

## Genetic structure of the xerophilous bromeliad *Pitcairnia geyskesii* on inselbergs in French Guiana – a test of the forest refuge hypothesis

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Inselbergs are isolated granitic rock outcrops that provide distinctive ecological conditions. In northern South America they rise above the surrounding rainforest. Among inselberg specialists, *Pitcairnia geyskesii* (Bromeliaceae) is restricted to these habitats in French Guiana. We studied populations from 12 inselbergs using 7 microsatellite loci to give a “reverse image” of the reduction-expansion of the rainforest in the context of the refuge hypothesis. Our analyses showed that populations are fragmented with dispersal occurring only over very short distances. Genetic diversity was higher in northern French Guiana, whereas specific alleles were observed in the south. The results point to the occurrence of a dry corridor in the north, as hypothesized by Tardy (1998) based on charcoal analyses, whereas de Granville’s (1982) hypothesis of a unique past refuge is not confirmed. Moreover, our data suggests the importance of Oyapock River as a pathway for range expansion, arguing against the potential role of the Inini-Camopi Mountains as a physical barrier. Finally, in spite of a strongly argued scenario in favour of a north-to-south migration history, a clear genetic isolation of *P. geyskesii* populations living on inselbergs of the Mitaraka archipelago suggests a distinct ancestry of the most southern populations.

The influence of past climatic changes in tropical rainforests, especially in South America, has been widely debated (Colinvaux et al. 2000, Willis and Whittaker 2000). Although climatic changes during the last Pleistocene glaciations (between 22 000 and 10 000 yr ago) were less pronounced in the tropics than at temperate latitudes, glacial periods in tropical zones were characterized by a lowering of rainfall and a drop in temperature by up to 5°C compared to the present (Dynesius and Jansson 2000). Consequently, species distributions may have been strongly affected (de Granville 1982, Rull 1998, Pennington et al. 2000, Bonaccorso et al. 2006). These large-scale environmental changes may have resulted in population fragmentation into smaller demes in some periods and in rapid range expansion in others. Both processes are expected to affect population genetic structure, but with opposite consequences. During fragmentation, the subdivided populations may be reduced in size, accelerating the loss of genetic variability at the species level through random genetic drift and increasing the risk of inbreeding (Hedrick 2000). During expansion periods, even if colonization of new habitats may produce local and temporal loss of genetic diversity through founder effects (Maruyama and Fuerst

1985), overall genetic diversity among the newly derived populations should at least be maintained as a result of limited genetic drift and increased gene flow (Hewitt 1999, Zenger et al. 2003).

In the Neotropics, the periodic glaciations of the Quaternary corresponded to drier climatic conditions (Hooghiemstra and Hammen 1998). It has been suggested that these periods would have led to the expansion of open vegetation types such as savannas and outcrop vegetation into areas in the Guiana Shield region, whereas such open vegetation in the present interglacial is restricted to inselbergs isolated in a forest matrix. Northern South American inselbergs are scattered across the Guiana Shield as isolated rock outcrops consisting mainly of Precambrian granites or gneisses. They rise abruptly from the surrounding landscape and constitute singular habitats in the tropical rain forest. Air temperature and relative air humidity on inselbergs show high diurnal variation (Sarthou 2001), and soils are generally very shallow with low water retaining capacity. Inselbergs thus represent edaphic as well as microclimatic xeric habitats, with a discontinuous vegetation cover, separated by open rock, creating “terrestrial xeric islands” in a humid forest matrix (Szarzynski 2000).

Inselbergs are presently sanctuaries for a herbaceous and heliophilous xeric flora (Hurault 1974, Descamps et al. 1978, de Granville 1982) and it has been suggested that studies of such non-rainforest species could elucidate the history of the forests themselves (Pennington et al. 2004, Würster et al. 2005), arguing that vicariant non-rainforest taxa separated by the Amazonian forest must have had a continuous distribution at some point in the past.

Here, we adopt this strategy by studying the spatial genetic structure of a herbaceous specialist species that requires the climatic conditions found on inselbergs. Studying the genetic structure to infer the past range dynamics of such fragmented populations of xerophilous plants strictly associated to inselbergs should thus give a “reverse image” of the past history, e.g. reduction-expansion dynamics, of the rainforest. Among the specialists restricted to inselbergs, *Pitcairnia geyskesii* (Bromeliaceae) represents a dominant member of the widespread epilithic plant community in French Guiana (Sarhou and Villiers 1998). This species is endemic to the Eastern Guyana Shield (French Guiana, Suriname, Guyana) and is protected in French Guiana. To evaluate past scenarios, we surveyed seven microsatellite loci (Sarhou et al. 2003) for genetic diversity among 413 adult plants growing on 12 inselbergs throughout French Guiana. Genetic variation within and among populations was quantified at three different spatial scales: regional (among isolated inselbergs or archipelagos of close inselbergs), landscape (among closely spaced inselbergs within archipelagos), and local (among demes separated by physical barriers on the same inselberg) in order to use patterns of genetic diversity in *P. geyskesii* to assess several divergent hypotheses regarding the past dynamics of dry, open areas and rainforest in this region.

According to the refuge hypothesis (RH) developed for French Guiana by de Granville (1982), a wide forested area remained as a humid refuge in the northern part of French Guiana during the dry glacial periods. In contrast, two forest refuges were suggested by Tardy (1998), in the north along the littoral between Matoury and Kaw, and in the south along the mountain range between Inini and Camopi (Fig. 1). Furthermore, Tardy (1998) suspected the occurrence of a wide dry corridor across the northern forest refuge during the early Holocene (9000–8000 BP) and middle Holocene (6000–4000 BP) as well as a generally more restricted humid zone in the north. Under both versions of RH, the actual populations living in these past refuge areas should be strongly differentiated from the remaining populations as they have remained isolated during glacial periods. Conversely, the existence of a wide dry corridor as suggested by Tardy (1998) should result in less differentiated populations in the suspected zone, as a consequence of continuous gene flow.

