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Genetic relationships and variation in reproductive strategies in four closely related bromeliads adapted to neotropical 'inselbergs': *Alcantarea glaziouana*, *A. regina*, *A. geniculata* and *A. imperialis* (Bromeliaceae)

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• *Background and Aims* Bromeliads (Bromeliaceae) adapted to rock outcrops or 'inselbergs' in neotropical rain forests have been identified as suitable plant models for studying population divergence and speciation during continental plant radiations. Little is known about genetic relationships and variation in reproductive strategies within and among inselberg-adapted species, yet knowledge of these parameters is important for understanding divergence processes and for conservation planning.
• *Methods* Nuclear microsatellites were used to assess the role of clonal reproduction, estimate genetic diversity and explore genetic relationships and variation in reproductive strategies for a total of 15 populations of four closely related *Alcantarea* inselberg species in south-eastern Brazil: *A. glaziouana*, *A. regina, A. geniculata* and *A. imperialis*.
• *Key Results* Clonal propagation is frequent in coastal populations of *A. glaziouana* and *A. regina*, but absent in the high-altitude species *A. geniculata* and *A. imperialis*. Considerable variation in clonal diversity, gene diversity (H_e), allelic richness, and Wright's inbreeding coefficient (F_{IS}) exists within and between species of *Alcantarea*. A Bayesian analysis of coastal inselberg species indicated pronounced genetic structure. A neighbor-joining analysis grouped populations of each species together with moderate bootstrap support, except for the high altitude species *A. imperialis*.
• *Conclusions* The coastal inselberg species *A. glaziouana* and *A. regina* tend to propagate asexually via vegetative clonal growth, and both reproductive strategies and breeding systems vary greatly between populations and species of *Alcantarea*. The microsatellite data indicate a history of hybridization and reticulation involving the high-altitude species *A. geniculata* and *A. imperialis* in areas of co-occurrence. The results highlight the need to understand similarities and differences in reproductive strategies both within and between related species for conservation planning and as a basis for understanding evolutionary processes in tropical radiations.

Key words: *Alcantarea*, Atlantic Rainforest, Bromeliaceae, clonality, gene flow, inselberg, microsatellites, reproductive strategy.

INTRODUCTION

Integrating genetics into the ecological theory of adaptive radiation has become an important task for evolutionary biologists in recent years (Schluter, 2000), and research on rapidly radiating plant lineages has played a crucial role in working towards this goal (Givnish *et al.*, 1997; Barrier *et al.*, 2001; review by Seehausen, 2004). The proven usefulness of oceanic islands as model systems for studies of species' radiations (Darwin, 1859; MacArthur and Wilson, 1967; Emerson, 2002; Stuessy *et al.*, 2006) provides concepts and tools that may also be applicable to evolutionary study of continental radiations (Porembski and Barthlott, 2000). Indeed, a growing number of studies explore plant species' evolution in archipelagos of 'terrestrial islands',

e.g. in continental mountain ranges (Schönswetter *et al.*, 2005; Hughes and Eastwood, 2006;) or other insular types of habitats (Givnish *et al.*, 1997; DeChaine and Martin, 2005). So far, most population genetic or phylogeographic studies of this type have focused on inferring historical range expansions and contractions associated with paleoclimatic cycles (e.g. Dechaine and Martin, 2005; Schönswetter *et al.*, 2005). Nevertheless, it is clear that terrestrial island archipelagos may also represent suitable models for studying ecological speciation during adaptive radiations, especially where populations are distributed across a range of different environments in an island-like manner (MacArthur and Wilson, 1967; Schluter, 2000).

Knowledge of natural variation in breeding systems and reproductive strategies within and between closely related species can help interpret the origin and maintenance of barriers to gene flow during speciation and species' radiations (Stebbins,

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1957; Wendt *et al.*, 2001, 2002; Coyne and Orr, 2004). The breeding system of a plant will determine how much variation is available to natural selection during bouts of ecological change and/or speciation (Seehausen, 2004). The breeding system will also have profound consequences for the build-up and maintenance of reproductive barriers during speciation (Stebbins, 1957), with obligate or facultative apomicts at one end of the continuum, and highly outcrossing, wind-pollinated species at the other. Two topics have aroused particularly great interest in the context of variation in breeding systems and reproductive strategies in recent years: the genetics of self-incompatibility (SI) in plants with mixed or outcrossing breeding systems, and the population dynamics of taxa with mixed sexual and asexual reproductive modes (Silvertown, 2008).

With respect to the population dynamics of mixed sexual/asexual taxa, new molecular and statistical tools to study clonal structure have led to a recent surge of studies on this topic (Arnaud-Haond *et al.*, 2008). These studies have focused on the maintenance of genetic diversity in species with mixed sexual and asexual reproduction, the role of environmental effects and intraspecific competition in shaping clonal structure, and the role of asexuality in plant invasions (Chapman *et al.*, 2004; Scheepens *et al.*, 2007; recent reviews by Arnaud-Haond *et al.*, 2008 and Silvertown, 2008). Despite the known importance of differences in breeding systems and reproductive strategies for population divergence and speciation alluded to above, few studies have addressed these topics for taxa that form part of well-described adaptive radiations (but see Whitkus *et al.*, 2000).

Bromeliaceae is a well characterized example of an adaptive radiation in the neotropics (Benzing, 2000; Barfuss *et al.*, 2005), comprising approx. 56 genera and 3000 species (Luther, 2004). Numerous adaptations and putative key innovations have been described for the family and have been the focus of much interest from botanists and evolutionary biologists, e.g. epiphytic and rupicolous growth habits (life on trees and bare rocks), the tank-habit (rosettes forming a tank-like structure able to hold large amounts of water), crassulacean acid metabolism (CAM), and several specialized strategies of nutrient uptake in nutrient-poor environments (Givnish *et al.*, 1997; Benzing, 2000; Crayn *et al.*, 2004). Systematic studies have been carried out on the family (e.g. Brown and Gilmartin, 1989; Givnish *et al.*, 1997; Smith and Till, 1999) including a recent molecular phylogeny of Tillandsioideae (Barfuss *et al.*, 2005), the subfamily that includes the species studied here. Population genetic studies are available only for a small number of bromeliads, e.g. for species of *Tillandsia* (Soltis *et al.*, 1987; Gonzalez-Astorga *et al.*, 2004), *Aechmea* (Murawski and Hamrick, 1990; Izquierdo and Pinero, 2000), *Pitcairnia* (Sarthou *et al.*, 2001), *Puya* (Sgorbati *et al.*, 2004) and *Encholirium* (Cavallari *et al.*, 2006). Only one of these was focused on rock-adapted species, namely that on *Pitcairnia geyskesii* from rock outcrops in French Guiana (Sarthou *et al.*, 2001).

Recently, we have begun to investigate within- and between-species patterns of genetic variability and gene flow in *Vriesea* and *Alcantarea*, two genera that have radiated into multiple species adapted to epiphytic life in continuous forest or rupicolous life on inselberg rock outcrops in the South American Atlantic Rainforest (Palma-Silva *et al.*, 2007; Barbará *et al.*, 2007). The 22 species of the genus *Alcantarea* are endemic to south-eastern Brazil, where they occur from 0–1900 m

above see level on inselbergs of the Atlantic Rainforest or in Campo Rupestre (rocky field) vegetation of the Espinhaço mountain range (Versieux and Wanderley, 2007*b*). *Alcantarea* is phylogenetically and morphologicaly closely related to *Vriesea* (Grant, 1995; Barfuss *et al.*, 2005) from which it is morphologically distinguished by its long spirales-cent petals and seeds with both basal and apical comas (Grant, 1995). Ongoing research on *Alcantarea* has lead to the description of three new species (Versieux and Wanderley, 2007*a*, *b*) and has confirmed the identity of the four species included in the present study. *Alcantarea regina* is now thought to cover a wider range than previously suspected, as it was found to be synonymous with another *Alcantarea* species, *A. edmundoi* (Versieux and Wanderley, 2007*c*).

