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Diversity and Genetic Structure of the Mexican Endemic Epiphyte *Tillandsia achyrostachys* E. Morr. ex Baker var. *achyrostachys* (Bromeliaceae)

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• *Background and Aims* The monoecious, bird-pollinated epiphytic *Tillandsia achyrostachys* E. Morr. ex Baker var. *achyrostachys* is an endemic bromeliad of the tropical dry forests of Mexico with clonal growth. In the Sierra de Huautla Natural Reserve this species shows a host preference for *Bursera copallifera* (Sessé & Moc ex. DC) Bullock. As a result of deforestation in the study area, *B. copallifera* has become a rare tree species in the remaining forest patches. This human-induced disturbance has directly affected the population densities of *T. achyrostachys*. In this study the genetic consequences of habitat fragmentation were assessed by comparing the genetic diversity, gene flow and genetic differentiation in six populations of *T. achyrostachys* in the Sierra de Huautla Natural Reserve, Mexico.
• *Methods* Allozyme electrophoresis of sixteen loci (eleven polymorphic and five monomorphic) were used. The data were analysed with standard statistical approximations for obtaining diversity, genetic structure and gene flow.
• *Key Results* Genetic diversity and allelic richness were: $H_E = 0{\cdot}21 \pm 0{\cdot}02$, $A = 1{\cdot}86 \pm 0{\cdot}08$, respectively. *F*-statistics revealed a deficiency of heterozygous plants in all populations ($F_{it} = 0{\cdot}65 \pm 0{\cdot}02$ and $F_{is} = 0{\cdot}43 \pm 0{\cdot}06$). Significant genetic differentiation between populations was detected ($F_{st} = 0{\cdot}39 \pm 0{\cdot}07$). Average gene flow between pairs of populations was relatively low and had high variation ($Nm = 0{\cdot}46 \pm 0{\cdot}21$), which denotes a pattern of isolation by distance. The genetic structure of populations of *T. achyrostachys* suggests that habitat fragmentation has reduced allelic richness and genetic diversity, and increased significant genetic differentiation (by approx. 40 %) between populations.
• *Conclusions* The *F*-statistic values (>0) and the level of gene flow found suggest that habitat fragmentation has broken up the former population structure. In this context, it is proposed that the host trees of *T. achyrostachys* should be considered as a conservation priority, since they represent the limiting factor to bromeliad population growth and connectivity.

Key words: Allozymic electrophoresis, bromeliad, conservation genetics, genetic structure, gene flow, habitat fragmentation, tropical dry forest, Sierra de Huautla Natural Reserve Mexico.

INTRODUCTION

Genetic population analysis of tropical plant species has the potential to address a range of evolutionary and ecological questions. It allows the measurement of variation within and between species (Hamrick, 1994; Alvarez-Bullya *et al*., 1996; Hamrick and Godt, 1996*a*, *b*), the determination of phylogenetic relationships among species (Gitzendanner and Soltis, 2000; Cole, 2003), and the distribution of genetic variation in widespread populations (Loveless and Hamrick, 1984). The genetic structure of plant populations reflects the interactions of different processes, including the long-term evolutionary and short-term ecological history of the species, such as the effects of habitat fragmentation, shifts in distribution and population isolation. In addition, several factors may influence the genetic structure of plant populations. Among these, the more important are the breeding systems (Lande and Schemske, 1985) and seed dispersal mechanisms (Hamrick *et al*., 1995; Hamrick and Godt, 1996*b*), as well as the action of natural selection (Endler, 1986) and inbreeding at the microhabitat level (Lande and Shannon, 1996).

Reduction in population size due to habitat fragmentation may produce a loss of allelic richness or gene diversity (Lande, 1999). This can occur through population bottlenecks at the time of the disturbance and genetic drift afterwards (Barrett and Kohn, 1991; Ellstrand and Elam, 1993). Thus, the study of the genetic consequences of habitat fragmentation in plant populations has implications for species conservation (Young *et al*., 1996; González-Astorga and Núñez-Farfán, 2001; González-Astorga and Castillo-Campos, 2004).

