

ORIGINAL ARTICLE

Within-population spatial genetic structure in four naturally fragmented species of a neotropical inselberg radiation, *Alcantarea imperialis*, *A. geniculata*, *A. glaziouana* and *A. regina* (Bromeliaceae)

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Studies of organisms on ‘terrestrial islands’ can improve our understanding of two unresolved issues in evolutionary genetics: the likely long-term effects of habitat fragmentation and the genetic underpinnings of continental species radiations in island-like terrestrial habitats. We have addressed both issues for four closely related plant species of the adaptive radiation Bromeliaceae, *Alcantarea imperialis*, *A. geniculata*, *A. regina* and *A. glaziouana*. All four are adapted to ancient, isolated inselberg rock outcrops in the Brazilian Atlantic rainforest and are thus long-term fragmented by nature. We used eight nuclear microsatellites to study within-population spatial genetic structure (SGS) and historical gene dispersal in nine populations of these species. Within-population SGS reflected known between-species differences in mating systems. The strongest SGS observed in *A. glaziouana* ($Sp=0.947$) was stronger than literature estimates available for plants. Analysis of short- and

long-distance components of SGS identified biparental inbreeding, selfing and restricted seed dispersal as main determinants of SGS, with restricted pollen dispersal by bats contributing in some localities. The ability of *Alcantarea* spp. to colonize isolated inselbergs probably stems from their flexible mating systems and an ability to tolerate inbreeding. Short-ranging gene dispersal (average $\sigma = 7\text{--}27$ m) is consistent with a loss of dispersal power in terrestrial island habitats. Population subdivision associated with sympatric colour morphs in *A. imperialis* is accompanied by between-morph differences in pollen and seed dispersal. Our results indicate a high potential for divergence with gene flow in inselberg bromeliads and they provide base-line data about the long-term effects of fragmentation in plants.

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Introduction

Genetic studies of natural ‘terrestrial islands’, that is, systems of terrestrial habitat patches that resemble oceanic islands in terms of spatial isolation and restriction of gene flow (MacArthur and Wilson, 1967; Porembski and Barthlott, 2000), promise to improve our understanding of at least two major unresolved issues in evolutionary genetics. One of these issues concerns the likely genetic long-term effects of habitat fragmentation, such as the fragmentation processes brought about worldwide by human activities over the last few thousands of years (Young *et al.*, 1996). The second issue refers to the genetic underpinnings of continental species radiations, including processes of population divergence and speciation during adaptive radiations in island-like

habitats (Givnish and Sytsma, 1997; Schluter, 2000; Campbell and Bernatchez, 2004; Gavrilets and Vose, 2005).

With respect to the first issue, a clear trend in the ecological genetics literature is for studies on fragmentation to focus on habitats that have been fragmented rather recently. In contrast, genetic studies of terrestrial organisms subjected to long-term fragmentation are rare. Most studies that do address this topic aim at understanding range contractions and expansions in response to paleoclimatic cycles in continental mountain ranges (Schönswetter *et al.*, 2005) or—more rarely—in other ‘insular’ types of terrestrial habitats (Knowles, 2001). Many important questions concerning the long-term genetic effects of fragmentation remain open, such as: how quickly will inbreeding depression occur in fragmented environments and how will organisms respond to it? In flowering plants, an important set of questions refers to the direction in which breeding systems, pollination syndromes and dispersal mechanisms will evolve in fragmented environments (Young *et al.*, 1996; Lowe *et al.*, 2005; Bittencourt and Sebbenn, 2007). Many

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aspects of these questions can be addressed by analysing the fine-scale spatial genetic structure (SGS) of naturally fragmented species using molecular markers (Vekemans and Hardy, 2004).

The second issue, regarding the genetic underpinnings of species radiations in terrestrial 'island' systems, has received great impetus from conceptual advances in studying evolutions on islands (reviewed by Savolainen *et al.*, 2006; Stuessy *et al.*, 2006) and studying ecological speciation in fragmented habitats (Schluter, 2000; Campbell and Bernatchez, 2004). Patchily distributed habitats, such as terrestrial lakes in the case of animals (Schluter, 2000; Campbell and Bernatchez, 2004), or isolated rock outcrops in the case of plants (Barbará *et al.*, 2007), can potentially provide 'replicated natural experiments' for studies of parallel selection pressures such as those found during adaptive radiations (Schluter, 2000; Gavrilets and Vose, 2005), or simply for testing the role of drift in population divergence and speciation. Here, fine-scale SGS analysis can provide initial clues about the non-random spatial distribution of genotypes or visually recognizable morphs within populations (Vekemans and Hardy, 2004; Van Rossum and Triest, 2007). This is especially important in studies of divergence with gene flow, a process thought to be common during adaptive radiations (Seehausen, 2004). Here, fine-scale SGS analysis can help identify microgeographic discontinuities that may have contributed to the origin or maintenance of reproductive barriers, a prerequisite for distinguishing sympatric and parapatric models of speciation.

With the advent of increasingly informative genetic markers, many empirical studies have addressed SGS in natural populations of plants using spatial autocorrelation methods (for example, Epperson and Allard, 1989; Hamrick *et al.*, 1993; Fenster *et al.*, 2003; Vaughan *et al.*, 2007). A widely used approach, reviewed by Vekemans and Hardy (2004), involves regressing pairwise kinship coefficients (F_{ij}) on spatial distances between individuals and estimating the logarithmic slope of the regression as a measure of SGS. Notably, SGS analysis not only allows the estimation of historical gene dispersal under the assumption of drift-dispersal equilibrium, it also facilitates the estimation of the curvature of the kinship-distance relationship. With only a modest amount of prior information about the dispersal biology of the species studied, this can provide information on the probable relative roles of pollen and seed dispersal in generating the observed spatial genetic patterns (Heuertz *et al.*, 2003). If combined with other, complimentary approaches to individual-based genetic analysis (for example, Corander and Marttinen, 2006), SGS studies make it possible to address many of the issues outlined earlier, including the genetic signatures of differences in breeding systems or dispersal biology in populations adapted to naturally fragmented habitats and the role of spatial or phenotypic discontinuities within such populations during divergence.

The granitic rock outcrops or inselbergs of the South American Atlantic rainforest represent a classical example for terrestrial habitat islands. Neotropical inselbergs are ancient, isolated outcrops embedded within a 'matrix' of tropical rainforest, harbouring a highly specialized fauna and flora. These outcrops are ecologically separated from their surrounding rainforest by steep gradients in irradiation, temperature, moisture and

nutrient availability (Porembski and Barthlott, 2000). The few available genetic studies of inselberg species indicate that inselbergs indeed behave like islands in terms of their effects on patterns of variability and gene flow in flowering plants (Sarhou *et al.*, 2001; Barbará *et al.*, 2007, in review). Members of Bromeliaceae (bromeliads) are particularly attractive models for investigating SGS in naturally fragmented inselberg plants: this family represents an increasingly well characterized continental adaptive radiation (Givnish and Sytsma, 1997; Benzing, 2000; Barfuss *et al.*, 2005), there are multiple species that are adapted to and occur only on inselbergs, closely related inselberg bromeliads sometimes differ in reproductive strategies and breeding systems (Barbará *et al.*, in review) and polymorphic microsatellite markers are available for SGS analysis (Boneh *et al.*, 2003; Sarhou *et al.*, 2003; Palma-Silva *et al.*, 2007).

