Published in "Molecular Ecology 20(15): 3185–3201, 2011"
which should be cited to refer to this work.

Sympatric bromeliad species (*Pitcairnia* spp.) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs

C. PALMA-SILVA,*† T. WENDT,‡ F. PINHEIRO,† T. BARBARÁ,*§ MICHAEL F. FAY,* S. COZZOLINO¶ and C. LEXER§

Jodrell Laboratory, Royal Botanic Gardens Kew, Richmond, Surrey TW9 3DS, UK, †Instituto de Botânica, 04301-902 São Paulo, Brazil, ‡Departamento de Botânica, CCS/IB/UFRJ, 21941-590 Rio de Janeiro, RJ, Brazil, §Unit of Ecology and Evolution, Department of Biology, University of Fribourg, Chemin du Musée 10, CH-1700 Fribourg, Switzerland, ¶Dipartimento di Biologia Strutturale e Funzionale, Università degli Studi di Napoli Federico II, 80100 Napoli, Italy

Abstract

The roles of intra- and interspecific gene flow in speciation and species evolution are topics of great current interest in molecular ecology and evolutionary biology. Recent modelling studies call for new empirical data to test hypotheses arising from the recent shift from a 'whole-genome reproductive isolation' view to a 'genic' view of species and speciation. Particularly scarce (and thus of particular interest) are molecular genetic data on recently radiated, naturally hybridizing species in strongly structured and species-rich environments. Here, we studied four sympatric plant species (*Pitcairnia* spp.; Bromeliaceae) adapted to Neotropical inselbergs (isolated outcrops resembling habitat 'islands' in tropical rainforests) using nuclear and plastid DNA. Patterns of plastid DNA haplotype sharing and nuclear genomic admixture suggest the presence of both, incomplete lineage sorting *and* interspecific gene flow over extended periods of time. Integrity and cohesion of inselberg species of *Pitcairnia* are maintained despite introgression and in the face of extremely low within-species migration rates ($N_em < 1$ migrant per generation). Cross-evaluation of our genetic data against published pollination experiments indicate that species integrity is maintained by the simultaneous action of multiple prezygotic barriers, including flowering phenology, pollinator isolation and divergent mating systems. Postzygotic Bateson–Dobzhansky–Muller incompatibilities appear to contribute to isolation, as suggested by asymmetric introgression rates of single loci. Our results suggest that incomplete lineage sorting, hybridization and introgression form integral aspects of adaptive radiation in Neotropical inselberg 'archipelagos'. Inselbergs with multiple closely related co-occurring species should be of special interest to students of speciation in mountain systems, and to ongoing conservation programmes in the Atlantic Rainforest biodiversity hotspot.

Keywords: biodiversity, bromeliad, chloroplast sharing, hybridization, intraspecific gene flow, introgression, microsatellite, population genetics

Introduction

The roles of intra- and interspecific gene flow in maintaining species cohesion and integrity are topics of great interest to molecular ecologists and evolutionary biologists (Ehrlich & Raven 1969; Wu 2001; Morjan & Rieseberg 2004; Currat *et al.* 2008; Petit & Excoffier 2009). Mayr's (1942) Biological Species Concept was based on crossability ('whole-genome reproductive isolation') between divergent populations, and thus its helpfulness in understanding these phenomena was limited. More

Correspondence: Clarisse Palma-Silva, Fax: +55 11 50733678; E-mail: clarissepalma@yahoo.com.br

recently, a genic view of species evolution and speciation has reached wide acceptance. In this view, species barriers are porous (i.e. semipermeable to gene flow) (Wu 2001), and populations within a species evolve collectively owing to a combination of gene flow and selective sweeps at different spatial scales (Morjan & Rieseberg 2004; Kane & Rieseberg 2007).

The genic view of species evolution has already facilitated tests of long-standing hypotheses in evolutionary biology (Barton & Hewitt 1985) by providing a framework for interpreting locus-to-locus patterns of gene exchange between divergent populations (reviewed by Lexer & Widmer 2008; Arnold & Martin 2010). Recent empirical and modelling studies also indicate a negative relationship between intra- and interspecific gene flow in many settings: species (or genomic compartments) with high levels of *intraspecific* gene flow (Currat *et al.* 2008; Petit & Excoffier 2009) are predicted to experience less *interspecific* gene exchange, because introgressed alleles will lose out against intraspecific variants during drift-based processes ('allele surfing') in colonizing populations (Petit & Excoffier 2009). If this is true, then reduced levels of gene flow within species represent a threat to species integrity in the face of hybridization and introgression (Petit & Excoffier 2009).

Species assemblages that are products of recent adaptive radiation are highly informative study systems for addressing the evolution of reproductive isolation and for evaluating the roles of drift and selection in maintaining species cohesion (Schluter 2000; Seehausen 2004). Of special interest are recently radiated taxa that occur in insular environments, which have long been recognized as natural laboratories for studying evolution (Losos & Ricklef 2009). Examples in the literature include cichlid fishes in African lakes (Barluenga *et al.* 2006), palm trees in oceanic islands (Savolainen *et al.* 2006) and endemic bromeliads occurring in terrestrial islands known as inselbergs (Sarthou *et al.* 2001; Barbará *et al.* 2007).

As for species radiations in general, the ensemble of evolutionary forces acting during radiations in island-like settings is contentious. Radiations in island-like environments may be adaptive or nonadaptive (Schluter 2000; Rundle & Nosil 2005; Rundell & Price 2009), may proceed via simple replacement of progenitor taxa (anagenesis) or by more complex sequences of evolutionary splits (cladogenesis) (Stuessy *et al.* 2006) and may or may not involve drift and founder effects during island colonization. Disentangling the deterministic and stochastic forces operating during island radiations thus represents a formidable challenge (Schluter 2000; Jorgensen & Olesen 2001; Rundell & Price 2009). One prominent recent hypothesis on the molecular ecology of adaptive radiations refers to the role of interspecific gene exchange: hybridization and introgression in isolated populations may effectively 'fuel' adaptive radiation by providing the standing genetic variation required for ecological speciation (Seehausen 2004). Recent genetic data on Darwin's finches on the Galápagos archipelago are indeed consistent with this prediction (Grant & Grant 2010). Assessing the role of hybridization during radiations also requires careful consideration of alternative explanations for allele sharing, such as homoplasy and shared ancestral polymorphism (incomplete lineage sorting; Martinsen *et al.* 2001).

With regard to shared ancestral polymorphism, recently radiated taxa often display similar genomes. This is the case because the process of speciation starts with only few differentially adapted loci, whereas much of the genome becomes differentiated only during later stages of divergence (Wu 2001; Via & West 2008; Nosil *et al.* 2009). Thus, descendant lineages are expected to share genetic polymorphism for some time (incomplete lineage sorting). Consequently, shared alleles in recently radiated taxa may be attributed to both, shared ancestral polymorphism *and* recurrent gene exchange between divergent populations, and distinguishing between these mechanisms is not a trivial task (Muir & Schlotterer 2005; Lexer *et al.* 2006). Knowing the genomic distribution of weakly differentiated chromosome segments would help, as pronounced block structure is expected under recurrent gene flow (Lexer *et al.* 2006). In the absence of genomic data, the geographical distribution of allele sharing may be used to address this issue. This is the case because the genetic signature of hybridization is expected to exhibit a strong local component (Olsen 2002; Petit *et al.* 2002; Palmé *et al.* 2004; Heuertz *et al.* 2006; : Hathaway *et al.* 2009; McKinnon *et al.* 2010; Pinheiro *et al.* 2010; among others). Direct evidence for or against hybridization among closely related species may also be won from crossing experiments, as demonstrated for the inselberg radiation that is at the centre of attention of the present study (Wendt *et al.* 2001, 2002; Rôças *et al.* 2004).

Inselbergs are isolated rock outcrops that rise abruptly from the surrounding forest matrix (Porembski 2007). Inselbergs, and Neotropical inselbergs in particular, are considered terrestrial habitat islands because of their strong spatial and ecological isolation, which provides a barrier against dispersal and migration. Inselbergs form centres of high diversity and endemism because of their considerable age (millions of years) and ecological peculiarity (Porembski 2007); steep gradients in climatic and edaphic factors separate these rock outcrops from the surrounding forest, and these factors help to explain their highly specialized flora (Meirelles *et al.* 1999).

Bromeliad species adapted to Neotropical inselbergs have recently attracted the interest of molecular ecologists and evolutionary biologists, because they represent extreme cases for understanding species cohesion and speciation in isolated environments (*Alcantarea* – southeastern Brazil: Barbará *et al.* 2007, 2008, 2009; *Pitcairnia* – French Guiana: Sarthou *et al.* 2001 and Boisselier-Dubayle *et al.* 2010). These studies revealed extremely high population differentiation and the absence of isolation by distance among inselbergs, thus indicating that these rock outcrops may resemble oceanic islands as arenas for evolutionary studies. In particular, their insular nature facilitates their use as extreme cases for testing the interplay of intra- and interspecific gene flow during plant speciation and radiation.

In the present study, we aim to address the interplay of microevolutionary forces operating within these radiating Neotropical inselberg species. In particular, we tackle the following questions regarding the mechanisms facilitating species cohesion and reproductive isolation in this group: (i) What do multi-species plastid DNA genealogies tell us about the presence or absence of interspecific gene flow (introgression) during inselberg radiations over extended periods of time? (ii) What are the potential roles of gene flow and selection in maintaining species cohesion in these strongly structured inselberg environments? (iii) What do patterns of genomic diversity and admixture tell us about the role of prezygotic *vs*. postzygotic barriers during speciation processes in inselberg bromeliads? We test hypotheses raised by recent crossing experiments (Wendt *et al.* 2001, 2002) and population genetic studies (Sarthou *et al.* 2001; Barbará *et al.* 2007; Boisselier-Dubayle *et al.* 2010) of inselberg taxa, which suggest a view of inselbergs as 'terrestrial islands' harbouring multiple sympatric species maintained by strong but permeable reproductive barriers.

