

Close genetic proximity between cultivated and wild *Bactris gasipaes* Kunth revealed by microsatellite markers in Western Ecuador

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Abstract

Bactris gasipaes Kunth (peach palm or Pejibaye) is the only domesticated palm of the Neotropics. The genetic relationships between the crop and its wild relatives are still unclear. We undertook field and laboratory work in order to describe differentiation and relationships between the wild and cultivated populations of the species in Western Ecuador, and their possible interactions. A volumetric study was undertaken on the fruits of both populations, as well as a population genetic analysis in order to clarify these relationships. Fruits from cultivated plants collected in the region of sympatry of wild and cultivated plants in North-West Ecuador showed intermediate volumes between those of reference samples for the wild and cultivated plants in allopatry. Using 8 microsatellite loci, we assessed 83 wild and cultivated individuals from Western Ecuador and cultivated plants from Amazonia and Central America as a reference for the cultivated form. We detected high polymorphism in the wild and cultivated samples and low, but significant level of genetic differentiation between wild and cultivated populations. The cultivated population in North-Western Ecuador showed close genetic proximity with the sympatric wild population, consistent with the volumetric study. These results have implications for hypotheses on evolution of this crop and for strategies of genetic conservation of the wild forms.

Introduction

Bactris gasipaes Kunth (peach palm or Pejibaye) is the only domesticated palm of the neotropics. It has been cultivated by Amerindian tribes for more than 2000 years (Corrales and Mora Urpi

1990; Ferreira 1999). The palm is mainly distributed in the Amazon region, but occurs from Bolivia to southern Mexico (Henderson 2000). Peach palm is an important component of the traditional way-of-life in Amazonia and Central America, not only as a fruit crop, but thanks to

a multitude of uses of the palm as a whole (Popenoe and Jimenez 1921; Patiño 1958; Clement and Mora Urpi 1987; Patiño 1992). Peach palm yields two food crops with commercial potential: the fruit and palm heart. The fruit, produced in traditional agricultural systems, is highly nutritious (Metzler et al. 1992). It provides pulp for direct consumption (once boiled), flour, baked goods, cooking oil, and fodder for farm animals. Peach palm is also the best crop for the modern production of palm heart. The processing of this gourmet product for the international market is growing into a major agro-industry in countries such as Costa Rica, Brazil, Ecuador, and other Latin American countries (Clement and Manshardt 2000). More recently plantations have started in other tropical countries such as Indonesia, Hawaii and the French island of La Reunion, making it a truly important crop worldwide. For the past three decades, peach palm has been rediscovered, with promising nutritional and commercial benefits for resource poor families in Latin America, and the exportation of the fine palm heart to Northern countries.

Peach palm has been speculated to have two wild progenitors: *Bactris macana* Martius found West of the Andes, and *Bactris dahlgreniana* Glassman, growing in the southern part of Peruvian Amazonia and Western Brazil (Clement 1988; Clement et al. 1999). Several cladistic studies (Salzman and Judd 1995; Ferreira 1999) have suggested a common ancestor between these wild species and the cultivated forms. These studies were only based on morphological similarities. Fruit size is the main morphological distinction between the cultivated and the wild genotype. The wild palm produces a very small fruit, uniform in size and shape, weighing 1 to 3 g. The cultivated fruit is quite variable and reflects the degree of domestication. There are many local races throughout South and Central America, and they have been classified into three groups according to fruit size (Clement and Mora Urpi 1988): 10–20 g microcarps; 20–70 g mesocarps and 70–200+ g macrocarps, the latter defined as advanced. A recent taxonomic treatment of the genus *Bactris* (Henderson 2000) considers *B. macana* and *B. dahlgreniana*, as well as the cultivated forms, as part of a single species, *Bactris gasipaes*. Henderson (2000) distinguishes two varieties within the *Bactris gasipaes* complex: the cultivated variety *Bactris gasipaes* var. *gasipaes*;

and the wild variety *Bactris gasipaes* var. *chichagui* (Karsten) Henderson. *Bactris gasipaes* var. *chichagui* includes the two previously recognized wild species, *B. macana* and *B. dahlgreniana*, in synonymy. We will adopt Henderson's nomenclature throughout the article. In Western Ecuador, var. *chichagui* (common Ecuadorean name *Chontilla*) grows at the Western edge of its geographic distribution. Populations are naturally distributed from a semi-dry climate South-West of the Andes, near the Peruvian border, to the seasonal, humid forest in the central and northern parts of Western Ecuador (Figure 1). They are absent from the high-rainfall tropical forest of the Chocó region near the Colombian border (Northern Esmeraldas, Imbabura and Carchi provinces). These wild plants have little use. Trunks are used occasionally as posts and the small fruits sometimes sold to prepare a beverage, called *chicha de Chontilla*, at Easter (Borchsenius et al. 1998). Peach palm (var. *gasipaes*; common Ecuadorian name *Chonta* or *Chontaduro*) is widely cultivated in Western Ecuador below 900 m (Figure 1), except in the dryer parts of the south. Peach palm cultures in Western Ecuador persist in a traditional way for fruit production only in Cayapas communities in the Chocó region of Northern Esmeraldas province (Figure 1). More recently the intensive modern culture for palm heart has developed from southern Esmeraldas to El Oro Provinces, and is promoted by the Ecuadorian Ministry of Agriculture since 1980 (David Vera, pers. comm). The presence of wild and cultivated populations in sympatry makes Western Ecuador an ideal region to conduct studies on the relationship between the two groups.