Here, we address the following questions regarding the fine-scale SGS of four closely related inselberg-dwelling bromeliad species of the genus *Alcantarea*: (1) Do populations of these species exhibit significant SGS, indicating isolation by distance within isolated inselberg populations and if yes, how great or small are historical gene dispersal distances? (2) What can the spatial genetic patterns tell us about the relative roles of pollen vs seed dispersal in mediating gene flow? (3) To what extent does intraspecific variation for fine-scale spatial genetic patterns reflect differences in biparental inbreeding or selfing rates between populations, or the presence of phenotypically divergent forms maintained in sympatry within populations? We use our data to obtain insights into microevolutionary patterns and processes in four closely related members of a neotropical inselberg radiation. We highlight the need to study historical gene dispersal patterns in naturally fragmented species with varying breeding systems, in order to better understand the likely long-term effects of habitat fragmentation currently induced in many taxa world-wide.

Materials and methods

Alcantarea inselberg species studied

The four *Alcantarea* species studied here are characterized by animal-based pollination syndromes and wind-based seed dispersal (Martinelli, 1994). *Alcantarea imperialis* and *A. geniculata* are two saxicolous bromeliads of subfamily Tillandsioideae endemic to high-altitude inselberg rock outcrops in the Atlantic rainforest of southeastern Brazil (Figure 1; Martinelli, 1994). The ranges of the two species overlap and they co-occur on several inselbergs (Barbará *et al.*, 2007). Both species exhibit outcrossing breeding systems with mixed mating (Martinelli, 1994; Barbará *et al.*, 2007). Both have hermaphrodite flowers, but they differ in their pollinators: *A. imperialis* is pollinated by bats, whereas *A. geniculata* is thought to be pollinated primarily by bees and sphingid moths (Martinelli, 1994). *A. imperialis* is partially self-incompatible as demonstrated by experimental studies of pollen tube growth and ovule penetration (Martinelli, 1994), but no such information is currently available for *A. geniculata*. Asexual reproduction via vegetative clonal growth appears to be absent or rare in both species (Barbará *et al.*, in review).

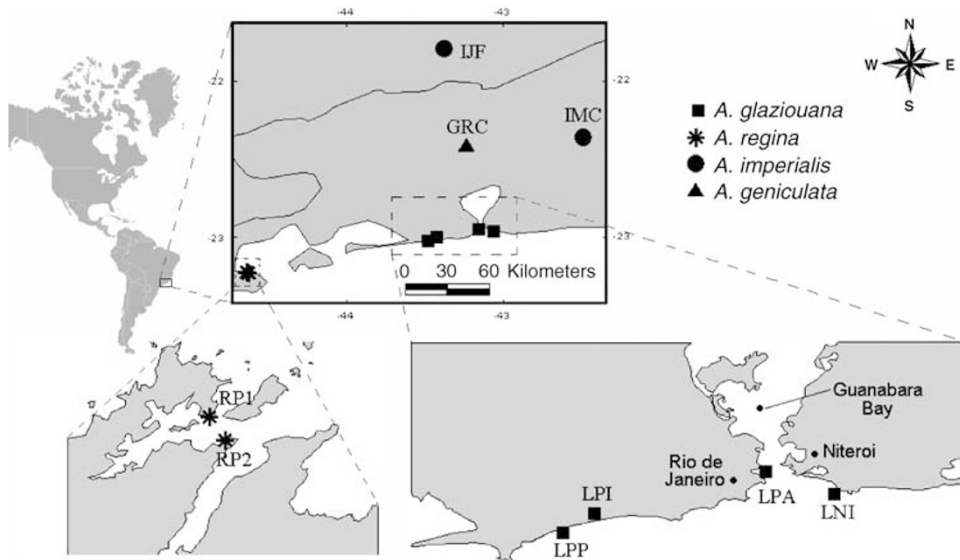


Figure 1 Distribution map of *Alcantarea* inselberg populations sampled in the Atlantic rainforest of Brazil. The sampled populations form part of a larger sample set for these species and represent all those populations for which detailed geographic coordinates were available, thus facilitating spatial analysis. For population abbreviations and details see Materials and methods.

A. glaziouana and *A. regina* are two species of coastal inselbergs endemic to the Atlantic rainforest (Figure 1; Martinelli, 1994). *A. regina* is currently undergoing taxonomic revision (L Versieux, unpublished data; PhD thesis at the University of São Paulo) and thus only two well-studied coastal populations (Martinelli, 1994) were included in the present study. *A. glaziouana* and *A. regina* do not co-occur in sympatry on any rock outcrop examined so far (Martinelli, 1994; Barbará, 2008; Lexer C, unpublished data). The hermaphrodite flowers of both species are thought to be pollinated by bats (Martinelli, 1994). Studies of pollen tube growth and ovule penetration in *A. regina* indicated full self-compatibility, however, marked protandry has been documented in this species (Martinelli, 1994). No information on self-compatibility is available for *A. glaziouana*, but most populations are highly inbred (Barbará *et al.*, in review). Varying levels of clonal reproduction have been documented for both coastal species (Barbará *et al.*, in review).

These four species were chosen because (1) they represent closely related inselberg species, and are thus suitable for a larger project on the use of inselbergs for studying microevolutionary processes during the continental adaptive radiation Bromeliaceae (Barbará *et al.*, 2007), (2) the ecological divergence between their two different habitats (high-altitude vs coastal inselbergs) is of the type normally thought to be conducive to ecological speciation and adaptive radiation (for example, clear differences in temperature regimes and salinity), (3) they facilitate studies of gene dispersal in naturally fragmented species, thus they can help close a knowledge gap in existing studies of the genetic effects of fragmentation, (4) although in general the four species represent consistent taxonomic units (Martinelli, 1994), one of them (*A. imperialis*) displays striking within-population phenotypic variability, most conspicuously, different colour morphs that differ in the colouration of rosettes and bracts (Barbará *et al.*, 2007). A large, polymorphic population of *A. imperialis* was included in

the present study, although other similarly polymorphic populations exist and may serve as replicates for future work (Barbará, 2008; Lexer C, unpublished data).

Population sampling

A total of nine populations of *A. imperialis*, *A. geniculata*, *A. glaziouana* and *A. regina* were sampled on coastal and high altitude granitic inselbergs located in the Atlantic rainforest of southeastern Brazil (states of Rio de Janeiro and Minas Gerais; Figure 1). These populations were sampled during a larger field expedition. They represent all those populations for which detailed GPS-based geographic coordinates were available, thus facilitating SGS analysis. Multiple 'replicate' populations were available for *A. imperialis*, *A. glaziouana* and *A. regina*, whereas only a single population with sufficient geographic information was available for the narrow endemic *A. geniculata*. The names, abbreviations and geographical coordinates of the sampled coastal inselberg populations are as follows: *A. imperialis*: Imperialis 'Macaé-de-Cima' or IMC (22°22.176'S, 42°29.774'W), Imperialis 'Juíz-de-Fora' or IJF (21°47.922'S, 43°22.243'W); *A. geniculata*: Genuculata 'Ricardo's Clearing' or GRC (22°25.044'S, 43°13.262'W); *A. glaziouana*: Glaziouana 'Niterói' or LNI (22°58.617'S, 43°2.825'W), Glaziouana 'Pão-de-Açúcar' or LPA (22°57.064'S, 43°09.092'W), Glaziouana 'Pedra de Itaúna' or LPI (23°00.223'S, 43°25.338'W), Glaziouana 'Pedra do Pontal' or LPP (23°2.105'S, 43°28.247'W); *A. regina*: Regina 'Parati 1' or RP1 (23°13.369'S, 44°37.616'W) and Regina 'Parati 2' or RP2 (23°14.071'S, 44°37.208'W) (Figure 1). Clonal copies detected in the two coastal species in a previous study (Barbará *et al.*, in review) were removed from the present dataset, thus allowing us to focus the present study on the genet level and to compare SGS in all four species. The sample sizes for all populations are given in Tables 1 and 2. For each plant, leaf material for DNA extraction was collected in silica gel.