The inselbergs of south-eastern Brazil (from the German *Insel* = island and *Berg* = mountain) are ancient granitic rock outcrops embedded within a matrix of tropical forest (Safford and Martinelli, 2000) which exhibit much of the 'insular' nature alluded to at the beginning of this paper. Population genetic work on two high-altitude inselberg species in this group, *Alcantarea imperialis* and *A. geniculata*, found no evidence of isolation by distance, high levels of population differentiation, and extremely low amounts of historical gene flow ($N_e m < 1$ for most pairs of populations), despite the predominantly outcrossing breeding systems of these animal-pollinated, wind-dispersed plants (Barbará *et al.*, 2007). These findings are in agreement with the long-standing hypothesis that 'insel-bergs' may resemble oceanic islands in their effects on patterns of variability and gene flow (Porembski and Barthlott, 2000).

Here, this earlier work is extended to two coastal inselberg species endemic to the same study area in the Brazilian Atlantic Rainforest, *Alcantarea glaziouana* and *A. regina*. To our knowledge, these two taxa have not previously been studied with any type of molecular genetic marker. Data are presented on patterns of genetic diversity and gene flow in coastal inselberg populations of these species, and we comprehensively analyse population and species relationships and compare variation in reproductive strategies across all four *Alcantarea* species studied to date, including the two high-altitude taxa studied previously, *A. imperialis* and *A. geniculata*. In particular, we ask the following questions. (1) How much variation in basic genetic parameters, such as clonal diversity, gene diversity (H_e), allelic richness and Wright's inbreeding coefficient (F_{IS}), is there within and between these four *Alcantarea* inselberg species, and what are the mechanisms responsible for variation in reproductive strategies among populations and species? (2) Do populations of each species group together when analysed in a phylogenetic context, and what is the likely biological significance of departures from congruence between the genetic data and traditional taxonomic groupings? (3) How weak or strong are genetic structures in populations of the two coastal species, and what are the practical implications for conservation?

MATERIALS AND METHODS

Population sampling

A total of 15 populations of *Alcantarea glaziouana*, *A. regina*, *A. geniculata* and *A. imperialis* were sampled on coastal

(0–20 m elevation) and high-altitude (900–1300 m elevation) granitic inselbergs located in the Atlantic Rainforest of south-eastern Brazil (states of Rio de Janeiro and Minas Gerais; Fig. 1). Of these, data for seven coastal inselberg populations of *A. glaziouana* and *A. regina* are reported for the first time here. For *A. glaziouana*, the entire species range was sampled in this study. For *A. regina*, the only two previously described populations were included (Martinelli, 1994). Ongoing taxonomic revision indicates that the distribution range of *A. regina* is likely to be larger than previously assumed – another *Alcantarea* species has recently been found to be synonymous with *A. regina* (Versieux and Wanderley, 2007*c*). Nevertheless, the type specimen for *A. regina* is from exactly the same locality that was sampled in the present study (Parati, RJ, Brazil). *Alcantarea imperialis* and *A. geniculata* were sampled range-wide except for one locality per species, approx. 20 km west of population IJF in the case of *A. imperialis* and a few kilometres east of population GIM in the case of *A. regina*. The names, abbreviations and geographical co-ordinates of the sampled coastal inselberg populations are as follows: *A. glaziouana*: Glaziouana 'Niterói' or LNI (S22°58·617′, W43°2·825′), Glaziouana 'Pão-de-Açúcar' or LPA (S22°57·064′, W43°09·092′), Glaziouana 'Pedra de Itaúna' or LPI (S23°00·223′, W43°25·338′), Glaziouana 'Pedra do Pontal' or LPP (S23°2·105′, W43°28·247′) and 'Glaziouana 'Prainha' or LPR (S23°2·376′, W043°30·258′); *A. regina*: Regina 'Parati 1' or RP1 (S23°13·369′, W44°37·616′) and Regina 'Parati 2' or RP2 (S23°14·071′, W44°37·208′) (Fig. 1). The geographic distances between

populations of the narrow endemic *A. glaziouana* ranged from 3·5 to 47 km, whereas the two well-referenced coastal populations of *A. regina* were separated by 1·5 km. The sample sizes for all populations are given in Table 1. For each plant, leaf material for DNA extraction was collected and stored in silica gel.

Eight populations of the high-altitude inselberg species *A. geniculata* and *A. imperialis* described previously (Barbará *et al.*, 2007) were included here to facilitate comparisons among high-altitude and coastal species (Fig. 1). Their names and abbreviations are as follows: Imperialis 'Irmã Menor' or IIM, Imperialis 'Macaé-de-Cima' or IMC, Imperialis 'Juíz-de-Fora' or IJF and Imperialis 'Vale das Videiras' or IVV; Geniculata 'Irmã Menor' or GIM, Geniculata 'Ricardo's Clearing' or GRC, Geniculata 'Ricardo's Rock' or GRR and Geniculata 'Reserva Privada' or GRP. In addition, two populations of *Vriesea gigantea* from the Atlantic Rainforest of Brazil (VG1, Florianópolis, Brazil; VG2, Santa Leopoldina, Brazil) were used for rooting the population tree of *Alcantarea* species.

Molecular markers and genotyping assays

The eight microsatellite markers used in this study (Table 2) were isolated from *Alcantarea imperialis* (Palma-Silva *et al.*, 2007) or developed for the related bromeliad genera *Tillandsia*, *Guzmania* (Boneh *et al.*, 2003) and *Pitcairnia* (Sarthou *et al.*, 2003). Repeat types and molecular size ranges for all eight markers in the coastal inselberg species
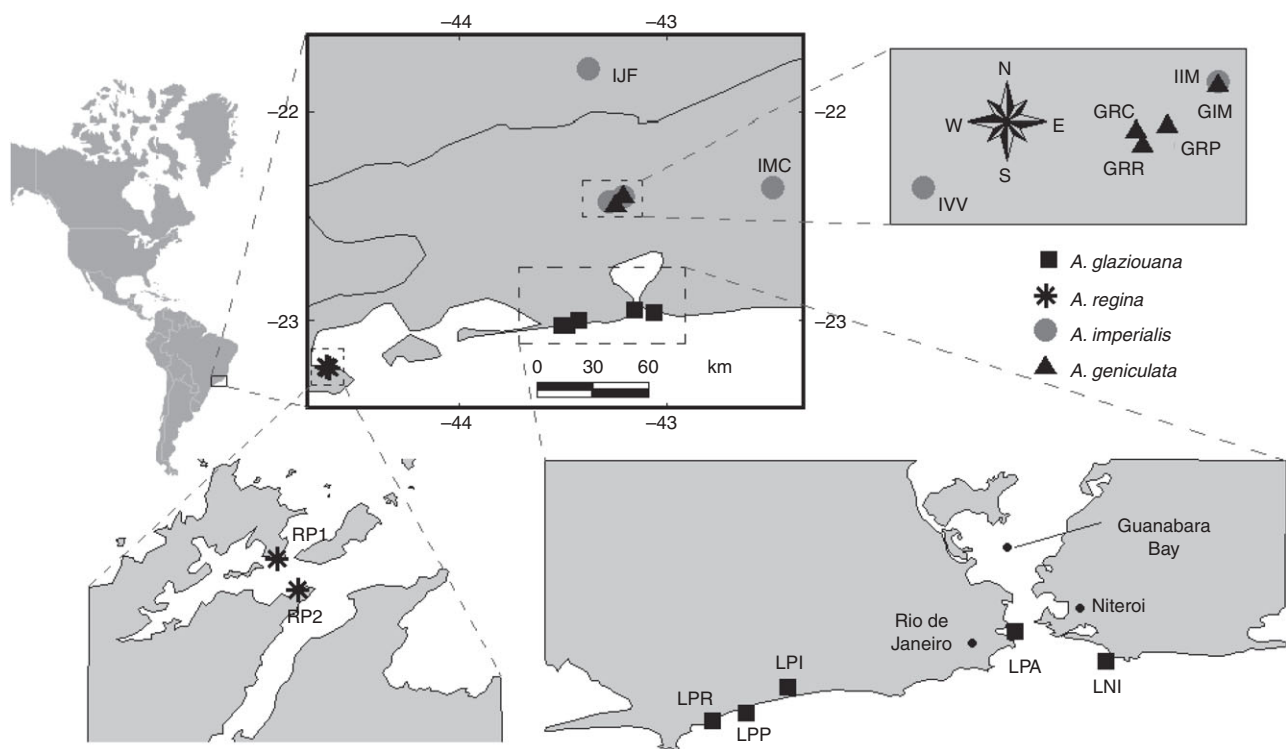