Although 10 % of the world's vascular flora is epiphytic (Kress, 1986), population genetic data for epiphytes are very scarce. Only a few epiphyte ferns (Ranker, 1992; Hooper and Haufler, 1997), orchids (Ackerman and Ward, 1999; Tremblay and Ackerman, 2001) and bromeliads (Soltis *et al*., 1987, Izquierdo, 1995) have been studied genetically. Epiphytes represent an ideal model to study population genetic structure and genetics of speciation, because of their colonization habit, their patchy ecological and geographical distribution patterns, as well as their specific pollination strategies (Ackerman, 1986; Ackerman and Ward, 1999; Benzing, 2000). In epiphytes with host-specificity,

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the action of genetic drift and founder events could determine their genetic structure (Tremblay and Ackerman, 2001). In fact, at a biogeographical level, the patchy distribution of epiphytes has been suggested to be a force that induces isolation between populations and promotes fast species evolution. However, this will depend on how low are the levels of gene flow, and whether the population was founded by few individuals with low effective population size (Gentry and Dodson, 1987; Tremblay and Ackerman, 2001).

In this study, the impact was assessed of recent habitat fragmentation on diversity and genetic structure of an epiphyte plant species, *Tillandsia achyrostachys* var. *achyrostachys*, in the tropical dry forest of central Mexico. In the study area, *T. achyrostachys* shows a clear host preference, indicated by its greater abundance on a rare tree species (Martínez, 1999). Allozyme loci were used to examine the population genetics of *T. achyrostachys*. The objectives were: (1) to evaluate the genetic diversity in a fragmented landscape; (2) to determine the amount and distribution of genetic diversity within and among the six populations; (3) to estimate the gene flow between populations, and (4) to evaluate the conservation status of the species, given the actual disturbance conditions.

METHODS

Study site

The study was carried out at the Sierra de Huautla Natural Reserve, during 1998–99. The site is located in the State of Morelos (18°29–18°32′N, 99°05′–99°08′W) in central Mexico (Fig. 1). The original vegetation is tropical dry forest (*sensu* Rzedowski, 1978), which is now highly fragmented as a result of recent human activities (Trejo and Dirzo, 2000). The Reserve has 114 forest fragments, which comprise 31·5 % of the total area (Dorado *et al*., in press). Tropical dry forest physiognomy is produced by marked seasonal changes. Most plants lose their leaves for periods of 6–7 months during the dry season (September–March). The climate is warm sub-humid (the driest of the sub-humid climates according to García, 1988). The precipitation/temperature ratio is less than 43·2, and less than 5 % of the rainfall occurs in winter. Mean annual temperature and precipitation for the study site are 24·5 °C and 1039 mm, respectively (García, 1988).

Target species

Tillandsia achyrostachys var. *achyrostachys* (*T. achyrostachys* hereafter) is a Mexican endemic epiphyte (Smith and Downs, 1977), with flowers borne in a glabrous, shell-pink inflorescence, up to 10 cm long. The actinomorphic flowers are citron-coloured, 42 mm long, and only one flower at a time per inflorescence is produced. The breeding system is unknown; however, the flower anatomy suggests an outcrossing, bird-mediated mating system. The exerted pistil is longer than exerted stamens and the flowers appear to be suited for hummingbird pollination, which are the most common bromeliad pollinators (Gardner, 1986; Sazima *et al*., 1995, 1996; Cruz-Angón, pers. obs.).