Although there are numerous articles on agronomical issues of *Bactris gasipaes* var. *gasipaes*, few studies have been undertaken on the evolutionary history, origin of domestication as well as Crop Wild Relatives (CWR). To date, only genetic diversity studies have been undertaken on Amazonian *Bactris gasipaes* var. *gasipaes* (Clement 1995; Clement et al. 2002; Adin et al. 2004), and none concerning its wild relatives and the genetic relationships between them. Understanding the genetic relationships between CWR is fundamental for the management of agrobiodiversity (Ellstrand et al. 1999) and will, in turn, be useful for genetic engineering of the species. The purpose of this study was primarily to assess the situation of wild and cultivated populations of

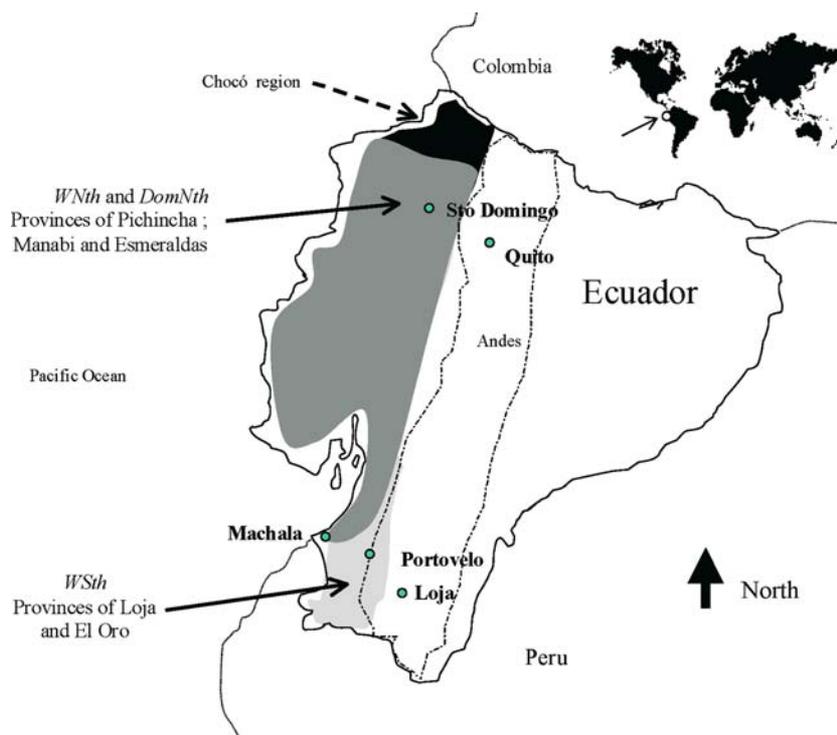


Figure 1. Map showing the distribution ranges of wild and cultivated *Bactris gasipaes* in Western Ecuador. Distribution range of wild populations in allopatry in light gray, traditional peach palm cultures range in black and region of sympatry between wild and cultivated plants in intermediate gray. *WStH* = wild, south Ecuador, *WNth* = wild, North Ecuador, *DomNth* = cultivated, North Ecuador.

Bactris gasipaes in Western Ecuador. We undertook a preliminary population genetic study including samples from wild and cultivated populations in order to estimate their relationships. Nuclear DNA microsatellite analysis was chosen to address these questions, because microsatellite loci are a powerful tool to study population genetics, due to their great allelic variability (Schlötterer and Pemberton 1998).

Materials and Methods

Field investigation

Field investigations were undertaken during several trips in 2003 and 2004 in the South and North of Western Ecuador. Farmers cultivating *Chonta* for palm heart production were asked a series of questions in Spanish in order to understand the origin of the cultivated plants and the perspectives of the farmers.

- What is the size of your plantation?
- How old is your plantation?
- Where do the seeds used for the plantation come from?
- Do you grow some *chonta* to maturity? If yes, for what purpose?
- Do you have phytosanitary problems in your plantation?
- Are you satisfied by the yield and income of your plantation?
- How do you perceive the *Chontilla*? Do you leave it growing freely in the fields? If yes, why?

These inquiries were made in five plantations in the municipalities of Puerto Quito, Los Bancos, Santo Domingo and La Concordia, all in the province of Pichincha in North-Western Ecuador. Inquiries were also made in South-Western Ecuador in El Oro and Loja provinces, in areas too dry for the cultivation of the *chonta* but where the *chontilla* is growing spontaneously. The

purpose was to know how do farmers perceive and use the wild plant when the cultivated one is not available.

