GENETIC DIVERSITY OF TRADITIONAL SOUTH AMERICAN LANDRACES OF CASSAVA (*MANIHOT ESCULENTA* CRANTZ): AN ANALYSIS USING MICROSATELLITES[1]

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*The extent and structure of the genetic variability of traditional varieties of cassava (*Manihot esculenta *Crantz) have been little documented, despite considerable evidence for this crop's great varietal diversity in traditional agroecosystems. We used microsatellite markers to assess the genetic structure of traditional landraces of sweet and bitter cassava collected from five South American sites. As reference, we used a sample of 38 accessions from a world collection of cultivated cassava. For a total of 10 loci examined, we found 15 alleles that were not represented in this sample. Ten of these had been previously detected in wild* Manihot *species. The geographical structure of genetic variability was weak, but the genetic differentiation between bitter and sweet landraces was significant, suggesting that each form had evolved separately after domestication. Our results showed that traditional landraces form an important source of genetic diversity and merit more attention from managers of crop genetic resources.*

DIVERSIDADE GENÉTICA DE VARIEDADES TRADICIONAIS SULAMERICANAS DE MANDIOCA (*MANIHOT ESCULENTA* CRANTZ): UMA ANÁLISE COM MARCADORES MICROSSATÉLITES. *A extensão e a estruturação da variabilidade genética de variedades tradicionais de mandioca (*Manihot esculenta *Crantz) têm sido pouco documentadas, apesar de existirem evidências sugerindo uma grande diversidade varietal desta cultura em agroecosistemas tradicionais. No presente trabalho, foram usados marcadores de DNA, do tipo microssatélite, para avaliar a estrutura genética de variedades tradicionais de mandioca brava e mandioca de mesa coletadas em cinco localidades da América do Sul. Como referência, usou-se um conjunto de 38 acessos de uma coleção mundial de germoplasma de mandioca. Entre as variedades tradicionais, foram encontrados 15 alelos que não estavam presentes nesta amostragem da coleção mundial. Dez destes alelos já haviam sido detectados em espécies silvestres de* Manihot*. Apenas uma leve estruturação geográfica da variabilidade foi observada. No entanto, foi evidenciada uma diferenciação genética entre variedades bravas e de mesa, sugerindo que cada forma tenha evoluído separadamente após a domesticação. Nossos resultados mostram que variedades tradicionais constituem uma importante fonte de diversidade genética e deveriam receber maior atenção no manejo de recursos genéticos de plantas cultivadas.*

Key Words: bitter cassava; genetic diversity; microsatellites; sweet cassava; traditional farming.

Conservation of genetic resources of crop plants, today regarded as an urgent priority, requires characterization of the genetic diversity of domesticated plants and understanding its origin and evolution under domestication (Miller et al. 1995). Traditional farming systems are privileged places in which to study these processes,

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because, despite the diversity of cultural practices among different regions or different groups, they have in common the fact that selective pressures may be closer to those that occurred under domestication than under modern farming (especially natural selection factors, which are still very important). Moreover, under traditional farming, varietal and genetic diversity has been shown to be high (Brush et al. 1994; Elias, Panaud, and Robert 2000; Elias, Rival, and McKey 2000; Emperaire, Pinton, and Second 1998; Kerr and Clement 1980; Louette, Charrier, and Berthaud 1997; Quiros et al. 1990; Second et al. 1997) and subject to complex dynamics.

Traditional landraces therefore merit more attention from geneticists, especially for defining strategies for conserving the genetic diversity of cultivated crops. In this paper, we present a study of the genetic diversity of traditional South American landraces of cassava (*Manihot esculenta* ssp. *esculenta* Crantz, Euphorbiaceae).