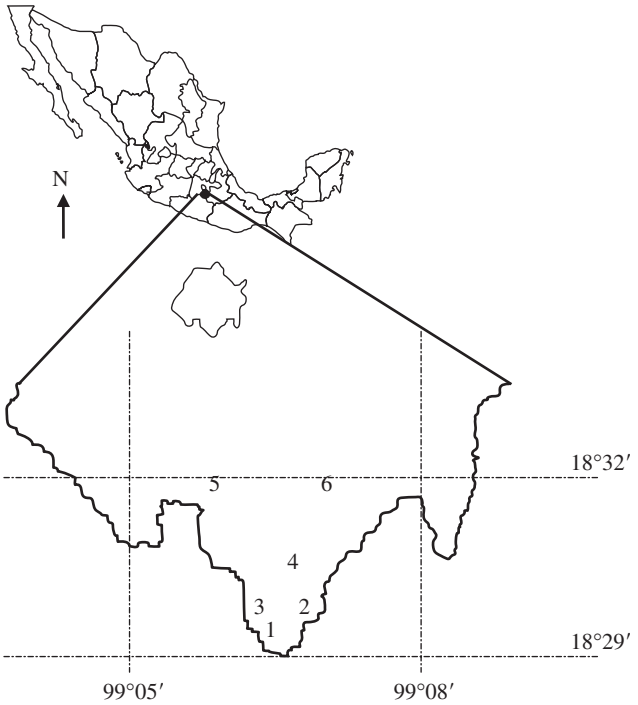

FIG. 1. Geographical distribution of *Tillandsia achyrostachys* in the Sierra de Huautla Natural Reserve, Morelos, Mexico. The numbers show the geographical position of each population evaluated.

In the study area, *T. achyrostachys* can be found in up to 16 different host tree species, but it shows a clear host preference (*sensu* Cornelissen and ter Steege, 1989) for *Bursera copallifera* ($\chi^2 = 1141{\cdot}33$, $P < 0{\cdot}0001$; Martínez, 1999). The *T. achyrostachys* individuals occur on 35·1 % of *B. copallifera* trees, which is a very rare species in the tropical dry forest of the Sierra de Huautla Natural Reserve. *Bursera copallifera* trees have a mean density (± SD) of 2 ± 4 trees ha^{-1}, while total forest tree density is 110 ± 81 trees ha^{-1}.

Sample collection

Tissue sampling was done in the only six populations of *Tillandsia achyrostachys* where the plant could be found in the Sierra de Huautla Natural Reserve, Morelos, Mexico. Distances ranging from 2·6 to 25·4 km separate populations. Fully expanded young leaves were collected from reproductive individuals in each population. To prevent the effect of sampling clonal growth, only one *T. achyrostachys* plant was sampled per individual host as recommended by Ranker (1992).

Electrophoretic procedure

To determinate the diversity and genetic structure of *T. achyrostachys* populations, allozyme variation was analysed using horizontal starch gel electrophoresis (12 % w/v) (Müller-Strack, 1998). Multilocus genotypes of 15–22 individuals from each population were obtained and allozymic variation was scored in 16 loci for each individual plant. The polymorphic loci were: malate-dehydrogenase

TABLE 1. *Allelic frequencies of 16 loci for six populations of bromeliad epiphyte* Tillandsia achyrostachys, *in the Sierra de Huautla Natural Reserve, Morelos, Mexico*

