuron Kunze and Pleopeltis crassi-
nervata (Fée) T. Moore are epiphytic ferns belonging to the
family Polypodiaceae. Chromosome numbers are 2n = 74
in Polypodium, and 2n = 70 in Pleopeltis (Mickel and
Smith 2004). Both species occur in tropical montane for-
est (classified as “bosque mesofilo de montaña” according
to Rzedowski 1986) between 800–2100 (Polypodium) and
700–1900 m.s.m. (Pleopeltis; Mickel and Smith 2004).
Polypodium has dry-deciduous leaves (Winkler et al.
2005), restricted to the trunks and lower branches of
forest trees (Tejero-Diez and Pacheco 2004) and is rare in
the area with only a few known populations. By contrast,
Pleopeltis can survive prolonged drought by curling its
leathery, scaly leaves and is common in forests as well as
on isolated trees and tree plantations (Hooper and Haufler
1997). Individual life spans are difficult to estimate, as both
species possess creeping rhizomes. In Mexico, both species
are distributed from the Sierra Madre Oriental to the Sierra
Madre de Chiapas. Beyond Mexico, Polypodium occurs in
Guatemala, Honduras and probably Nicaragua (Tejero-
Diez and Pacheco 2004), Pleopeltis is found south to Costa
Rica (Mickel and Smith 2004). The study species belong
to different lineages (Polypodium and Pleopeltis clade; Otto
et al. 2009) of a large neotropical clade within the Poly-
podiaceae (Schneider et al. 2004). The relationship
between the two lineages remains ambiguous (Otto et al.
2009).

Sampling

Polypodium and Pleopeltis were sampled from six and 17
localities, respectively, on the eastern slopes of the Sierra
Madre Oriental around Xalapa, and additionally from one
site (Los Tuxtlas) near the southern coast of the Gulf of
Mexico in the Mexican state of Veracruz (Fig. 1; Table 1).
For Polypodium these were all populations in the area we
were able to find consulting herbarium registers and a local
fern specialist (K. Mehltreter).

According to the most accurate survey data available
(calculated from satellite-image data (Muñoz-Villers and
López-Blanco 2007), forests, secondary habitats with
trees (shaded coffee plantations, agroforestry) and non-
arboreal vegetation (grassland, sugar cane and sun coffee
plantations) covered about 21, 38 and 33%, respectively,
of the study area in central Veracruz in 2003 (Fig. 1). The
mean size of forest patches was 1.06 ha (ranging
between 0.04 and 2528 ha). Only between 1990 and
2003, a third of these forests were replaced by grassland
and agricultural crops (Muñoz-Villers and López-Blanco
2007). In the Los Tuxtlas area, there are two larger and
one smaller patch of humid montane forest on three
isolated volcanoes, covering about 9600 ha in the core of
the Los Tuxtlas Biosphere Reserve (Laborde 2004). The
Los Tuxtlas mountains are separated from the Sierra

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Madre Oriental by about 200 km of coastal lowlands, where the two species do not occur.

Wherever possible, leaves of 15 individuals per site and located on different trees were collected, their surface cleaned from epiphylls and dried in silica gel. Voucher specimens were deposited at the herbarium of the Institute of Ecology, A.C. Mexico (XAL).

DNA extraction and AFLP fingerprinting

Total genomic DNA was extracted from similar amounts of dried tissue (ca. 20 mg) with the DNeasy 96 plant mini kit (Qiagen, Hilden, Germany) following the manufacturer’s protocol. AFLP profiles were generated following established procedures (Vos et al. 1995) with minor modifications (Gugerli et al. 2008). Genomic DNA (ca. 200 ng) was digested with 1 U MseI (New England BioLabs, Ipswich, USA) and 5 U EcoRI (Promega, Madison, USA) and ligated (with 1.2 U of T4 DNA-Ligase; Promega) to double-stranded adapters in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems, Foster City, CA, USA) for 3 h at 37°C. Preselective amplification was performed using primer pairs with a single selective nucleotide. Two selective primer combinations were chosen after a primer trial (fluorescent dye in brackets): EcoRI ATA (6-FAM)-MseI CTG; EcoRI AGG (HEX)-MseI CAG (Pleopeltis), and EcoRI ATA (6-FAM)-MseI CTC; EcoRI AGG (HEX)-MseI CTC (Polypodium). For each individual, 5 µl of each selective PCR product was Sephadex-purified. 1 µl of the purified product was combined with 0.25 µl MegaBace Et-550 R (GE Healthcare) internal size standard and 4.75 µl water, and separated on a capillary sequencer MegaBace 500 (GE Healthcare).