Fruit sampling and volumetric study

Because fruit size is the main characteristic that differentiates wild and cultivated plants, we undertook a volumetric study on a total of 48 fruit bundles representing various origins of wild and cultivated plants (Table 1): cultivated individuals collected in the plantations of N-W Ecuador (14); wild individuals from North and South-Western Ecuador (13); and fruit bundles purchased on the Amazon markets of Iquitos and Nauta, Peru (16). Volume of ten fruits (if possible) per bundle was measured by water displacement in graduated cylinders of appropriate size. The mean and standard deviation were calculated for each group, and a two-sample unequal *T*-test was performed between mean values of each group using STATISTICA 6.0 (StatSoft 2001, www.StatSoft.com).

DNA sampling

A total of 83 individuals of wild and cultivated *Bactris gasipaes* were analyzed (Table 1). Among them, 69 individuals were collected in Western Ecuador during the 2003 field trips. The sampled localities correspond to two geographical regions (Figure 1): North-Western Ecuador where wild and cultivated plants grow in sympatry, and South-West Ecuador where the wild type occurs alone in a dryer climate. In North-Western Ecuador, we collected wild individuals in the Pichincha,

Table 1. Number of bundles and individuals sampled in the four defined groups.

Volumetric sample		Genetic sample	
Group	Number of bundles	Group	Number of individuals
<i>Bgc North</i>	8	<i>WNth</i>	32
<i>Bgc South</i>	7	<i>WSth</i>	24
<i>Bgg North</i>	14	<i>DomNth</i>	13
<i>Bgg America</i>	16	<i>DomAm</i>	14

Manabí and Esmeraldas provinces (group *WNth*). We sampled cultivated individuals in a palm heart plantation at El Placer de Toachi near Santo Domingo (Pichincha; group *DomNth*). Wild-type individuals were collected from two localities in the South-Western Ecuadorian province of Loja (group *WSth*), where no cultivated plants can be found. In addition, 14 cultivated peach palms representing various landraces from Amazonia and Central America, presumably not in contact with wild plants, were included in this study to provide a reference group for the cultivated type (group *DomAm*). These samples were obtained from germplasm collections in Peru (INIA Yurimaguas) and French Guyana (CIRAD).

DNA extraction, PCR and microsatellite genotyping

Fresh leaf samples were dried out in the field with silica gel for 3 days and then preserved in a dry place. Total genomic DNA was extracted from dry leaves using DNeasyreg Plant MINI Kit (Qiagen) and then stored at -20°C .

Eight microsatellite loci (listed in Table 2) were PCR-amplified in 10- μL reaction volumes. Seven loci were originally isolated from the cultivated peach palm (Billotte et al. 2004) and one isolated from the wild type (mBgCIR204, forward primer TGGCAGTTCAAAGTAGTATCAAT, reverse primer TAAGCCACCACCAAGCAGTCC). The reactions were done with 30–50 ng of genomic DNA, 0.01 μM of the forward primer extended

Table 2. Microsatellite loci used and summary of the allelic variation in *Bactris gasipaes* (wild and cultivated individuals lumped together) (from Billotte et al. 2004 except for mBgCIR204).

Locus ID	No. of alleles ^a	H _E ^b
mBgCIR010	13	0.86
mBgCIR057	17	0.88
mBgCIR058	23	0.89
mBgCIR062	25	0.90
mBgCIR071	11	0.84
mBgCIR087	17	0.89
mBgCIR094	17	0.76
mBgCIR204	21	0.90

^aTotal numbers of alleles.

^bH_E is the expected heterozygosity (Nei 1987) on all individuals by locus as computed using FSTAT.

with a M13 tail (Boutin-Ganache et al. 2001), 0.1 μ M of the reverse primer, 0.2 mM dNTP PCR mix (Promega, Madison, WI), 2.5 μ M MgCl₂, 1X PCR buffer (Promega, Madison, WI), 0.15 μ M of 700 or 800 nm infrared-fluorescent M13 primer, and 0.5 U of *Taq* DNA polymerase (Promega, Madison, WI). The PCR program was as follows: 35 thermal cycles at 94 °C for 1 min, 50–55 °C for 30 s, 72 °C for 30 s and a final extension at 72 °C for 3 min. PCR products were analyzed on a Li-Cor IR² automated DNA sequencer (Li-Cor, Lincoln, NE, USA). Allele scoring was done using the Li-Cor IR² software SAGA-GTTM (Li-Cor, Lincoln, NE, USA).

Analysis of genetic variation

The total number of alleles, observed heterozygosity, and non-biased expected heterozygosity (Nei 1987) were calculated for each group using the program GENETIX 4.04 (Belkhir et al. 2000). We also calculated allelic richness, which is a measure of the number of alleles independent of sample size, using FSTAT 2.9.1 (Goudet 1995). A Mann–Whitney test was used to evaluate differences in allelic richness between wild and cultivated groups. All tests were conducted separately for each measure of diversity, using STATISTICA 6.0 (StatSoft Inc. 2001). The number of private alleles (PA; defined as alleles found in a single group throughout the region studied) was also calculated using the software CONVERT (Glaubitz 2004). In order to determine the genetic structure among and within the groups, hierarchical analysis of molecular variance (AMOVA, Michalakis and Excoffier 1996) was calculated using the Arlequin program, version 2.0 (Schneider et al. 2000). With microsatellite data, the estimation of the fixation index (Φ_{ST}) incorporates variance in allele size and distribution of alleles in each population. Significance levels for the overall values were determined after 1023 permutations. Finally, we used the programs GENEPOP 3.1 (Raymond and Rousset 1995) to test pairwise linkage equilibria (LE) at all microsatellite loci over the four groups (batches: 2000; iterations: 1000). Significance levels were adjusted using the Bonferroni method to take into account multiple tests on the same data set (Rice 1989).