Table 1 Characterisation of spatial genetic structure in four *Alcantarea* inselberg species, including for each population the mean distance of the first distance class (DC), kinship coefficient ($F_{ij(1)}$) for that class using species data as a reference, logarithmic regression slope b_{\log} including standard error and one-sided probability and S_p statistic

Species	Population	Number of pairs	Mean distance (m) of 1st DC	$F_{ij(1)}$	b_{\log} (s.e.)	P of b_{\log} 1-sided	Sp
<i>A. geniculata</i>	GRC	496	7.0	0.054	−0.034 (0.011)	***	0.0359
<i>A. imperialis</i>	IMC	1540	9.0	0.102	−0.0118 (0.009)	*	0.0131
	IMC green	253	11.0	0.149	−0.0495 (0.012)	**	0.0581
	IMC red	435	9.2	0.175	0.0045 (0.013)	NS	NC
	IJF	325	3.0	0.353	−0.0064 (0.015)	NS	NC
<i>A. regina</i>	RP1	325	4.0	0.111	−0.0266 (0.014)	**	0.0299
	RP2	153	3.0	0.217	−0.0064 (0.014)	NS	NC
<i>A. glaziouana</i>	LPI	351	1.1	0.169	0.0010 (0.006)	NS	NC
	LPP	435	1.4	0.194	−0.0170 (0.005)	***	0.0211
	LNI	136	3.3	0.408	−0.0817 (0.036)	***	0.1381
	LPA West	55	3.3	0.683	−0.3003 (0.102)	*	0.9468
	LPA East	91	4.9	0.246	−0.0816 (0.072)	**	0.1083

Abbreviations: NC, not calculated; NS, not significant.

See text for details.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.**Table 2** Characterization of genetic diversity (H_E) and mating system parameters in four *Alcantarea* inselberg species

Species	Population	N	H_E	F_{IS}	$F_{ij(1)}$	$P(F_{IS} > F_{ij(1)})$
<i>A. geniculata</i>	GRC	64	0.357	0.192***	0.054	***
<i>A. imperialis</i>	IMC	112	0.392	0.078	0.039	**
	IMC Green	46	0.361	−0.010	0.055	NS
	IMC red	60	0.381	0.064	0.052	NS
	IJF	52	0.441	0.113***	−0.005	*
<i>A. regina</i>	RP1	52	0.514	0.009	0.051	NS
	RP2	36	0.415	−0.097	0.059	NS
<i>A. glaziouana</i>	LPI	54	0.242	0.265***	−0.011	***
	LPP	60	0.445	0.084**	0.107	NS
	LNI	34	0.383	0.205**	0.235	NS
	LPA West	22	0.576	0.556***	0.625	NS
	LPA East	28	0.432	0.127	0.047	NS

Abbreviation: NS, not significant.

See text for details.

$P(F_{IS} > F_{ij(1)})$ is the P -value of a one-sided Kolmogorov–Smirnov test with alternative hypothesis that the cumulated frequency distribution of the inbreeding coefficient F_{IS} lies under that of the corresponding pairwise kinship coefficient at short distance, $F_{ij(1)}$, using population data as a reference. The test is significant if F_{IS} is stochastically larger than $F_{ij(1)}$, indicating that selfing is the main factor responsible for inbreeding. N is the number of chromosomes, which is twice the number of individuals sampled in each population.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Two of the populations were analysed at the level of sub-populations occurring on the same inselberg rock, for different reasons. Population LPA of *A. glaziouana* was subdivided into an eastern and a western sub-population for SGS analysis, because there was a clear sampling discontinuity of > 800 m between them and an initial exploratory microsatellite analysis reflected this partitioning (not shown). On the other hand, population IMC of *A. imperialis* was subdivided according to the presence of two different colour morphs in the population: one morph with green and one with red rosettes and bracts. The two morphs were found at roughly equal frequency and without any obvious spatial pattern. Inspection of progeny surrounding individual maternal plants indicated that the colour polymorphism segregated in a discrete fashion, suggesting a simple mode of

inheritance (Barbar   *et al.*, 2007). Red and green plants were often found at distances of < 1 m of one another in the extremely uniform environment of the rock face, thus making it very unlikely that trait expression was influenced strongly by environmental variance. The frequent presence of segregating colour polymorphisms in natural populations is well known to bromeliad breeders and collectors.

Molecular marker genotyping

The eight nuclear microsatellite markers used in this study were isolated from *Alcantarea imperialis* (loci Ai4.10 and Ai4.3; Palma-Silva *et al.*, 2007) and from other bromeliad genera (loci E19, E6, CT5, E6b, P2p19, Boneh *et al.*, 2003; locus Pit8, Sarthou *et al.*, 2003). The markers

have been used for studies of population divergence and interpopulation gene flow in these four *Alcantarea* species previously (Barbará *et al.*, 2007, in review) and were characterized for their information content there. Here, the same set of markers was used for within-population spatial genetic analysis at the individual level, in combination with the spatial coordinates.

For molecular genotyping, total genomic DNA was extracted from silica gel-dried leaves using a modified approach based on Doyle and Doyle (1987). The eight loci were polymerase chain reaction (PCR)-amplified and genotypes were resolved on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) as described by Palma-Silva *et al.* (2007) with modifications by Barbará *et al.* (2007). Pairs of markers were multiplexed in a single capillary, making use of the fluorescent dyes FAM and JOE (Applied Biosystems).

Data analysis

The microsatellite marker loci were already characterized in these and other populations of these four species (Barbará *et al.*, 2007, in review). For the present study, expected heterozygosities (H_E) and inbreeding coefficients (F_{IS}) for each population were reestimated, and the significance of F_{IS} was tested by exact tests in GENEPOP (Raymond and Rousset, 1995). This seemed appropriate because some individuals sampled did not have geographic coordinates available, so the population samples analysed here differ slightly in size and composition from those studied previously.