FIG. 1. Distribution map of *Alcantarea* inselberg populations sampled in the Atlantic Rainforest of Brazil. The present study is focused on the two coastal inselberg species seen at the bottom of the figure, *A. glaziouana* and *A. regina*. Data for the high-altitude inselberg species *A. imperialis* and *A. geniculata* were presented in detail elsewhere (Barbará *et al.*, 2007) and are only used for comparative purposes here. For population abbreviations and details see Materials and Methods.

TABLE 1. *Characterization of microsatellite markers in 'inselberg' populations of* Alcantarea glaziouana *and* A. regina, *including marker source, repeat type, molecular size range in each species in basepairs (bp), and the following diversity and breeding system parameters calculated at the genet level (clones removed): number of alleles (A), expected (H_e) and observed (H_o) heterozygosity in each species, within-population inbreeding coefficient F_{IS} and total-population inbreeding coefficient F_{IT}*

		A. glaziouana						*A. regina*					
Locus	Repeat type	Size range (bp)	A	H_e	H_o	F_{IS}	F_{IT}	Size range (bp)	A	H_e	H_o	F_{IS}	F_{IT}
Ai4.10[1]	di-	186–189	3	0·364	0·257	+0·229*	+0·312	184–188	3	0·633	0·705	−0·149	−0·080
E19[2]	di-	111–119	4	0·273	0·102	+0·562****	+0·644	111–123	4	0·244	0·227	+0·040	+0·096
E6[2]	tri-	106–166	13	0·820	0·419	+0·362****	+0·520	108	1	–	–	–	–
Ai4.3[1]	di-	185–191	2	0·019	0	+1·00****	+1·00	191–195	3	0·317	0·341	−0·104	−0·052
CT5[2]	di-	161–199	17	0·871	0·644	+0·151***	+0·290	165–189	10	0·887	0·727	+0·118	+0·237
E6b[2]	tri-	131–143	3	0·364	0·308	+0·060	+0·181	131–143	4	0·715	0·682	−0·193	+0·209
P2p19[2]	tri-	194–205	4	0·548	0·381	+0·147	+0·343	199–214	5	0·758	0·682	+0·007	+0·180
Pit8[3]	di-	282–305	4	0·521	0·281	+0·230*	+0·510	268–308	5	0·632	0·512	−0·027	+0·340

Microsatellite markers: [1]isolated from *A. imperialis* in the Jodrell Laboratory at RBG Kew (Palma-Silva *et al.* 2007); [2]isolated by Boneh *et al.* (2003); [3]isolated by Sarthou *et al.* (2003).

Departures of within-population inbreeding coefficients (F_{IS}) from HWE are indicated as follows: * $P < 0.05$, *** $P < 0.005$, **** $P < 0.001$.

TABLE 2. *Characterization of coastal 'inselberg' populations of* Alcantarea glaziouana *and* A. regina *with eight nuclear microsatellite markers, including sample size, genotypic diversity (G/N) and Simpson's index to document clonal diversity, and the following diversity and breeding system parameters calculated at the genet level: variance in allele size (Var), allelic richness, expected (H_e) and observed (H_o) heterozygosity, and within-population inbreeding coefficients F_{IS}*

Species	Population	n	G/N	Simpson's D	Var	Allelic richness	H_e	H_o	F_{IS}
A. glaziouana	LPR	11	0·22	0·6	0·36	1·21	0·085	0·094	−0·125
	LPP	68	0·42	0·94	22·2	2·23	0·440	0·404	+0·083*
	LPI	29	0·81	0·98	9·52	1·66	0·221	0·178	+0·197*
	LPA	31	0·83	0·97	38·2	2·62	0·557	0·338	+0·397***
	LNI	31	0·42	0·77	12·4	2·07	0·365	0·283	+0·231*
	Overall:	170	0·55	0·98	23·53	6·13	0·472	0·299	–
A. regina	RP1	26	1	1	26·2	3·514	0·505	0·505	+0·001
	RP2	29	0·59	0·96	15·1	3·478	0·412	0·453	−0·104
	Overall:	55	0·79	0·99	25·7	4·369	0·523	0·484	–
A. imperialis[1]	Overall:	124	1	1	27·96	6·250	0·615	0·362	–
A. geniculata[1]	Overall:	84	1	1	23·84	5·250	0·429	0·357	–

[1] Overall values for *A. imperialis* and *A. geniculata* from Barbará *et al.* (2007) are shown for comparative purposes.

Departures from Hardy–Weinberg equilibrium are indicated as follows: * $P < 0.05$, *** $P < 0.005$.

A. glaziouana and *A. regina* are given in Table 2. We chose to use the same set of markers also used for our recent study of the high-altitude species *A. imperialis* and *A. geniculata* (Barbará *et al.*, 2007). This was done to facilitate direct comparisons among coastal and high-altitude populations and species and to be able to combine the datasets for construction of a phylogenetic tree of all populations.

For molecular genotyping, total genomic DNA was extracted from silica-gel-dried leaves using a modified approach based on Doyle and Doyle (1987), and DNA was quantified using an Eppendorf BIO photometer. The eight nuclear microsatellites were amplified using polymerase chain reaction (PCR) as in Palma-Silva *et al.* (2007), making use of a standard touchdown cycling program with an annealing temperature (T_a) of 48°C and either FAM or JOE labelled forward primers, or a three-primer protocol including unlabelled M13-tagged forward and unlabelled/untagged reverse primers for each marker and a third 'universal' M13-primer labelled with one of the fluorescent dyes, FAM or JOE (Applied Biosystems, Foster City, CA, USA).

Microsatellite genotypes were resolved on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems), making use of the different fluorescent dyes for duplexing. Molecular sizes in base pairs were determined using the GENESCAN-500 ROX size standard (Applied Biosystems), and result files from the sequencers were analysed using GENESCAN and GENOTYPER software (Applied Biosystems).

Data analysis

Detection of clones and identification of genets for subsequent analysis. Because clonal reproduction had previously been suspected in coastal *Alcantarea* inselberg populations (Martinelli, 1994), we checked for the presence of identical genotypes in all populations of *A. regina* and *A. glaziouana*. Individuals with identical genotypes were identified using a simple neighbor-joining (NJ) tree in Phylip (Felsenstein, 2004) based on the proportion of shared alleles between individuals (Bowcock *et al.*, 1994) and later confirmed using the GENCLONE software (Arnaud-Haond and Belkhir, 2007).

In order to explore the power of our marker loci for detecting clones we calculated the probability of identity, P_{ID} (Waits *et al.*, 2001), for each locus in *A. regina* and *A. glaziouana* using the GIMLET software (Valière, 2002) and the probability of clonal identity, P_{sex}, using GENCLONE (Arnaud-Haond and Belkhir, 2007). In addition, all samples with identical genotypes were checked by eye for their geographic location using maps produced by GIS software and GPS co-ordinates of sampled specimens. This was done to check whether identical genotypes aggregated in patches as expected for vegetatively derived clonal copies of a genet. The detection of clones allowed us to focus all subsequent analyses at the genet level.