Clustering and ordination plots of genotypes

To depict the genetic relationships among individuals, phylogenetic trees were generated using the Neighbor-Joining (NJ) algorithm (Saitou and Nei 1987) with the program PHYLIP v.3.6 (Felsenstein 1995). The allele-sharing distance D_{AS} (Chakraborty and Jin 1993) was used because it is thought to be more appropriate for recently diverged populations (Goldstein and Pollock 1997). The genetic distance D_{AS} was calculated using MSA 3.10 (Dieringer and Schlötterer 2003). Two *Bactris setulosa* individuals (Couvreux et al. unpublished) were added as outgroup. Individual microsatellite genotype scores were also ordinated in a multi-dimensional space by principal coordinate analysis (PCO), using the shared alleles distance, computed with STATISTICA 6.0 (StatSoft 2001, www.StatSoft.com).

Bayesian clustering analyses and population assignment

We undertook these analyses with a sampling restricted to the sympatric North-West area (45 individuals). We first used a Bayesian method implemented in GENECLASS (Cornuet et al. 1999). This method uses prior population information (cultivated/wild) to assign plants to clusters. This exclusion method permits calculation of the probabilities for each individual of belonging to the wild and the cultivated compartment. Admix individuals will have probabilities of belonging to both compartments. The simulation was run with 10,000 simulated individuals and a threshold of 0.05.

In a second analysis, we used another Bayesian method implemented in the software STRUCTURE (Pritchard et al. 2000) to detect individuals with possible admix ancestries assuming two populations ($K=2$). Prior (USE-POPINFO=1 and MIGRPRIOR=0.001) and no prior information on the origin (wild or cultivated) of the plants were used. The results were based on 1,000,000 iterations following a burn-in period of 100,000 iterations. Individual proportions, q_i , were estimated with their 90% probability intervals (ANCESDIST=1; BOXES=10,000).

Results

Field investigation

The palm heart plantations of the Pichincha province investigated are of small to moderate size (5–50 ha) and mostly recent (less than 10 years old) to relatively recent (less than 20 years old). The plantations are owned by businessmen having several agro-industrial activities or are part of large *haciendas*. It is generally not an activity of small individual farmers as it is labor-demanding and profitable only through price agreement with processing factories which purchase the production for canning and export.

All the plantations investigated were initially established independently from different seeds purchased on Amazon markets or from seedlings produced in Amazon nurseries. Consequently, the plantations are very heterogeneous and produce many different fruit shapes. However, nurseries are now producing seedlings in Santo Domingo (Pichincha) from the material of Amazon origin established there. Renewal of plantings is either made by importation of new Amazon seeds or seedlings, or from seedlings provided by local nurseries or from seeds produced directly in the plantation. These options partly depend on the method of management of the cultivated plants. In palm heart plantations, *Bactris gasipaes* is cultivated at very high densities, and maintained in a juvenile, sub-herbaceous state by cutting stems every 12–18 months. In some plantations, the palm is never grown to maturity, since adult plants are considered potential pests reservoirs. In other plantations, cultivated palms are left growing in the margin of the fields to delimit them and serve as seed-holders for the next generation. The wild plants are very commonly left in and around the fields, either for simple ornamental purpose or for fear that cutting them would induce a massive spread of pests hosted in those trees. Moreover, palm heart farmers in North-Western Ecuador indicated that they do not recognize the close affinity between *Bactris gasipaes* var. *gasipaes* (*chonta*) and var. *chichagui* (*chontilla*). As the former is introduced from Amazonia, it appears to the farmers as an entity totally distinct from the autochthonous wild var. *chichagui*. It is important to consider however that farmers in Pichincha province come mostly from the *Sierra* (Quito) and have little experience and knowledge of the palms.

In the Loja and El Oro provinces, on the contrary, the *chontilla* is interpreted as a useless, wild form of *chonta*. The use of fruits for preparing *chicha de chontilla*, which is rather common in N-W Ecuador, is unknown in the South. On the other hand, the affinity of *chontilla* with *chonta* is recognized because the cultivated plant is well-known from the nearby Amazon province of Zamora-Chinchipe. Many farmers of Loja and El Oro actually come from Zamora-Chinchipe and often conserve the *chontilla* in their fields as a remainder of the useful palm of the Amazon, which is unable to grow in the drier climate of the southern Pacific slopes of the Ecuadorian Andes.