Blind samples were included to test for contamination. To test the reproducibility of AFLP fingerprinting, 20 plants of Polypodium were replicated between PCR plates, and seven samples of Polypodium and eight of Pleopeltis were replicated more than twice, resulting in a total of 55 and 168 replicates, respectively. The error rate was calculated as the number of mismatches divided by the number of phenotypical comparisons (Bonin et al. 2004).

Fragments in the range of 65–550 bp were aligned, visualized, scored and exported as binary presence/absence matrix using DAx 8.1 (Van Mierlo Software Consultancy, The Netherlands).

Analysis of AFLP data

All monomorphic fragments and those present or absent in all minus the number of individuals corresponding to the error rate (N×error rate/100) were removed from the data set to avoid biased parameter estimates (Bonin et al. 2004). Seven individuals of Polypodium produced non-reproducible AFLP patterns and were excluded from analyses. We calculated the following statistics of genetic diversity at the population level:

(i) the number of fragments present in a population;
(ii) the number of private fragments restricted to a given population;
(iii) the proportion of polymorphic markers;

Fig. 1 Location of sampled populations of the epiphytic ferns Polypodium rhodopleuron and Pleopeltis crassinervata in Veracruz, Mexico. Land-use types are derived from satellite-images from the year 2003 (Muñoz-Villers and López-Blanco 2007). For white areas no land-use information was available. The lower inset shows the location of the Los Tuxtlas population (Tux). Contour lines were derived from the CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad) GIS portal http://www.conabio.gob.mx/informacion/gts/, and GTOPO30 from the US Geological Survey (http://eros.usgs.gov/#/Find_Data/Products_and_Data_Available/gtopo30_info)
the frequency of rare markers as frequency-down-weighted marker values, calculated for each individual according to (Scho¨ nswetter and Tribsch 2005):

\[
R_x = \frac{\sum_{i=1}^{n} s_{ix}}{\sum_{i=1}^{n} s_{i}}
\]

where \( n \) is the number of markers, \( s_{ix} \) is the state of the \( i \)-th marker in individual \( x \) (either 1 or 0 in AFLPs), and \( k \) is the total number of individuals in the data set. In the denominator the number of occurrences of the \( i \)-th marker in the total data set is calculated. To control for differences in the number of markers, \( R_x \) was divided by \( n/100 \).

Population values (in the following termed \( DW \)) were estimated as the average of the individual values. \( DW \) is expected to be high in populations with a high frequency of rare markers, and is independent of the number of individuals. Calculations for i–iv were carried out in R