Material and methods

Biological samples and DNA extraction

We studied populations of four morphologically distinct *Pitcairnia* (Bromeliaceae) species, exclusively saxicolous (rock-dwelling) and endemic to gneissic-granitic rock outcrops in southeastern Brazil: *P. albiflos*, *P. staminea*, *P. corcovadensis* and *P. flammea*. The first two species are endemic to outcrops in the cities of Rio de Janeiro and Niterói; *P. corcovadensis* is endemic to the inselbergs of Rio de Janeiro city and its surroundings, whereas *P. flammea* has a somewhat larger distribution range in southern and southeastern Brazil. All four species are self-compatible but their mating systems are divergent, ranging from predominantly outcrossing in *P. albiflos* (pollinator dependent) to highly selfing in *P. corcovadensis* and *P. staminea* (Wendt *et al.* 2001, 2002). The species exhibit divergent pollination syndromes (Wendt *et al.* 2001, 2002): the diurnal taxa *P. staminea*, *P. flammea* and *P. corcovadensis* have red, scentless flowers, whereas the nocturnal species *P. albiflos* has white, scented flowers. Nectar is produced by all species except *P. corcovadensis*. Hummingbirds and halictid bees frequently visit *P. flammea* but only rarely visit *P. corcovadensis*, whereas *P. staminea* is mainly visited by halictid and trigonid bees and butterflies. The predominant nocturnal visitors of *P. albiflos* are bats and hawk moths (Wendt *et al.* 2001, 2002). Although these differences in pollination syndromes suggest that prezygotic, ecological isolation between species is strong, hybrid swarms frequently form where these species co-occur (Wendt *et al.* 2001). For example, morphologically intermediate individuals were found in sympatric populations of *P. albiflos* and *P. staminea* (Wendt *et al.* 2000, 2001), and synthetic hybrids were obtained in crossing experiments involving these four taxa (Wendt *et al.* 2001, 2002; Rôças *et al.* 2004). Despite these reports of natural and experimental hybridization, the magnitude and direction of gene exchange in natural populations have never been demonstrated with molecular genetic markers.

Samples of inselberg species of *Pitcairnia* (*P. albiflos*, *P. staminea*, *P. corcovadensis* and *P. flammea*, 368 samples in total) were collected on eight granitic inselbergs located in the Atlantic Rainforest in southeastern Brazil, where these four species co-occur (Table 1; Fig. 1). For the narrow endemics *P. albiflos* and *P. staminea*, the sampled populations represent the entire geographical range of each species. Individuals were collected randomly on each inselberg rock outcrop. Geographical distances between the sampled inselbergs ranged from ~2 km (ITA – ANDO) to ~30 km (NHA-ITA). The altitude of the sampled populations ranged from 18 to 600 m above sea level. For each plant, leaves were collected and stored in silica gel. Total genomic DNA was extracted following Doyle & Doyle (1990).

Identification of plastid DNA markers

To facilitate the analysis of maternally inherited DNA lineages across multiple closely related species, six plastid microsatellite markers (plastid SSR, simple sequence repeats) were developed for semi-automated genotyping using fluorescently labelled primers (FAM, NED, VIC, PET – Applied Biosystems), following the protocols of Palma-Silva *et al.* (2009). One of these markers has previously been described for other bromeliad species (Locus VgCP4; Palma-Silva *et al.* 2009; Table S1, Supporting information). The remaining five plastid SSR were isolated *de novo* from *P. albiflos* and *P. staminea*

Population/species (code)	GPS position	Altitude (m)	Sample size	
			Nuclear	Plastid
Itacoatiara	S22 58.392, W43 02.477	120	27	15
P. staminea (sta-ITA)				
Andorinha	S22 58.441, W43 01.586	112	27	12
P. staminea (sta-ANDO)				
Forte Imbuhy	S22 56.673, W43 06.984	53	22	21
P. albiflos (alb-IMB)				
Pão de açucar	S22 57.178, W43 09.532	30		
P. staminea (sta-PAO)			40	38
P. albiflos (alb-PAO)			47	46
Hybrids (hyb-PAO)			33	32
Chacrinha	S22 57.388, W43 10.478	120		
P. staminea (sta-CHA)			25	25
P. albiflos (alb-CHA)			13	13
Hybrids (hyb-CHA)			5	5
Itanhangua	S22 59.769, W43 18.739	18		
P. staminea (sta-NHA)			—	28
Corcovado	S22 56.976, W43 13.356	600		
P. albiflos (alb-COR)			21	21
P. flammea (fla-COR)			—	24
P. corcovadensis (corco-COR)			—	22
Grajaú	S22 55.660, W43 16.248	275		
P. albiflos (alb-GRU)			31	15
P. flammea (fla-GRU)			—	3

Table 1 Population names, localities, geographical coordinates and sample sizes of *Pitcairnia* in the gneissic-granitic inselbergs of the Atlantic Rainforest in southeastern Brazil. Hybrid individuals were classified based on Bayesian admixture coefficients from STRUCTURE (see Results for details)

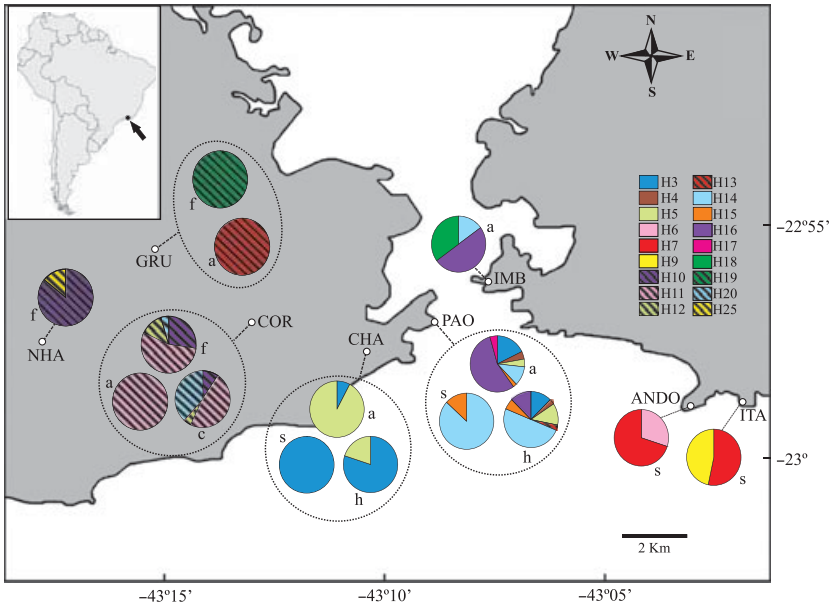

Fig. 1 Geographical distribution of *Pitcairnia* species in southeastern Brazil (Rio de Janeiro - left - and Niterói - right - cities) and plastid DNA haplotype frequencies. (a) *Pitcairnia albiflos*; (s) *Pitcairnia staminea*; (f) *Pitcairnia flammea*; (c) *Pitcairnia corcovadensis;* (h) hybrids between *Pitcairnia albiflos* and *Pitcairnia staminea* identified with STRUCTURE based on nuclear markers.

plastid DNA sequences. Primer pairs were designed by sequencing noncoding regions using the universal primers described by Taberlet *et al.* (1991), Demesure *et al.* (1995), Sang *et al.* (1997), Small *et al.* (1998) and Shaw *et al.* (2007). Amplification, sequencing, primer design and the screening of polymorphisms were carried out following Pinheiro *et al.* (2009). The characteristics and sequences of the primers designed for each plastid SSR marker are reported in Table S1 (Supporting information).

Nuclear microsatellite markers

To study the patterns of genomic diversity and admixture in these inselberg species, 15 nuclear microsatellite loci (nuclear SSR) isolated from *P. albiflos* (Paggi *et al.*

2008) and other bromeliads (Boneh *et al.* 2003; Sarthou *et al.* 2003; Palma-Silva *et al.* 2007) were genotyped in the studied populations. Genotyping reactions made use of fluorescently labelled primers (6-FAM or JOE) and protocols described by Palma-Silva *et al.* (2007). Both nuclear and plastid SSR alleles were resolved on an ABI PRISM 3100 DNA Analyzer (Applied Biosystems). Molecular sizes in base pairs were precisely sized against ROX (500) molecular size standard (Applied Biosystems) using GENEMAPPER v4.1 software (Applied Biosystems).

Data analysis

Analyses of plastid DNA lineages. In each individual, genetic variants at all plastid SSR sites were combined into haplotypes. Then, each population was characterized for its plastid DNA diversity using the number of haplotypes detected and gene diversity estimated using the program CONTRIB (Petit *et al.* 1998).

A median-joining haplotype NETWORK (Bandelt *et al.* 1999) was constructed based on the plastid DNA haplotypes using the program NETWORK 4.5.1.6 (http://www.fluxus-engineering.com) following the procedures described by Lexer *et al.* (2005). In addition, analysis of molecular variance (AMOVA) in ARLEQUIN 3.11 (Excoffier *et al.* 2005) was used to assess patterns of plastid DNA differentiation in hierarchical models, including either *species* or *geographical region* as the highest level in the hierarchy, and significance of each model was tested using 10 000 permutations. The single population of *P. corcovadensis* was not included in the hierarchical AMOVA to avoid 'species' groups with a single population.

Analysis of nuclear DNA microsatellites

Nuclear DNA diversity. The extent of interspecific gene flow in *Pitcairnia* species was addressed by using nuclear markers focusing exclusively on populations of *P. albiflos* and *P. staminea* (in which putative hybrids were characterized morphologically by Wendt *et al.* 2000, 2001). Thus, allopatric and sympatric populations of *P. albiflos* and *P. staminea* were characterized for their genetic diversity by calculating allelic richness (El Mousadik & Petit 1996), the variance in allele size, observed and expected heterozygosity, using the software FSTAT 1.2 (Goudet 1995). Departures from Hardy–Weinberg equilibrium (HWE) were tested with GENEPOP 3.5 (Raymond & Rousset 1995). GIMLET software (Valière 2002) was employed to detect and remove multi-ramet genets ('clones') in both species and their hybrids. In addition, all nuclear SSR loci were characterized for standardized differentiation measure G'_{ST} (Hedrick 2005) to check the extent to which interlocus differences in heterozygosity affected estimates of F_{ST}. The relationship between G'_{ST} and F_{ST} was explored using Spearman's rank correlation in SPSS.

As for plastid DNA, AMOVA in ARLEQUIN 3.11 (Excoffier *et al.* 2005) was used to test for nuclear genomic differentiation in hierarchical models including both species and *geographical region* as highest level in the hierarchy (inland *vs.* coastal, as revealed by plastid DNA). In addition, AMOVA was used to calculate intraspecific genetic structuring of the nuclear DNA and plastid DNA data in each species. Isolation by distance (Wright 1965) in *Pitcairnia* inselberg species was tested by calculating the correlation between geographical and genetic distance matrices with a standardized Mantel test (Sokal & Rohlf 1995), using 10 000 randomizations to determine significance.