Cassava is the fourth most important starchy root crop cultivated in the world. Although it originates from South America (Allem 1994; Olsen and Schaal 1999), it is now widely grown throughout the tropics. Cassava is cultivated for its starchy roots, and is vegetatively propagated by means of stem cuttings. Traditional cassava cultivation is performed in slash-and-burn agricultural systems (Boster 1984a; Cury 1993; Dufour and Wilson 1996; Elias, Rival, and McKey 2000; Peroni 1998; Sambatti, Martins, and Ando 2001). An old fallow or a new area in the forest is cut, and the vegetation is burned after drying under the hot sun for two or three weeks. The field is then cleaned, and cassava stem cuttings are planted sequentially. Up to 40 landraces can be planted in the same field (Emperaire, Pinton, and Second 1998). Depending on its landrace, a plant can take between seven months and up to two years to produce harvestable roots. During harvest, the whole plant is cut, its roots are collected in a basket, and its stems are carefully kept for planting a second crop in the same area, or for planting in a new field. Two to five cassava crops can be planted successively in the same field, depending on soil quality and weeds. After the last crop, the field is left fallow for 5 to more than 20 years, before being cultivated again.

Thousands of cassava landraces exist, which can be classified into two main groups, according to the degree of cyanogenic glucoside content of their roots (hereafter referred to as cyanide content) and their uses (Dufour 1988, 1995; McKey and Beckerman 1993). These cyanogenic glucosides in their roots (and in other organs) are believed to act as a defense against herbivores (McKey and Beckerman 1993). When the cyanide content exceeds 100 ppm of fresh weight, cassava roots are toxic for humans (Bolhuis 1954) and must be detoxified before consumption through a complex and time-consuming process (Dufour 1989; Hugh-Jones 1979; McKey and Beckerman 1993). Such landraces are called ''bitter'' (*amarga* in Spanish and *brava* in Portuguese), and are processed into products such as coarse-grained flour (*farinha, goma,* ''tapioca,'' or *farine*), flat breads (*beiju*), cassava bread, or fermented drinks (Dufour 1989; Elias, Rival, and McKey 2000; Mowat 1989). Roots of so-called ''sweet'' or ''cool'' landraces (Chiwona-Karltun et al. 1998) (*dulce* in Spanish and *de mesa, macaxeira,* or *aipim* in Portuguese) are characterized by low cyanide contents (below 100 ppm), and can be eaten safely, whether boiled, roasted, or even raw.

In traditional cassava-farming systems, both types of cassava landraces are cultivated, but the types are usually separated at the regional and local levels. Sweet landraces are mostly cultivated in the Andean foothills, whereas bitter cassava is the staple crop for indigenous or mixed-race people living in the lowlands of the Amazonian Basin (Renvoize 1972). Bitter-cassava farmers also cultivate a few sweet landraces, which are planted either in the farms with the bitter cassava, or separately, closer to the house.

The causes of this uneven distribution, as well as the reasons motivating the cultivation of toxic landraces despite the risks of poisoning and the time spent in detoxification, are discussed by McKey and Beckerman (1993) and Chiwona-Karltun et al. (1998). McKey and Beckerman (1993) also raise the question of the evolutionary origins of the sweet and bitter landraces, proposing four scenarios for the existence of both forms: (1) Cassava was first domesticated from a sweet wild progenitor. Bitter forms, believed to be more productive in harsh environments (Wilson and Dufour 2002), evolved later from the domesticated sweet cassava. (2) Cassava was first domesticated from a bitter wild progenitor. Sweet forms evolved later, as a result of selection for low cyanide content. (3) Sweet and bitter forms were domesticated indepen-

dently, from sweet and bitter wild progenitors, respectively. (4) Sweet and bitter forms were domesticated from the same wild ancestor, of intermediate cyanide content. Sweet forms are the result of selection for low cyanide content, while bitter forms evolved simultaneously as the product of selection for increased cyanide content.

Until now, few studies have been published on the cyanide content of wild *Manihot* species. The roots of most wild species have intermediate or high cyanide content (McKey and Beckerman 1993). Moreover, unpublished data at the Centro Internacional de Agricultura Tropical (CIAT, Cali, Colombia) show that the roots of *M. esculenta* ssp. *flabellifolia,* now regarded as the wild progenitor of cassava (Allem 1994; Olsen and Schaal 1999) have intermediate or high cyanide content. Although these data clearly do not support scenario (1), they are not sufficient to discard it.