To analyse SGS, pairwise kinship coefficients (F_{ij}) were estimated according to Loiselle *et al.* (1995) and regressed on the logarithm of the spatial distance between individuals in each population using Spagedi 1.2. (Hardy and Vekemans, 2002; Vekemans and Hardy, 2004). The significance of SGS was tested through permutation tests implemented in Spagedi 1.2, which involved 10 000 permutations of spatial positions among individuals to obtain the frequency distribution of the logarithmic regression slope b_{\log} under the null hypothesis that F_{ij} and interindividual distances were unrelated. Standard errors of b_{\log} were obtained by jack-kniving over loci. The magnitude of SGS was estimated using the Sp statistic (Vekemans and Hardy, 2004) as $Sp = -b_{\log} / (1 - F_{ij(1)})$, where $F_{ij(1)}$ is the mean kinship coefficient in the first distance class, which varied from 1.1 to 11 m depending on the population analysed. The kinship coefficient F_{ij} of Loiselle *et al.* (1995) was used for estimates because it performed best with respect to bias and sampling variance in a comparison of relatedness statistics, especially with markers displaying low polymorphism (Vekemans and Hardy, 2004). Kinship coefficients F_{ij} are relative measurements, that is, they are estimated relative to the allele frequencies in a reference sample. The main determinant of SGS, the slope b_{\log} , is not influenced by the choice of the reference sample, and Sp is fairly robust to variation in $F_{ij(1)}$ resulting from different reference samples (Vekemans and Hardy, 2004). To allow identical scaling of kinship-distance plots between populations within species facilitating direct graphical comparison of b_{\log} , allele frequencies of the respective whole-species datasets were used as reference allele frequencies for each population to test and quantify SGS. For graphical representation of SGS in

each population, average F_{ij} values between pairs of individuals were computed for eight classes of increasing distance between individuals. Distance classes were defined in such a way that each contained the same number of pairs of individuals.

Comparisons of F_{IS} and $F_{ij(1)}$ were used to assess the contribution of biparental inbreeding to total inbreeding. We used a one-sided non-parametric Kolmogorov–Smirnov test with alternative hypothesis that the cumulative frequency distribution of F_{IS} lay under that of $F_{ij(1)}$. A significant test establishes that F_{IS} is stochastically larger than $F_{ij(1)}$, indicating that inbreeding is essentially due to selfing. If both statistics are similar, mating between geographically close relatives (biparental inbreeding) contributes a great deal to total inbreeding. To avoid inflation of absolute F_{ij} values due to great genetic divergence between populations (F_{ST} in the four species ranged between 0.111 and 0.434; Barbará *et al.*, 2007, in review), within-population references were used for inferring the contribution of biparental inbreeding to total inbreeding.

Historical gene dispersal under migration-drift equilibrium was estimated in each population that displayed significant SGS as the standard deviation of gene dispersal distance $\sigma_g = (1 / (4Sp\pi D_e))^{0.5}$, where D_e is the effective population density. The effective density was estimated as 0.1–0.5 times the census density. Estimates of σ_g were obtained from Sp resulting from kinship-distance regressions (1) over the whole distance range as described above and (2) using an iterative approach in order to restrict regression to the appropriate range between σ_g and $20\sigma_g$ where linearity of the regression is expected (Fenster *et al.*, 2003; Vekemans and Hardy, 2004). Gene dispersal estimates from SGS assume that the sampling range includes distances up to $20\sigma_g$ (Vekemans and Hardy, 2004). At a distance larger than approximately $20\sigma_g$, mutations are expected to influence the shape of the kinship-distance plot, whereas at short distance, deviation from linearity can be caused by the ratio of pollen vs seed dispersal.

To investigate the curvature of the kinship-distance plots at short distance and obtain first insights into the ratio of pollen vs seed dispersal σ_p / σ_s from the SGS data, a polynomial regression of degree 3 of the form $y = a + b\ln(x) + c(\ln(x))^2 + d(\ln(x))^3$ was fitted to the residuals of F_{ij} after linear regression on the logarithm of distance (Heuertz *et al.*, 2003). The curvature of this polynomial regression is given by its second derivative $k = 2c + 6d\ln(x)$. A concave shape or a positive k in the first distance class indicates a restricted short-distance component of gene dispersal, most commonly due to restricted seed dispersal, whereas a convex shape or negative k indicates no restriction of this component.

To better understand spatial patterns in population IMC of *A. imperialis*, the population with two colour morphs (plants with red and plants with green rosettes and bracts), simple and partial Mantel tests with 10 000 randomizations were performed with the software zt (Bonnet and Van de Peer, 2002) using matrices of pairwise kinship coefficients, pairwise geographic distances and a binary matrix in which pairs of individuals were attributed '1' when belonging to the same colour morph and '0' otherwise. These analyses allowed us to

test (1) whether the spatial distribution of colour morphs deviated from random expectations, (2) whether plants within colour morphs were more genetically related than plants of different colour morphs and (3) whether this remained the case when controlling for geographic distance. Further, a Bayesian genetic structure analysis was carried out at the individual level using BAPS 4.14 (Corander and Marttinen, 2006). This approach makes use of geographic coordinates to assign a biologically non-uniform prior distribution over the space of clustering solutions. BAPS 4.14 was run as recommended in the user manual using 100 iterations to estimate individual-based admixture coefficients.

Results

Fine-scale spatial genetic structure in *Alcantarea* inselberg populations

The regression of pairwise kinship values on the logarithm of geographic distance had a significantly negative slope b_{\log} indicative of SGS in three out of four populations studied for *A. glaziouana* (populations LPP, LNI and both sub-populations of LPA), in one out of two populations sampled for *A. regina* (RP1) and *A. imperialis* (IMC) and in the single population of *A. geniculata* (GRC) for which geographic data were available. In population IMC of *A. imperialis* where two different colour morphs co-occur in sympatry, the green morph exhibited a significant SGS pattern, whereas the red one did not. The logarithmic regression slopes (b_{\log}) along with the significance values of permutation tests, the SGS statistic S_p for each population and kinship coefficients (F_{ij}) for the first distance class are shown in Table 1. SGS (S_p) ranged from 0.013 to 0.947, the highly inbred *A. glaziouana* exhibiting the highest values of S_p . Correlograms depicting kinship coefficients (F_{ij}) and their 95% confidence intervals as a function of geographic distance are shown in Figure 2 and, for ease of comparison, their regression slopes (b_{\log}) with standard errors are also shown in graphical form in Figure 3.

In three out of six populations with significant inbreeding (population LPI of *A. glaziouana*, IJF of *A. imperialis* and GRC of *A. geniculata*), inbreeding coefficients F_{IS} were stochastically greater than kinship coefficients F_{ij} for the first distance class (Table 2). This indicates that inbreeding in these populations is more likely to be due to selfing than to biparental inbreeding (matings among relatives). Only for one of these three populations, significant SGS was observed (population GRC of *A. geniculata*; Table 1). For the remaining three populations with significant F_{IS} (populations LPP, LNI and LPA of *A. glaziouana*), F_{IS} was similar to F_{ij} for the first distance class, thus suggesting that matings between geographically close relatives contributed to inbreeding and in all three of these cases SGS was significant (Table 2).

Historical gene dispersal distances

Estimates of gene dispersal distances (σ_g) were obtained for all populations or sub-populations that exhibited significant SGS (Table 3). Estimates of σ_g obtained from the conservative estimate of effective density $D_e=0.1D$ were on average two to three times larger than those

from $D_e=0.5D$; the intermediate results for $D_e=0.2D$ are discussed here. Estimates of σ_g were higher for populations of *A. geniculata*, *A. imperialis* and *A. regina* (27.1, 23.3 and 27.2 m, respectively) than for the highly inbred *A. glaziouana* (2.6–11.8 m). The green colour morph of population IMC of *A. imperialis* displayed fairly restricted dispersal (11.1 m). Iterative estimation of σ_g converged in six out of eight populations/sub-populations and generally yielded estimates that were similar to those calculated over the whole distance range (Table 3).