| Population | Land-use type | Forest fragment size (ha) | Coordinates | N | \( N_{frag} \) | \( N_{priv} \) | \( P_{poly} \) | \( H_j \) (±SE) | DW |
|-------------|---------------|--------------------------|-------------|---|-----------|-----------|------------|-------------|-----|
| Polypodium rhodopleuron | Aca-Acatlán | Forest | 579 | 96.85/19.68 | 15 | 163 | 5 | 0.764 | 0.216 ± 0.011 | 1.088 |
| | Agu-Aguita Fría | Forest | 100 | 96.95/19.60 | 12 | 160 | 7 | 0.768 | 0.211 ± 0.011 | 1.039 |
| | Ban-Banderilla | Forest | 5 | 96.96/19.58 | 15 | 141 | 4 | 0.679 | 0.193 ± 0.011 | 0.87 |
| | Can-Las Cañadas | Forest | 299 | 96.99/19.52 | 12 | 172 | 10 | 0.801 | 0.244 ± 0.011 | 1.377 |
| | Jil-Jilotepec | Forest | 20 | 96.94/19.51 | 8 | 129 | 3 | 0.589 | 0.221 ± 0.011 | 1.105 |
| | Par-Parque Ecológico, INECOL | Forest | 32 | 96.98/19.18 | 14 | 143 | 2 | 0.642 | 0.182 ± 0.011 | 0.855 |
| | Tux-Los Tuxtles | Forest | 9356 | 95.20/18.56 | 14 | 173 | 10 | 0.78 | 0.241 ± 0.011 | 1.488 |
| Means | | | | 154.4 | | 5.9 | | 0.718 | 0.215 | 1.117 |
| Pleopeltis crassinervata | Aca-Acatlán | Forest | | 96.85/19.68 | 15 | 144 | 0 | 0.673 | 0.218 ± 0.012 | 0.285 |
| | Agu-Aguita Fría | Forest | | 96.95/19.60 | 14 | 165 | 0 | 0.774 | 0.267 ± 0.012 | 0.396 |
| | Ato-El Atoron | Shade coffee plantation | | 96.95/19.58 | 15 | 157 | 0 | 0.744 | 0.255 ± 0.013 | 0.321 |
| | Ban-Banderilla | Forest | | 96.99/19.52 | 14 | 165 | 0 | 0.769 | 0.276 ± 0.012 | 0.416 |
| | Bol-Bola de Oro | Shade coffee plantation | | 96.94/19.52 | 15 | 164 | 0 | 0.754 | 0.265 ± 0.012 | 0.376 |
| | Can-Las Cañadas | Forest | | 96.97/19.50 | 15 | 154 | 0 | 0.709 | 0.228 ± 0.012 | 0.305 |
| | Cin-Cinco Palos | Pasture | | 96.95/19.50 | 14 | 162 | 1 | 0.769 | 0.249 ± 0.013 | 0.383 |
| | Jil-Jilotepec | Forest | | 96.95/19.49 | 15 | 165 | 0 | 0.774 | 0.252 ± 0.012 | 0.374 |
| | Mas-La Mascota | Forest | | 97.00/19.50 | 15 | 162 | 0 | 0.754 | 0.246 ± 0.013 | 0.362 |
| | Ord-La Orduña | Shade coffee plantation | | 97.00/19.48 | 15 | 170 | 0 | 0.799 | 0.272 ± 0.012 | 0.428 |
| | Par-Parque Ecológico, INECOL | Forest | | 96.96/19.47 | 15 | 165 | 0 | 0.754 | 0.269 ± 0.012 | 0.398 |
| | Pit-La Pitahaya | Shade coffee plantation | | 97.00/19.46 | 15 | 153 | 0 | 0.683 | 0.241 ± 0.012 | 0.329 |
| | Ris-El Riscal | Forest | | 96.93/19.47 | 15 | 161 | 1 | 0.754 | 0.262 ± 0.012 | 0.379 |
| | Tex- Texolo | Forest | | 96.95/19.43 | 15 | 162 | 0 | 0.764 | 0.246 ± 0.012 | 0.331 |
| | Tix-Rancho Tixtla | Forest | | 96.99/19.40 | 15 | 154 | 0 | 0.709 | 0.244 ± 0.013 | 0.325 |
| | Tot-Totutla | Forest | | 96.95/19.21 | 15 | 164 | 0 | 0.769 | 0.260 ± 0.012 | 0.41 |
| | Tux-Los Tuxtles | Forest | | 96.98/19.18 | 15 | 172 | 2 | 0.789 | 0.265 ± 0.012 | 0.516 |
| | Zim-Zimpizahua | Shade coffee plantation | | 95.18/18.58 | 15 | 163 | 0 | 0.749 | 0.252 ± 0.012 | 0.346 |
| Means | | | | 161.2 | | 0.2 | | 0.749 | 0.254 | 0.371 |

Different lower-case letters indicate significant differences between species (\( t \) test, \( P < 0.01 \)), with \( a \) representing the lower and \( b \) the higher value, respectively.

\( N \) number of samples per population, \( N_{frag} \) number of AFLP fragments in the population, \( N_{priv} \) number of private fragments in the population, \( P_{poly} \) proportion of polymorphic fragments in the population, \( H_j \) Nei’s gene diversity, \( DW \) within-population rarity of markers