Pop	Allele	*Mdh1*	*Mdh2*	*Pgi*	*Pgm*	*Est1*	*Est2*	*Adh*	*Amp1*	*Amp2*	*G-6-pdh1*	*G-6-pdh2*	*Idh*	*Dia1*	*Dia2*	*Lap*	*Apx*
1	A	0·8000	0·5000	0·9667	0·9231	0·9333	0·6000	0·7857	0·6000	0·8667	0·9231	0·0333	1·0000	1·0000	1·0000	1·0000	1·0000
	B	0·0333	0·2500	0·0333	0·0769	0·0667	0·4000	0·2143	0·1000	0·1333	0·0769	0·7000	0·0000	0·0000	0·0000	0·0000	0·0000
	C	0·1667	0·2500						0·3000			0·2667					
2	A	0·3438	0·7667	0·5333	0·5938	0·9000	0·8571	0·0133	0·0333	0·9667	1·0000	0·0937	1·0000	1·0000	1·0000	1·0000	1·0000
	B	0·5625	0·2333	0·4667	0·4063	0·1000	0·1429	0·9687	0·3333	0·0333	0·0000	0·2813	0·0000	0·0000	0·0000	0·0000	0·0000
	C	0·0938	0·0000						0·9334			0·6250					
3	A	0·9063	0·6667	0·6563	0·5938	0·7813	0·7813	0·8667	0·0133	0·9667	0·9000	0·0333	1·0000	1·0000	1·0000	1·0000	1·0000
	B	0·0000	0·3333	0·3438	0·4062	0·2187	0·2187	0·1333	0·0625	0·0333	0·1000	0·1000	0·0000	0·0000	0·0000	0·0000	0·0000
	C	0·0937	0·0000						0·9062			0·8667					
4	A	0·0588	0·2353	0·7333	0·5333	0·5000	0·7353	0·7941	0·9063	0·9706	0·8667	0·0937	1·0000	1·0000	1·0000	1·0000	1·0000
	B	0·7059	0·5294	0·2667	0·4667	0·5000	0·2847	0·2059	0·0625	0·0294	0·1333	0·0313	0·0000	0·0000	0·0000	0·0000	0·0000
	C	0·2353	0·2353						0·0312			0·8750					
5	A	0·0294	0·6000	0·9706	0·8235	0·9118	0·2941	0·2353	0·0625	0·9687	0·1765	0·2059	1·0000	1·0000	1·0000	1·0000	1·0000
	B	0·0588	0·0667	0·0294	0·1765	0·0882	0·7059	0·7647	0·7500	0·0313	0·8235	0·6765	0·0000	0·0000	0·0000	0·0000	0·0000
	C	0·9118	0·3333						0·1875			0·1146					
6	A	0·5556	0·5000	0·5278	0·9444	0·3611	0·5294	0·0882	0·9412	0·0278	0·2647	0·0588	1·0000	1·0000	1·0000	1·0000	1·0000
	B	0·0000	0·1471	0·4722	0·0556	0·6389	0·4706	0·9118	0·0588	0·9722	0·7353	0·9412	0·0000	0·0000	0·0000	0·0000	0·0000
	C	0·4444	0·3529						0·0000			0·0000					
χ^2 observed		53·26*	25·55*	44·85*	43·19*	44·18*	34·56*	29·96*	43·81*	51·20*	40·01*	50·84*	–	–	–	–	–

*$P < 0·0001$.

(E.C. 1.1.1.37, loci *Mdh1* and *Mdh2*), phosphoglucoisomerase (E.C. 5.3.1.9, locus *Pgi*), phosphoglucomutase (E.C. 5.2.2, locus *Pgm*), esterase (E.C. 3.1.1, loci *Est1* and *Est1*), alcohol-dehydrogenase (E.C. 1.1.1.1. locus *Adh*), aminopeptidase (E.C. 3.4.11.1, loci *Amp1* and *Amp2*), and glucose-6-phosphate-dehydrogenase (E.C. 1.1.1.49, loci *G-6-pdh1* and *G-6-pdh 2*). The monomorphic loci were: isocitrate-dehydrogenase (E.C. 1.1.1.41, locus *Idh*), diaphorase (E.C. 1.6.99.-, loci *Dia1* and *Dia2*), leucine aminopeptidase (E.C. 3.4.11.1, locus *Lap*) and peroxidase anodic (E.C. 1.11.1.7, locus *Apx*). The enzymes were extracted from the young leaves using extraction buffer according to Wendel and Weeden (1989; i.e. Tris–HCl pH 7.5, sucrose, PVP-40, mercaptoethanol, ascorbic acid, diethyldithiocarbamate, bovine serum albumin, sodium metabisulphite and sodium tetraborate) and the resulting solution was absorbed onto filter paper wicks and stored at −70 °C until analyses. The buffers (gel and electrode) used were histidine to pH 5.7 and citric acid (Soltis *et al*., 1983, 1987). Electrophoresis was carried out at 4 °C over 8 h (constant current of 60 mA at 250 V).