Another question to be addressed is whether sweet or bitter forms have evolved once or several times from wild progenitors or other forms, depending on the evolutionary scenario. Up to now, no clear answer to these questions has been proposed.

Cassava varietal diversity in traditional farming systems is high (Boster 1984b; Carneiro 1983; Chernela 1987; Dufour and Wilson 1996; Elias, Rival, and McKey 2000; Emperaire, Pinton, and Second 1998; Grenand 1993; Kerr and Clement 1980; Salick, Cellinese, and Knapp 1997). The first factor may be exchange of cassava landraces. Exchange has been reported for all ethnic groups studied (Boster 1985, 1986; Chernela 1987; Elias, Rival, and McKey 2000; Emperaire, Pinton, and Second 1998; Peroni 1998; Salick, Cellinese, and Knapp 1997; Sambatti, Martins, and Ando 2001), and sometimes occurs over distances of more than 300 km (Chernela 1987). A second factor is that, although cassava is asexually propagated, farmers often incorporate into their harvest, and sometimes as parts of their propagation material, plants originating from sexually produced seeds (Boster 1984b; Chernela 1987; Chiwona-Karltun et al. 1998; Elias, Rival, and McKey 2000; Elias et al. 2001; Emperaire, Pinton, and Second 1998; Sambatti, Martins, and Ando 2001). Indeed, seeds are produced in the fields before the plants are harvested. Autochory (explosive dehiscence of the drying fruit) and myrmecochory (dispersal by ants) move seeds over short distances, leading to the constitution of a seed bank (Elias and McKey 2000). The seeds germinate when the same area is cut after a fallow period and burned for cultivation, and the seedlings grow at the same time as do plants from cuttings.

Both processes—the widespread exchange of planting material and incorporation of plants grown from sexually produced seeds—reflect the farmers' strong interest in diversity for its own sake (Carneiro 1983; Elias, Rival, and McKey 2000; Emperaire, Pinton, and Second 1998; Kerr and Clement 1980). That is, farmers eagerly acquire new landraces, leading to high varietal diversity on their farms. The genetic studies published on traditional South American landraces (Colombo, Second, and Charrier 2000; Elias, Panaud, and Robert 2000; Faraldo et al. 2000; Mühlen, Martins, and Ando 2000; Sambatti, Martins, and Ando 2001; Second et al. 1997) have all shown that local farmers manage high genetic diversity. In two cases (Elias, Panaud, and Robert 2000; Second et al. 1997), cassava genetic diversity, assessed with AFLP markers in one community and even in one field, was almost as high as a representative sample of a core collection (''core of the core,'' Roa et al. 1997) maintained at CIAT. These studies also showed that, despite vegetative propagation, many cassava landraces are polyclonal.

Recently, codominant and highly polymorphic microsatellite markers have been developed for the study of cassava (Chavarriaga-Aguirre et al. 1998). Ten microsatellite loci were used to assess allelic polymorphism and degree of relationship within the genus *Manihot* (Roa et al. 2000). Cultivated cassava was represented by the ''core of the core'' (Roa et al. 2000). That study confirmed results obtained previously with AFLP markers on the same samples (Roa et al. 1997), which showed that (1) cultivated forms are genetically homogeneous (i.e., each accession of cultivated cassava was closer to all other cultivated landraces than to any accession of wild *Manihot,* and each taxon had some alleles that were specific to it); and (2) cassava and the wild forms *M. esculenta* ssp. *flabellifolia* and *M. peruviana* are sufficiently genetically close to be considered synonymous (Roa et al. 1997). Moreover, Roa et al. (2000) confirmed that cassava was highly heterozygous.