Curvature of kinship-distance relationships

The initial curvature of the kinship-distance curve (shown as the polynomial regression of the third degree of residuals ($F_{ij}-F_{ij\text{ exp}}$) on the logarithm of geographic distance; Figure 4) was concave (positive k) for five of the eight populations/sub-populations with significant SGS (Table 4). The concave curvature (positive k) indicated that the short-distance component of dispersal was restricted in these populations or sub-populations, resulting in leptokurtic dispersal (Figure 4; Table 4). For three populations (RP1 of *A. regina*, LNI of *A. glaziouana* and IMC of *A. imperialis*), the polynomial regression at short distance was convex (negative k), indicating that gene dispersal was not leptokurtic and that the long-distance component of dispersal was restricted instead (Figure 4; Table 4).

Based on known dispersal patterns in flowering plants in general and Bromeliaceae in particular (Benzing, 2000), the short-distance component of gene dispersal was attributed to seed and the long-distance component to pollen dispersal. In simulations to determine pollen/seed dispersal ratios (σ_p/σ_s), estimates of σ_p/σ_s for a curvature of $k=0$ converged to 5.7 across the full range of parameter values tested (Heuertz *et al.*, 2003). This implies that populations with a curvature of $k>0$ have σ_p/σ_s ratios of at least 5.7, whereas populations with $k<0$ will exhibit σ_p/σ_s ratios smaller than that. Thus, in the majority of populations of the *Alcantarea* species studied and in which SGS was significant, wind-based seed dispersal is more restricted than animal-based pollen dispersal, the exceptions being two coastal populations of *A. regina* and *A. glaziouana* (RP1 and LNI) and population IMC of the high altitude species *A. imperialis*.

Within-population genetic structure and sympatric colour morphs in *A. imperialis*

The Mantel test between the matrices of pairwise colour morph membership and geographic distance was not significant ($r=0.027$, $P=0.079$), indicating that colour morphs were not spatially aggregated in the population (Figure 5a). Individuals belonging to the same colour morph were genetically more related than individuals belonging to different colour morphs (simple Mantel test, $r=0.258$, $P<0.001$) and this remained the case when controlling for geographic distance (partial Mantel test, $r=0.261$, $P<0.001$).

Bayesian-based mixture analysis of population IMC with BAPS (Corander and Marttinen, 2006) using prior information on spatial coordinates revealed that, unlike the random pattern apparent for the two colour morphs co-occurring in this population (Figure 5a), the best

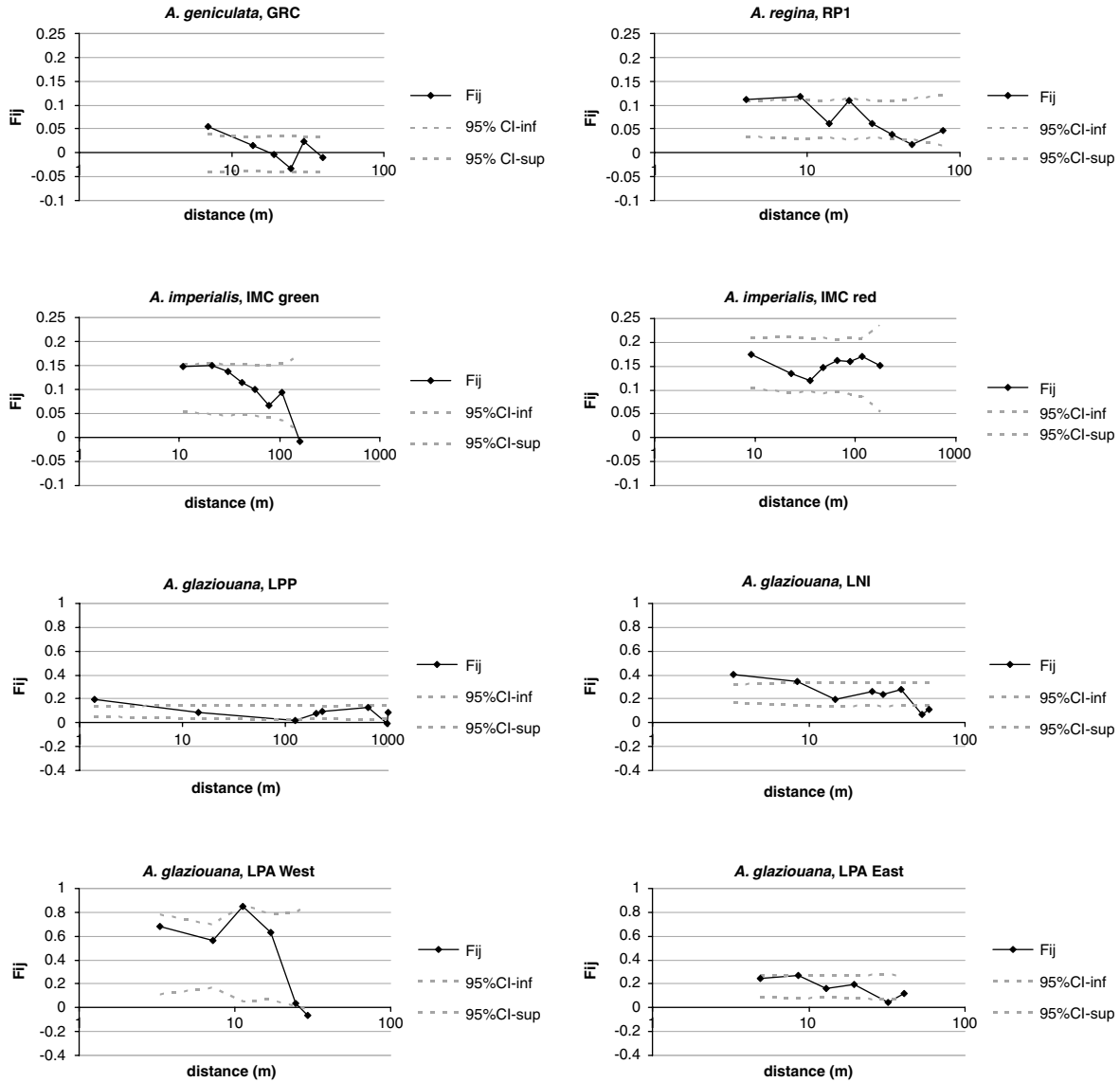


Figure 2 Kinship (F_{ij}) values (connected by solid black lines) and their 95% confidence intervals (dotted lines) as a function of geographic distance between pairs of individuals (logarithmic scale) for seven populations of four different *Alcantarea* species. The eight panels represent all those populations or sub-populations for which significant SGS was detected. The red morph of population IMC is shown for comparative purposes, although SGS for this sub-population was not significant.

genetic partition within the population had a clear spatial component (Figure 5b). The two genetic clusters detected by BAPS overlapped entirely at the north-western edge, whereas only one of the clusters extended to the southeastern edge of the population (Figure 5b). Individual-level admixture analysis in BAPS (50% criterion of inferred ancestry) resulted in cluster 1 having an average ancestry of 0.938 ± 0.354 (s.e.) and cluster 2 having an average ancestry of 0.945 ± 0.016 (s.e.). Genetic divergence (F_{ST}) between the two clusters found by BAPS was 0.198 ($P < 0.001$). The green morph had on average higher ancestry in cluster 1 (47%) than the red morph (18%) and this difference was significant (ANOVA, $F = 7.313$, $P < 0.01$). The spatial genetic data allowed us to formulate hypotheses on the origin or breakdown of reproductive isolation between divergent forms present in a large, polymorphic population of the inselberg species *A. imperialis*.