Genetic diversity of the sampled loci and populations. In order to characterize the microsatellite loci in coastal inselberg populations of *A. glaziouana* and *A. regina*, the number of alleles (A), expected heterozygosity (H_e), observed heterozygosity (H_o), within-population inbreeding coefficient (F_{IS}) and total population inbreeding coefficient (F_{IT}) were calculated at the genet level (clones removed) for each locus using the computer programs MSA (Dieringer and Schlötterer, 2003) and FSTAT (Goudet, 1995). In addition, within each population, each locus was tested for departure from Hardy–Weinberg equilibrium (HWE) using FSTAT. This was done to explore whether departures from HWE are due to locus-specific effects or rather due to systematic factors, i.e. deviations from random mating of the sampled genets. We note that the absence of null-alleles for these loci in *Alcantarea* spp. has been established previously (Barbará *et al.*, 2007; Palma-Silva *et al.*, 2007).

Subsequently, each coastal inselberg population was characterized using clonal diversity (G/N) and Simpson's diversity index calculated with Genclone (Arnaud-Haond and Belkhir, 2007) and the following diversity parameters at the genet level (clones removed): variance in allele size (Var), H_e and H_o calculated by MSA and allelic richness in FSTAT (Goudet, 1995). All of these genetic diversity parameters were corrected for sample size in MSA and FSTAT. Departures from HWE for each population at the genet level were identified using exact tests in GENEPOP (Raymond and Rousset, 1995).

Tests of basic assumptions for interpreting F-*statistics in terms of historical gene flow.* In order to determine if the sampled coastal inselberg populations are likely to meet the equilibrium conditions required for the interpretation of *F*-statistics in terms of historical gene flow, the possibility of founder effects due to recent colonization (bottlenecks) was tested using the 'sign test' and 'Wilcoxon sign-rank' test in the BOTTLENECK program (Piry *et al.*, 1999). Both tests are able to detect recent reductions in effective population size due to genetic bottlenecks. The analyses were carried out both for the 'infinite allele model' (IAM) and for the 'two-phased mutation model' (TPM) recommended for microsatellites in the user manual. The detection of recent bottlenecks may indicate that *Alcantarea* inselberg populations are not in an equilibrium between gene flow and genetic drift, which would render the indirect estimation of gene flow via F_{ST} difficult (Whitlock, 1992). In many plant species, equilibrium between migration and drift can also be tested by studying correlations between genetic and geographic distance ('isolation by distance'; Slatkin, 1993). This test is not suitable for the species studied here, since isolation by distance is not necessarily expected for species with island-like distribution patterns, including species on inselbergs (Barbará *et al.*, 2007). The absence of isolation by distance in the coastal inselberg species *A. glaziouana* was established using non-parametric Mantel tests in FSTAT with 10 000 randomizations. This test could not be carried out for *A. regina* as only two populations were available.

Indirect analysis of gene flow via F-*statistics and population phylogeny.* Analysis of molecular variance (AMOVA) in GENALEX (Peakall and Smouse, 2006) was used to obtain *F*-statistics for microsatellites at different hierarchical levels using the genet-level data. We tested the hierarchies 'among species', 'among populations within species' and 'within populations' for the entire dataset. Subsequently, separate AMOVA models were analysed to test the distribution of genetic variance among and within populations of each species. The significance of each *F*-statistic was tested through 9999 permutations in GENALEX at the appropriate hierarchical level. In addition, F_{ST} was also estimated for pairs of coastal *Alcantarea* inselberg populations and gene flow (N_em) between pairs of populations was estimated from F_{ST} as $(1/F_{ST} -1) \times 0.25$ under the assumption of migration-drift equilibrium.

In order to depict relationships between populations and species in a graphical way, a neighbor-joining (NJ) tree was constructed based on Nei's (1987) genetic distance. One thousand bootstrap replicates of the distance matrix were obtained in MSA, and NJ trees were generated and analysed in PHYLIP 3·6 (Felsenstein, 2004). To gain a more complete picture of genetic relationships among *Alcantarea* inselberg populations and species, eight previously described (Barbará *et al.*, 2007) populations of the high-altitude species *A. imperialis* and *A. geniculata* were added to the dataset. In addition, two populations of *Vriesea gigantea* were included for comparative purposes.

Bayesian genetic structure analysis. Bayesian analysis in STRUCTURE v2 (Pritchard *et al.*, 2000) was used to obtain insights into patterns of gene flow and population subdivision within coastal *Alcantarea* inselberg populations. Our aim was to determine the most likely number of populations (K) for each species, and to estimate admixture proportions (Q) for individuals of each population. A 'burn-in' period of 5×10^4 iterations and data collection for 10^6 iterations were identified as being appropriate based on the diagnostic tools available in STRUCTURE. The analyses were carried out under the admixture model for independent allele frequencies. All possible models from $K = 1$ to $K = 10$ were evaluated for *A. glaziouana* and all models from $K = 1$ to $K = 7$ for *A. regina*, based on the natural logarithm of their probability and on their variances. Individual admixture proportions (Q) for each sampled population in each genetic cluster found by STRUCTURE were recorded for the model that best explained the data. The STRUCTURE results were subsequently confirmed by analysis with BAPS (Corander *et al.*, 2004) without the use of baseline information for population origin. In contrast to STRUCTURE, BAPS estimates K and

Q separately, which may increase precision of parameter estimation in some situations. One hundred iterations, 200 reference individuals and 20 iterations for reference individuals were used in the admixture analysis, following the software manual.

RESULTS

Information content of microsatellite loci in coastal Alcantarea inselberg populations

All eight microsatellite loci were polymorphic in coastal *Alcantarea* inselberg populations, with up to 17 alleles per locus in *A. glaziouana* and up to ten alleles per locus in *A. regina* (Table 2). One locus, *E6*, was fixed for a single allele in *A. regina* but was polymorphic in *A. glaziouana*. The combined probability of identity (P_{ID}) for the eight microsatellite loci was $8 \cdot 44 \times 10^{-6}$ for *A. regina* and $2 \cdot 32 \times 10^{-5}$ for *A. glaziouana*, thus indicating a low probability that two unrelated individuals drawn from each of these two species' gene pools will share the same multi-locus genotype. Likewise, the probability of a particular genotype being present more than once as a result of sexual reproduction (as opposed to being part of the same genetic individual), P_{sex}, was on average $2 \cdot 10 \times 10^{-3}$ for *A. glaziouana* and $6 \cdot 0 \times 10^{-4}$ for *A. regina*. Based on these values it was considered appropriate to use the microsatellite genotype data for the identification of clones for subsequent analysis.

Clonal reproduction, genetic diversity, and variation in reproductive strategies in coastal Alcantarea inselberg populations

Inspection of microsatellite genotypes using individual genetic distances and GENCLONE revealed the presence of clones in all populations of *A. glaziouana* and in one population of *A. regina*, resulting in values for clonal diversity (*G*/*N*) and Simpson's diversity lower than 1 for these populations (Table 1). Individual genets comprised up to 12 ramets in *A. glaziouana* and up to three ramets in *A. regina*. Graphical inspection of geo-referenced, mapped individuals showed that ramets (clonal copies) were always spatially clustered, as expected if identical genotypes are due to vegetative clonal reproduction (Fig. 2). The largest distance observed between plants belonging to the same genet (= the same clone) was 9 m (rosettes of individual ramets typically have a diameter of up to 1 m). A detailed fine-scale spatial genetic structure analysis of all *Alcantarea* inselberg populations was presented previously (Barbará *et al.*, 2008). Here, identification of clones allowed us to assess variation in reproductive strategies and to focus our analysis of genetic diversity and structure at the genet level.