Volumetric study of fruits

Mean fruit volume was small as expected for the wild individuals in the South ($BgcS$, $x = 2.13 \text{ cm}^3$) and in the North ($BgcN$, $x = 5.451 \text{ cm}^3$) of Western Ecuador. $BgcN$ showed however a much higher variance than $BgcS$ fruits (Figure 2). The mean fruit volume of the Amazonian individuals ($BggA$, $x = 70.04 \text{ cm}^3$) was more than 10 times the mean fruit volume of the wild individuals, and the largest Amazon fruit measured (189 cm^3) was almost 100 times bigger than the smallest wild

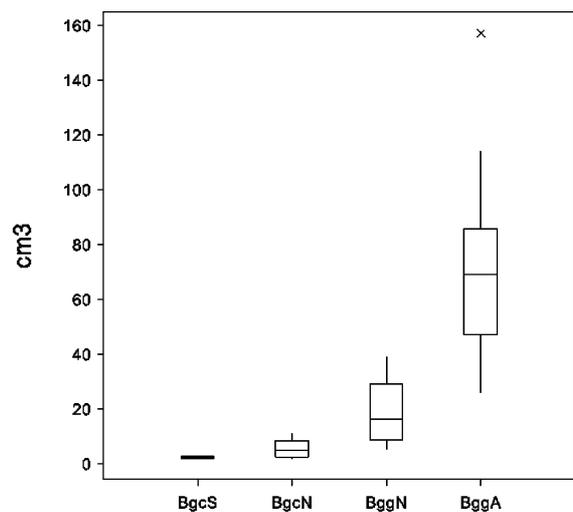


Figure 2. Mean volume size of *Bactris gasipaes* fruit. ($BgcS$ = wild South; $BgcN$ = Wild North; $BggN$ = cultivated, North; $BggA$ = cultivated, Amazon).

fruit (2 cm³). However, the mean fruit volume of the cultivated individuals of North-Western Ecuador (*BggN*, $x = 18.84$ cm³), is intermediate between the autochthonous wild group (*BgcN*) and the Amazon group (*BggA*). Fruit size in *BggN* actually encompasses the upper size range of *BgcN* and the lower size range of *BggA*. All groups had highly significant mean volume differences from one another ($p < 0.001$), except between *BgcS* and *BgcN* which were only significantly different at $p < 0.03$.

Analysis of genetic diversity

We determined the individual genotypes at 8 loci for 83 *Bactris gasipaes* samples. All microsatellite loci were polymorphic with a diallelic pattern, homozygote or heterozygote. The number of alleles varied from 10 (*Bg 71*) to 25 (*Bg 62*). The average number of alleles per locus over all individuals was 17.5 (± 2.6). Expected heterozygosity (H_E) values varied from 0.7 to 0.9 (Table 2). Distribution of allele frequencies (results not shown) varied across all loci. Some loci showed marked differences between wild and crop genotypes and even between South-West (*WSth*) and North-West (*WNth*) origins of wild genotypes. The two cultivated groups (*DomNth* and *DomAm*) had significantly more alleles (Mann–Whitney test $p < 0.05$) than both wild groups *WNth* and *WSth* (Table 3). Expected heterozygosity (H_E) was not significantly different among the four groups ($p > 0.05$). The number of private alleles (PA) ranged from 8 (*DomNth*) to 20 (*DomAm*) but did not differ between wild and cultivated forms ($p > 0.05$). No disequilibrium was significant at any pair of loci over all groups ($p > 0.05$, after Bonferroni correction for 112 comparisons). This

Table 3. Genetic diversity indices of *Bactris gasipaes* in Western Ecuador.

Variety	Wild <i>Bactris gasipaes</i>		Cultivated peach palms	
	<i>WNth</i>	<i>WSth</i>	<i>DomNth</i>	<i>DomAm</i>
Geographical region:				
No. of private alleles	17	12	8	21
Average no. of alleles	10.20	8.50	7.50	10.70
Observed heterozygosity	0.58	0.56	0.65	0.52
Expected heterozygosity	0.80	0.75	0.74	0.80

Individuals are grouped according to phenotypic and geographical criteria. Cultivated peach palm from Amazonia and Central America: *DomAm*; *DomNth*, *WNth* and *WSth* as in Figure 1.

Table 4. Analysis of molecular variance components (AMOVA, Michalakis and Excoffier 1996) and their significance (1023 permutations) for the four geographical groups.

	Genetic variance	Probability
Among geographical groups	9.43	<0.0001
Within geographical groups	90.57	<0.0001

suggests that the loci were sampled in independent regions of the genome.

Genetic differentiation

The hierarchical AMOVA amova analysis based on allele frequencies over all loci indicated that 90.6% of the total genetic variance was found within the groups (Table 4), and that 9.4% was found among groups (Table 4). This indicates low genetic structure between the groups, which is also shown by the low, but highly significant value of the fixation index estimator ($\Phi_{ST} = 0.09$, $p < 0.001$).