The objectives of the present study were to use the ''core of the core,'' characterized by mi-

crosatellite loci, as a basis for comparing and thus characterizing the genetic diversity of traditional South American landraces of cassava. Traditional landraces originate from Brazilian Amazonia (Tukâno Amerindians and mixed-race riverside communities), from the coastlands of São Paulo State, Brazil (Caiçara farmers), and from Guyana (Makushi Amerindians, bitter-cassava farmers). The specific questions addressed were: (1) What degree of genetic diversity was present locally, given local cultivation practices? (2) On a larger scale, what is the geographic structure within cassava's molecular diversity? (3) Is the ex situ collection representative of traditional landraces?

Materials and Methods

Materials

We analyzed 117 accessions of cultivated cassava, of which 23 were sweet and 94 bitter. Samples from Brazil (Table 1) were collected in 1992–1993 from traditional farming communities that practice slash-and-burn agriculture. The Amazonian accessions came from 11 communities, comprising either mixed-race riverside groups (*ribeirinhos*) or Tukâno Amerindians, living near three major rivers: Negro (State of Amazonas), Branco (Roraima), and Solimões (Amazonas). The accessions from the Brazilian southeastern coastlands came from two Caiçaras communities, at Cananéia and in the Ilha Comprida (State of São Paulo) (Fig. 1). ''Mantiqueira,'' a commercial variety that has been frequently used as reference in cassava genetic research, was included (synonyms for this variety are IAC 24–2, CMC 40, M Col 1468, and DG 137).

Samples from central Guyana (Table 1) were collected in April–May 1998 from among the Makushi Amerindians (Fig. 1). The Makushi belong to the Carib-speaking family, and are, traditionally, bitter-cassava farmers, who practice slash-and-burn agriculture (Elias, Rival, and McKey 2000). Samples were collected mainly from Rewa Village, a Makushi community of 30 households. Some accessions came from Toka, another Makushi village in the same region.

Only one individual per landrace was collected (except for a few landraces, when synonymous landraces were identified a posteriori). Indeed, in this study, we focused on intervarietal and intergeographic diversity, and neglected intravarietal diversity (although we were aware of it). For reasons of simplicity, from here on, each sample will be called a ''population,'' even though none represents a biological population.

The cultivated accessions of the ''core of the core'' are presented in Roa et al. (1997). Data on the cyanide content of these accessions were not available.

DNA Extraction

DNA of cultivated cassava was extracted from Brazilian accessions by drying leaves for 24 hours at 50°C, and then using the protocol based on CTAB extraction buffer (Dellaporta, Wood, and Hicks 1983); and from Guyanese accessions by drying leaves for 48 hours at 35°C, and using the protocol based on the MATAB buffer (Colombo et al. 1998).

Characterization, Using Microsatellites

Ten microsatellite loci, named GAGG5, GA12, GA13, GA16, GA21, GA126, GA131, GA134, GA136 and GA140, were used to characterize cassava accessions. The sequences of the primers are available in Chavarriaga-Aguirre et al. (1998). Multiplex PCR reactions and electrophoresis were performed with phosphoramidite-labeled primers, following the methods described by Chavarriaga-Aguirre et al. (1998) and Roa et al. (2000).

Statistical Analysis

Allelic frequencies, heterozygosity, Nei index of diversity (Nei 1978), genetic distances between populations, and F statistics (Weir and Cockerham 1984) for all populations were computed, using the program BIOSYS (Swofford, Selander, and Black 1997). An UPGMA dendrogram, representing the genetic distances between populations assessed by the pairwise Nei genetic distances, was constructed, using the program PHYLIP (Felsenstein 1993).

The principal coordinate analysis (PCO) was performed with NTSYS (Rohlf 1994). Graphics were edited with STATISTICA (1997).

Results

Allelic Diversity in Each Population and in the Whole Sample

The number of alleles and allelic frequencies for each population studied, as well as for the ''core of the core'' (adapted from Roa et al.

TABLE 1. ORIGIN, NAME, AND TOXICITY OF THE CASSAVA SAMPLES STUDIED FOR THEIR GENETIC DIVERSITY.