Genetic diversity evaluated over all loci at the genet level was generally higher in populations of *A. regina* than in *A. glaziouana* when estimated via the variance in allele size (Var), expected (H_e) or observed heterozygosity (H_o; Table 1), and only allelic richness was higher in *A. glaziouana*. The data facilitate a graphical comparison of genetic diversity (H_e, allelic richness, clonal diversity) and inbreeding coefficients (F_{IS}) among populations of all four
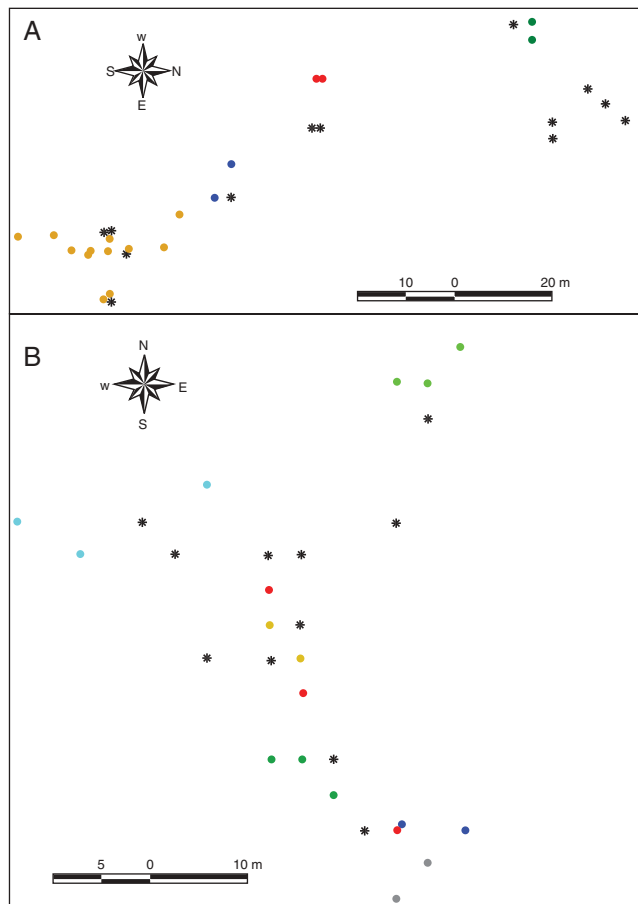

FIG. 2. Spatial distribution of clones (genets) in two coastal inselberg populations of *Alcantarea*: (A) population LNI of *A. glaziouana*; (B) population RP2 of *A. regina*. In each graph, colours indicate different clones as identified with eight nuclear microsatellite markers, and stars indicate plants that had unique genotypes for these markers. The two graphs exemplify the spatial clustering of plants belonging to the same clone in populations of these species, which supports the hypothesis of asexual reproduction via vegetative clonal growth rather than agamospermy.

Alcantarea species studied to date, *A. glaziouana*, *A. regina*, *A. geniculata* and *A. imperialis* (Fig. 3), which allows us to discuss variation in breeding systems and reproductive strategies.

Tests for departure from Hardy-Weinberg equilibrium (HWE) at the genet level indicated significant heterozygote deficits (= significantly positive F_{IS}) for most loci and populations in *A. glaziouana*, whereas in *A. regina* no significant departure from HWE was found (Tables 1 and 2). Total-population inbreeding coefficients F_{IT}, included for completeness in Table 2, were positive for all loci in *A. glaziouana* and for most loci in *A. regina*, indicating species-level homozygote excess due to population subdivision.

Neither the sign test nor the Wilcoxon sign-rank test for recent population bottlenecks was significant for any of the populations, regardless of the mutation model used (data not shown), which allows us to interpret population differentiation (F_{ST}) in terms of inter-population gene flow. The Mantel correlation between geographic and genetic distance was not
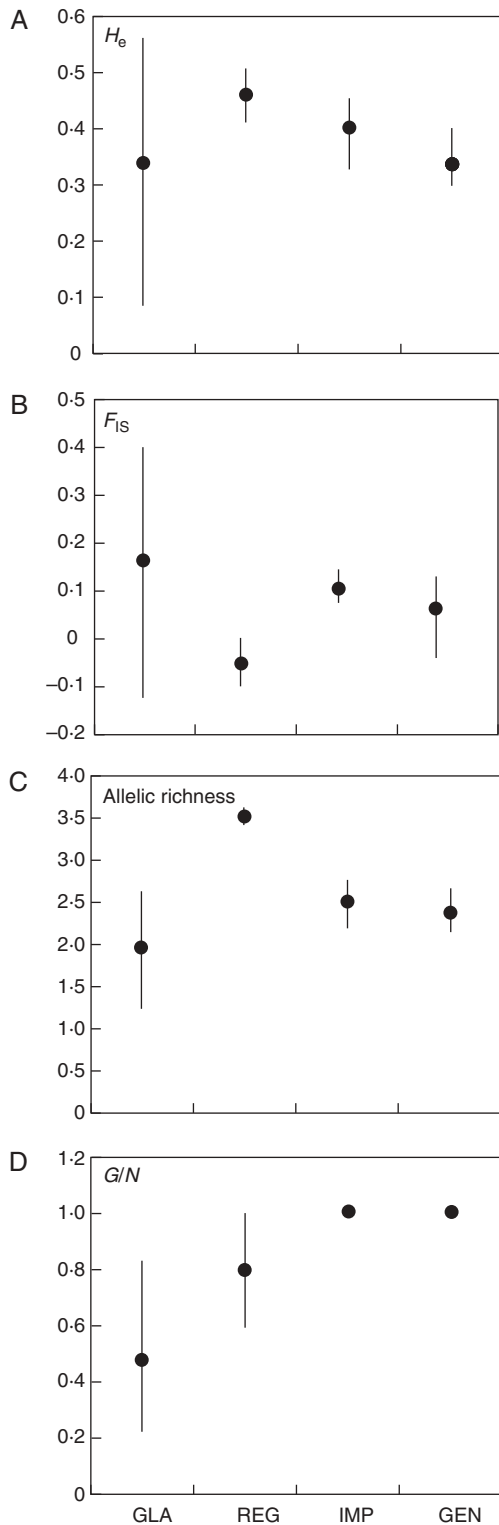
FIG. 3. Comparative analysis of genetic diversity and breeding system parameters in four *Alcantarea* inselberg species, *A. glaziouana*, *A. regina*, *A. imperialis* and *A. geniculata*. (A) Expected heterozygosity (H_e); (B) within-population inbreeding coefficient (F_{IS}); (C) allelic richness, (D) genotypic diversity (G/N). Data are the averages and ranges of genetic parameter estimates across populations of each species. The increased range of F_{IS}, H_e, and allelic richness across populations of *A. glaziouana*, and the total absence of clonal structure in *A. imperialis* and *A. geniculata*, are clearly visible.

significant (data not shown), thus suggesting the absence of isolation by distance among inselberg populations of *A. glaziouana*, as previously observed for high-altitude inselberg species of this genus (Barbará *et al.*, 2007).

Genetic divergence and relationships among inselberg populations and species of Alcantarea

Analysis of molecular variance (AMOVA) of coastal inselberg species attributed a significant proportion of the genetic variance (18 %) to the 'among population within species' level, and a similar and significant proportion to the 'among species' (16 %) level, whereas most of the variance resided within populations (67 %, Table 3; all *P*-values < 0·001). Separate AMOVA models for each species revealed that similar proportions of the genetic variance resided among populations in *A. regina* (20 %) and *A. glaziouana* (22 %) and a high and significant proportion was observed within populations of each species (80 % and 78 %, respectively; Table 3). Individual F_{ST} estimates between pairs of populations of *A. glaziouana* ranged from 0·116 to 0·315 (Table 4; all *P*-values < 0·001). The pairwise F_{ST} values translate into estimates of gene flow ($N_e m$) ranging from 0·54 to 1·91 for coastal inselberg populations of *A. glaziouana* and $N_e m = 1·03$ for the pair of coastal populations sampled for *A. regina*, respectively (Table 4).