Clustering and multivariate analysis

The NJ tree (Figure 3) based on shared allele distance (D_{AS}), showed weakly supported clusters (bootstrap values < 65%), but coincided well with the phenotypic and geographical classification. Most of the northern sympatric cultivated individuals (*DomNth*) joined the northern wild cluster (*WNth*). Three *DomNth* individuals clustered with the *DomAm* group, and one *DomNth* individual grouped with the wild south (*WSth*) cluster. Finally, two *DomAm* clustered with the *WNth* group.

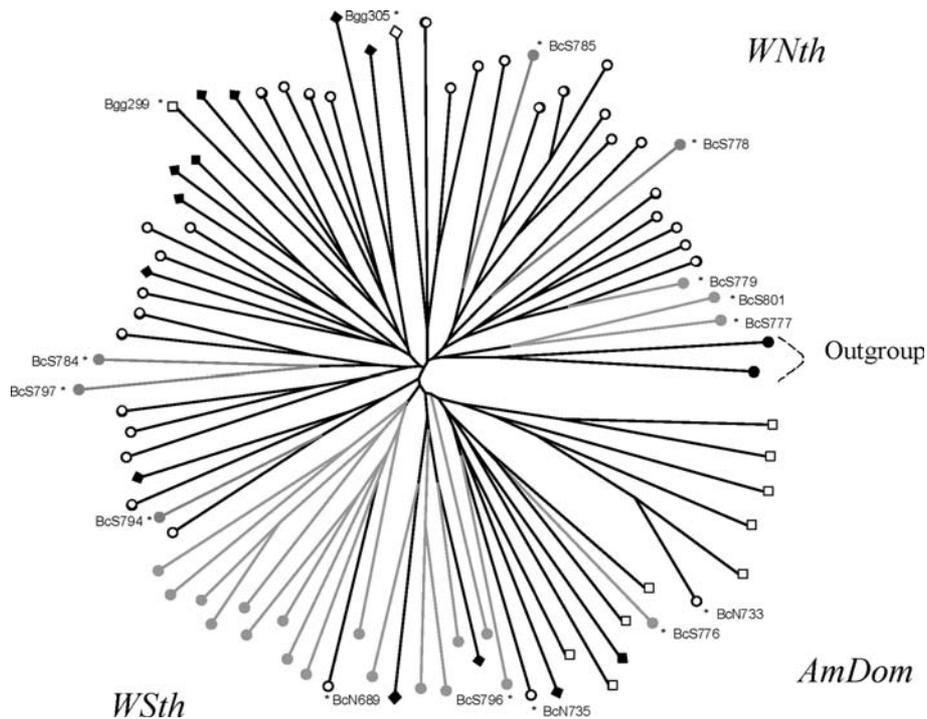


Figure 3. Unrooted neighbor-joining tree with microsatellites genotypes using Shared Alleles distance of the *Bactris gasipaes* complex in Western Ecuador. Each branch represents a single individual. Cultivated individuals in NW Ecuador (*DomNth*) are represented by ◆. Cultivated peach palm from Amazonia and Central America (*DomAm*, open squares), wild individuals from NW Ecuador (*WNth*, open circles), wild individuals from SW Ecuador (*WSth*, gray circles). Outliner individuals are indicated by asterisk and their names.

Principal component analysis explained 17.6% of the total genetic diversity on the two first axes (Figure 4). The first axis showed a marked difference between the wild Ecuadorian plants and the cultivated individuals. The second axis separated northern and southern wild Ecuadorian populations. A majority of cultivated individuals of North-West Ecuador (*DomNth*) overlapped into the northern wild group which is also shown by the NJ analysis.

Analysis in the area of sympatry

A first Bayesian method was used to calculate individual probabilities of belonging to both the cultivated and the wild group using GENECLASS (Table AI). Of the 32 wild plants, 18 were exclusively assigned to the wild group and 14 were significantly assigned to both wild and cultivated groups. Assignment probabilities to the wild group were always greater than to the cultivated one. For the cultivated palms (*DomNth*), 7 of 13 individuals

were assigned to the cultivated group and 6 were assigned to both groups. For these plants, assignment probabilities were always greater for the cultivated group except for individual Bgg759.

We then used another Bayesian method implemented in STRUCTURE assuming two populations ($K = 2$). A first group was formed of cultivated plants (10/13). The second group was formed of wild plants (15/32). 18 plants showed ancestry in more than one cluster. The 90% confidence intervals associated with the q_i estimator was large encompassing both theoretically possible extremes (0–1). This shows that the data gives only little information on intermediate q_i values.