Nuclear migration rates. Effective population sizes (N_e) and pairwise migration rates (N_em) were estimated following a coalescent theory and maximum-likelihood-based approach using MIGRATE 3.0.3 (Beerli & Felsenstein 1999) as described by Barbará *et al.* (2007). The computations were carried out under both the infinite allele model (IAM) and the stepwise mutation model (SMM), and mutation rates (μ) among loci were estimated from data. In addition, bidirectional single-locus migration rates (N_em) between *P. albiflos* and *P. staminea* were estimated for PAO population, the hybrid zone with the greatest sampling effort. Our rationale was to test for asymmetric introgression of single loci, which can point to negative interactions among nuclear loci as a mechanism maintaining reproductive isolation (Coyne & Orr 2004).

Patterns of genomic admixture. STRUCTURE 2.3.2 (Pritchard *et al.* 2000) was used to assign individuals to genetic clusters (K) and to identify hybrids between *P. albiflos* and *P. staminea*. The analyses were carried out under the admixture model assuming independent allele frequencies and using a burn-in period of 50 000, run lengths of 100 000 and 10 replicates per K ranging from 1 to 10. The method of Evanno *et al.* (2005) was employed to determine the most likely number of clusters (K) present in the data, based upon the *ad hoc* measure ΔK that evaluates the second-order rate of change of the likelihood function with respect to K. In addition, the clustering method of Anderson & Thompson (2002) implemented in NEWHYBRIDS version 1.1 was used to test assignments of individuals to different genotypic classes (pure parental species 1 or 2, F1's, F2's and backcrosses). Following the procedure described by Burgarella *et al.* (2009), STRUCTURE was first employed to classify individuals using a threshold of $Q = 0.90$, then

NEWHYBRIDS was used to assign plants to different hybrid classes using a threshold value of $Q = 0.75$.

Cytonuclear disequilibrium. To explore patterns and consequences of interspecific gene flow in *Pitcairnia* inselberg species more deeply, departures from random cytonuclear associations (Arnold 1993) were tested using the CNDm program (Basten & Asmussen 1997). The analyses were carried out by encoding nuclear markers in the form of synthetic alleles (S, alleles typical of *P. staminea*; A, alleles typical of *P. albiflos*) and by encoding plastid DNA alleles as synthetic haplotypes (Hs, haplotypes typical of *P. staminea*; Ha, haplotypes typical of *P. albiflos*). Only the eight most species-informative nuclear loci were used in this analysis. Normalized cytonuclear disequilibria (CND) were calculated following Asmussen & Basten (1994) for allelic and genotypic associations, and significance levels were tested using Fisher's exact test.

Significantly positive and negative values of CND indicate positive and negative associations between nuclear and cytoplasmic genomes, respectively. Significant disequilibria involving heterozygous nuclear loci (AS) – represented by D_2 (AS/Hs) – point to nonrandom mating in hybrid zones and unidirectional hybridization, whereas significant disequilibria involving homozygous genotypes D_1 and D_3 (involving nuclear genotypes SS and AA, respectively) point to barriers to introgression, effectively maintaining species integrity in the face of gene flow (Asmussen *et al.* 1989; Latta *et al.* 2001).

Results

Multi-species plastid DNA genealogies

The characterization of six plastid SSR loci in 13 populations of four *Pitcairnia* species yielded from two to six alleles per locus (Table S1, Supporting information), resulting in a total of 25 haplotypes (Table 2; Fig. 1; note that hybrids in sympatric populations of *P. albiflos* and *P. staminea* were inferred from nuclear SSR and STRUCTURE analysis). The distribution of the haplotypes revealed surprising patterns of haplotype sharing: seven recurrent (nonsingleton) haplotypes were shared among species: H3, H14 and H15 between *P. albiflos* and *P. staminea*, H12 and H20 between *P. flammea* and *P. corcovadensis*, H10 among *P. staminea*, *P. flammea* and *P. corcovadensis*, and H11 among *P. albiflos*, *P. flammea* and *P. corcovadensis* (Table 2, Fig. 1). In contrast, only a single haplotype was shared between the two geographical areas sampled, namely coastal and inland inselbergs (Table 2). Accordingly, a highly significant proportion of the molecular variance (11%; $p < 0.001$)

Table 2 Distribution of plastid DNA haplotypes in populations of *P. staminea*, *P. albiflos*, *P. flammea* and *P. corcovadensis*. (A) populations ordered by species; (B) populations ordered by geographical region (inland *vs.* coastal). Hybrids detected based on nuclear admixture analysis in STRUCTURE are not included in the table

Haplotype	*N*	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23	H24	H25
(A)																										
Taxonomic level																										
P. staminea	118		X	X			X	X	X	X	X				X	X										X
P. albiflos	116	X		X	X	X						X		X	X	X	X	X					X			
P. flammea	27										X	X	X							X	X			X	X	
P. corcovadensis	22										X	X	X								X	X				
(B)																										
Geographical level																										
Inland (GRU, COR, NHA)	113	X									X	X	X	X						X	X	X		X	X	X
4 species																										
Coastal (PAO, CHA, IMB, ITA, ANDO) 2 species	170		X	X	X	X	X	X	X	X				X	X	X	X	X	X				X			

Table 3 Analysis of molecular variance (AMOVA) of plastid DNA markers for two different hierarchical models, one with 'species' and one with 'geographical regions' as highest levels in the hierarchy

		Plastid microsatellites		
	Source of variation	Variation %	*F*-statistics	*P*-value
Species				
By species†	Among species	0	FCT = 0.00	n.s.
	Among population within species	61	FSC = 0.61	<0.001
	Within populations	39	FST = 0.60	<0.001
Geographical regions				
Inland *vs.* coastal inselbergs	Among regions	11	FCT = 0.11	<0.001
	Among population within regions	50	FSC = 0.56	<0.001
	Within populations	39	FST = 0.61	<0.001

†The single population of *Pitcairnia corcovadensis* was not included in the hierarchical AMOVA to avoid 'species' groups with a single population.

in the plastid DNA data resided *among geographical regions*, whereas none resided *among species* (Table 3).

Median-joining analysis revealed a multi-species haplotype network with two major haplogroups: one group containing primarily haplotypes typical of the inland inselbergs (populations NHA, COR and GRU; see Table 1 for species and population codes) and the other containing mainly haplotypes typical of the coastal inselbergs (populations ITA, ANDO, IMB, PAO, CHA) (Fig. 2). The extensive haplotype sharing among species indicates interspecific gene flow, homoplasy or retention of ancestral polymorphism predating the divergence of the species. Phylogenetic relationships of haplotypes revealed close genetic affinity among haplotypes belonging to the same or close inselbergs (Fig. 2a), regardless of the species of origin (Fig. 2b).

Nuclear microsatellite diversity

All 15 nuclear SSR analysed were polymorphic with up to 24 alleles segregating in each species and with observed (H_O) and expected (H_E) heterozygosities of up to 0.88 and 0.92, respectively (Table S2, Supporting information). Few loci displayed significant departures

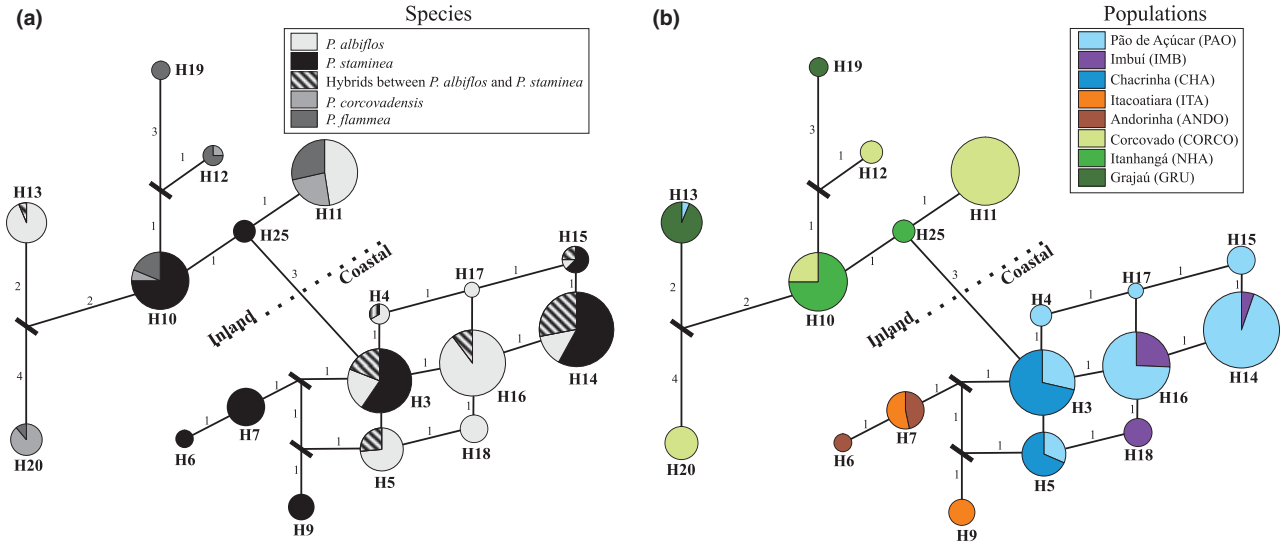

Fig. 2 Median-joining network of plastid DNA haplotypes of *Pitcairnia* species in southeastern Brazil. The number of mutations required to explain transitions among haplotypes is indicated along the lines connecting the haplotypes. Filled circles indicate the haplotypes, the size of each circle being proportional to the observed frequency of each haplotype. (a) Network labelled by species sampled, each colour representing one of the four species and hybrids between *P. albiflos* and *P. staminea* identified by nuclear admixture analysis in STRUCTURE. (b) Network labelled by populations sampled, each colour representing one population. For population abbreviations, see Table 1.

from HWE in *P. albiflos* and in hybrids (five and six loci, respectively; Table S2, Supporting information). In contrast, almost all markers (12 loci) departed from HWE in *P. staminea*, consistent with the predominantly selfing mating system of this species. Most loci displayed high numbers of unique alleles or striking shifts in allelic frequencies, with 53 private alleles (out of 116 alleles) in *P. albiflos* and 56 private alleles (out of 136 alleles) in *P. staminea*.