Bayesian genetic structure analysis confirmed clear population structure in both coastal species and provided additional insights into patterns of inter-population gene flow in *A. glaziouana*. Analysis in BAPS and STRUCTURE yielded comparable results, and the latter are presented here. A $K = 5$ population model was chosen to represent the data for *A. glaziouana* and a $K = 2$ model was chosen for *A. regina*, based on the pointers provided in the software manual (probabilities and variances of each model). For *A. glaziouana*, the Bayesian analysis indicated occasional gene exchange events beyond neighbouring populations (Fig. 4). Population LNI (Niteroi; yellow cluster) will be given particular attention in the discussion as it provides information on the potential of Guanabara bay, the bay that separates LNI from all other populations, to act as a barrier to gene flow (Fig. 1, bottom right).

A phylogenetic tree of populations based on microsatellite genetic distances (Fig. 5) revealed relationships among inselberg populations of *A. glaziouana*, *A. regina*, *A. geniculata* and *A. imperialis* and two populations of *Vriesea gigantea* included for comparative purposes. Populations of all *Alcantarea* species except for *A. imperialis* were grouped together with moderate-to-high bootstrap support (100 for *A. geniculata*, 73 for *A. glaziouana* and 66 for *A. regina*, respectively). Populations of *Alcantarea imperialis* did not cluster together, with populations IIM and IVV being more closely related to *A. geniculata* than to their remaining conspecific populations IMC and IJF (Fig. 5). Populations IIM of *A. imperialis* and GIM of *A. geniculata*, connected by a dotted line in Fig. 5, co-occur on the same inselberg and were previously suspected to have experienced low levels of historical interspecific gene flow based on coalescent-based estimates of migration rates (Barbará *et al.*, 2007).

TABLE 3. *Results of analysis of molecular variance (AMOVA) for the coastal inselberg species* A. glaziouana *and* A. regina *calculated at the genet level using three different hierarchical models: a three-level model including both species, and separate two-level models for each species*

Model	Partitioning	d.f.	SS	MS	Variation (%)	F-statistic
Three levels – two species	Among species	1	70·719	70·719	16	$F_{RT} = 0.158$****
	Among populations within species	5	96·580	19·316	18	$F_{SR} = 0.210$****
	Within populations	291	480·054	1·650	67	$F_{ST} = 0.334$****
Two levels – *A. glaziouana*	Among populations	4	75·395	18·849	22	$F_{ST} = 0.217$****
	Within populations	205	319·398	1·558	78	
Two levels – *A. regina*	Among populations	1	21·185	21·185	20	$F_{ST} = 0.195$****
	Within populations	86	160·657	1·868	80	

The significance of each *F*-statistic was tested through 9999 permutations at the appropriate hierarchical level. **** $P < 0.001$.

TABLE 4. *Genetic divergence (F_{ST}; below diagonal) and gene flow (N_em; above diagonal) for pairs of populations of the coastal inselberg species* Alcantarea glaziouana, *estimated at the genet level*

	LPI	LPP	LNI	LPR	LPA
LPI	–	1·03	0·59	0·54	0·63
LPP	0·196	–	0·92	0·69	1·91
LNI	0·299	0·214	–	0·59	1·10
LPR	0·315	0·266	0·296	–	0·600
LPA	0·285	0·116	0·185	0·294	–

Gene flow was estimated from F_{ST} under the assumption of migration-drift equilibrium. Pairwise F_{ST} and N_em for the only pair of coastal populations sampled for *A. regina* were 0·195 and 1·03, respectively. All F_{ST} values were significant at the 0·001 level.
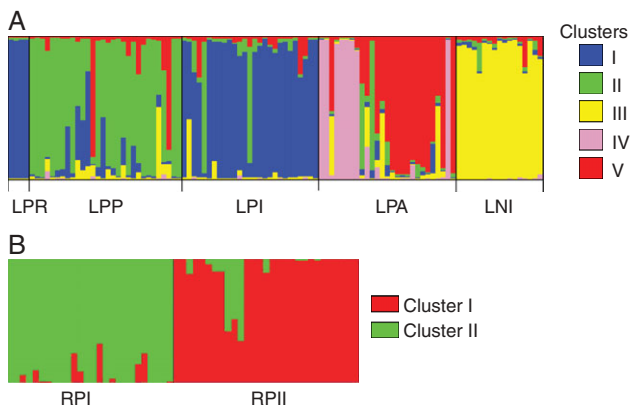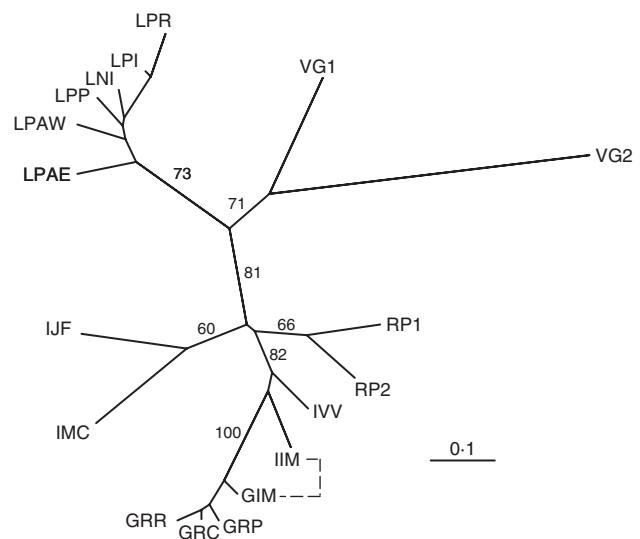

FIG. 5. Genetic distance-based neighbor-joining tree of populations of four *Alcantarea* inselberg species, *A. glaziouana*, *A. regina*, *A. imperialis* and *A. geniculata*, calculated at the genet level and including two populations of the closely related taxon *Vriesea gigantea* for comparative purposes. Bootstrap support values >60 % are shown for nodes of interest to the discussion of the data. Populations starting with V = *Vriesea gigantea*; starting with I = *A. imperialis*; starting with G = *A. geniculata*; starting with R = *A. regina*; starting with L = *A. glaziouana*. For details of population abbreviations see Materials and Methods. The two populations connected by a dashed line occur in sympatry on the same inselberg and were suspected to have experienced historical interspecific gene flow at the outset of the study (Barbará *et al.*, 2007).

FIG. 4. Bayesian admixture proportions (*Q*) of individual plants of (A) *A. glaziouana* for a *K* = 5 population model, and (B) *A. regina* for a *K* = 2 population model, calculated at the genet level. The five and two 'genetic clusters' identified by STRUCTURE for *A. glaziouana* and *A. regina*, respectively, are indicated by different colors. For details of population abbreviations see Materials and Methods.

DISCUSSION

Variation in clonal diversity within and among Alcantarea *inselberg species*

The molecular marker-based estimates of clonal diversity, genetic variability and inbreeding provide a first glimpse at variation in reproductive strategies within and among four closely related *Alcantarea* bromeliad species endemic to 'inselberg' rock outcrops in the Atlantic Rainforest of Brazil. The results indicate great differences in clonal diversity between these four closely related inselberg species, with 100 % sexual reproduction in the high-altitude species *A. imperialis* and *A. geniculata*, resulting in clonal diversities equal to 1, and a considerable proportion of clonally derived rosettes in the two coastal species *A. glaziouana* and *A. regina*, resulting in clonal diversities lower than 1 (Figs 2 and 3D). As expected from the literature (see review by Ellstrand and Roose, 1987), increased levels of cloning, i.e. decreased clonal diversities, were not necessarily reflected by decreased genetic variability (Fig. 3, Table 1).