Apart from the glacial-interglacial dynamics, the spatial distribution of genetic diversity in *P. geyskesii* could also be influenced by the Inini-Camopi Mountains that are expected to act as a barrier to gene flow between northern and southern populations. If so, current genetic diversity should thus be influenced by this barrier and by the presence of the Oyapock River as a dispersal corridor between northern and southern populations. This can be addressed by comparing isolation by distance patterns using Euclidian geographic distances (i.e. “as the crow flies”) and

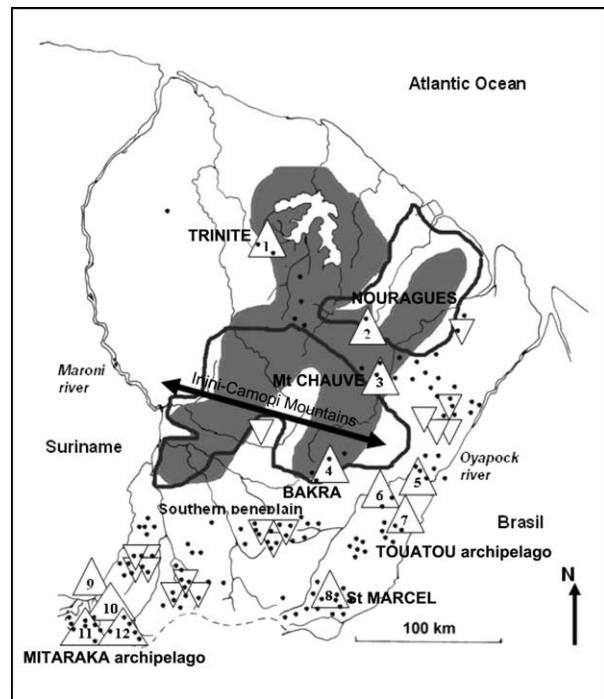


Figure 1. Location of the 28 inselbergs visited in French Guiana. White downward-pointing triangles correspond to inselbergs and archipelagos where *Pitcairnia geyskesii* is absent. White triangles pointed upward and numbered correspond to the 12 inselbergs where *P. geyskesii* was present and sampled (Table 1). Black dots: major inselbergs according to Descamps et al. (1978). Grey area: rainforest refuges according to Granville (1982). Bold lines in dark grey: rainforest refuges according to Tardy (1998). Double-headed arrow: Inini-Camopi Mountain Range.

“ecological” distances taking into account barriers and corridors.

To detail as much as possible the past-range dynamics of the presently fragmented populations, we used the “isolation with migration” model (Nielsen and Wakeley 2001) under which an ancestral population is split into two populations that are subsequently connected by gene flow. The coalescent process for this model was implemented by Hey and Nielsen (2004) for several unlinked loci using a Markov chain Monte Carlo method. This method provides estimates of multiple demographic parameters (including divergence time, migration rates, effective population sizes of the three populations). These estimates were then used to support or refute different historical scenarios concerning Pleistocene ice age forest refuges as well as colonization of *P. geyskesii* in French Guiana.

## Material and methods

### Studied species

*Pitcairnia geyskesii* reproduces both sexually and asexually (Sarhou et al. 2001) and forms dense mats on sun-exposed rock surfaces as its creeping rhizomes produce new shoots, each bearing an erect rosette of spiny leaves. The apical bud of the parent rosette yields a flowering shoot of 50–200 cm height with tubular zygomorphic, red, nectariferous flowers.

Flowering occurs in March–April, during the short dry season. Hummingbirds act as the primary pollinators. The dehiscent capsules produce small, light dry seeds (Varadarajan and Gilmartin 1988), and wind is the main dispersal agent. Before flowering, the plant forms two secondary rosettes which replace the parent rosette, forming two lateral ramets.

## Sites and sampling design

In French Guiana, inselbergs occur mainly south of 4° latitude, with altitudes between 50 and 800 m. In northern French Guiana, inselbergs are scarce and isolated compared to the south, where they sometimes form archipelagos (Descamps et al. 1978, Fig. 1). Because inselbergs are hard to reach, samples were collected during four helicopter expeditions. Twenty-eight inselbergs were visited (Fig. 1), covering the total distribution within French Guiana. Among those visited, *Pitcairnia geyskesii* was found on 12 inselbergs distributed among seven geographic sets (I–VII, Table 1). Five inselbergs are clearly isolated from each other (mainly in the north) whereas the 7 other inselbergs are included into two archipelagos (Touatou and Mitaraka) (Fig. 1). In total, 14 populations were sampled (Table 1, Fig. 1).

Trinité, Nouragues and three inselbergs of the Mitaraka archipelago were visited in 1998, 1999 and 2001, respectively, whereas all other inselbergs were sampled in 2002. Sampling covered the entire area of each population of *P. geyskesii*, except on Mt. Chauve, where the helicopter could only stay for one hour and plants therefore were collected in the most accessible parts. Sampling was performed on a grid pattern adjusted to the population size (from 10 to 35 m) and resulting in a sample size of at least 30 individuals, except on the very small inselbergs (15 individuals on site ii and iii of Touatou archipelago). At St. Marcel, two populations 100 m apart and separated by a small forest were identified, as well as a pair of populations separated by a 100 m cliff at Nouragues. From all populations, except Trinité and Nouragues where samples

were frozen, young leaves were desiccated and preserved in silica gel.

The geographic distances between inselbergs belonging to the same archipelago ranged from 1.2–2.4 km at Mitaraka and 8.1–12.3 km at Touatou and the maximum distance in the total sampling was found between the Trinité and Mitaraka South inselbergs (292.2 km).

## Molecular and statistical analyses

Genomic DNA from 413 individuals was extracted with the DNeasy Plant Mini Extraction Kit (Qiagen). Seven micro-satellite loci were analyzed according to the protocols of Sarthou et al. (2003). Primers were tagged with fluorochromes supplied by Applied Biosystems. Amplified fragments were separated according to size by an Abi Prism 310 DNA sequencer and analyzed with the GeneScan software (Applied Biosystems).