1 Derived from aerial photographs from the year 1993 (Williams-Linera et al. 2000)
2 (Manson et al. 2008)
3 Derived from satellite images from the year 2000 (Mendoza et al. 2005)
4 Estimation M. Winkler
A principal co-ordinate analysis (PCoA) based on a matrix of Jaccard distances among individuals was calculated using the modules ‘Dcenter’ and ‘Eigen’ from NTSYS-pc 2.2 (Rohlf 1997). The software STRUCTURE v.2.2 (Falush et al. 2007; Pritchard et al. 2000) was employed to complement the distance-based analysis with a Bayesian clustering approach. We used an admixture model with uncorrelated allele frequencies and recessive alleles. Ten replicate runs for \( K \) (number of groups) ranging from 1 to 10 were carried out at the Biorportal of the University of Oslo (http://www.bioportal.uio.no/), using a burn-in of 100,000 iterations followed by 1,000,000 additional MCMC iterations. We identified the number of optimal groups as the value of \( K \) where the increase in likelihood started to flatten out, the result of replicate runs was similar and the clusters were non-empty. The replicate runs of the best \( K \) were then merged with CLUMPP 1.1.1. (Jakobsson and Rosenberg 2007) using the full-search algorithm.

**Results**

The two AFLP primer combinations yielded 246 and 199 clear polymorphic fragments after the removal of ten and eleven invariable markers in *Polypodium* and *Pleopeltis*, respectively. In the AFLP profiles from replicated samples, 777 differences were observed out of 20,515 phenotypic comparisons, resulting in an error rate of 3.79% in *Polypodium*, and of 3.72% in *Pleopeltis* (1,550 differences, 41,664 comparisons). All phenotypes of both species were unique. There were no significant differences in the proportion of polymorphic markers (\( P_{\text{poly}} \)) between *Polypodium* and *Pleopeltis*. Genetic diversity (\( H_i \)) was significantly higher in populations of *Pleopeltis* compared to *Polypodium*, whereas genetic rarity (\( DW \)) and the number of private markers (\( N_{\text{prev}} \)) were significantly higher in *Polypodium* (\( t \) tests, \( P < 0.01 \); Table 1).

There was no significant correlation of \( H_i \), the \( P_{\text{poly}}, N_{\text{prev}} \) or \( DW \) with forest fragment size in *Polypodium* (Spearman rank correlation, all \( P > 0.05 \)). Of the measures of genetic diversity and rarity, only \( H_i \) and \( DW \) (\( \rho = 0.96, P < 0.001 \)), and \( N_{\text{prev}} \) and \( P_{\text{poly}} \) (\( \rho = 0.96, P < 0.001 \)) were significantly correlated. In *Pleopeltis*, \( H_i \) and \( P_{\text{poly}} \) (\( \rho = 0.69, P < 0.001 \)), \( H_i \) and \( DW \) (\( \rho = 0.85, P < 0.001 \)), and \( DW \) and \( P_{\text{poly}} \) (\( \rho = 0.85, P < 0.001 \)) were correlated, respectively.

Within-population variation contributed at least 89.5% to overall genetic variation in *Polypodium*, and 92.1% in *Pleopeltis* (\( G_{\text{ST}}, F_{\text{ST}}, H_i \); Table 2). In the Population Graphs network of both species, the distant Tux population was connected with two central Veracruz populations each by extended edges indicating long-distance dispersal (Fig. 2). The covariance structure of central Veracruz
Table 2  Partitioning of genetic diversity within and among populations of the epiphytic ferns *Polypodium rhodopleuron* and *Pleopeltis crassinervata*

|                 | $H_t$ | $H_w$       | $H_b$    | $G_{ST}$ | $Nm$ | AMOVA          |
|----------------|-------|-------------|----------|----------|------|----------------|
| **Polypodium rhodopleuron** |       |             |          |          |      | Source of variation d.f. Sum of squares Components of variance Percentage variation $F_{ST}$ $P$ |
| Among populations | 0.223 | 0.215 ± 0.009 | 0.008 ± 0.001 | 0.105 | 4.263 | Among populations 6 363.2 2.4 7.7 0.077 <0.001 |
| Within populations |       |             |          |          |      | Within populations 83 2427.5 29.2 92.3 |
| Total |       |             |          |          |      | Total 89 2790.7 31.7 |
| **Pleopeltis crassinervata** |       |             |          |          |      | Source of variation d.f. Sum of squares Components of variance Percentage variation $F_{ST}$ $P$ |
| Among populations | 0.257 | 0.254 ± 0.004 | 0.003 ± 0.001 | 0.079 | 5.815 | Among populations 17 638.8 0.8 3.2 0.031 <0.001 |
| Within populations |       |             |          |          |      | Within populations 249 6312.7 25.4 96.9 |
| Total |       |             |          |          |      | Total 266 6951.5 26.2 |

$H_t$, total gene diversity, $H_w$, average gene diversity within populations, $H_b$, average gene diversity among populations in excess of that observed within populations, $G_{ST}$, proportion of total variation that is distributed among populations (Nei 1973), $Nm$, gene flow estimated as $Nm = 0.5(1 - G_{ST}) / G_{ST}$ (McDermott and McDonald 1993; Slatkin and Barton 1989); results of AMOVA.