With regard to diversity at the population level, the number of multi-ramet genets ('clones') detected in the studied populations was low, ranging from zero to three per population (Table 4), pointing to the importance of sexual reproduction (seed recruitment) in population persistence. Population-level inbreeding coefficients (F_{IS}) were higher, and significant heterozygote deficits were more prevalent in *P. staminea* than in *P. albiflos* (F_{IS} = 0.24 and 0.11, respectively; Table 4), consistent with the per-locus results.

Patterns of nuclear divergence and gene flow

Strong nuclear genomic differentiation was detected between *P. albiflos* and *P. staminea* (22% in AMOVA, $P < 0.001$; Table S3, Supporting information; and G'_{ST} between species of 0.65, $P < 0.001$), in contrast to the extensive sharing of plastid DNA haplotypes across species boundaries. The genetic divergence parameters G'_{ST} and F_{ST} were correlated across loci (Spearman's $r = 0.88$, $P < 0.001$), thus indicating that F_{ST} was little affected by interlocus differences in heterozygosity. The separate AMOVA model for each species and genome revealed that genetic differentiation within species was higher for plastid DNA than for nuclear DNA in both *P. albiflos* (nuclear DNA = 34%, plastid DNA = 57%; both $P < 0.001$) and *P. staminea* (27% and 74%; both $P < 0.001$). Genetic and geographical distances between populations were not significantly correlated in either species, indicating the absence of isolation by distance in these inselberg *Pitcairnia* species (Fig. 3). Graphical inspection revealed the likely cause: genetic divergence between most populations was great even at short geographical distances, with genetic divergence for intraspecific comparisons approaching that for interspecific ones (Fig. 3).

Maximum-likelihood-based estimates of migration rates (N_em) for sympatric populations of *P. albiflos* and *P. staminea* (PAO and CHA, the two 'hybrid zone' localities) were extremely low (Fig. 4), indicating restricted gene flow between inselberg populations and species. In fact, intraspecific N_em estimates were <1 N_em, often regarded as the minimum required for species cohesion (Table S4 (Supporting information) for most pairwise comparisons).

Interspecific migration rates were asymmetric towards *P. albiflos*, with higher N_em values from *P. staminea* to *P. albiflos* (Fig. 4). Single-locus estimates of gene flow, obtained for the larger of the two hybrid

Table 4 Characterization of *Pitcairnia staminea*, *P. albiflos* and hybrid populations with fifteen nuclear microsatellites, including sample size (*N*), number of clones (*N* clones), allelic richness (*Rs*), variance in allele size (Var), observed heterozygosity (H_O), expected heterozygosity (H_E) and inbreeding coefficient (F_{IS})

Species/Populations	*N*	*N* clones	*Rs*	Var	H_O	H_E	F_{IS}
P. albiflos							
Alb-COR	21	1	2.80	15.3	0.317	0.425	0.260***
Alb-GRU	31	1	2.43	5.30	0.315	0.334	0.056
Alb-IMB	22	1	3.78	37.1	0.522	0.528	0.010
Alb-CHA*	13	1	3.32	13.9	0.348	0.380	0.087
Alb-PAO*	47	0	3.69	19.9	0.413	0.477	0.134***
Overall/average	134	0.8	3.20	18.3	0.383	0.429	0.109
P. staminea							
Sta-ANDO	27	0	3.98	31.9	0.411	0.449	0.087**
Sta-ITA	27	3	4.47	64.9	0.499	0.560	0.111***
Sta-CHA*	25	0	2.81	28.8	0.273	0.345	0.212***
Sta-PAO*	40	0	3.59	43.3	0.206	0.454	0.548***
Overall/average	121	0.75	3.71	42.2	0.347	0.452	0.240
Hybrid							
Hyb-CHA*	5	1	3.20	7.10	0.333	0.333	−0.139
Hyb-PAO*	33	1	3.72	38.7	0.528	0.535	0.146***
Overall/average	38	0.5	3.46	22.9	0.431	0.434	0.003

Departures of within-population inbreeding coefficients (F_{IS}) from Hardy–Weinberg equilibrium (HWE) are indicated by asterisks.
**$P < 0.01$; ***$P < 0.001$.

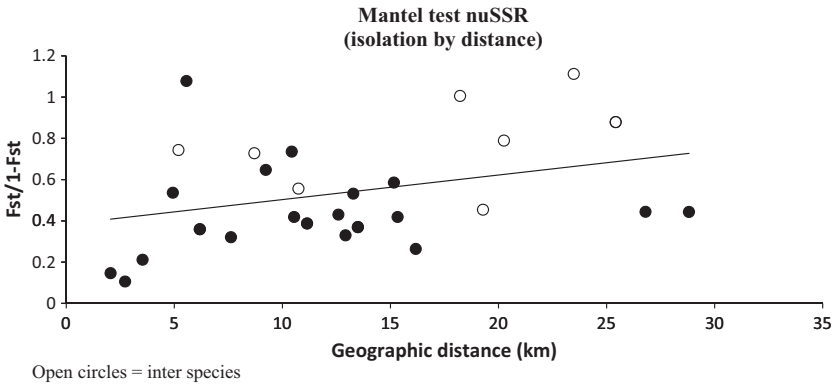

Fig. 3 Relationship between genetic divergence, based on F_{ST} for nuclear microsatellites, and geographical distances in kilometres among populations of four *Pitcairnia* species (Mantel test correlation: r = 0.109; P = 0.061). Filled circles: within-species comparisons; open circles: between-species comparisons.

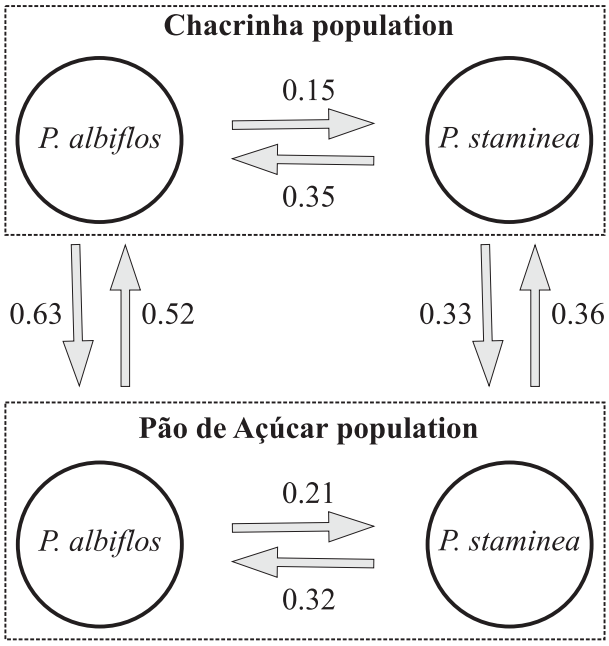

Fig. 4 Bidirectional migration rates (effective number of migrants, N_em) between two sympatric inselberg populations of *Pitcairnia albiflos* and *P. staminea*. For population abbreviations, see Table 1.

zones (PAO), revealed two loci (Pit5 and Pit8) with particularly high and asymmetrical N_em from *P. staminea* towards *P. albiflos*, and two additional loci with zero gene flow in the opposite direction (PaC05, Pad07; Table S5, Supporting information).

Patterns of genomic admixture

Genomic admixture analysis with STRUCTURE 2.2.3 identified K = 2 as the most likely number of genetic clusters (Figs 5 and Fig. 1S), based on threshold values of $Q \geq 0.90$ (*P. albiflos*) or $Q \leq 0.10$ (*P. staminea*). Individuals showing intermediate assignment probabilities were found in sympatric populations PAO and CHA (38 individuals with admixture coefficients Q ranging from 0.353 to 0.873; Table 5). Further analysis carried out with NEWHYBRIDS indicated that recombinant hybrid classes (F2s and backcrosses) were more frequent than F1s (Fig. S2, Supporting information). At a threshold of Q = 0.75, NEWHYBRIDS detected six F1s, 12 F2s and two backcrosses towards *P. albiflos* (Table 5), whereas no backcrosses towards *P. staminea* were observed.

Tests for cytonuclear associations

Interspecific hybrids carried haplotypes from both parental species: haplotypes typical of *P. staminea* (e.g. H3 and H14) and haplotypes typical of *P. albiflos* (e.g. H5 and H16) (Fig. 2b, Table 5). This indicates that *hybridization* occurs in both directions (with both species acting as male and female parents). Accordingly, tests for cytonuclear interactions in hybrids were nonsignificant (Table 6; coefficient D_2 involving nuclear genotypes AS and plastid DNA haplotypes Hs). In contrast, extensive CND were detected for homozygous nuclear genotypes (D_1 and D_3; Table 6). In agreement with the nuclear DNA results, plastid DNA introgression was asymmetric towards *P. albiflos*, with no evidence of plastid DNA introgression occurring into pure individuals of *P. staminea* (Figs 1 and 2b; Table 2 and 5). The most common haplotypes of *P. staminea* (H3 and H14) were detected in pure *P. albiflos* and in hybrids of all genotypic classes (F1, F2, and backcrosses; Table 5), again pointing to a porous but strongly asymmetric species barrier.

Discussion

Persistent interspecific gene flow during adaptive radiation of Pitcairnia *spp. on Neotropical inselbergs*

Recent modelling and review studies on the interplay of intra- and interspecific gene flow in evolution (e.g. Morjan & Rieseberg 2004; Currat *et al.* 2008; Petit &

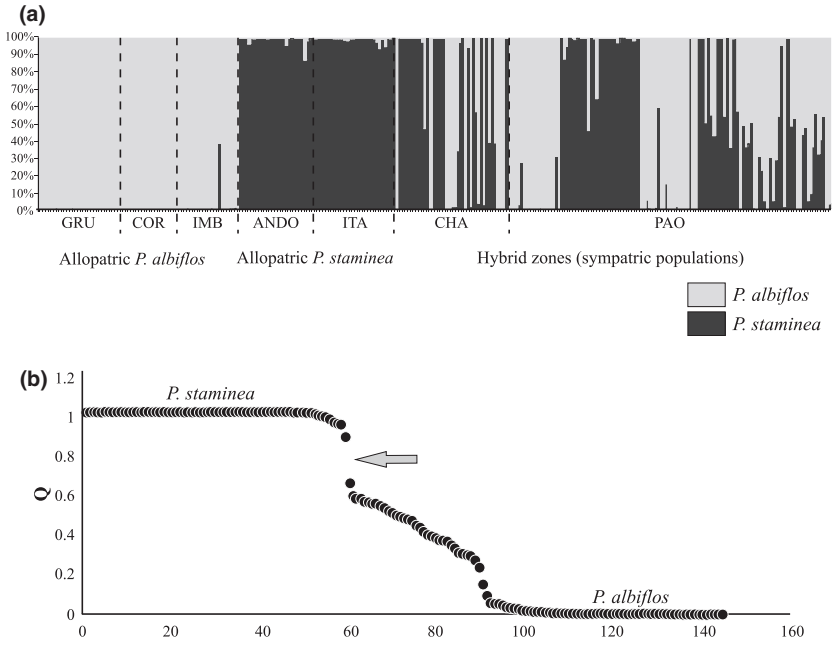

Fig. 5 Bayesian admixture proportions (Q) of each *P. albiflos* and *P. staminea* individual estimated by STRUCTURE, assuming $K = 2$. (a) All allopatric and sympatric populations. (b) Sympatric populations (hybrid zones) only. Arrow indicates the gap between admixed individuals and *P. staminea* For population abbreviations, see Table 1.