Statistical methods

The data matrix of individual genotypes was analyzed using the TFPGA 1.3 program (Miller, 1997). Genotypic frequencies of each population were obtained and used to calculate observed mean heterozygosity (H_O) and allelic frequencies. The observed allelic frequencies for each population were used to estimate the mean number of alleles per locus (A), the average proportion of polymorphic loci (P), and expected mean heterozygosity (H_E), based on Hardy–Weinberg expectations (Hartl and Clark, 1997). Significance of estimators was obtained by Monte Carlo methods (Weir, 1990; Guo and Thompson, 1992).

To quantify levels of allelic variation within and among populations (population subdivision), F-statistics were calculated (Wright, 1965, 1978). To determine whether F_{is} and F_{it} estimations for each locus were significantly different from zero, Chi-square statistics [$\chi^2 = F(2N)(k - 1)$] were obtained, with $k(k - 1)/2$ degrees of freedom, where N is the sample size, and k the number of alleles (Weir, 1990). To determine the significance of the F_{st} statistic per locus, the Chi-square statistic was used: $\chi^2 = (2N)F_{st}(k - 1)$, with $(k - 1)(s - 1)$ degrees of freedom, where s is the number of populations sampled (Workman and Niswander, 1970). The F-statistic confidence intervals (at 95 %) were obtained by bootstrapping over loci for the multilocus estimate, and jackknifing over populations for the single-locus estimates (Weir and Cockerham, 1984; Weir, 1990). The average gene flow among populations (Nm) was estimated from F_{st} values, as $F_{st} = 1/(4\mathrm{Nm}a + 1)$, where $a = [n/(n - 1)]^2$ and n is the number of populations (Crow and Aoki, 1984). Nm is interpreted as the number of migrants per generation between two given populations and can be correlated with geographical distances (Slatkin, 1993, 1994). The significance of this relationship was evaluated through a Mantel's test (1967), because no other statistical methods can be used since Nm estimates are not independent (Smouse *et al*., 1986; Sokal and Rohlf, 1995).

RESULTS

Genetic diversity

Allelic frequencies for the sixteen loci (*Mdh*, *Est*, *Amp*, *G-6-pdh* and *Dia*, with two loci each; *Pgi*, *Pgm*, *Adh*, *Idh*, *Lap* and *Apx* with one locus each) scored for each individual plants are given in Table 1. The test for Hardy–Weinberg equilibrium of the entire data set indicated significant differences between a number of individuals for each observed and expected genotype (for polymorphic loci), as determined by a χ^2 test with one degree of freedom and $a = 0·0001$ (Table 1).

TABLE 2. *Levels of genetic variation of six populations of* Tillandsia achyrostachys *in the Sierra de Huautla Natural Reserve, Morelos, Mexico. A, mean number of alleles per locus; P, percentage of polymorphic loci; N_i, average sample size; H_O and H_E are the observed and expected mean heterozygosity, respectively*

Population	*A*	*P*	N_i	H_O	H_E
1	1·94	62·50	14·6	0·1764	0·2159
2	1·81	50·00	15·5	0·1178	0·1933
3	1·81	62·50	15·7	0·0880	0·1949
4	1·94	62·50	16·5	0·1112	0·2402
5	1·94	56·25	16·7	0·1678	0·2005
6	1·75	62·50	17·6	0·1007	0·2179
Mean (± SD)	1·86 ± 0·08	59·38 ± 5·23	16·1 ± 1·05	0·1270 ± 0·04	0·2105 ± 0·02

Mean number of alleles per locus was 1·86 ± 0·08. Percentage of polymorphic loci per population varied from 50 % (population 2) to 62·5 % (populations 1, 3, 4 and 6), with an overall mean of 59·4 % ± 5·2 %. Observed mean heterozygosity was 0·127 ± 0·04 (range 0·088–0·176). Similarly, expected mean heterozygosity was 0·210 ± 0·02 (range 0·195–0·218) (Table 2).