Brazilian population			Guyanese population		
Site and Code	Name	Toxicity*	Site and Code	Name	Toxicity*
Rio Negro			Makushi		
DG038	Maniva Inajá	B	E3-1	akuriu ye'	B
DG039	Macaxeira Branca	S	E4-1	amo'ko pîye'	B
DG040	Branquinha	B	E5-2	amuru pîye'	B
DG041	no identification	B	E6-1	anra pîye'	B
DG042	Mandioca São João	B	E7-1	fatpoi pîye'	B
DG043	Pretinha	B	E7-2	fatpoi pîye'	B
DG044	Mandioca do Antonio	B	E8-1	kraiwa pîye'	B
DG045	Macaxeira	S	E12-1	eti pîye'	B
DG046	Orelha de Burro	B	E12-2	eti pîye'	B
DG047	Mamaroca	B	E15-1	esekwipo ye'	B
DG048	Tartaruga	B	E17-1	sona pîye'	B
DG049	Amarela II	B	E14-2	eri pîye'	B
DG050	Olho Roxo	B	E18-1	eri pîye'	B
DG051	Pretinha	B	E18-3	eri pîye'	B
DG052	Seis Meses	B	E19-1	kasiri pîye'	B
DG054	Antinha	B	E21-1	kini' pîye'	B
DG055	Anará	B	E22-1	kuraatuma pîye'	B
DG056	Arapari	B	E23-1	kurarî pîye'	B
DG058	Amarela I	B	E24-1	kuraswa pîye'	B
DG059	Samuauma	B	E25-1	santra pîye'	B
DG060	no identification	B	E26-1	paranakîrî pîye'	B
DG061	Macaxeira	S	E27-1	rio pîye'	B
DG062	no identification	B	E28-1	maka pîye'	B
			E29-1	marasî pîye'	B
Rio Branco			E30-1	mauri pîye'	B
DG065	Anará	B	E31-1	mai pîye'	B
DG067	Roxinha	B	E32-2	meekoro pîye'	B
DG068	Macaxeira Pão	S	E35-2	papîro ye'	B
DG069	Camarão	B	E36-1	pakaima ye'	B
DG070	Tala Encarnada	B	E37-1	parakîrî pîye'	B
DG073	Socó	B	E38-1	pinkîu ye'	B
			E39-2	pîrîk̃wa pîye'	B
Rio Solimões			E40-2	paranakîrî pîye'	B
DG111	Geoató	B	E41-2	reni pîye'	B
DG112	Pretinha	B	E43-1	sapîrî pîye'	B
DG113	Antinha	B	E44-1	seruak pîye'	B
DG114	(from seed)	B	E45-2	siya pîye'	B
DG115	Maguari	B	E48-1	supra pîye'	B
DG116	Ourinho	B	E48-2	supra pîye'	B
DG118	Macaxeira	S	E16-2	tarekaya pîmoi pîye'	B
DG138	Antinha	B	E49-2	tarekaya pîmoi pîye'	B
DG117	Caneová	B	E52-1	usariu'ye'	B
DG119	Macaxeira	S	E53-2	u'wi pîye'	B
DG120	Antinha	B	E54-1	wo'ye'	B
DG121	Turuna	B	58-21	kana ye'	S
DG122	Marreca (seed)	B	58-24	kana ye'	S
DG123	Antinha × Marreca	B	58-25	kana ye'	S
DG124	Macaxeira	S	58-26	kana ye'	S
DG125	no identification	B	58-28	kana ye'	S

TABLE 1. CONTINUED.

Brazilian population			Guyanese population		
Site and Code	Name	Toxicity*	Site and Code	Name	Toxicity*
			58-29	kana ye'	S
São Paulo coast			E102-1	kunani pîye'	B
DG126	Aipim Roxo	S	E104-1	kanaima ye'	B
DG127	Aipim Roxo (seed)	S	E115-1	kompani pîye'	B
DG128	Manteiga (aipim)	S	E116-1	ko'ko pîye'	B
DG129	Aipim Mata Fome	S	E125-1	paapa ye'	B
DG130	Vassourinha	S	E201-1	wayar pîye'	B
DG131	Manteiguinha	S	E00-1	prona pîye'	B
DG132	Aipim Roxo	S	H1**	kitum pîye'	B
DG133	Manteiga	S	H1B**	kitum pîye' perurupe	B
DG134	Aipim Roxo	S	H3	kari'na pîye'	B
IAC commercial			S1	waakîri pîye'	B
variety			S2	paranakîrî pîye' perurupe	B
DG137	Mantiqueira	S			

* Toxicity = root toxicity where B = bitter; S = sweet.
** Individuals H1 and H1B were collected in Toka; all other individuals were collected in Rewa.