Table 5 Plastid DNA haplotypes found in two hybrid zones of *Pitcairnia albiflos* and *Pitcairnia staminea*, including indication about haplotype sharing among taxa, haplotype frequencies in both species and hybrids based on STRUCTURE and NEWHYBRIDS assignment probabilities and range of STRUCTURE nuclear admixture proportions (Q) for hybrids plants carrying each haplotype

		Frequencies (Structure)				Frequencies (NewHybrids)		
Haplotype	Taxon	*P. albiflos*	Hybrids	*P. staminea*	Hybrid nuclear admixture (Q)	F1	F2	BC *P. albiflos*
H1	Alb	1						
H2	Sta		1		0.105			
H3	Alb/Hyb/Sta	9	8	25	0.437–0.709	2	3	2
H4	Alb/Hyb	2	1		0.447		1	
H5	Alb/Hyb	17	3		0.457–0.705	1	1	
H6	Sta			3				
H7	Sta			15				
H9	Sta			7				
H13	Alb/Hyb	15	1		0.353		1	
H14	Alb/Hyb/Sta	8	16	33	0.403–0.777	3	4	
H15	Alb/Hyb/Sta	1	2	5	0.392–0.544		2	
H16	Alb/Hyb	35	4		0.452–0.873		1	
H17	Alb	2						
H18	Alb/Hyb	7	1		0.54			1

STRUCTURE assignments: pure *P. albiflos*, pure *P. staminea* and hybrids were defined by posterior probabilities of $Q \geq 0.90$; $0.90 > Q \geq 0.10$; and $Q \leq 0.10$, respectively.
NEWHYBRIDS assignments: only hybrid classes with individuals showing posterior probabilities of $Q \geq 0.75$ were considered.

Excoffier 2009) call for more empirical studies of this topic, including extreme spatial settings and species-rich environments, as is the case for Neotropical inselbergs (Porembski & Barthlott 2000). One outstanding pattern detected in our study was the extensive plastid DNA haplotype sharing among multiple sympatric inselberg species, indicating both interspecific gene flow (introgression) and shared ancestral polymorphism (incomplete lineage sorting) persisting over extended periods of time (Figs 1 and 2; Tables 2, 3, and 5). Also remarkable was the strong geographical structuring of plastid DNA haplotype sharing at small spatial scales (even at distances <11 km), with little to no plastid DNA differentiation among species but strong differentiation among inselbergs or narrowly defined geographical regions (coastal *vs*. inland; Figs 1 and 2; Table 2 and 3).

Table 6 Cytonuclear disequilibria (standard deviations) observed in two hybrid zones of *Pitcairnia albiflos* and *P. staminea*

PAO population				
Loci	D_1 (SS/Hs)	D_2 (AS/Hs)	D_3 (AA/Hs)	D (S/Hs)
PaD07	0.8603 (0.0172)***	0.4177 (0.0174)	−0.6943 (0.0195)***	0.7581 (0.0180)***
VgA04	0.6895 (0.0192)***	0.4760 (0.0146)	−0.6335 (0.0203)***	0.6572 (0.0196)***
PaA05	0.8736 (0.0168)***	0.2276 (0.0157)	−0.6861 (0.0193)***	0.7639 (0.0184)***
PaA10	0.7440 (0.0188)***	0.7525 (0.0128)	−0.7462 (0.0197)***	0.7452 (0.0200)***
PaB11	0.5868 (0.0213)***	−0.1905 (0.0189)	−0.3686 (0.0223)***	0.4671 (0.0202)***
E6b	1.0000 (0.0147)***	0.3821 (0.0151)	−0.7861 (0.0181)***	0.8707 (0.0173)***
ct5	0.8646 (0.0178)***	0.1962 (0.0193)	−0.6166 (0.0206)***	0.7124 (0.0189)***
PaC05	0.9306 (0.0161)***	0.4183 (0.0155)	−0.7651 (0.0189)***	0.8319 (0.0177)***
CHA population				
Loci	D_1 (SS/Hs)	D_2 (AS/Hs)	D_3 (AA/Hs)	D (S/Hs)
PaD07	1.0000 (0.0295)***	0.5385 (0.0312)	−0.8558 (0.0281)***	0.9145 (0.0334)***
VgA04	0.8728 (0.0298)***	−0.0444 (0.0183)	−0.7719 (0.0317)***	0.8196 (0.0309)***
PaA05	1.0000 (0.0272)***	−0.0444 (0.0248)	−0.8806 (0.0316)***	0.9372 (0.0289)***
PaA10	1.0000 (0.0272)***	0.1731 (0.0194)	−0.8859 (0.0300)***	0.9387 (0.0295)***
PaB11	1.0000 (0.0266)***	−0.6296 (0.0338)	−0.8148 (0.0339)***	0.9242 (0.0292)***
E6b	0.8677 (0.0297)***	1.0000 (0.0151)	−0.8897 (0.0300)***	0.8797 (0.0295)***
ct5	1.0000 (0.0272)***	−0.0444 (0.0183)	−0.8819 (0.0299)***	0.9376 (0.0301)***
PaC05	1.0000 (0.0281)***	0.0714 (0.0279)	−1.0000 (0.0330)***	1.0000 (0.0294)***

D_1; D_2; D_3, genotypic disequilibria; D, allelic disequilibria; SS, nuclear genotypes typical of *P. staminea*; AS, nuclear genotypes heterozygous for A and S alleles; AA, nuclear genotypes typical of *P. albiflos*; Hs, plastid DNA haplotype typical of *P. staminea*; Fisher's exact test ***$P < 0.0001$.

This is in line with the expectation of island-like patterns of diversification in inselberg taxa (strong isolation *among* isolated inselbergs coupled with increased opportunity for species interactions *within* inselbergs; Porembski & Barthlott 2000; Sarthou *et al.* 2001; Barbará *et al.* 2007; Boisselier-Dubayle *et al.* 2010). Our data support the idea that intraspecific gene flow among inselbergs is unusually low (Sarthou *et al.* 2001; Barbará *et al.* 2007; Boisselier-Dubayle *et al.* 2010) and that populations of closely related inselberg species have frequently been maintained in sympatry (=in the face of gene flow) confined to narrow habitat islands.

Haplotype sharing can potentially be explained by different processes: local gene flow (recent introgressive hybridization), the retention of ancestral polymorphism (incomplete lineage sorting), homoplasy (evolutionary convergence) or a combination of these. The positioning of haplotypes within the network (Fig. 2) and spatial patterns of haplotype sharing (Figs 1 and 2: Table 2) are highly informative in distinguishing between these scenarios. The fact that shared haplotypes are both central (H3, H14, H10) and peripheral (H11, H12, H15, H20) in the plastid DNA haplotype network (Fig. 2), present at both high (H3, H14, H10, H11) and low frequencies (H12, H15, H20), supports the hypothesis that many of the shared haplotypes are because of introgression, i.e. ancestral polymorphism is not the only cause for haplotype sharing. The strong spatial pattern of haplotype sharing among inselbergs and regions (Fig. 1; coastal *vs.* inland) is also in line with locally restricted introgression rather than homoplasy or ancestral polymorphism. Finally, experimental cross-pollination studies also indicate that hybridization in these taxa occurs easily (Wendt *et al.* 2001, 2002; Rôças *et al.* 2004). It would thus appear that interspecific hybridization and introgression in inselberg taxa of *Pitcairnia* are more widespread than previously believed (Wendt *et al.* 2002).

Hybridization and introgression have been identified as the main causes of geographically structured sharing of plastid DNA in many temperate plant species complexes (*Quercus*: Petit *et al.* 2002; *Betula*: Palmé *et al.* 2004; *Fraxinus*: Heuertz *et al.* 2006; *Silene*: Hathaway *et al.* 2009; *Eucalyptus*: McKinnon *et al.* 2010; among others), but relatively few examples are available from the tropics (*Manihot*, Olsen 2002; *Epidendrum*, Pinheiro *et al.* 2010). Here, this spatial pattern was recovered at surprisingly small geographical scales (Figs 1 and 2), indicating a great potential for interspecific gene flow to contribute to the spatial dynamics of diversity in Neotropical inselberg taxa.

Ancestral polymorphism may contribute to haplotype sharing in these taxa, but it cannot explain its strong spatial component (Fig. 1) and is even less likely to explain patterns of allele sharing at rapidly evolving nuclear microsatellites (below). Quantifying the contribution of ancestral polymorphism to patterns of DNA sharing in these inselberg taxa will require a robust species-level molecular phylogeny of the genus (~350 species), which is not currently available. A phylogenetic approach is crucial for understanding diversification in island-like environments (and elsewhere) (Givnish & Sytsma 1997, Givnish *et al.* 2007). A species-level phylogeny would facilitate tests of the mode and tempo of speciation and the prevalence of geographical *vs*. ecological speciation trajectories (Barraclough & Nee 2001). Time-calibrated, saturated phylogenies (e.g. Savolainen *et al.* 2006) would also reveal the age of species splits during the radiation of *Pitcairnia* spp., which would provide us with estimates of the time these species may have been in contact on inselbergs. Of course, correct dating would require the identification of phylogenetic markers that are little affected by interspecific gene exchange (Wu 2001), which appears feasible with the use of comparative genomics and bioinformatics tools now available (Duarte *et al.* 2010).