Other population genetic studies of bromeliads have also detected clonality, including studies on the rock-outcrop species *Pitcairnia geyskesii* (Sarthou *et al.*, 2001), the terrestrial taxa *Aechmea magdalenae* (Murawski and Hamrick, 1990), *Aechmea tuitensis* (Izquierdo and Pinero, 2000) and *Puya raimondii* (Sgorbati *et al.*, 2004), and the epiphyte *Guzmania monostachia* (Cascante-Martin, 2006). The degree of clonality detected by these studies varied greatly and was clearly highest in *Puya raimondii*, the 'Queen of the Andes' (Sgorbati *et al.*, 2004). These authors reported a clonal diversity of only 0·09, based on a genetic analysis with amplified fragment length polymorphisms (AFLP). Indeed, studies reporting exclusive or predominant sexual reproduction in bromeliads, as exemplified by species of *Encholirium* (Cavallari *et al.*, 2006), appear to be the exception rather than the rule. Clonal reproduction is thus an important reproductive strategy in bromeliads with different geographic distributions, habitats and life histories.

A limiting aspect of previous studies on clonality in bromeliads is that they used genetic markers and spatial sampling strategies that differed in their ability to detect clones. In addition, some studies deliberately avoided sampling clones as they were designed to address other questions (Soltis *et al.*, 1987; Gonzalez-Astorga *et al.*, 2004). Considering the increasing interest in population divergence, micro-evolution and speciation in bromeliads (Soltis *et al.*, 1987; Murawski and Hamrick, 1990; Sarthou *et al.*, 2001; Wendt *et al.*, 2001, 2002; Barbará *et al.*, 2007; Palma-Silva *et al.*, 2007), more studies such as ours are needed that estimate clonal diversity and structure with highly informative molecular markers and that apply the same markers and sampling strategies to several closely related species in a comparative way.

It is of great botanical interest to know whether or not clonal reproduction in a given species may be due to agamospermy (apomixis; Richards, 2003). In bromeliads, controlled crossing experiments including experimental manipulations of a large number of flowers and plants suggested that agamospermy occurs in species of the genus *Pitcairnia* that live in the same type of habitat (inselberg rock outcrops) and in the same study area (south-eastern Brazil) as the species studied here. Agamospermy, in combination with high levels of selfing, may contribute to the maintenance of species-barriers in sympatric *Pitcairnia* species in the absence of temporal, geographic and post-pollination barriers in this group (Wendt *et al.*, 2001, 2002). It is thus of relevance to test for the possibility of agamospermy in the inselberg *Alcantarea* species studied here, and our data allow us to do so.

In light-seeded, wind-dispersed bromeliads such as *Alcantarea* spp., agamospermy would be expected to result in the spread of clonally propagated seeds within populations (Richards, 2003). In contrast, asexual reproduction via vegetative clonal growth would result in spatial aggregation of clones. In the two coastal *Alcantarea* species studied here, ramets of the same clone were always spatially clustered, and neighbouring rosettes of the same genotype were never separated by more than 9 m (examples shown in Fig. 2). This is close to the maximum distance of 10 m observed between neighbouring vegetatively derived rosettes of *Aechmea magdalene*, a bromeliad with asexual propagation through vegetative clonal growth (Murawski and Hamrick,

1990). Thus, we tentatively conclude that asexual reproduction in coastal *Alcantarea* inselberg species is due to clonal growth rather than agamospermy. Crossing experiments of the type carried out by Wendt *et al.* (2001, 2002) would provide further information about the presence or absence of agamospermy in coastal *Alcantarea* species.

Variation in inbreeding coefficients within and among Alcantarea *inselberg species*

Estimates of clonal diversity and within-population inbreeding coefficients (F_{IS}) estimated at the genet level (after the removal of clonal copies) differed between populations of *Alcantarea* species (Table 1, Fig. 3B). *Alcantarea glaziouana* was the species with the highest inbreeding coefficients and the lowest clonal diversities (Fig. 3), suggesting that positive F_{IS} values may stem in part from reduced mate availability (i.e. biparental inbreeding) due to clonal growth (Honnay and Jacquemyn, 2008). Nevertheless, populations with a propensity to form clones were not always those with high inbreeding coefficients (Table 1), and thus we may assume that differences in F_{IS} at the genet level capture other factors in addition to clonal propagation.

The coastal species *A. glaziouana* clearly displays the genetic signature of a primarily inbreeding species, with consistently positive inbreeding coefficients for all loci (Table 2) and most populations (Table 1), in contrast to the remaining three *Alcantarea* species studied (Fig. 2B). There also appears to be considerable variation in the strength of inbreeding among populations of *A. glaziouana* (Fig. 3B). Some – but certainly not all – of this intraspecific variation is due to population LPR for which F_{IS} was neither significant nor positive (Table 1). This population is very small (almost all extant plants were sampled), and is thus subject to stochastic factors and offers low power for detecting inbreeding. Nevertheless, even if the ranges for F_{IS} in Fig. 3 are corrected for the low inbreeding coefficient of population LPR, the range of inbreeding estimates for *A. glaziouana* is still greater than those of all other species studied (data not shown). Thus, there appears to be considerable variation in inbreeding levels not only between different *Alcantarea* species, but also within the coastal species *A. glaziouana*.

The evolution of breeding systems in plants with mixed mating has received great interest in recent years (Charlesworth and Charlesworth, 1987; Stephenson *et al.*, 2000; Vogler and Kalisz, 2001). In bromeliads, the question has been raised as to whether evolution towards selfing may sometimes be driven by the need to avoid the fitness costs associated with hybridization between related species with incomplete pre- or postzygotic barriers (Wendt *et al.*, 2001, 2002). If so, then an important question is to what extent differences in selfing rates are controlled by heritable factors versus ecological factors such as pollinator or resource limitation in particular localities (Wendt *et al.*, 2002; Paggi *et al.*, 2007). It is noteworthy in this context that two of the species studied here, *A. imperialis* and *A. regina*, have been the subject of controlled pollination experiments and observations of pollen tube growth (Martinelli, 1994). For *A. imperialis*, these experiments showed that ovule penetration was much higher after cross-pollination (range 52–75 %) than

after self-pollination (range 0–29 %), suggesting partial self-incompatibility. Although no such difference in ovule penetration was observed for *A. regina*, marked protandry was observed in this species (Martinelli, 1994). Both observations are congruent with our population genetic data, since both *A. imperialis* and *A. regina* present the genetic signatures of near-random mating with low or non-significant inbreeding coefficients (Barbará *et al.*, 2007; Fig. 3, Tables 1 and 2). No information is currently available on the timing of flowering or ovule penetration for the other two species studied, *A. glaziouana* and *A. geniculata*. Clearly, more work is needed to assess the role of variation in reproductive strategies and mating systems on the evolution and maintenance of reproductive barriers in these species.