Fig. 2  Population graphs network (Dyer and Nason 2004) illustrating the genetic covariance structure in Mexican populations of the epiphytic ferns *Polypodium rhodopleuron* (a), and *Pleopeltis crassinervata* (b). Populations are connected by edges if their genetic structures are conditionally dependent. Normal edges (black lines) are those where the physical distances are proportional to the genetic distances. If they are not, then the populations are either closer (compressed edges, blue lines) or further apart (extended edges, red lines) than expected given the genetic distances.
populations was characterized by compressed and normal edges (with the exception of one extended edge in *Pleopeltis*). Geographically close *Pleopeltis* population pairs were either not connected or by compressed edges. Graph distances and physical distances were not correlated (Mantel tests; *Polypodium*: $R = 0.02$, $P = 0.418$; *Pleopeltis*: $R = 0.06$, $P = 0.272$).

Both the distance-based approach (PCoA; Fig. 3) and the Bayesian clustering approach (STRUCTURE) showed no substructure in the dataset of both species. The optimal number of groups in STRUCTURE was $K = 1$ in both species, because there were empty clusters in $K = 2$ to $K = 10$ (data not shown).

**Discussion**

Genetic consequences of man-made habitat fragmentation

Despite being affected by different degrees of habitat loss and fragmentation, both the colonizing and the forest fern exhibited high genetic diversity and low differentiation from which we infer that levels of contemporary gene flow are high. The number of migrants per generation ($N_m$) in both ferns by far exceeded the benchmark of one migrant per generation as a minimum to prevent genetic divergence (Slatkin 1985; Wright 1931; Table 2). These results suggest that populations of both species were sufficiently connected by gene flow to prevent genetic divergence. Although indirect $N_m$ estimation from $F_{ST}$ or $G_{ST}$ probably violates several assumptions of the underlying model, $N_m$ values may be correct within a few orders of magnitude (Whitlock and McCauley 1999) and thus constitute a useful and widely used measure of overall gene flow levels for comparisons with other species. Our gene flow estimates are in the range of those recorded for 25 terrestrial and epiphytic fern species (reviewed in Ranker and Geiger 2008). Interestingly, none of the epiphytic species in this compilation had a $N_m$ value $<1$. In agreement with high levels of interpopulational gene flow (historic or recent) no clustering of populations was detected in either species (Fig. 2; optimal $K = 1$ in STRUCTURE)—not even the Los Tuxtlas populations at a distance of about 200 km were grouped separately but were connected with central Veracruz via long-distance dispersal (Fig. 2). Furthermore, most genetic variability by far was located within rather than among populations (Table 2) with no isolation by distance (Mantel-tests).

Our study confirms the notion that ferns are exceptionally good at long-distance dispersal. Their dust-like spores have been found in the jet streams (e.g., Caulton et al. 2000), and are capable of surviving intense UV radiation and low temperatures in the upper atmosphere (Gradstein and Van Zanten 1999). As a result, ferns are often among the first plants to colonize new habitats, often repeatedly (Ranker et al. 1994), and are overrepresented in island floras (Kessler 2010; Ranker and Geiger 2008).

Human impact was comparatively moderate in the study area until the end of the 19th century when coffee plantations began to replace montane forests. In the 1960s demographic pressure in the region around Xalapa increased dramatically and rates of forest destruction and conversion to pastures accelerated (Marchal and Palma 1985). Today, about 20% of our study area is still covered by tropical montane forest (Muñoz-Villers and López-Blanco 2007; Fig. 1), but less than half of these fragments can be considered as undisturbed (CONABIO 2010; Williams-Linera 2002) and are...
thus suitable habitat for *Polypodium*. The habitat of *Pleopeltis*, in contrast, is disrupted in those 40% of the study area only, where no trees are available at all (Muñoz-Villers and López-Blanco 2007; Fig. 1).