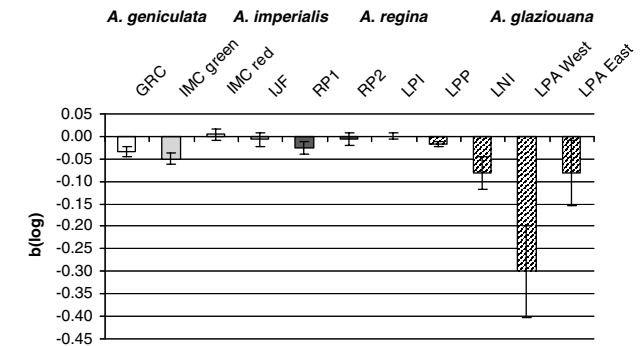


Figure 3 Comparison among populations of different *Alcantarea* species of b_{\log} , the slope of the regression of F_{ij} on the logarithm of distance, with standard errors determined by jack-kniving over loci. For ease of visualization, bars for different species are indicated by different fill patterns (white: *A. geniculata*; grey: *A. imperialis*; black: *A. regina*; hatched: *A. glaziouana*).

Table 3 Estimates of historical gene dispersal (σ_g) in four *Alcantarea* inselberg species, including population density (D) estimated from geographical data and maps, maximum sampling distance and σ_g estimated for three different effective densities (D_e) over the whole distance range or using the iterative procedure as described in text

Species	Population	Number of pairs	D (individuals per km ²)	Maximum sampling distance (m)	σ_g estimate from $D_e=0.1 D$		σ_g estimate from $D_e=0.2 D$		σ_g estimate from $D_e=0.5 D$	
					Whole range	Iterative ^a	Whole range	Iterative ^a	Whole range	Iterative ^a
<i>A. geniculata</i>	GRC	496	15 070	89.2	38.3	No conv	27.1	28.1	17.1	22.7
<i>A. imperialis</i>	IMC	1540	56 000	217.2	32.9	22.9	23.3	16.0	14.7	11.0
	IMC green	253	56 000	194.7	15.6	13.9	11.1	10.0	7.0	8.1
	IMC red	435		217.2						
	IJF	325		49.9						
<i>A. regina</i>	RP1	325	17 930	90.7	38.5	No conv	27.2	No conv	17.2	15.3
	RP2	153		33.3						
<i>A. glaziouana</i>	LPI	351		203.9						
	LPP	435	500 000	994.2	8.7	6.4	6.1	No conv	3.9	No conv
	LNI	136	20 730	63.6	16.7	14.0	11.8	10.9	7.5	6.5
	LPA West	55	60 000	30.4	3.7	3.3	2.6	2.6	1.7	1.6
	LPA East	91	60 000	51.9	11.1	10.6	7.8	10.6	4.9	4.7

Abbreviation: No conv: no convergence of iterative procedure.

^aValues in bold indicate convergence of the iterative procedure, the remaining σ estimates are averages of those estimates around which the iterative procedure cycled.

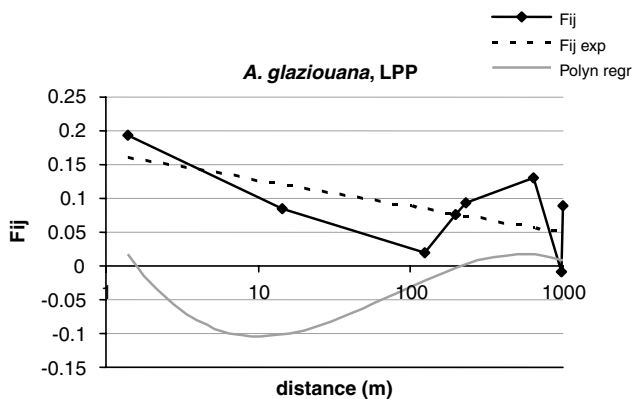


Figure 4 Illustration of the polynomial regression curve of order 3 (polyn regr; grey line) of F_{ij} residuals on the logarithm of distance. Observed F_{ij} values are on average higher than their expectation from linear regression (F_{ij} exp) at short distance in this population. This results in a concave polynomial regression line at short distance (at the first distance class), indicating that seed dispersal is restricted compared to pollen dispersal. A convex shape would indicate restricted pollen dispersal (see also text and Table 4).

Discussion

Variation in SGS patterns across four naturally fragmented *Alcantarea* inselberg species with varying breeding systems

Our analysis of nine populations from four naturally fragmented *Alcantarea* inselberg species revealed great variation in the strength and extent of within-population genetic structure (Table 1; Figures 2 and 3), the spatial structure statistic Sp varying by almost two orders of magnitude (Table 1). In populations for which significant SGS was observed, spatial structure (quantified as Sp) was fairly strong compared to SGS literature estimates by

Vekemans and Hardy (2004) and the highest Sp value observed in population LPA West of *A. glaziouana*, $Sp=0.9468$, was much higher than the maximum value found in 47 plant datasets, $Sp=0.2632$ for the highly inbred Costa Rican bean species *Phaseolus lunatus* (Zoro Bi et al., 1997). We note that the pronounced SGS in population LPA is unlikely to be due to null alleles, because no indications for null alleles were found for these loci and species previously (Barbará et al., 2007, in review). The apparent potential for strong within-population SGS mirrors the great population differentiation and extremely low levels of between-population gene flow found in these highly fragmented inselberg species ($N_e m < 1$ migrant per generation between most pairs of populations; Barbará et al., 2007, in review). Further, as three of the species studied here were represented by two or more populations, our data can be interpreted in terms of variation in dispersal biology and breeding systems among populations and species in these naturally fragmented taxa.

Within-population spatial structure, quantified either by the Sp statistic or the regression slope b_{\log} , was clearly strongest in populations of the coastal inselberg dweller *A. glaziouana* (Table 1; Figure 3). Consequently, estimates of historical gene dispersal (σ_g) were much lower in *A. glaziouana* than in the other three species (on average $\sigma_g = 7.1$ m when estimated over the whole distance range and for intermediate effective densities, compared to 27.1, 23.3 and 27.2 m for populations of *A. geniculata*, *A. imperialis* and *A. regina*, respectively; Table 3). This species also had consistently positive inbreeding coefficients F_{IS} (Table 2), whereas the other two species with population replicates, *A. imperialis* and *A. regina*, generally had lower F_{IS} values (Table 2). The latter two species have previously been characterized as mixed outcrossers whereas *A. glaziouana* has been characterized as a predominant inbreeder (Martinelli, 1994; Barbará et al., 2007, in review). So differences in breeding systems appear to be reflected by differences in SGS patterns and

Table 4 Estimates of the initial curvature of kinship-distance graphs including d , the coefficient of $(\ln(x))^3$ of the polynomial regression of third degree of residuals ($F_{ij}-F_{ij\text{ exp}}$) on the logarithm of distance ($y=a+b*\ln(x)+c*(\ln(x))^2+d*(\ln(x))^3$) and k , the second derivative of this equation at the average distance of the first distance class (1st DC), or at a distance of 2 m, which corresponds to neighbouring individuals