Genetic structure

The Wright's *F*-statistics, F_{it} and F_{is}, were positive and significantly different from zero for all polymorphic loci ($P < 0·05$) in all populations, indicating inbreeding (Table 3). In addition, all loci showed estimates that were positive and significantly different from zero F_{st} ($P < 0·05$). The means of the global F_{it} and local F_{is} inbreeding were 0·651 ± 0·021 and 0·433 ± 0·058, respectively, which suggests that genetic drift and inbreeding have been the dominant differentiating processes. The magnitude of genetic differentiation among populations indicated that on average approx. 40 % of *T. achyrostachys* genetic variation was due to differences between populations (Table 3).

TABLE 3. *Wright's F-statistics in six populations of* Tillandsia achyrostachys*, in the Sierra de Huautla Natural Reserve, Morelos, Mexico*

Loci	F_{it}	F_{st}	F_{is}
Mdh 1	0·641*	0·380*	0·422*
Mdh 2	0·666*	0·424*	0·420*
Pgi	0·648*	0·407*	0·407*
Pgm	0·649*	0·409*	0·406*
Est 1	0·650*	0·400*	0·414*
Est 2	0·655*	0·413*	0·413*
Adh	0·657*	0·374*	0·452*
Amp 1	0·655*	0·356*	0·464*
Amp 2	0·643*	0·361*	0·441*
G-6-pdh 1	0·648*	0·375*	0·437*
G-6-pdh 2	0·656*	0·383*	0·442*
Idh	0·651	0·389	0·429
Dia1	0·651	0·389	0·429
Dia2	0·651	0·389	0·429
Lap	0·651	0·389	0·429
Apx	0·651	0·389	0·429
Mean (± SD)	0·651 (0·021)*	0·391 (0·068)*	0·433 (0·058)*
Confidence interval to 95 %	0·614–0·689	0·255–0·505	0·313–0·531

*$P < 0·05$.

Gene flow

An average of 0·46 ± 0·21 migrant individuals per generation between pairs of populations was obtained (Table 4). The lowest estimate was obtained between populations 3 and 6 (Nm = 0·22) separated by approx. 25·4 km, and the highest was found between populations 1 and 3 (Nm = 0·94) separated by approx. 2·6 km. Correlations between Nm estimates and the geographical distance matrices between population pairs indicated that genetic differentiation has occurred as expected under an isolation-by-distance model, as demonstrated using the Mantel's test ($r = -0·74$; $P = 0·009$). Moreover, the relationship between gene flow and geographical distances between pairs of populations was negative and statistically significant ($\beta = -0·472$; $P = 0·002$; $R^2 = 52·8$ %) (Fig. 2).

TABLE 4. *Number of individual migrants per generation (Nm; above diagonal) and geographical distance (km; below diagonal) between pairs of populations of* Tillandsia achyrostachys*. The Mantel test indicated a significant relationship between Nm and geographical distances ($r = -0·74$; $P = 0·009$)*

Population	1	2	3	4	5	6
1	–	0·51	0·94	0·70	0·46	0·40
2	9·7	–	0·77	0·49	0·32	0·25
3	2·6	9·3	–	0·58	0·27	0·22
4	17·9	3·2	10·2	–	0·32	0·29
5	11·3	15·7	20·3	16·8	–	0·36
6	12·5	21·6	25·4	22·1	17·1	–

DISCUSSION

Genetic diversity

Tillandsia achyrostachys exhibits moderate diversity and genetic variability even though it has a restricted distribution in Mexican tropical dry forests. This is probably a consequence of habitat fragmentation. The results presented here suggest that the effect of inbreeding and genetic drift on genetic structure in *T. achyrostachys* might be counteracted by the influence of clonal growth. This growth helps to maintain *T. achyrostachys* genets and the genetic variability at both local and regional levels, as suggested for the