Still, *Polypodium* retains high genetic diversity (mean expected heterozygosity $H_e = 0.223$) compared to the mean reported for 486 seed plants ($H_e = 0.113$; Hamrick and Godt 1990), terrestrial ferns ($H_e = 0.117$), or other epiphytic ferns ($H_e = 0.149$; reviewed in Ranker and Geiger 2008), and was little lower than in the successful colonizer *Pleopeltis* ($H_e = 0.257$). Krauss (2000) showed that genetic diversity estimates from dominant marker data derived with a method introduced by Lynch and Milligan (1994) are accurate in outcrossing species, therefore estimates should be comparable to the allozyme data from the studies cited above. Furthermore, expected heterozygosity for *Pleopeltis* obtained with AFLP fingerprinting (0.254) was nearly identical to the value obtained using allozymes (0.252; Hooper and Haualer 1997). Breeding systems of both species help maintain high levels of genetic diversity in small and isolated populations. *Pleopeltis crassinervata* was shown to be outcrossing (Hooper and Haualer 1997). For *Polypodium rhodopleuron*, no information on breeding system is available, but all epiphytic fern species presented in a review by Ranker and Geiger (2008) are outcrossing.

Despite weak genetic structure and evidence for high gene flow in both species there are subtle differences in the distribution of rare markers. $DW$ and the number of private fragments were significantly higher in populations of the forest species compared to the colonizer with most rare markers present in Los Tuxtlas, the remotest population, in both species (Table 1). A high proportion of rare markers can be explained in two ways: First, rare markers accumulate in fragmented populations due to mutations. This scenario appears unlikely because not enough time has elapsed since fragmentation (ca. 50 years, which translates into very few generations in a long-lived species; Aguilar et al. 2008) to produce the observed genetic rarity levels, especially given the high level of gene flow, via mutations. The Los Tuxtlas population is the only exception because the surrounding lowlands do not constitute suitable habitat for the fern species suggesting a longer-lasting isolation of this particular population. Neither $DW$ nor the number of private fragments were negatively correlated with fragment size, on the contrary, the measures of genetic rarity were highest in the largest forest patches and increased significantly with increasing genetic diversity. Furthermore, genetic drift in small remnant populations puts rare markers at a greater risk of disappearing than frequent ones (Frankham et al. 2002; Young et al. 1996). Secondly, genetic rarity is expected to be high in long-term isolated populations (Schönswetter and Trübsch 2005), suggesting that populations of *Polypodium* have been more isolated than those of *Pleopeltis* already before anthropogenic habitat fragmentation due to differing habitat requirements. Suitable habitat for *Polypodium* may have been patchy and restricted to the most humid parts of continuous forests of the past.

Conservation messages

Anthropogenic habitat fragmentation so far had no apparent effect on genetic diversity of an epiphyte restricted to forests compared to a widespread species thriving in secondary habitats. In determining the genetic effects of fragmentation, plant longevity, mating system and ability for dispersal play an important role (Hamrick and Godt 1996). Similar to tropical trees (Nason 2002), epiphytes seem to be more resistant to adverse genetic consequences of habitat fragmentation (e.g., Alcantara et al. 2006; Avila-Diaz and Oyama 2007; Murren 2003; but see González-Astorga et al. 2004) than terrestrial herbs. Most epiphytes are wind-dispersed (Benzing 1990), and especially epiphytic ferns and orchids with their dust-like diasporas can bridge even long distances between remnant forest patches, which efficiently reduces genetic isolation. As both study species are also rhizomatous and long-lived, genetic consequences of fragmentation may in fact be delayed and only visible after several generations (Kramer et al. 2008).

Not genetic but ecological consequences of habitat loss and forest fragmentation might be crucial (Kramer et al. 2008; Lande 1988) for the fate of *Polypodium* populations. Microclimatic conditions in secondary habitats (Scheffknecht et al. 2010) and forest fragment edges (Murcia 1995) are drier compared to the interior of undisturbed forests. Epiphytes growing in exposed positions in the crowns of forest trees like *Pleopeltis* are supposed to be better adapted to these conditions than shade-loving and drought-sensitive species such as *Polypodium* (Hietz 2010). If the availability of safe sites for recruitment declines, population sizes will decrease, eventually leading to Allee effects and demographic stochasticity (Lande 1988; Ouborg et al. 2006). Therefore, conservation efforts should not be limited to preserve as many forests as possible but should also aim at preventing the deterioration of these fragments.

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