Introgression of different genomic compartments

Our comparative analysis of plastid DNA and nuclear genetic data indicate that the plastid genome is more prone to introgression than the nuclear genome, as observed in other groups of plants. In our case, the differential introgression of genomes appears to be a direct consequence of differences in levels of gene flow characteristic of plastid DNA and nuclear DNA (Petit & Excoffier 2009). The use of nuclear markers allowed us to assess the prevalence of gene flow via pollen *vs*. seeds in both species. Plastid DNA showed stronger genetic structure (*P. albiflos:* $\theta = 0.56$, *P. staminea:* $\theta = 0.74$,) than nuclear DNA markers ($\theta = 0.26$ and 0.34, respectively). This implies that in *Pitcairnia* species, the dispersal of seeds (which carry the maternally inherited plastid genome) is expected to be restricted, as has recently been shown for other bromeliads (Barbará *et al.* 2008; Palma-Silva *et al.* 2009; Paggi *et al.* 2010). So female propagules (seeds) represent the sex with poorer dispersibility, and this may explain the higher levels of introgression observed for maternally inherited plastid DNA (Currat *et al.* 2008; Du *et al.* 2009; Petit & Excoffier 2009). One important implication of this finding is that phylogenetic studies of species diversification in *Pitcairnia* spp. should make use of single or low copy nuclear loci instead of plastid markers, which may be true for the entire Bromeliaceae family. Very large sets of informative single/low copy nuclear genes for this purpose are rapidly becoming available from bioinformatics comparisons of sequenced plant genomes (Duarte *et al.* 2010).

The role of gene flow in species cohesion

Our study of four sympatric species adapted to inselberg habitat islands indicates that the level of intraspecific neutral gene exchange is too low to hold populations of these species together (Fig. 4; <1 N_em per generation) and interspecific gene flow surprisingly common. This pattern is remarkably similar to patterns of intra- and interspecific gene flow seen in *Geospiza* finches from the Galápagos islands (Grant & Grant 2010). In fact, estimates of nuclear genetic divergence (F_{ST}) among populations of the same bromeliad species are so great that they fit almost perfectly into the larger distribution including all interspecific divergence estimates (Fig. 3; isolation by distance relationship not significant). This indicates that other forces (other than within-species gene flow) are operating to maintain species cohesion (Rieseberg & Burke 2001; Morjan & Rieseberg 2004). Similarly, low levels of intraspecific gene flow as reported for Brazilian *Pitcairnia* inselberg species here ($N_em = 0.15$–0.35; Fig. 4) have also been reported for other bromeliads adapted to similar island-like environments: *Pitcairnia geyskesii* ($N_em = 0.2$; Sarthou *et al.* 2001; Boisselier-Dubayle *et al.* 2010) and *Alcantarea* species (N_em from 0.45 to 0.75; Barbará *et al.* 2007). These findings challenge the idea that species are cohesive entities held together primarily by gene flow (Mayr 1942) and indicate that natural selection, i.e. the spread of advantageous alleles, contributes to species cohesion in strongly structured inselberg environments (Morjan & Rieseberg 2004; Barbará *et al.* 2007). Of course, a minimum level of gene exchange will be required for the spread of advantageous alleles, coupled with selection coefficients of sufficient magnitude ($s > 0.05$; Slatkin 1976). Thus, gene flow at particular loci appears to be crucial for maintaining species cohesion, even if overall rates of neutral gene exchange are low.

Strong but permeable reproductive barriers

So far, we have discussed genetic interactions among sympatric *Pitcairnia* inselberg species primarily in terms of neutral processes (Currat *et al.* 2008; Petit & Excoffier 2009), but understanding patterns of interspecific gene flow also requires the explicit consideration of reproductive barriers (Barton & Hewitt 1985; Pialek & Barton 1997; Coyne & Orr 2004). Our data on nuclear admixture revealed pronounced genomic cohesion of the

studied species pair, *P. albiflos* and *P. staminea* (Figs 5 and 2S), indicating a strong 'genomic filter' *sensu* Martinsen *et al.* (2001), despite a long history of gene exchange as indicated by plastid DNA. Thus, multiple pre- and postzygotic barriers appear to contribute to reproductive isolation between these species, as previously shown for other groups of plants (Widmer *et al.* 2009). Nevertheless, these reproductive barriers are permeable and introgression is possible at least in one direction (towards *P. albiflos*; Figs 5 and 2S). Whether introgression among these species is more likely to be neutral or adaptive remains to be clarified. It should be noted, however, that this distinction is artificial, because introgressed alleles that are initially neutral may become adaptive in certain environments and genetic backgrounds ('adaptation from standing variation'; Barrett & Schluter 2008).

Maintenance of sympatric Pitcairnia *inselberg species: pre- or postzygotic barriers?*

Bromeliads represent a textbook example for adaptive radiation (Benzing 2000), and this implies an important role for ecological isolating barriers in speciation (Schluter 2000). In animal-pollinated plants such as bromeliads, such ecological barriers are often prezygotic (Widmer *et al.* 2009), and the divergent floral traits, flowering times and mating systems in these *Pitcairnia* species (see Introduction) are consistent with this pattern. Nevertheless, populations of inselberg-adapted bromeliads also experience extreme geographical isolation (Sarthou *et al.* 2001; Barbará *et al.* 2007; Boisselier-Dubayle *et al.* 2010; this study), which should facilitate the accumulation of postzygotic incompatibilities (Coyne & Orr 2004); note that in plants, postzygotic barriers sometimes evolve rapidly, as indicated by the frequent polymorphism of postzygotic isolation factors within species (Scopece *et al.* 2010). Indeed, a recent study of 42 sympatric bromeliad species suggested that prezygotic isolation is weak (Wendt *et al.* 2008), suggesting an important role for postzygotic factors in species isolation. Nevertheless, that study did not include representatives of Pitcairnioideae, the subfamily studied here, leaving the question of the nature of reproductive barriers maintaining these sympatric bromeliads open. Our molecular genetic data allow us to address this issue.

In the larger (=more informative) of the *Pitcairnia* hybrid zones studied with nuclear markers (Pão de Açucar; Fig. 2S), F1 hybrids were rare compared to F2s and backcrosses to *P. albiflos* (BCPa). Note that the NEWHYBRIDS classification of hybrid classes used in Table 5 is very stringent – Fig. 2S provides a more complete account of the relative probabilities of different recombinant hybrid classes. The results are consistent with recent cross-pollination experiments in these species and hybrids (Wendt *et al.* 2001, 2002). These experiments indicated that traits associated with assortative mating such as flowering time, pollinator specificity, and mating systems result in prezygotic isolation in these species. Although *P. albiflos* and *P. staminea* show some overlaps in their flowering times, hybrids clearly start to flower first and have a longer flowering period, followed by *P. albiflos* and only then by *P. staminea*, the species with the shortest flowering period (Wendt *et al.* 2001). The 'transgressive' tendency of hybrids to flower earlier than their parental species may explain why there are more F2's than backcrosses, and asymmetrical introgression towards *P. albiflos* may be explained by the greater overlap in flowering times (Figs 5 and 2S).

Pollinator isolation is also likely to play a role in prezygotic isolation of these species, judging by the differences in flower colour and the presence of diurnal pollination in one species and nocturnal pollination in the other (Wendt *et al.* 2001). Furthermore, the two species differ greatly in their mating systems (selfing/outcrossing rates): although both are self-compatible, only *P. staminea* can self-pollinate spontaneously (Wendt *et al.* 2001). Accordingly, our genetic data revealed consistently positive inbreeding coefficients (F_{IS}) for *P. staminea* (Table 4). So we would expect that *P. staminea* is more strongly protected from interspecific introgression than its outcrossing, closely related congener *P. albiflos*, which is exactly the pattern recovered by the present study (Figs 5 and 2S).

Divergent mating systems are known to contribute to strong reproductive barriers in plants (Sweigart & Willis 2003; Abbott & Lowe 2004; Fishman & Stratton 2004; Brandvain & Haig 2005; Martin & Willis 2007). Phenotypic changes associated with shifts in mating systems can also influence the degree of reproductive isolation between selfers and their outcrossing relatives (Levin 1971; Sweigart & Willis 2003). This is the case because the floral traits that are typical of selfers (reduction or absence of nectar production and flower scent) tend to diminish pollinator visitation and, therefore, cross-pollination (Sweigart & Willis 2003). Clearly, our genetic data are compatible with any of these premating barriers: phenology, pollinator isolation and divergent mating systems, and in fact all three may act in combination.

Our data are also informative regarding the potential role of postzygotic barriers between these two sympatric species. Postzygotic barriers may be exogenous (hybrids fall between the habitats of the parental species) or intrinsic, i.e. incompatibilities because of cytonuclear or purely nuclear (Bateson–Dobzhansky–Muller or BDM) interactions (Orr 1996; Tiffin *et al.* 2001; Coyne

& Orr 2004; Turelli & Moyle 2007). We have little evidence for purely exogenous postzygotic factors (these species and hybrids grow intermingled on the same rocks). Based on our data, cytonuclear interactions do not appear to play an important role either, because cytoplasmic DNA of both species is found in hybrids, and CNDs involving heterozygous genotypes are not significant (Table 6). Thus, the significant CNDs involving homozygotes (Table 6: coefficients D_1 and D_3) indicate the action of other (presumably prezygotic) isolating mechanisms, rather than cytonuclear incompatibilities *per se* (Asmussen *et al.* 1989). The fact that these significant coefficients are of the *same sign* and of *similar magnitude* across all loci also indicates that CNDs arose by migration and assortative mating, rather than by selection for particular cytonuclear combinations.

Weak support for nuclear (BDM) incompatibilities stems from estimates of bidirectional migration rates for nuclear microsatellites (Table S5, Supporting information). Two loci show strongly asymmetrical migration (>1 migrant per generation towards *P. albiflos* and zero migration towards *P. staminea*; Table S5, Supporting information), suggestive of linkage to loci involved in BDM incompatibilities (Table S5, Supporting information). Asymmetries in reproductive isolation are expected under the BDM model because of stochastic differences in the accumulation of these incompatibilities (Coyne & Orr 2004; Turelli & Moyle 2007; Scascitelli *et al.* 2010). Similar indications for BDM incompatibilities were recently revealed by asymmetrical migration rates in *Helianthus annuus* × *H. debilis* hybrid zones (Scascitelli *et al.* 2010). The detection of potential BDM interactions is consistent with the observation of reduced germination rates in artificial crosses between these species (Wendt *et al.* 2001).