Genetic diversity within each population was estimated as percentages of polymorphic loci (P), mean number of alleles per locus (A), mean observed and expected heterozygosities ( $H_o$ ,  $H_e$ ) and  $F_{is}$  using Genetix (Belkhir et al. 2000). Allelic richness per population was computed with a rarefaction method in order to correct values for unequal sampling sizes (El-Mousadik and Petit 1996). As the plant reproduces both sexually and asexually, we used the program MLGSim (Stenberg et al. 2003) to assess if identical genotypes found within a population were likely to have occurred there by chance given sexual reproduction. Departures from Hardy-Weinberg equilibrium (HWE) were assessed with a global test using the exact HWE test and heterozygote deficit or excess was further checked using a U test. Genotypic linkage disequilibrium was tested within each population and for each pair of loci, using Fisher's exact test. All tests were performed with GENEPOP (Raymond and Rousset 1995). A sequential Bonferroni correction (Rice 1989) was used for all tests performed for the same population, for the different loci or pairs of loci.

Table 1. Location, date, and sample size of *Pitcairnia geyskesii*. Numbers in the first column refer to the inselberg labeling in Fig. 1. Inselbergs were numbered from north to south. Where grid size is not indicated, all mats were sampled.

Geographic set	Inselberg	Sub-population	Geographical location	Altitude (m)	Sampling date	Grid size (m)	Sample size	No. of individuals analyzed
I	1 - Trinité		4°38'N, 53°22'W	501	1998	20	59	44
II	2 - Nouragues		4°06'N, 52°41'W	410				
		summit			1999	20	32	31
		south			1999	20	50	37
III	3 - Mt. Chauve		3°49'N, 52°44'W	265	2002	–	80	29
IV	4 - Bakra		3°18'N, 52°57'W	550	2002	10	55	31
V	Touatou archipelago							
	5 - Site iii		3°01'N, 52°32'W	200	2002	–	15	15
	6 - Site ii		2°57'N, 52°37'W	200	2002	–	15	15
	7 - Roche Touatou		2°57'N, 52°33'W	300	2002	–	30	28
VI	8 - St. Marcel		2°23'N, 53°00'W	500				
		St M 1			2002	30–35	87	32
		St M 2			2002	20	34	31
VII	Mitaraka archipelago							
	9 - Petit Mitaraka		2°18'N, 54°32'W	490	2002	–	30	30
	10 - Mitaraka North		2°17'N, 54°32'W	660	2001	20	40	30
	11 - Mitaraka South		2°17'N, 54°32'W	690	2001	25	70	31
	12 - Arlésienne		2°16'N, 54°31'W	480	2001	20	40	29

Delimitation of homogeneous population clusters and detection of barriers to gene flow was addressed using an unsupervised clustering method implemented in Structure ver. 2.1 (Pritchard et al. 2000). The procedure uses several Markov Chains Monte Carlo (MCMC) with different values of  $K$  (number of genetic groups) to infer which  $K$  is the most likely from the approximate posterior probability of the data. We also ran several MCMC for each value of  $K$  to check convergence and consistency of the results in finding the best estimate of the number of genetic groups present in our dataset: 20 replicates for each values of  $K$ , ranging from 1 to 10, under the model with admixture and correlated allele frequencies. MCMC runs of 1 000 000 iterations and 50 000 iterations of burn-in were selected. Results were used to conduct the subsequent analyses.

Exact tests of genetic differentiation were performed on each population pair with a Bonferroni correction. Level of genetic differentiation between populations was assessed by estimating  $F_{st}$  over all populations, between locations, and between each pair of populations, using the method of Weir and Cockerham (1984). Tests and estimations of  $F_{st}$  were performed using GENEPOP. Deviation of pairwise  $F_{st}$  from zero was tested by a permutation method using GENETIX. We also computed global and pairwise  $R_{st}$  statistics (differentiation based on allelic size differences, Michalakis and Excoffier 1996) and applied the allele size mutation tests of Hardy et al. (2003) in order to provide insights into the main causes of population differentiation (i.e. drift/migration vs mutation). These tests were performed with SPAGeDi (Hardy and Vekemans 2002) using 10 000 permutations to estimate  $pR_{st}$ .

Isolation by distance was analyzed by regressing genetic differentiation computed as  $F_{st}/(1 - F_{st})$  between pairs of population over the logarithm of the geographic distances (Rousset 1997), and Mantel tests were performed on these two matrices. Two different matrices of geographical distances were constructed in order to test the influence of the Oyapock River corridor: we first considered Euclidian distances between populations (i.e. “as the crow flies”), and then considered more “ecological” distances corresponding to the shortest path not crossing the putative geographic barriers of the Inini-Camopi Mountains (Fig. 1).

Finally, in order to discriminate between the relative effects of divergence and gene flow, we analysed our data set under the isolation-with-migration model using the IM software (Hey and Nielsen 2004). The model assumes that an ancestral population splits into two descendant populations with gene flow possibly continuing between the diverging populations. To fit the IM model to our data, we used a Bayesian coalescent method that approximates the integration over the possible genealogies using a MCMC simulation. The method estimates posterior probability distributions for demographic parameters including divergence time, two-directional migration rates, and effective population sizes of two current populations and the ancestral population. As convergence to the stationary posterior distributions and exploration of the parameter space is a critical issue with MCMC, we took special care to run sufficiently long runs by looking at different criteria such as autocorrelation values, parameters update rates and similarity between posterior distributions of independent runs. After a few runs to check convergence, we finally

performed very long runs of  $4 \times 10^8$  iterations with a burn-in of  $5 \times 10^6$  steps. Because we were interested in analysing the dynamics of past connectivity between populations in the context of the RH (de Granville 1982, Tardy 1998), we explored the data by pooling some sampled sites according to both their geographic locations and the results of Structure. Several comparisons were conducted on a 32 node cluster of 64bit AMD processor at 2.5 GHz hosted by the Museum National d'Histoire Naturelle (MNHN). Each run took ca 900–1000 h.