Species	Population	Mean distance (1st DC)	k^a (1st DC)	k^a (2 m)	d	Sign of k^a	σ_p/σ_s estimate ^a
<i>A. geniculata</i>	GRC	7.0	0.011	0.000	0.002	+	> 5.7
<i>A. imperialis</i>	IMC	9.0	-0.014	-0.005	-0.000	-	< 5.7
	IMC green	11.0	0.029	0.133	-0.011	+	> 5.7
	IMC Red	9.2					
	IJF	3.0					
<i>A. regina</i>	RP1	4.0	-0.027	-0.036	0.003	-	< 5.7
	RP2	3.0					
<i>A. glaziouana</i>	LPI	1.1					
	LPP	1.4	0.094	0.084	-0.004	+	> 5.7
	LNI	3.3	-0.024	-0.014	-0.005	-	< 5.7
	LPA	3.3	0.191	0.524	-0.147	+	> 5.7
	LPA	4.9	0.115	0.233	-0.024	+	> 5.7

^aA gross estimate of pollen vs seed dispersal (σ_p/σ_s) can be obtained from k (Heuertz *et al.*, 2003; Vekemans and Hardy, 2004): simulations showed that the ratio σ_p/σ_s equals 5.7 at $k=0$ and is larger for $k>0$ (concave shape) and smaller for $k<0$ (convex shape).

gene dispersal in closely related inselberg bromeliads. In addition, great among-population variation in spatial genetic patterns was observed in *Alcantarea* inselberg taxa (Table 1; Figure 3), in line with available theory of the dynamics of mixed mating in plants (Lande and Schemske, 1985; Barrett, 2003). Variation in spatial and dispersal parameters will be even more pronounced in inselberg taxa, because genetic divergence among inselberg populations appears to be greater than normally expected from the plant literature (Barbará *et al.*, 2007, in review).

In three out of six populations with significant inbreeding, inbreeding coefficients F_{IS} were similar in magnitude to kinship coefficients F_{ij} for the first distance class (LPP, LNI and LPA West; Table 2), thus indicating that inbreeding may be due to biparental inbreeding rather than selfing. This is in contrast to populations with significant inbreeding for which F_{IS} was stochastically greater than F_{ij} for the first distance class (LPI of *A. glaziouana*, IJF of *A. imperialis* and GRC of *A. geniculata*; Table 2), which suggests that inbreeding was caused by selfing in these cases. Knowledge of the distribution of pollen dispersal distances for cross-pollinations would allow a more refined estimation of the relative roles of selfing and biparental inbreeding (Fenster *et al.*, 2003), but this type of information is not currently available for *Alcantarea* spp. Also, to our knowledge, nothing is currently known about genetic variation in breeding systems within and among naturally fragmented, radiating bromeliad species, although this general topic is certainly of great interest to plant evolutionary genetics (Lande and Schemske, 1985; Barrett, 2003; Charlesworth, 2003).

The role of pollen vs seed dispersal in naturally fragmented *Alcantarea* inselberg species

Ennos (1994) has shown that information on the relative roles of pollen vs seed dispersal in natural populations of plants can be gleaned by comparing patterns of differentiation at biparentally inherited nuclear vs maternally inherited organellar markers. However, in many organismal groups, organellar markers are not

sufficiently variable to allow reliable estimation of maternal gametes at the relevant spatial scale (McCauley, 1997). This is expected for many species of Bromeliaceae, a family with extremely slow rates of plastid DNA evolution (Barfuss *et al.*, 2005). Indeed, a search of nine plastid DNA regions known to be variable among species of Bromeliaceae and/or related plant families yielded no within-species polymorphism in the plastid genome of *A. imperialis* (Barbará, 2008). In the absence of sufficiently variable organellar markers, the likely role of pollen vs seed dispersal in highly fragmented *Alcantarea* inselberg species was inferred via the curvature of the kinship-distance curve, following an approach first described by Heuertz *et al.* (2003).

For five out of eight *Alcantarea* inselberg populations/sub-populations with significant SGS, the curvature (k) of the kinship-distance regression at short distance was positive, indicating restricted seed dispersal (Table 4). The positive k translates into a pollen vs seed flow (σ_p/σ_s) ratio of >5.7 (Heuertz *et al.*, 2003). In contrast, for the other three populations the curvature k was negative, suggesting that pollen dispersal was restricted instead. This was the case for populations IMC of *A. imperialis*, RP1 of *A. regina* and LNI of *A. glaziouana* (Table 4). The finding of more frequent or consistent restriction of seed dispersal is important, as it suggests an important role for pollinating animals in enabling gene flow in these highly fragmented inselberg species of the South American Atlantic rainforest (Martinelli, 1994; Sazima *et al.*, 1999). A conflicting signal was obtained for population IMC of *A. imperialis*—the sign of k changed when only the green colour morph present in this population was considered. This polymorphic population is of special interest for our discussion of divergence processes in *Alcantarea* inselberg species (further below).

The genetic legacy of long-term fragmentation

The spatial patterns observed in *Alcantarea* inselberg species can be interpreted in terms of microevolution in populations subject to long-term fragmentation. Clearly,

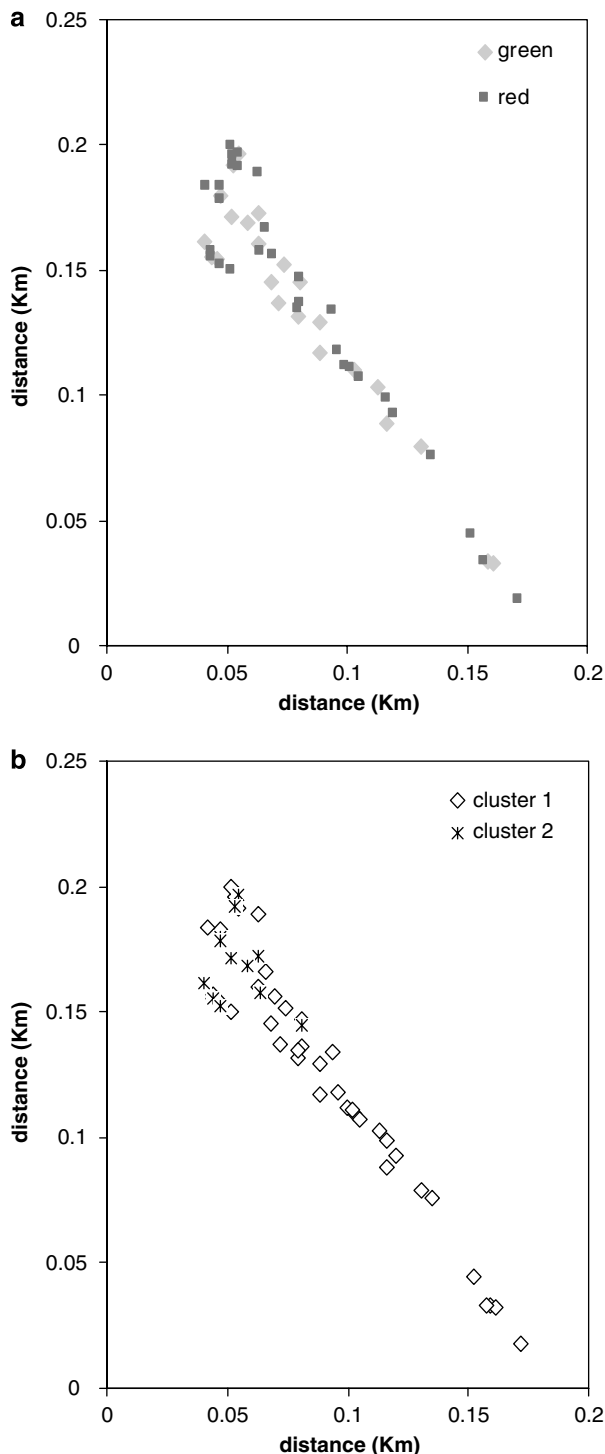


Figure 5 Spatial positions of individuals sampled in population IMC of *A. imperialis*, the population with two different colour morphs occurring in sympatry (plants with red and plants with green rosettes and bracts). (a) Spatial positions of plants belonging to the green and red morph, respectively. (b) Membership of individual plants in the two genetic clusters identified by Bayesian-based mixture analysis in BAPS 4.14.