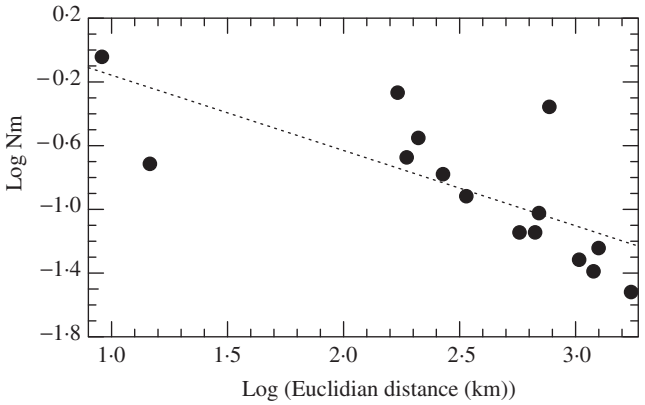

FIG. 2. Linear regression between individual migrants per generation (Nm) and Euclidian distances (*d*) among pairs of populations (both transformed to natural logarithms), for six populations of *Tillandsia achyrostachys*. (log(Nm) = 0·315 − 0·472 log*d*; $P = 0{\cdot}002$, $R^2 = 52{\cdot}8$ %).

saxicolous bromeliad *Pitcairnia geykesii* (Sarthou *et al*., 2001) and the terrestrial bromeliad *Chevaliera* (=*Aechmea*) *magdalenae* (Murawski and Hamrick, 1990). The percentage of polymorphic loci in *T. achyrostachys* was similar (59·4 %) to other values reported for several plant species of similar geographic range and mixed-animal breeding systems (52·9 % and 40 %, respectively; Hamrick and Godt 1996*a*). Mean expected heterozygosity within populations for *T. achyrostachys* was higher (0·21) than that reported for other plant species with similar distribution (0·15; Hamrick, 1994).

On the other hand, the results indicated higher levels of variability in *T. achyrostachys* when compared with those previously reported for three other bromeliad species, the epiphytes *Tillandsia ionantha* and *T. recurvata* (Soltis *et al*., 1987) and the terrestrial *Chevaliera magdalenae* (Murawski and Hamrick, 1990). However, we found similar results to those reported for the saxicolous bromeliad *Pitcairnia geyskesii* (Sarthou *et al*., 2001) and the terrestrial bromeliad *Ursulaea* (=*Aechmea*) *tuitensis* (Izquierdo and Piñero, 2000). The species studied by Soltis *et al*. (1987), Murawski and Hamrick (1990) and Izquierdo and Piñero (2000) had smaller population sizes than *T. achyrostachys*. Thus, the moderate genetic diversity and variability exhibited by *T. achyrostachys* is similar to those found in other bromeliad species.

Genetic structure and gene flow

The populations of *T. achyrostachys* show homogeneity in F_{st} values for all the analysed loci ($F_{st} = 0{\cdot}391 \pm 0{\cdot}068$), which may indicate that genetic drift and inbreeding are responsible for the deficiency of heterozygous individuals both at the population and sub-population level. Our results show that the percentage of variation among *T. achyrostachys* populations is larger than that reported for the outcrossing epiphytic bromeliad *T. ionantha* (0·043; Soltis *et al*., 1987), and also for the self-incompatible orchid *Tolumnia variegata* (0·060; Ackerman and Ward, 1999), and the outcrossing ferns *Adenophorus tamarisinus* (0·024), *A. tripinatifidus* (0·122), *Grammitis hookeri* (0·161), *G. tenella* (0·070; Ranker, 1992), *Pleopeltis astrolepis* (0·021), *P. complanata* (0·039), *P. crassinervata* (0·035), *P. polylepis* var. *polylepis* (0·069), and *P. wiesbaurii* (0·032; Hooper and Haufler, 1997) where sporophyte or seedling establishment is independent of host availability.