Conclusions and conservation implications

In summary, multiple prezygotic barriers (phenology, pollinator isolation and divergent mating systems) are responsible for genomic cohesion in *Pitcairnia* inselberg species, resulting in a highly selective 'genomic filter', and postzygotic (BDM) incompatibilities may contribute to species barriers in this group. The presence of multiple barriers and their interactions have allowed these species to persist in sympatry for extended periods of time. Despite the presence of this 'filter', the gene pools of these species have been affected by hybridization and introgression over long timescales, as indicated by both nuclear and plastid DNA. Thus, interspecific gene exchange is an integral aspect of this inselberg radiation, rather than a product of recent disturbance. The strong 'filter' for nuclear alleles suggests that there is no great danger of genetic assimilation, at least in *P. staminea* and *P. albiflos*. Rather, hybridization will contribute to bromeliad biodiversity by enriching local gene pools on inselbergs in the face of extremely low intraspecific gene flow. Consequently, inselbergs with multiple sympatric bromeliads deserve special attention by both biologists interested in plant speciation in mountain systems and agencies involved with the implementation of conservation programmes in the Brazilian Atlantic Forest.

Acknowledgements

We thank Daniel Medina Corrêa Santos, Denise Dias da Cruz, Leandro de Oliveira Furtado de Sousa, Márcia Goetze and Miriam Valli Büttow for helping in field work, and Giovanni Scopece, Valentina Tranchida and Barbara Fussi for helpful discussions. We thank IBAMA for processing of collection and export permits. This work was supported by grants from CNPq (152310/2007-0; 471775/2010-0), CAPES (2500-08) and FAPESP (2009/527253; 2009/174118).

References

Abbott RJ, Lowe AJ (2004) Origins, establishment and evolution of new polyploid species: *Senecio cambrensis* and *S. eboracensis* in the British Isles. *Biological Journal of the Linnean Society*, **82**, 467–474.

Anderson EC, Thompson EA (2002) A model-based method for identifying species hybrids using multilocus genetic data. *Genetics*, **160**, 1217–1229.

Arnold J (1993) Cytonuclear disequilibria in hybrid zones. *Annual Review of Ecology and Systematics*, **24**, 521–554.

Arnold ML, Martin NH (2010) Hybrid fitness across time and habitats. *Trends in Ecology and Evolution*, **25**, 530–536.

Asmussen MA, Basten CJ (1994) Sampling theory for cytonuclear disequilibria. *Genetics*, **138**, 1351–1363.

Asmussen MA, Arnold J, Avise JC (1989) The effects of assortative mating and migration on cytonuclear associations in hybrid zones. *Genetics*, **122**, 923–934.

Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Barbará T, Martinelli G, Fay MF, Mayo SJ, Lexer C (2007) Population differentiation and species cohesion in two closely related plants adapted to Neotropical high-altitude 'inselbergs', *Alcantarea imperialis* and *A. geniculata*. *Molecular Ecology*, **16**, 1981–1992.

Barbará T, Lexer C, Martinelli G *et al.* (2008) 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). *Heredity*, **101**, 285–296.

Barluenga M, Stolting KN, Salzburger W, Muschick M, Meyer A (2006) Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature*, **439**, 719–723.

Barraclough TG, Nee S (2001) Phylogenetics and speciation. *Trends in Ecology and Evolution*, **16**, 391–399.

Barrett RDH, Schluter D (2008) Adaptation from standing genetic variation. *Trends in Ecology and Evolution*, **23**, 38–44.

Barton NH, Hewitt GM (1985) Analysis of hybrid zones. *Annual Review of Ecology Evolution and Systematics*, **16**, 113–148.

Basten CJ, Asmussen MA (1997) The exact test for cytonuclear disequilibria. *Genetics*, **146**, 1165–1171.

Beerli P, Felsenstein J (1999) Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. *Genetics*, **152**, 763–773.

Benzing D (2000) *Bromeliaceae: Profile of An Adaptive Radiation*. Cambridge University Press, Cambridge, UK.

Boisselier-Dubayle MC, Leblois R, Samadi S, Lambourdiere J, Sarthou C (2010) Genetic structure of the xerophilous bromeliad *Pitcairnia geyskesii* on inselbergs in French Guiana – a test of the forest refuge hypothesis. *Ecography*, **33**, 175–184.

Boneh L, Kuperus P, Van Tienderen PH (2003) Microsatellites in the bromeliads *Tillandsia fasciculata* and *Guzmania monostachya*. *Molecular Ecology Notes*, **3**, 302–303.

Brandvain Y, Haig D (2005) Divergent mating systems and parental conflict as a barrier to hybridization in flowering plants. *American Naturalist*, **166**, 330–338.

Burgarella C, Lorenzo Z, Jabbour-Zahab R *et al.* (2009) Detection of hybrids in nature: application to oaks (*Quercus suber* and *Q. ilex*). *Heredity*, **102**, 442–452.

Coyne JA, Orr HA (2004) *Speciation*. Sinauer Associates, Sunderland, MA.

Currat M, Ruedi M, Petit RJ, Excoffier L (2008) The hidden side of invasions: massive introgression by local genes. *Evolution*, **62**, 1908–1920.

Demesure B, Sodzi N, Petit RJ (1995) A set of universal primers for amplification of polymorphic noncoding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology*, **4**, 129–131.

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus*, **12**, 13–15.

Du FK, Petit RJ, Liu JQ (2009) More introgression with less gene flow: chloroplast vs. mitochondrial DNA in the *Picea asperata* complex in China, and comparison with other conifers. *Molecular Ecology*, **18**, 1396–1407.

Duarte JM, Wall PK, Edger PP *et al.* (2010) Identification of shared single copy nuclear genes in *Arabidopsis*, *Populus*, *Vitis* and *Oryza* and their phylogenetic utility across various taxonomic levels. *BMC Evolutionary Biology*, **10**, 61.

Ehrlich PR, Raven PH (1969) Differentiation of populations. *Science*, **165**, 1228–1232.

El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree *Argania spinosa* (L) Skeels endemic to Morocco. *Theoretical and Applied Genetics*, **92**, 832–839.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics*, **1**, 47–50.

Fishman L, Stratton DA (2004) The genetics of floral divergence and postzygotic barriers between outcrossing and selfing populations of *Arenaria uniflora* (Caryophyllaceae). *Evolution*, **58**, 296–307.

Givnish TJ, Sytsma KJ (1997) *Molecular Evolution and Adaptive Radiation*. Cambridge University Press, New York.

Givnish TJ, Millam KC, Berry PE, Sytsma LJ (2007) Phylogeny, adaptive radiation, and historical biogeography of Bromeliaceae inferred from ndhF sequence data. *Aliso*, **23**, 3–26.

Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485–486.

Grant PR, Grant BR (2010) Conspecific versus heterospecific gene exchange between populations of Darwin's finches. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **365**, 1065–1076.

Hathaway L, Malm JU, Prentice HC (2009) Geographically congruent large-scale patterns of plastid haplotype variation in the European herbs *Silene dioica* and *S. latifolia* (Caryophyllaceae). *Botanical Journal of the Linnean Society*, **161**, 153–170.

Hedrick PW (2005) A standardized genetic differentiation measure. *Evolution*, **59**, 1633–1638.

Heuertz M, Carnevale S, Fineschi S *et al.* (2006) Chloroplast DNA phylogeography of European ashes, *Fraxinus* sp. (Oleaceae): roles of hybridization and life history traits. *Molecular Ecology*, **15**, 2131–2140.

Jorgensen TH, Olesen JM (2001) Adaptive radiation of island plants: evidence from *Aeonium* (Crassulaceae) of the Canary Islands. *Perspectives in Plant Ecology Evolution and Systematics*, **4**, 29–42.

Kane NC, Rieseberg LH (2007) Selective sweeps reveal candidate genes for adaptation to drought and salt tolerance in common sunflower, *Helianthus annuus*. *Genetics*, **175**, 1823–1834.

Latta RG, Linhart YB, Mitton JB (2001) Cytonuclear disequilibrium and genetic drift in a natural population of ponderosa pine. *Genetics*, **158**, 843–850.

Levin D (1971) The origin of reproductive isolating mechanisms in flowering plants. *Taxon*, **20**, 91–113.

Lexer C, Widmer A (2008) The genic view of plant speciation: recent progress and emerging questions. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **363**, 3023–3036.

Lexer C, Kremer A, Petit RJ (2006) Shared alleles in sympatric oaks: recurrent gene flow is a more parsimonious explanation than ancestral polymorphism. *Molecular Ecology*, **15**, 2007–2012.

Lexer C, Fay MF, Joseph JA, Nica MS, Heinze B (2005) Barrier to gene flow between two ecologically divergent *Populus* species, *P. alba* (white poplar) and *P. tremula* (European aspen): the role of ecology and life history in gene introgression. *Molecular Ecology*, **14**, 1045–1057.

Losos JB, Ricklef RE (2009) Adaptation and diversification on islands. *Nature*, **457**, 830–836.

Martin NH, Willis JH (2007) Ecological divergence associated with mating system causes nearly complete reproductive isolation between sympatric *Mimulus* species. *Evolution*, **61**, 68–82.

Martinsen GD, Whitham TG, Turek RJ, Keim P (2001) Hybrid populations selectively filter gene introgression between species. *Evolution*, **55**, 1325–1335.

Mayr E (1942) *Systematics and the Origin of Species*. Columbia University Press, New York.

McKinnon GE, Smith JJ, Potts BM (2010) Recurrent nuclear DNA introgression accompanies chloroplast DNA exchange between two eucalypt species. *Molecular Ecology*, **19**, 1367–1380.

Meirelles ST, Pivello VR, Joly CA (1999) The vegetation of granite rock outcrops in Rio de Janeiro, Brazil, and the need for its protection. *Environmental Conservation*, **26**, 10–20.

Morjan CL, Rieseberg LH (2004) How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. *Molecular Ecology*, **13**, 1341–1356.

Muir G, Schlotterer C (2005) Evidence for shared ancestral polymorphism rather than recurrent gene flow at microsatellite loci differentiating two hybridizing oaks (Quercus spp.). *Molecular Ecology*, **14**, 549–561.

Nosil P, Funk DJ, Ortiz-Barrientos D (2009) Divergent selection and heterogeneous genomic divergence. *Molecular Ecology*, **18**, 375–402.

Olsen KM (2002) Population history of *Manihot esculenta* (Euphorbiaceae) inferred from nuclear DNA sequences. *Molecular Ecology*, **11**, 901–911.