biparental inbreeding, selfing and restricted seed dispersal are the main factors responsible for strong spatial structure in fragmented inselberg populations of *Alcantarea* and restricted pollen dispersal by bats contributes to SGS in some localities (Table 4; negative k). Biparental

inbreeding contributes to strong SGS more frequently than selfing, as visible from our comparison of inbreeding coefficients (F_{IS}) and short-distance kinship coefficients (F_{ij} ; Table 2). Nevertheless, there is no indication of inbreeding depression in isolated inselberg populations of *Alcantarea*: population densities are generally large (Table 3) and effective population sizes (N_e) estimated with microsatellites are in the order of hundreds (Barb  ra et al., 2007), as corroborated by our observation of regular flowering and fruit/seed set in natural populations (Martinelli, 1994; Barb  ra, 2008; Martinelli G and Lexer C, unpublished data). Data on fruit set upon cross- and self-pollination are available for *A. imperialis* and *A. regina* and the results indicate no significant difference between cross- and self-pollination in either species (Martinelli, 1994). We note that population IJF (Table 2), located in a disturbed setting near a mining site, was not included in Martinelli's (1994) experiments.

More extensive data on reproductive output (= fitness) are available for the closely related *Vriesea gigantea* (note that *Vriesea* and *Alcantarea* have previously been classified as a single genus; Benzing, 2000). Manual self-pollination in the predominant inbreeder *V. gigantea* resulted in 73–100% fruit set and seed set was generally higher after self-pollination than open pollination, thus suggesting the absence of inbreeding depression in this self-compatible, predominantly inbreeding species (Paggi et al., 2007). Flexible mating systems (Barb  ra et al., in review) and the apparent ability of members of the *Vriesea/Alcantarea* species complex to tolerate selfing may explain their potential to colonize and succeed on isolated inselberg rock outcrops—selfing will effectively provide 'reproductive assurance' upon colonization of new 'terrestrial islands', as also observed for successful colonizers of oceanic islands (Baker's law; Baker, 1967). Also, a tendency to self will effectively translate into a loss of 'dispersal power' in island species. This is predicted by classical island phylogeography (MacArthur and Wilson, 1967) and the relatively strong SGS and short gene dispersal (σ_g) in *Alcantarea* inselberg species compared to the plant literature (Vekemans and Hardy, 2004) are consistent with this prediction.

As alluded to in the introduction, little is known about the genetic effects of long-term fragmentation in plants (Young et al., 1996; Lowe et al., 2005; Bittencourt and Sebbenn, 2007) and most available knowledge of this topic concerns phylogeographic structure arisen from paleoclimatic cycles rather than the evolution of fitness-related genetic variation. We eagerly await in-depth genetic studies of other species adapted to 'terrestrial islands' in different parts of the world and we anticipate that inselberg rock outcrops will provide useful models for comparative studies in this context (Porembski and Barthlott, 2000; see Byrnes and Hopper, 2008). Such studies may also provide us with a better understanding of the long-term impacts of selective pressures (that is, evolutionary change) in fragmented populations in human-altered environments, which is a topic of great current concern in conservation genetics (Tseng, 2007).

Fine-scale spatial structure and divergence with gene flow in inselberg bromeliads

Bromeliaceae are becoming a popular plant group for studying evolutionary processes during continental

adaptive radiations, including radiations in 'terrestrial island'-like environments (Givnish and Sytsma, 1997; Benzing, 2000; Sarthou *et al.*, 2001; Barbará *et al.*, 2007). Theoretical and literature work has shown that adaptive radiation often involves divergence in the face of gene flow (Seehausen, 2004; Gavrillets and Vose, 2005) and empirical work on oceanic islands suggests that sympatric speciation may indeed contribute to diversification in island plants (Savolainen *et al.*, 2006). It is thus of interest to ask whether divergence with gene flow (that is, in sympatry or parapatry) may also contribute to speciation in fragmented populations situated in terrestrial island-like environments.

Our results point at a potential for reproductive barriers to arise—or to be maintained—in sympatry or parapatry in fragmented *Alcantarea* inselberg populations. Our data indicate significant fine-scale spatial structure within populations, not only in the predominant inbreeder *A. glaziouana* but also in populations of the mixed outcrossers *A. imperialis*, *A. geniculata* and *A. regina* (Table 1; Figure 2). Another line of evidence stems from our data on population IMC of *A. imperialis*, the population with two colour morphs occurring in sympatry.

Bayesian-based spatial analysis revealed the presence of two 'genetic clusters' that overlapped in the north-western corner of the population (Figure 5b). It is not unexpected that phenotypic (Figure 5a) and genotypic (Figure 5b) maps differ from one another, as the microsatellite markers are unlikely to be linked to the colour polymorphism. Interestingly, SGS was five times stronger in the green morph compared to the overall population, which was reflected by shorter gene dispersal distance in this morph ($\sigma_g = 11$ vs 23 m, estimates are for intermediate densities; Table 3). These differences were accompanied by differences in dispersal biology, because pollen/seed dispersal ratios appear to be higher in the green morph (= positive k) than in the overall population (Table 4), which demands an explanation.

It is unlikely that the absence of SGS in the red morph is due to a more recent origin of this form, that is, a lack of time to reach migration-drift equilibrium; the red morph has a greater proportion of membership in the more wide-spread genetic cluster, which extends all the way to the southeastern corner of the population (Figure 5b). Thus, we consider it more likely that between-morph differences in spatial structure and pollen/seed flow ratios (=curvatures) are due to differences in the ability to attract pollinating bats. Alternatively, dispersal may also differ due to other, genetically correlated plant traits not measured in our study.

Clearly, our SGS data indicate a high potential for the build-up or maintenance of reproductive barriers in sympatry or parapatry in fragmented *Alcantarea* inselberg species, but the exact mechanisms underlying these processes remain to be determined (for example, ecological or pollinator-mediated selection). More empirical genetic work is needed on these and related species of Bromeliaceae in island-like environments, to assess the relative roles of divergence in geographic isolation vs divergence with gene flow during the adaptive radiation that gave rise to this large and successful family of flowering plants. An increasing number of population genetic studies are becoming

available for bromeliads (for example, Soltis *et al.*, 1987; Murawski and Hamrick, 1990; Sarthou *et al.*, 2001; Sgorbati *et al.*, 2004; Barbará *et al.*, 2007). We look forward to see more studies that specifically address the genetics of speciation in this group.

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