On the other hand, the F_{st} values found in the autogamous bromeliads *T. recurvata* (0·906; Soltis *et al*., 1987) and *Podaechmea* (=*Aechmea*) *mexicana* (0·56; Izquierdo, 1995) were greater than the F_{st} value of *T. achyrostachys* populations. This pattern is expected since autogamy promotes reproductive isolation, even in sympatric congeneric bromeliad species (Wendt *et al*., 2002). The F_{st} values we report for *T. achyrostachys* are similar to those reported for other three autogamous and terrestrial bromeliad species, *Chevaliera* (=*Aechmea*) *magdelaneae* (0·356; Murawski and Hamrick, 1990), *Pitcairnia geyskesii* (0·320; Sarthou *et al*., 2001) and *Podaechmea* (=*Aechmea*) *lueddemanina* (0·31, Izquierdo, 1995). Whilst in these three species autogamy and inefficient seed dispersal by birds could explain the population genetic structure, in the case of *Tillandsia achyrostachys* it is probably caused by outcrossing and long distance seed dispersal by wind, and so the F_{st} value should be correlated with host abundance (see below).

On average, gene flow was low between population pairs in *T. achyrostachys* ($Nm = 0{\cdot}46 \pm 0{\cdot}21$). This is consistent with the high genetic differentiation between populations and is evidence for a high host-dependence in the study area. Our results differ from those reported for epiphyte ferns (Ranker, 1992; Hooper and Haufler, 1997) and for 213 plant species with wind-dispersed seeds (Hamrick and Godt, 1996*a*). The species in the latter study were mostly annual and short-lived perennials, with no host-dependency (micro-habitat dependency) reported. However, epiphyte species depend upon host availability to develop populations. Thus, in order to increase the chances of exploiting hosts, we would expect epiphytes to develop both abundant and highly viable seeds (Callaway *et al*., 2002); otherwise, the population will depend on the abundance of an adequate host, as is the case for *T. achyrostachys* in the Sierra de Huautla Natural Reserve. Moreover, if tree hosts become scarce and distant from each other (as occurs with fragmentation), then isolation by distance should be expected, because populations would be founded by local seed-pools, not by regional ones. In accordance with this, Nm was negatively correlated to geographic distance. A similar pattern of isolation by distance has been found for the saxicolous/epiphyte *Podaechmea mexicana* (Izquierdo, 1995), and the epiphyte orchid *Tolumnia variegata* (Ackerman and Ward, 1999). Whilst the maximum distance between pairs of populations was 25 km for *T. achyrostachys*, the maximum distance for *T. variegata* was 1800 km (Ackerman and Ward, 1999), and 1748 km for *P. mexicana* (Izquierdo, 1995). Thus the populations of *T. achyrostachys* have the smallest geographical degree of isolation by distance reported for an epiphyte species (cf. Izquierdo, 1995; Ackerman and Ward, 1999).

Conservation implications

Habitat fragmentation reduces the sizes of a species' populations, increases isolation and modifies the abiotic

environment (Rathcke and Jules, 1993). As a direct outcome of this, genetic variation is reduced and interpopulation genetic divergence increases due to increased random genetic drift, elevated inbreeding and reduced gene flow (Young *et al*., 1996; Lande, 1999; González-Astorga and Núñez-Farfán, 2001; González-Astorga and Castillo-Campos, 2004).

In our study site, more than 60 % of the original forest area has been clear-cut (Trejo and Hernandez, 1996; Trejo and Dirzo, 2000; Dorado *et al*., in press) and the removal of trees associated with fragmentation reduces the colonization possibilities for any phorophyte-dependent species, thus affecting its population numbers. This phenomenon is more pronounced in *T. achyrostachys* because of its host preference. To date, all available information indicates that habitat fragmentation may also affect plant pollinators' abundance and diversity (Rathcke and Jules, 1993; Allen-Wardel *et al*., 1998; Kearns *et al*., 1998; Packer and Owen, 2001). Further research would clearly be desirable to assess the ecological consequences (e.g. effects on pollinator guild) of the decline in the population of *T. achyrostachys* at the study site.

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