## Results

### Diversity within populations

For the 413 individuals examined, we obtained 94% successful amplifications, although the rate differed among populations (from 84 to 100%, Table 2). The rate of non-amplification also differed among loci, from <3% for most loci to 11–15% for Pit12 and Pit4, respectively. However, when excluding the frozen samples, most loci were successfully amplified for all individuals suggesting that non-amplification was due to the fixation methods used (frozen vs desiccated). A total of 107 alleles were detected over all seven loci, with per population allele numbers ranging from 12 to 57. Loci Pit2 and Pit5 exhibited the highest polymorphism with 25 and 22 alleles, respectively, over all populations studied; locus Pit6 showed the lowest level, with only 6 alleles, whereas 12–14 alleles were scored for the other loci.

Within the 14 populations, the mean number of alleles per polymorphic loci ( $A$ ) ranged from 1.7 to 8.0 (mean  $A = 3.6$ ), allelic richness ranged from 1.5 to 5.2 (mean  $A(16) = 2.7$ ) and the percentage of polymorphic loci from 43 to 100% (mean  $P = 67\%$ ). The mean observed heterozygosity per population ( $H_o$ ) and gene diversity ( $H_e$ ) were 0.293 and 0.325, respectively. The smallest  $H_o$  value was observed for Petit Mitaraka and the highest was detected at Trinité. The number of different multilocus genotypes identified within populations was generally close to the sample size and in the few cases where we found identical genotypes, the MLGSim tests confirmed that they were likely to have occurred by chance given sexual reproduction.

The number of identified genotypes, as well as the observed and expected heterozygosities computed on the 7 locations, decreased from north to south (Table 2, Fig. 2). Interestingly, the decrease of heterozygosity with the geographic distance between each population and Trinité was very strong and a slight effect of the type of distance considered was detected (i.e. direct Euclidian distance vs. “ecological” distance taking into account potential barriers and corridors,  $R^2$  values of 0.90 vs 0.87, respectively). Over all loci, 47 alleles (44%) were exclusive to the three northern locations and 19 (18%) were specific to the four southern locations (delimited by the Inini-Camopi Mountains). Seven alleles were specific to the most southern Mitaraka archipelago.

No pair of loci showed significant genotypic disequilibrium in any population. Tests of genotypic disequilibrium computed for each locus pair across all populations were likewise not significant. The global HWE test for each

Table 2. Genetic variability of *Pitcairnia geyskesii* on the 12 sampled inselbergs and defined populations. In bold, the data obtained for the complete inselberg or archipelago. N=sample size, A=mean number of alleles per locus, A(16)=allelic richness standardized on the minimum sample size of 16 genes, P=percentage of polymorphic loci (at 0.95), H<sub>o</sub> and H<sub>e</sub>=observed and expected heterozygosities (with standard error), F<sub>is</sub>=inbreeding coefficient, HWE=loci showing a significant departure from Hardy-Weinberg equilibrium with a global test at 5% level and after a sequential Bonferroni correction (\*indicates loci with a heterozygote deficit). Loci in italics had no significant global test, but a significant test for heterozygote deficit. G=number of different genotypes identified within the population. AS=% of successful PCR amplification over the seven microsatellite loci.

12 Inselbergs (14 pops)	N	A	A(16)	P	H <sub>o</sub>	H <sub>e</sub>	F <sub>is</sub>	HWE	G	AS
<b>1 - Trinité</b>	<b>44</b>	<b>8.00</b>	<b>5.17</b>	<b>86</b>	<b>0.522 (0.299)</b>	<b>0.657 (0.298)</b>	<b>0.220</b>	<b>Pit4*, Pit5, Pit12*</b>	<b>43</b>	<b>85%</b>
<b>2 - Nouragues-total</b>	<b>68</b>	<b>7.86</b>	<b>4.15</b>	<b>86</b>	<b>0.395 (0.261)</b>	<b>0.498 (0.282)</b>	0.215	<b>Pit2*, Pit4*, Pit5, Pit12*</b>	<b>64</b>	<b>89%</b>
Nouragues summit	31	5.43	3.82	86	0.373 (0.310)	0.486 (0.285)	0.251	Pit4*, Pit12*	29	84%
Nouragues South	37	6.71	4.11	86	0.401 (0.247)	0.492 (0.285)	0.198	Pit2*, Pit12*	35	93%
<b>3 - Mt. Chauve</b>	<b>29</b>	<b>5.14</b>	<b>3.57</b>	<b>100</b>	<b>0.348 (0.274)</b>	<b>0.476 (0.258)</b>	<b>0.285</b>	<b>Pit2*, Pit4*, Pit12*</b>	<b>29</b>	<b>96%</b>
<b>4 - Bakra</b>	<b>31</b>	<b>2.86</b>	<b>2.47</b>	<b>86</b>	<b>0.288 (0.317)</b>	<b>0.290 (0.271)</b>	<b>0.023</b>	<b>Pit9</b>	<b>26</b>	<b>98%</b>
<b>Touatou Archipelago</b>	<b>58</b>	<b>2.71</b>	<b>1.96</b>	<b>57</b>	<b>0.275 (0.264)</b>	<b>0.274 (0.258)</b>	<b>0.007</b>	—	<b>39</b>	<b>93%</b>
5 - Site iii	15	1.71	1.67	43	0.176 (0.219)	0.166 (0.251)	-0.180	—	10	87%
6 - Site ii	15	2.14	1.99	57	0.307 (0.316)	0.251 (0.255)	0.145	—	9	87%
7 - Roche Touatou	28	2.43	1.99	57	0.312 (0.285)	0.301 (0.271)	-0.019	—	27	99%
<b>8 - St. Marcel</b>	<b>63</b>	<b>4.71</b>	<b>2.68</b>	<b>43</b>	<b>0.269 (0.325)</b>	<b>0.295 (0.331)</b>	<b>0.098</b>	<b>Pit2, Pit4*, Pit5, Pit12*</b>	<b>58</b>	<b>98%</b>
St. Marcel 1	32	4.00	2.69	57	0.263 (0.298)	0.296 (0.333)	0.125	<i>Pit2</i>	30	99%
St. Marcel 2	31	3.57	2.60	57	0.275 (0.358)	0.288 (0.323)	0.064	—	28	98%
<b>Mitaraka archipelago</b>	<b>120</b>	<b>3.00</b>	<b>1.86</b>	<b>57</b>	<b>0.209 (0.171)</b>	<b>0.241 (0.206)</b>	<b>0.136</b>	—	<b>54</b>	<b>97%</b>
9 - Petit Mitaraka	30	1.71	1.51	57	0.152 (0.205)	0.173 (0.219)	0.138	—	14	98%
10 - Mitaraka North	30	2.57	2.07	71	0.232 (0.166)	0.231 (0.178)	0.012	—	21	98%
11 - Mitaraka South	31	2.43	1.94	57	0.277 (0.243)	0.267 (0.236)	-0.021	—	22	100%
12 - Arlésienne	29	1.71	1.50	43	0.174 (0.220)	0.180 (0.234)	0.050	—	14	99%
Mean on the 14 populations	30	3.6	2.65	67	0.293	0.325				94%