Orr HA (1996) Dobzhansky, Bateson, and the genetics of speciation. *Genetics*, **144**, 1331–1335.

Paggi GM, Palma-Silva C, Bered F *et al.* (2008) Isolation and characterization of microsatellite loci in *Pitcairnia albiflos* (Bromeliaceae), an endemic bromeliad from the Atlantic Rainforest, and cross-amplification in other species. *Molecular Ecology Resources*, **8**, 980–982.

Paggi GM, Sampaio JAT, Bruxel M *et al.* (2010) Seed dispersal and population structure in *Vriesea gigantea*, a bromeliad from the Brazilian Atlantic Rainforest. *Botanical Journal of the Linnean Society*, **164**, 317–325.

Palma-Silva C, Cavallari MM, Barbará T *et al.* (2007) A set of polymorphic microsatellite loci for *Vriesea gigantea* and *Alcantarea imperialis* (Bromeliaceae) and cross-amplification in other bromeliad species. *Molecular Ecology Notes*, **7**, 654–657.

Palma-Silva C, Lexer C, Paggi GM *et al.* (2009) Range-wide patterns of nuclear and chloroplast DNA diversity in *Vriesea gigantea* (Bromeliaceae), a Neotropical forest species. *Heredity*, **103**, 503–512.

Palmé AE, Su Q, Palsson S, Lascoux M (2004) Extensive sharing of chloroplast haplotypes among European birches indicates hybridization among *Betula pendula*, *B. pubescens* and *B. nana*. *Molecular Ecology*, **13**, 167–178.

Petit RJ, Excoffier L (2009) Gene flow and species delimitation. *Trends in Ecology and Evolution*, **24**, 386–393.

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conservation Biology*, **12**, 844–855.

Petit RJ, Csaikl UM, Bordacs S *et al.* (2002) Chloroplast DNA variation in European white oaks – phylogeography and patterns of diversity based on data from over 2600 populations. *Forest Ecology and Management*, **156**, 5–26.

Pialek J, Barton NH (1997) The spread of an advantageous allele across a barrier: the effects of random drift and selection against heterozygotes. *Genetics*, **145**, 493–504.

Pinheiro F, Palma-Silva C, de Barros F, Cozzolino S (2009) Cross-amplification and characterization of microsatellite loci for the Neotropical orchid genus *Epidendrum*. *Genetics and Molecular Biology*, **32**, 337–339.

Pinheiro F, de Barros F, Palma-Silva C *et al.* (2010) Hybridization and introgression across different ploidy levels in the Neotropical orchids *Epidendrum fulgens* and *E. puniceoluteum* (Orchidaceae). *Molecular Ecology*, **19**, 3981–3994.

Porembski S (2007) Tropical inselbergs: habitat types, adaptive strategies and diversity patterns. *Revista Brasileira de Botânica*, **30**, 579–586.

Porembski S, Barthlott W (2000) *Inselbergs. Biotic Diversity of Isolated Rock Outcrops in Tropical and Temperate Regions*. Springer-Verlag, Berlin, Heidelberg, New York.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Raymond M, Rousset F (1995) GENEPOP (Version-1.2) – population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rieseberg LH, Burke JM (2001) The biological reality of species: gene flow, selection, and collective evolution. *Taxon*, **50**, 47–67.

Rôças G, Klein DE, De Mattos EA (2004) Artificial hybridization between *Pitcairnia flammea* and *Pitcairnia corcovadensis* (Bromeliaceae): analysis of the performance of parents and hybrids. *Plant Species Biology*, **19**, 47–53.

Rundell RJ, Price TD (2009) Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends in Ecology and Evolution*, **24**, 394–399.

Rundle HD, Nosil P (2005) Ecological speciation. *Ecological Letters*, **8**, 336–352.

Sang T, Crawford DJ, Stuessy TF (1997) Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany*, **84**, 1120–1136.

Sarthou C, Samadi S, Boisselier-Dubayle MC (2001) Genetic structure of the saxicole *Pitcairnia geyskesii* (Bromeliaceae) on inselbergs in French Guiana. *American Journal of Botany*, **88**, 861–868.

Sarthou C, Boisselier-Dubayle MC, Lambourdiere J, Samadi S (2003) Polymorphic microsatellites for the study of fragmented populations of *Pitcairnia geyskesii* L. B. Smith (Bromeliaceae), a specific saxicolous species of inselbergs in French Guiana. *Molecular Ecology Notes*, **3**, 221–223.

Savolainen V, Anstett MC, Lexer C *et al.* (2006) Sympatric speciation in palms on an oceanic island. *Nature*, **441**, 210–213.

Scascitelli M, Whitney KD, Randell RA *et al.* (2010) Genome scan of hybridizing sunflowers from Texas (*Helianthus annuus* and *H. debilis*) reveals asymmetric patterns of introgression and small islands of genomic differentiation. *Molecular Ecology*, **19**, 521–541.

Schluter D (2000) *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.

Scopece G, Lexer C, Widmer A, Cozzolino S (2010) Polymorphism of postmating reproductive isolation within plant species. *Taxon*, **59**, 1367–1374.

Seehausen O (2004) Hybridization and adaptive radiation. *Trends in Ecology and Evolution*, **19**, 198–207.

Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany*, **94**, 275–288.

Slatkin M (1976) The rate of spread of an advantageous allele in a subdivided population. In: *Population Genetics and Ecology* (eds Karlin S, Nevo E), pp. 767–780. Academic Press, Inc., New York.

Small RL, Ryburn JA, Cronn RC, Seelanan T, Wendel JF (1998) The tortoise and the hare: choosing between noncoding

plastome and nuclear ADH sequences for phylogeny reconstruction in a recently diverged plant group. *American Journal of Botany*, **85**, 1301–1315.
Sokal R, Rohlf F (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*. Freeman and Company, New York.
Stuessy TF, Jakubowsky G, Gomez RS *et al*. (2006) Anagenetic evolution in island plants. *Journal of Biogeography*, **33**, 1259–1265.
Sweigart AL, Willis JH (2003) Patterns of nucleotide diversity in two species of *Mimulus* are affected by mating system and asymmetric introgression. *Evolution*, **57**, 2490–2506.
Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three noncoding regions of chloroplast DNA. *Plant Molecular Biology*, **17**, 1105–1109.
Tiffin P, Olson MS, Moyle LC (2001) Asymmetrical crossing barriers in angiosperms. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **268**, 861–867.
Turelli M, Moyle LC (2007) Asymmetric postmating isolation: Darwin's corollary to Haldane's rule. *Genetics*, **176**, 1059–1088.
Valière N (2002) GIMLET: a computer program for analysing genetic individual identification data. *Molecular Ecology Notes*, **2**, 377–379.
Via S, West J (2008) The genetic mosaic suggests a new role for hitchhiking in ecological speciation. *Molecular Ecology*, **17**, 4334–4345.
Wendt T, Paz NPL, Rios RI (2000) A morphometric analysis of a putative hybrid between *Pitcairnia albiflos* and *Pitcairna staminea* (Bromeliaceae). *Selbyana*, **21**, 132–136.
Wendt T, Canela MBF, De Faria APG, Rios RI (2001) Reproductive biology and natural hybridization between two endemic species of *Pitcairnia* (Bromeliaceae). *American Journal of Botany*, **88**, 1760–1767.
Wendt T, Canela MBF, Klein DE, Rios RI (2002) Selfing facilitates reproductive isolation among three sympatric species of *Pitcairnia* (Bromeliaceae). *Plant Systematics and Evolution*, **232**, 201–212.
Wendt T, Coser TS, Matallana G, Guilherme FAG (2008) An apparent lack of prezygotic reproductive isolation among 42 sympatric species of Bromeliaceae in southeastern Brazil. *Plant Systematics and Evolution*, **275**, 31–41.
Widmer A, Lexer C, Cozzolino S (2009) Evolution of reproductive isolation in plants. *Heredity*, **102**, 31–38.
Wright S (1965) The interpretation of population structure by *F*-statistics with special regards to system of mating. *Evolution*, **19**, 395–420.
Wu CI (2001) The genic view of the process of speciation. *Journal of Evolutionary Biology*, **14**, 851–865.

This study is part of a research project on hybridization, speciation and the maintenance of species boundaries in Neotropical plants, based at the Instituto de Botânica in São Paulo, Brazil. C.P.S. is interested in the evolution and diversification of Neotropical plants. F.P. studies aspects of plant evolution in different Neotropical biomes. T.W. has long-standing interests in the ecology and evolution of Bromeliaceae. M.F.F. studies population genetic and cytogenetic aspects of plant evolution with a focus on conservation. S.C.'s main interests are in the ecology, evolution and conservation of orchids. C.L. leads a research programme on the evolutionary genomics of plant adaptation and speciation, and T.B. is a postdoctoral research fellow in his group.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Primer sequences and characteristics of plastid DNA microsatellite loci in *Pitcairnia* spp., including locus name, plastid DNA region, primer sequences, repeat type, allele size range, and no. of alleles.

Table S2 Genetic variability at 15 microsatellite loci in *P. staminea*, *P. albiflos*, and their hybrids, including locus name, number of alleles (A), observed heterozygosity (H_O), expected heterozygosity (H_E), standardized measure of differentiation (G'_{ST}), fixation index (F_{ST}), total inbreeding coefficient (F_{IT}), and within-population inbreeding coefficient (F_{IS}).

Table S3 Analysis of Molecular Variance (AMOVA) based on nuclear microsatellite data for *P. staminea* and *P. albiflos* for two different hierarchical models. (A) species level. (B) geographical level (inland *vs*. coastal inselbergs). Percentages of variation in parentheses refer to models in which hybrids were nested with *P. albiflos*.

Table S4 Genetic divergence (F_{ST}; below diagonal) and gene flow (N_em; above diagonal) for pairs of populations of the inselberg bromeliads *Pitcairnia albiflos* and *P. staminea* and their hybrids. $N_em = (1/F_{ST} - 1)/4$.

Table S5 Locus-specific, bidirectional introgression rates between *Pitcairnia albiflos* and *P. staminea* on PAO population.

Fig. S1 Magnitude of ΔK from STRUCTURE analysis as a function of K (mean ± SD over 10 replicates), calculated following the ΔK method proposed by Evanno *et al*. (2005), for *Pitcairnia albiflos* and *P. staminea* microsatellite data.

Fig. S2 Posterior probabilities (q) of *Pitcairnia albiflos* and *Pitcairnia staminea* for the two hybrid zones analyzed with NEWHYBRIDS.