Discussion

The Western Ecuadorian setting

In Western Ecuador, wild and cultivated *Bactris gasipaes* varieties are in sympatry, except in the extreme south of the country where only the wild

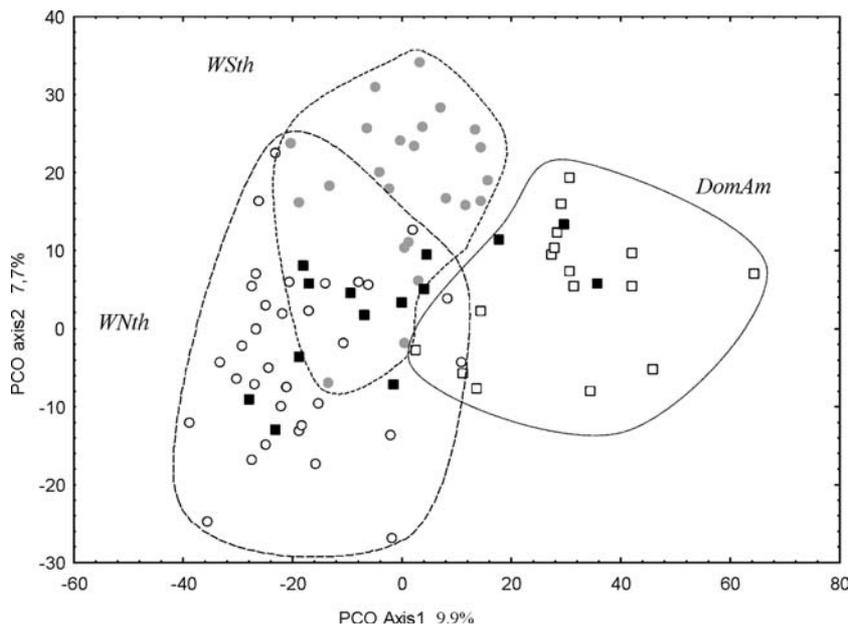


Figure 4. Principle coordinate analysis scores of individual microsatellite genotypes in the *Bactris gasipaes* complex. *WNth*, open circles; *WSth*, gray circles; *DomAm*, open squares and peach palm cultivated in NW Ecuador in black squares.

type grows and in the extreme north where only the cultivated type is to be found. Our field work allowed us to describe a very unusual and original management of *Bactris gasipaes*. Farmers in the North-Western part of Ecuador 'create', by importing Amazonian seeds, a mixture of traditional fruit races, and a contact zone between wild and cultivated palms otherwise inexistent. This has been ongoing for the past 30 years. *Bactris gasipaes* management in palm heart plantation is however variable and we have recognized different types of cultures (Table 5). Type I cultures are established from seeds directly imported from the Amazon. We can distinguish two sub-types: Ia corresponds to plantations where individuals are never left growing to maturity, and thus do not interact with nearby growing wild individuals. In Ib cultures, individuals are left growing to maturity along the plantations but are not used for seed production. These individuals could possibly hybridize with the wild genotypes. Type II cultures are established from individuals which are locally produced, and which could thus be hybrids with the wild plants growing nearby. Intermediate fruit size of the cultivated individuals in North-West Ecuador could indicate that hybridization is already ongoing.

Genetic relationships between wild and cultivated individuals

This is to our knowledge the first genetic study conducted in order to differentiate wild and cultivated *Bactris gasipaes* using multiple codominant markers. High levels of genetic diversity ($0.76 < H_E < 0.90$) were detected across all 8 microsatellite loci of *Bactris gasipaes* as expected for a self-incompatible species. This high genetic diversity was similar to that reported for other neotropical palms such as *Astrocaryum mexicanum* (Eguiarte et al. 1992) and *Euterpe edulis* (Gaiotto et al. 2003). Our results are also in accordance with other studies where most tropical trees show high levels of genetic diversity (Hamrick and Godt 1989; White et al. 1999). These markers can thus be considered as useful for genetic studies within *Bactris gasipaes*. This study contrasts strongly with two isozyme studies (Clement 1995; Clement et al. 1997) and a recent AFLP study (Adin et al. 2004) on different Amazonian peach palm races, where low expected heterozygosity was found ($0.07 < H_E < 0.19$; $0.06 < H_j < 0.2$, respectively). However, the lower diversity with isozymes compared to microsatellites has also been noted in other species, such as in *Quercus robur* (Degren et al. 1999).

Table 5. Types of palm heart culture in Western Ecuador and their consequences.

Type of culture	Origin of cultivated plants	Management of cultivated plants	Management of wild plants	Possible interactions between cultivated and wild compartments
Type Ia	Amazonia	Never grown to maturity	Conserved in and around fields	None
Type Ib	Amazonia	Grown to maturity on field margins (fences). Not used for seed production	Conserved in and around fields	Introgression of the wild genotypes
Type II	Locally produced	Grown to maturity on field margins (fences). Used for seed production	Conserved in and around fields	Reciprocal introgression of cultivated and wild genotypes

In Henderson's (2000) revision of the genus *Bactris*, wild and cultivated plants of *Bactris gasipaes* are considered as conspecific. The main morphological difference between wild and cultivated forms is fruit size (Mora Urpi et al. 1997). Our study shows that there is a low, but significant genetic differentiation between wild and cultivated palms. Such differentiation is shown by the existence of many private alleles restricted to either wild Ecuadorian or Amazonian/Central American crop peach palms. Differentiation, based on AMOVA and Φ_{ST} , is also statistically significant between wild and cultivated populations. Finally, the multivariate analysis was able to detect genetic structure among the three geographic groups: North-West Ecuador, South-West Ecuador and the Amazonian/Central American region. These groups also appear clearly in the NJ distance tree. This genetic differentiation is however less evident between cultivated and wild plants in North-Western Ecuador.