population indicated significant deviation from equilibrium in 6 out of the 14 populations, corresponding to five inselbergs (Table 2). These departures concerned few loci, principally for inselbergs located in the north, including two that correspond to frozen samples (Trinité and Nouragues).

### Genetic structure and differentiation among populations

For the 413 individuals, the Structure analyses revealed clear clustering of individuals corresponding to the different geographic sets, even if amongst the different runs different sampled populations clustered together. The most homogeneous result amongst the different runs was obtained for K=5 (95% of the runs showed a similar clustering of samples, Fig. 3). In this case, the five clusters correspond from north to south 1) Trinité, 2) Nouragues and

Mt. Chauve, 3) Bakra and Roche Touatou archipelago, 4) St. Marcel and finally 5) Mitaraka archipelago. The global Structure analyses provided information for different values of K and three main patterns were identified. Firstly, the plants collected on the four inselbergs of the Mitaraka archipelago in the south are always grouped together and clearly recognized as a separate and unique cluster in 30, 50 and 100% of the tests performed for K=2, 3 and for higher values of K, respectively. Secondly, in the north, the plants living on Trinité are associated in equivalent high proportions either to individuals collected on Nouragues and Mt. Chauve or to Bakra and Roche Touatou archipelago (respectively 40 and 30% for K=3, and 40 and 60% for K=4, Fig. 3). Thirdly, the plants coming from Bakra and Roche Touatou archipelago always belong to the same cluster for all K values. Lastly, all results suggest no recent migrants and very little introgression between populations and this lack of introgression is especially marked for K=5. The results obtained for K=5 are the most robust. The corresponding clustering was used to group or exclude populations in some further analyses (below).

Exact tests of genetic differentiation for each population pair showed that the sampled populations are extremely differentiated, even at small geographical scales (Table 3): all differentiation tests were highly significant when performed between different geographic sets and, at the population level, the five non significant tests involved populations from a single inselberg (Nouragues, St. Marcel) or archipelago (Touatou). Tests performed between pairs of populations within the Mitaraka archipelago yielded significant results.

Pairwise F<sub>st</sub> estimates between all population pairs are presented in Table 3 (upper part) and are concordant with the previous results of exact tests of differentiation. Among the 14 populations, pairwise F<sub>st</sub> estimates ranged from

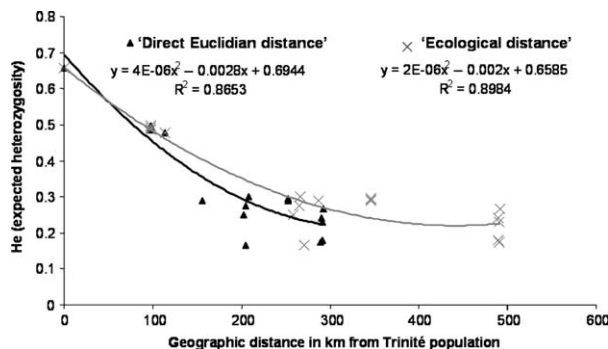


Figure 2. Decrease of expected heterozygosity with geographic distance between each sampled population and Trinité. Black triangles correspond to direct “Euclidian distance” and the grey crosses correspond to “ecological distance” taking into account the dry corridor and the Inini-Camopi Mountains.

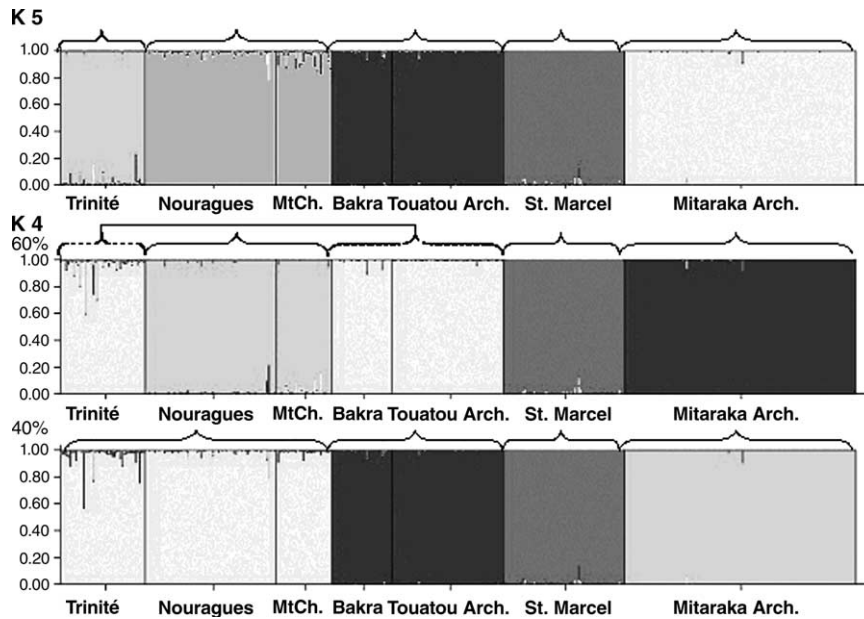


Figure 3. Barplots of the individual accessions of *Pitcairnia geyskesii* within the five and four groups inferred using Structure. Each individual is represented as a vertical bar comprising different grey levels on the X-axis. Each group is represented with a different grey level.