Genetic relationships and gene flow among Alcantarea inselberg populations

Our study detected great genetic divergence and low levels of gene flow among inselberg populations of the coastal species *A. glaziouana* and *A. regina*, as also observed in our earlier analysis of the high-altitude species *A. imperialis* and *A. geniculata* (Barbará *et al.*, 2007). This is reflected in a high and significant proportion of among-inselberg variation in our AMOVA analysis of coastal species (Table 3; 22 % and 20 % for *A. glaziouana* and *A. regina*, respectively). Indeed, gene flow (N_em) was lower than 1 for most pairs of populations of *A. glaziouana* and close to 1 migrant per generation for the pair of populations studied for *A. regina* (Table 4). In addition, departure from an isolation-by-distance model of migration was established for *A. glaziouana*, as shown previously for the two high-altitude species *A. imperialis* and *A. geniculata*. Thus, as alluded to in our earlier study on high-altitude species (Barbará *et al.*, 2007), neotropical inselbergs indeed appear to resemble archipelagos of 'terrestrial islands' in terms of their effect on restriction of gene flow and structuring of genetic variability, as predicted by ecologists (Porembski and Barthlott, 2000). This opens the way to address the genetic underpinnings of adaptive radiation in bromeliads based on the well-developed conceptual framework for studying radiations on islands (MacArthur and Wilson, 1967; Emerson, 2002), in order to address the origin and evolution of bromeliad species adapted to the very different ecological conditions prevailing on coastal versus high-altitude inselbergs of south-eastern Brazil (Safford and Martinelli, 2000). In particular, the insular nature of these species facilitates the identification of independent 'replicate' populations for studies of population divergence and speciation. This has been discussed in more detail elsewhere (Barbará *et al.*, 2007), and therefore the remainder of this discussion will focus on aspects that are specific to coastal inselberg species of *Alcantarea*, and on genetic relationships among populations of coastal and high-altitude species.

The Bayesian genetic analysis confirms pronounced genetic structure in coastal inselberg species, and it also reveals additional fine-scale spatial genetic patterns (Fig. 4). For instance, population subdivision becomes apparent in population LPA (clusters IV and V), whereas populations LPR and LPI are both dominated by genetic material from the same genetic cluster (cluster I; Fig. 4). The Bayesian analysis

also suggests the possibility of occasional long-distance gene dispersal in the coastal species *A. glaziouana*, a species that is probably pollinated by bats (Martinelli, 1994). Perhaps most conspicuously, recent long-distance dispersal is indicated from population LNI of *A. glaziouana*, sampled on an outcrop near the city of Niteroi across the Guanabara bay from Rio de Janeiro (Fig. 1), into populations sampled to the west of the bay. A small number of immigrants with an approximately 50 % contribution of nuclear DNA from population LNI was detected in populations LPA, LPI and LPP of *A. glaziouana*. Assuming these intermediate genotypes are first-generation immigrants, they indicate recent long-distance dispersal (Fig. 4A, yellow bars). Considering the increasing interest in animal pollination syndromes in neotropical species in general and bat pollination in particular (I. Sazima *et al.*, 1989; M. Sazima *et al.*, 1999), it would be of great value to know whether long-distance dispersal across the bay was facilitated by dispersal of pollen or seeds. This question may be addressed by a comparison of genetic patterns for biparentally inherited nuclear and uniparentally inherited organellar markers (Ennos, 1994). Alternatively, and considering the low chance of detecting sufficient polymorphism in the plastid genomes of *Alcantarea* species (Barfuss *et al.*, 2005; Barbará, 2008), the question may be answered by using nuclear markers only. In the absence of two-generation genotypic data, this can be achieved by an analysis of slope and shape components of the curve that describes the decrease of genetic relatedness between individual plants with geographic distance (Heuertz *et al.*, 2003). Given sufficient replication, this type of analysis can reveal whether gene flow is more restricted by dispersal of pollen or seeds.

A NJ tree of populations allowed us to test genetic relationships between populations of coastal and high-altitude inselberg species of *Alcantarea*, including two populations of the related *Vriesea gigantea* that were included for comparative purposes (Fig. 5). Two trends are clearly visible from this analysis. Firstly, populations of *A. geniculata*, *A. glaziouana* and *A. regina* each group together with moderate bootstrap support. Thus, microsatellite data provide a picture of population relationships that is congruent with traditional taxonomic species' delimitation for these taxa (Martinelli, 1994). Secondly, populations of *A. imperialis* do not group together, as two populations from an area of sympatry with the closely related *A. geniculata* show a clear affinity with that species (Fig. 5). In general, bootstrap support values of branches that involve populations of *A. imperialis* tend to be low. Population IIM of *A. imperialis* co-occurs sympatrically with population GIM of *A. geniculata* on the same inselberg (Fig. 5, dashed line), and it has been shown previously that historical gene flow (N_em) between these two morphologically well-differentiated species on this outcrop is greater than that detected between most populations of *A. imperialis* (Barbará *et al.*, 2007). Population IVV of *A. imperialis*, also affected by this affinity to *A. geniculata*, is from an outcrop just a few kilometres away (Fig. 1). This reticulate pattern (Fig. 5) suggests past hybridization between *A. imperialis* and *A. geniculata*. Our finding is in agreement with the hypothesis that recombination of genetic material from related species represents an important aspect of species' radiations (Seehausen, 2004).

The successful genotyping of populations of the closely related *V. gigantea* with the same set of nuclear microsatellites (Fig. 5) indicates a potential for microsatellites or other nuclear markers to resolve phylogenetic relationships among species of these two closely related genera. The long branches of the *V. gigantea* populations probably stem from the fact that they originate from different parts of the species' range – these populations also fall into different genetic clusters in a Bayesian analysis of the gene pool structure of *V. gigantea* (Palma-Silva, 2008). Note that *V. gigantea* has a mixed mating system with high levels of inbreeding due to selfing and/or biparental inbreeding (Paggi *et al.*, 2007; Palma-Silva, 2008) and is placed next to the highly inbred *A. glaziouana* in the unrooted NJ tree (Fig. 5). This suggests a potential for the use of well-resolved molecular phylogenies to understand the evolution of mating systems in this group. Molecular systematic work with multiple plastid and nuclear DNA markers including all known species of *Alcantarea* and multiple species of *Vriesea* is currently in progress in our lab (L. M. Versieux *et al.*, unpubl. res.).

Relevance for conservation and concluding remarks

The results not only provide information on population relationships and variation in reproductive strategies, they also have clear implications for conservation management. Firstly, the great and significant genetic differentiation between coastal inselberg populations of *Alcantarea* (Tables 3 and 4, Fig. 4) implies that efficient *in situ* or *ex situ* conservation strategies need to be based on a sufficient number of inselbergs to represent the gene pools of each species. Secondly, the strong genetic differentiation between inselberg populations opens the opportunity for the use of so-called genetic 'assignment tests' to match individual plants to particular populations (Cornuet *et al.*, 1999; Pritchard *et al.*, 2000). This type of test requires great among-population differences in allelic composition and frequencies, a precondition that is met by these species. This would allow conservation geneticists to test whether plant and seed material currently available in botanical gardens around the world is representative for the gene pool of each of these inselberg species, or whether more fieldwork is required to achieve adequate coverage of species' gene pools. Assignment tests would also allow a tracking of the population origin of plant material used in horticulture, e.g. to detect illegal extractions from natural populations. Thirdly, our results indicate that *in situ* conservation of coastal *Alcantarea* species must account for clonal reproduction – sampling plants at distances less than 10 m from each other would almost certainly result in clonal duplicates, thus decreasing the amount of genetic variability captured in *ex situ* collections.

In conclusion, reproductive strategies and breeding systems vary greatly both within and between *Alcantarea* inselberg species. Asexual reproduction via vegetative clonal growth occurs frequently in coastal inselberg populations whereas sexual reproduction prevails in high-altitude species. Great variation also exists for levels of inbreeding, but the relative contributions of genetic and ecological factors to this variation remain to be determined. Population relationships based on nuclear microsatellites, although supported only by moderate bootstrap support, are largely congruent with species' delimitation based on traditional taxonomy, except for indications of past hybridization and reticulation in areas where the two high-altitude species *A. imperialis* and *A. geniculata* overlap. The strong genetic structure observed for gene pools of coastal and high-altitude species provides an excellent basis for the application of genetic markers to conservation management in these ecologically and horticulturally important bromeliads. Our molecular marker study also provides a basis for future work on the genetic underpinnings of adaptive radiation in this group of Bromeliaceae.

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