Indeed, most of the *DomNth* individuals cluster with the *WNth* individuals in both multivariate and distance analyses which indicates overlapping genetic diversity between wild and cultivated forms in sympatry. This close genetic relationship in the area of sympatry can be explained by at least two hypotheses. The first possible explanation is a close affinity between the wild palms of North-Western Ecuador and the cultivated Amazon palms introduced there. The palm-heart farmers indicated that the plantations are based on fruits imported from different Amazonian regions. Indeed, some *DomNth* and *DomAm* individuals have a similar genetic structure, as shown by the multivariate analysis and NJ tree (Figures 3 and 4). Two cultivated individuals from Amazonian Peru (Bgg299 and Bgg305) are closer to the wild Ecuadorian *B. gasipaes* (Figure 3) than to other Amazon accessions. This result indicates that cultivated Amazonian individuals can show close genetic relationships with wild populations of Western Ecuador. We cannot, however, exclude that they originated by hybridization with the wild compartment also present in Peru. Indeed, a second likely hypothesis of this close genetic relationship is gene flow between wild and cultivated plants. The majority of the cultivated individuals in North-Western Ecuador are genetically closer to the sympatric wild genotypes than to cultivated plants of other American regions. Hybridization

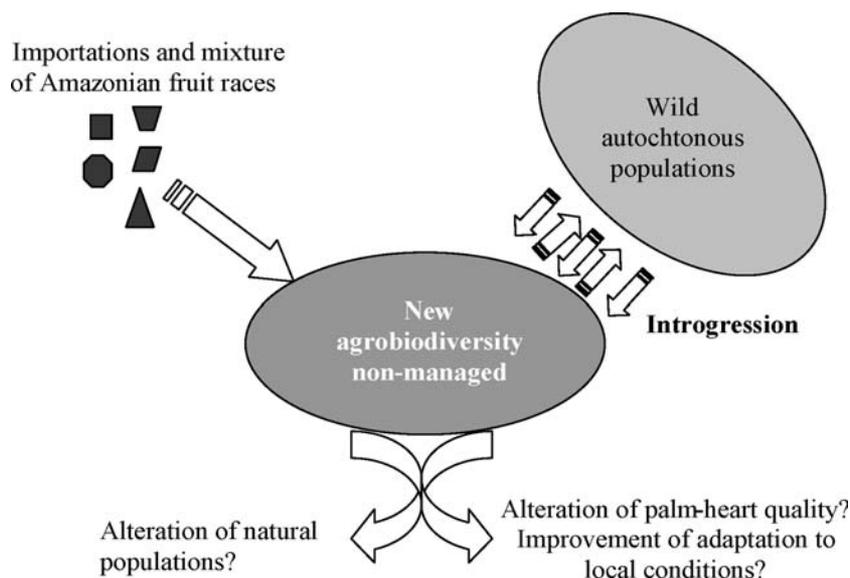


Figure 5. Possible interactions and consequences of *Bactris gasipaes* management in palm-heart plantations in Western Ecuador.

could be ongoing in this region since the beginning of palm heart culture. In the field, this hypothesis is highly coherent at the morphological level, based on the variation of fruit size. Northern cultivated individuals (*BggN*) present intermediate fruit sizes between northern wild (*BgcN*) and Amazonian (*BggA*) individuals (Figure 2), although they originated in the Amazon. This observation is also corroborated at the genetic level. The first Bayesian method showed 'intermediate' individuals between the cultivated and the wild gene pool. One individual (*Bgg730*), found growing spontaneously in a disturbed forest near a palm heart plantation, was genetically detected as admix. In the field, this individual showed intermediate fruit size characteristics also suggesting a hybrid origin. The high variance of fruit volume in wild individuals of North-Western Ecuador, compared to individuals from the South which have very homogeneous small fruits, is also an indication of possible hybridization in the area of sympatry. The fact that cultivated individuals in N-W Ecuador (such as *Bgg759*) are also associated with the sympatric wild cluster could indicate that reciprocal gene flow between wild and cultivated compartments occurs. The generalized practice of leaving wild plants within and around the palm heart fields, and of allowing cultivated plants to

grow to maturity on field borders, could allow for hybridization between wild and cultivated plants, with varied consequences (Figure 5). In order to confirm the presence of hybrids, more molecular markers must be added to obtain better statistical support.

The peach palm, cultivated in a traditional way, has been qualified as an underutilized crop for the Neotropics (Hernández Bermejo and León 1994) and a new potential resource for tropical nutrition. Palm heart production from peach palm is reaching international standards nowadays as it is becoming an important crop for developing countries. For these reasons it is important to be able to clarify and determine what is the closest wild relative of the cultivated plants, and if the two varieties interact when in contact (CWR). This will not only help preventing genetic pollution of cultures coming from the wild group, but it will also open the doors to genetic engineering of the species. In this perspective, conservation of the wild type appears important, especially with respect to the threat on genetic integrity of wild populations when cultures are established nearby. This preliminary study sets the grounds and the hypotheses for further analysis on the relationships between wild and cultivated *Bactris gasipaes*.

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