0.002 to 0.809 and all pairwise  $F_{st}$  were significantly different from zero except 1) between the two populations sampled on the same inselberg (Nouragues and St. Marcel), and 2) among the three Touatou archipelago inselbergs. Differentiation among populations of the same inselberg and among inselbergs within archipelago was low. In contrast, in agreement with the Structure results, pairwise  $F_{st}$  estimates amongst the seven geographic sets showed high values, ranging from 0.23 (between Nouragues and Mt. Chauve) to 0.75 (between Touatou and Mitaraka archipelagos). At this scale all pairwise  $F_{st}$  were significantly different from zero.  $R_{st}$  values are also presented in Table 3 (lower part) and values significantly higher than mean  $pR_{st}$  are indicated in bold. Our analyses first showed that  $R_{st}$  values are concordant with  $F_{st}$  values: population pairs showing high  $F_{st}$  also show high  $R_{st}$  values and reciprocally. Secondly, most of the  $R_{st}$  values that are significantly higher than  $pR_{st}$  involve inselbergs from the southern archipelago of Mitaraka, suggesting a phylogeographic pattern and longer historical isolation with limited dispersal events between these, and other, populations (Hardy et al. 2003).

All results of isolation by distance (IBD) tests are presented in Table 4. A strong and significant IBD pattern was found for the global treatment including all populations. However, analyses done without the Mitaraka samples showed lower and less significant values for the slope, suggesting that isolation is not homogeneous along the distances from Trinité to Mitaraka. There is probably a strong resistance to gene flow between Mitaraka and the other geographic sets. In fact, analyses conducted without Mitaraka inselbergs showed more concordant results with slope values around 0.2–0.3, a quite realistic value for such low dispersing plants, and the non significant Mantel test probably reflects the small number of populations considered. Taking into account the potential barrier effect of the Inini-Camopi Mountains (i.e. “ecological” distance)

did not greatly improve the correlation between genetic and geographic distances, except when St. Marcel sample is also excluded from the analysis. Even in this case, the improvement is not substantial ( $r^2$  values of 0.408 with direct Euclidian distance and 0.486 with ecological distance).

The different groups of population suggested by Structure and  $F_{st}$  analyses were used in the IM software to generate estimates of the isolation with migration model parameters (e.g. TMRCA-time of the most recent common ancestor;  $q$  – the population sizes scaled by mutation rate,  $t$  and  $m$  – respectively the time and migration rates scaled by mutation rate, Table 5). Posterior densities, autocorrelation parameters and parameter update rates showed good properties after running the analysis for 900–1000 h; thus, we are confident of the results given by IM (data not shown). However, estimates of population sizes and divergence times do not seem biologically realistic. We believe that errors in the estimation of the mutation rate parameter seriously biased those estimates and that such misspecification of the mutation rate can explain the very low estimates of population sizes and divergence times. We thus compare estimates between different runs without emphasizing the absolute values. The IM results first showed that estimates of actual population size scaled by mutation rate (i.e.  $q1$  and  $q2$  estimates) were significantly, and consistently among the different runs, much smaller than estimates of ancestral population sizes scaled by mutation rate (i.e.  $qA$ ). Secondly, estimates of population sizes of the Bakra, Touatou, St. Marcel and Mitaraka localities appeared to be lower than estimates of population sizes for the three northern inselbergs (Trinité, Nouragues, Mt. Chauve). Thirdly, the most interesting results from IM were in terms of migration rates between populations. Our results clearly showed that the northern populations were exchanging many more migrants than they were with the southern ones (St. Marcel and Mitaraka). Moreover, we

Table 3. Genetic differentiation among populations tested at the population level. Above are the pairwise  $F_{st}$  (Weir and Cockerham 1984), with values not significantly different from zero in italic. \*\* = significance values of 0.01. Below are the pairwise  $R_{st}$  values, with values significantly different from the permuted  $pR_{st}$  as calculated by Spagedi in bold.

Trinité	Nouragues		Mt. Chauve		Bakra		Touatou archipelago			St. Marcel		Mitaraka archipelago				
	T	Nsum	NS	MC	MC	BK	BK	Siii	Sii	RT	Ma1	Ma2	PM	MN	MS	Arl
T	–	0.345**	0.347**	0.299**		0.362**		0.357**	0.316**	0.343**	0.491**	0.494**	0.527**	0.503**	0.486**	0.518**
Nsum	0.435	–	0.002	0.215**		0.534**		0.564**	0.529**	0.516**	0.585**	0.588**	0.653**	0.623**	0.601**	0.650**
NS	0.330	0.0411	–	0.232**		0.534**		0.563**	0.532**	0.524**	0.590**	0.591**	0.647**	0.621**	0.601**	0.644**
MC	0.323	0.186	0.112	–		0.479**		0.487**	0.442**	0.423**	0.598**	0.600**	0.657**	0.621**	0.614**	0.657**
BK	0.447	<b>0.668</b>	<b>0.577</b>	0.564		–		0.446**	0.419**	0.409**	0.691**	0.695**	0.759**	0.732**	0.715**	0.758**
Siii	0.273	0.369	0.225	0.342		0.557		–	0.051	0.060	0.706**	0.714**	0.805**	0.774**	0.745**	0.809**
Sii	0.294	0.357	0.217	0.243		0.497		0.0667	–	0.015	0.688**	0.698**	0.786**	0.749**	0.722**	0.784**
RT	0.280	0.413	0.258	0.324		0.472		–0.011	0.329	–	0.671**	0.678**	0.759**	0.729**	0.712**	0.756**
Ma1	0.439	0.608	0.486	0.682		<b>0.855</b>		0.549	0.697	0.552	–	–	0.707**	0.684**	0.661**	0.711**
Ma2	0.389	0.572	0.445	0.647		<b>0.829</b>		0.494	0.646	0.505	0.0194	–	0.713**	0.687**	0.665**	0.717**
PM	<b>0.606</b>	<b>0.847</b>	<b>0.795</b>	<b>0.867</b>		<b>0.953</b>		0.887	<b>0.932</b>	0.845	<b>0.846</b>	0.831	–	0.157**	0.087**	0.222**
MN	<b>0.571</b>	<b>0.830</b>	<b>0.774</b>	<b>0.846</b>		<b>0.934</b>		0.858	<b>0.908</b>	0.824	<b>0.806</b>	0.794	0.130	–	0.136**	0.116**
MS	<b>0.593</b>	<b>0.843</b>	<b>0.792</b>	<b>0.860</b>		<b>0.943</b>		<b>0.882</b>	<b>0.925</b>	0.845	<b>0.829</b>	0.816	0.054	0.0432	–	0.099**
Arl	<b>0.595</b>	<b>0.847</b>	<b>0.796</b>	<b>0.866</b>		<b>0.954</b>		<b>0.897</b>	<b>0.939</b>	0.854	<b>0.844</b>	0.831	0.238	0.0750	0.0153	–

T = Trinité, N = Nouragues (summit and south), MC = Mt. Chauve, BK = Bakra, Siii = Site iii, Sii = Site ii, RT = Roche Touatou inselberg, Ma = St. Marcel (1 and 2), PM = Petit Mitaraka, MN = Mitaraka North, MS = Mitaraka South, Arl = Arlésienne.

observed that migration rates were always asymmetric with more migrants coming from the north and going to the south than inversely. Especially, the IM results showed that 1) St. Marcel and the northern population have strongly asymmetric migration rates with more migrants coming from the north to St. Marcel than inversely (m values of 103 vs 7, respectively); 2) St. Marcel and Mitaraka have lower asymmetric migration rates with few migrants from St. Marcel to Mitaraka and none in the opposite direction; and 3) Mitaraka only exchange very few migrants with the northern populations.

## Discussion

### What the present-day population structure tells us about the forest refuge hypotheses?

The aim of this study was to evaluate the influence of Pleistocene climatic oscillations on the demography and the genetic structure within species in the Neotropical region. Indeed, the forest refuge hypothesis has been proposed to explain the high species diversity in Amazonia, although it remains controversial (reviewed in Bush and Oliveira 2006). Concerning the Amazonian Basin and the Guianas, alternating periods of rainforest expansion and regression during the Pleistocene and Holocene have been described based on biogeographical, geomorphological and paleoecological data (Hooghiemstra and Hammen 1998, Pennington et al. 2004). Several long drought-associated fire events have also been recorded during the Holocene (Charles-Dominique et al. 1998, Rull 1998, Tardy 1998, Mayle et al. 2000). These results were supported by a paleoclimatic study of fossil charcoal in the soils of French Guiana (Tardy 1998) indicating successive wet and dry events during the Holocene.

In accordance with the fragmentation of the present-day xeric habitat of *P. geyskesii* in French Guiana we detected a high genetic differentiation among populations, confirming previous results based on isozyme polymorphism (Sarhou et al. 2001). A similar level of genetic differentiation has also been reported for Brazilian inselberg species of the *Alcantarea* genus (Barbara et al. 2007). Among the analysed inselbergs, some are located in areas proposed by different authors as potential refuges for rainforest during dry events. Indeed, de Granville (1982) considered that during these dry events the rainforest was restricted to the area that included Trinité, Nouragues, Mt. Chauve and Bakra. Later, Tardy (1998) showed that a wide dry corridor existed between the two forest refuges in the north during both the early Holocene (9000–8000 BP) and middle Holocene (6000–4000 BP) (Fig. 1), and that dry vegetation occurred at the same times in the south, where present annual rainfall is <2000 mm. Thus, according to Tardy (1998), during the dry events the rainforest was more restricted and included Bakra and maybe Nouragues. Considering either of these two hypotheses, during the dry periods, the inselbergs that are included in the rainforest refuges should have been isolated from the remaining dry areas. However, our results cannot fully support these hypotheses. Indeed, the populations from the two potential past rainforest refuges are presently not strongly differentiated from that of

Table 4. Isolation by distance tests performed on different sub samples. The results of the regression between genetic distance ( $F_{st}/(1 - F_{st})$ ) and the logarithm of the distance computed as the direct "Euclidian distance" or as the "ecological distance" taking into account the barrier due to the Inini-Camopi Mountains are reported in terms of intercept, slope,  $r^2$ , and Mantel test probabilities, testing the significance of the correlation between genetic and geographic distance.

Populations	Distance measure	Intercept	Slope	$r^2$	Mantel p
All	Euclidian distance	-0.589	0.47	0.356	0.002
	Ecological distance	-0.266	0.386	0.268	0.008
Without Mitaraka	Euclidian distance	-0.362	0.297	0.169	0.114
	Ecological distance	-0.0876	0.223	0.124	0.111
Without Mitaraka and St. Marcel	Euclidian distance	-0.314	0.224	0.408	0.159
	Ecological distance	-0.317	0.212	0.486	0.068

the inselbergs in the past extensive dry area in the south. Nevertheless, our analyses suggest a close relationship between the northern populations. Indeed, the plants sampled on Nouragues and Mt. Chauve inselbergs are always associated in the clustering analyses. These two inselbergs are located in the middle of the unique Pleistocene rainforest refuge described by de Granville (1982) or marginal to one of the two rainforest refuges and in the middle of the dry corridor suggested by Tardy (1998) in his paleoecological study based on charcoal analyses. Moreover, when evaluating the genetic structure with small number of clusters, plants living on the northern inselberg Trinité are associated in equivalent high proportions either to individuals collected on Nouragues and Mt. Chauve or to Bakra and Roche Touatou archipelago. Thus, these data argue for a past continuous gene flow between these populations, and therefore support the existence of dry vegetation making a corridor, as hypothesized by Tardy (1998).

### Some insights into the colonization history of *P. geyskesii* in French Guiana

Physical geographic barriers and corridors usually affect the current dynamics of plants and animals in contrasted way, respectively by breaking and enhancing gene flow. Such physical geographic constraints are also potentially represented in French Guiana, by the Inini-Camopi Mountains and the Oyapock River (Fig. 1). Our results, however, suggest that the Inini-Camopi range has been easily bypassed by stepping stones occurring along the Oyapock River (Fig. 1). The past existence of a dry corridor between two northern refuges and the impact of the river corridor in the dispersal events of *P. geyskesii* was confirmed by comparing the decrease of heterozygosity with the geographic distance between each population and Trinité. In contrast, no physical geographic constraint is suspected in the southern part of French Guiana. However, our analyses allowed us to detect a quite strong barrier between the Mitaraka archipelago and the rest of the *P. geyskesii* populations, based on isolation-by-distance tests and private allele's occurrence. *Pitcairnia geyskesii* from the Mitaraka archipelago inselbergs thus appeared genetically different from all the other analyzed populations, a result corroborated by the clustering analysis and isolation-with-migration models (e.g. with no migration estimated from Mitaraka archipelago to the nearest St. Marcel inselberg).

Under the scenarios of climatic oscillations, the high level of genetic diversity observed in the northern popula-

tions can be explained either by their continuous presence, the north being the potential centre of diversification of *P. geyskesii*, or by the admixture of different genetic pools during colonization events from the south, as illustrated by Petit et al. (2003). During glacial episodes, dryer conditions would have permitted expansion and present-day populations can be considered as relics of such successful migration events, involving a few individuals and therefore likely to have caused reduced genetic variability in the newly founded populations (Hewitt 2000). Colonization may have occurred across stepping stones, a process that would accentuate the loss of genetic diversity of any newly founded population (McCauley 1993). In agreement herewith, heterozygosity decreased with the geographic distance between each population and Trinité, the most northern inselberg. Moreover, differentiation among inselbergs within archipelagos was much lower, suggesting that gene flow occurred mainly at local scales (i.e. among populations of the same inselberg or from the same archipelago), consistent with the low dispersal ability of *P. geyskesii*.

### Conclusion

The forest refuge hypothesis applied to Amazonian lowlands has been questioned (Colinvaux et al. 2000, Willis and Whittaker 2000). However, this debate did not concern the forests that cover the Guiana Shield. The hypothetical role of past climatic events and their genetic consequences in plants and animals have been rarely tested and examined in a context of the forest refuge hypothesis in this area (Caron et al. 2000, Dutech et al. 2003, 2004, Noonan and Gaucher 2005, Fouquet et al. 2007). However, the patterns documented here are consistent with restriction-expansion dynamics of tropical forest during the Quaternary.

The strategy of Würster et al. (2005) to use vicariant non-rainforest taxa, such as *P. geyskesii*, a xerophytic plant strictly associated to inselbergs, to give a "reverse image" of the reduction-expansion of the rainforest brought new insight on the past forest dynamics in French Guiana. Firstly, the results differ from the premise of the forest refuge hypothesis of de Granville (1982) because the populations living in the proposed unique past forest refuge were not more genetically differentiated than the remaining populations. In contrast, our data indicated the occurrence of a dry corridor in the north as hypothesized by Tardy (1998). Secondly, the Oyapock River appears as an important pathway for subsequent expansion and more recent colonisation, by-passing the physical barrier of the Inini-Camopi Mountains. Thirdly, in spite of a strongly



Table 5. Maximum likelihood estimates for the effective population sizes of the two current populations (q1, q2) and for the ancestral population (qA), the migration parameters (m1: from group 2 to group 1 and m2: from group 1 to group 2), the time of population divergence (t) and mean expected time to the most recent common ancestor (TMRCA) evaluated on the seven microsatellite loci analyzed on *Pitcairnia geyskesii*. The comparisons are made according to the genetic groups identified using Structure. 95% credibility intervals are reported in brackets.

Compared groups	TMRCA	q1 = 4 × N <sub>1</sub> × μ	q2 = 4 × N <sub>2</sub> × μ	qA = 4 × N <sub>A</sub> × μ	t = T × μ	m2 = m <sub>21</sub> /μ	m1 = m <sub>12</sub> /μ
1							
2							
Trinité vs							
Nouragues and Mt. Chauve	159	0.04 [0.0217; 0.1320]	0.04 [0.0207; 0.1263]	170 [110.7695; 288.5049]	0.004 [0.0023; 0.0131]	34 [12.6500; 94.2500]	22 [4.3250; 48.5250]
Bakra and	159	0.05 [0.0299; 0.1959]	0.009 [0.0045; 0.0276]	160 [106.6062; 260.3241]	0.003 [0.0015; 0.0104]	39 [8.6750; 49.0750]	15 [3.3250; 47.2250]
Nouragues and Mt. Chauve vs							
St. Marcel vs	81	0.03 [0.0144; 0.1403]	0.015 [0.0069; 0.0600]	90 [50.5372; 128.5389]	0.006 [0.0024; 0.0230]	4 [1.0100; 18.4500]	0.01 [0.0118; 0.4863]
Trinité,							
Nouragues,							
Mt. Chauve,							
Bakra and Touthou archipelago vs	179	0.05 [0.0350; 0.2051]	0.006 [0.0039; 0.0238]	205 [126.9689; 342.5843]	0.003 [0.0019; 0.0111]	103 [32.9000; 192.5000]	7 [1.9100; 19.1500]
Trinité,							
Nouragues,							
Mt. Chauve,							
Bakra and Touthou archipelago vs	207	0.07 [0.0190; 0.2186]	0.009 [0.0026; 0.0321]	250 [167.3968; 393.1192]	0.004 [0.0011; 0.0125]	7 [2.3250; 43.6750]	4 [1.4750; 34.4750]

argued scenario in favour of a north-to-south migration history, a clear genetic isolation of populations of *P. geyskesii* living on inselbergs of the Mitaraka archipelago suggests a distinct origin of the southern-most populations, probably in Suriname and/or Guyana.

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