2000), are presented in Tables 2a–2j. Allelic frequencies in sets of sweet and bitter landraces are also shown.

In total, among seven of the ten loci studied, 15 alleles not present in the ''core of the core'' were detected in some accessions from the Brazilian populations (Rio Negro, Rio Branco, and Rio Solimões) or Guyanese Amazonia (Makushi). Nine of these alleles had been detected in accessions of wild *Manihot* species or subspecies (Table 3). Six alleles had never been detected before.

Fig. 1. Map of South America showing the different sites at which the samples analyzed in this study were collected. In Brazil: RB = Rio Branco; RN = Rio Negro; RS = Rio Solimões; SP = São Paulo coastlands. In Guyana: MK = Makushi.

Table 4 gives, for each population, for the sets of bitter and sweet landraces, and for the ''core of the core,'' the average number of alleles per locus, the proportion of heterozygous individuals, and the Nei index of diversity (Nei 1978). Individuals from the São Paulo coastlands, which were all sweet, were much more heterozygous (at 0.740) than individuals from any other population (average heterozygosity = 0.506), and from the ''core of the core'' (0.566). Accessions of sweet cassava are also more heterozygous than accessions of bitter cassava (0.678 versus 0.465).

DIFFERENTIATION BETWEEN POPULATIONS

Figure 2 shows representations in the principal coordinate analysis of all individuals. Axes 1 and 2 explain 8.96% and 6.50% of the total variance, respectively. Figure 2a shows the genetic differentiation between the five populations, whereas Fig. 2b shows the genetic differentiation between sets of sweet and bitter individuals. Both figures include the ''core of the core.''

TABLE 2. NUMBER OF ALLELES, SIZE IN BASE PAIRS, AND ALLELIC FREQUENCIES FOR EACH STUDY POPULATION, ALL SWEET INDIVIDUALS, ALL BITTER INDIVIDUALS, AND FOR A CORE COLLECTION (''CORE OF THE CORE''; ROA ET AL. N.D.), ACCORDING TO 10 MICROSATELLITE LOCI. ALLELES IN BOLD ARE THOSE THAT DO NOT EXIST IN THE ''CORE OF THE CORE.''

a. GAGG5

Population	Number of alleles	Allelic frequencies, size in base pairs		
		117	**119**	127
Rio Negro	3	0.609	0.217	0.174
Rio Branco	3	0.667	0.167	0.167
Rio Solimões	3	0.563	0.031	0.406
São Paulo coast	2	0.450	0	0.550
Makushi	3	0.460	0.194	0.347
Total sweet	3	0.370	0.130	0.500
Total bitter	3	0.548	0.165	0.287
Total	3	0.513	0.158	0.329
Core of the core	2	0.697	0	0.303

b. GA12

Population	Number of alleles	Allelic frequencies, size in base pairs			
		137	**143**	145	147
Rio Negro	3	0.652	0	0.174	0.174
Rio Branco	3	0.333	0	0.333	0.333
Rio Solimões	3	0.563	0	0.281	0.156
São Paulo coast	3	0.400	0	0.550	0.050
Makushi	4	0.177	0.008	0.637	0.177
Total sweet	3	0.478	0	0.326	0.196
Total bitter	4	0.319	0.005	0.511	0.165
Total	4	0.350	0.004	0.474	0.171
Core of the core	3	0.395	0	0.95	0.211

c. GA13

Population	Number of alleles	Allelic frequencies, size in base pairs	
		136	138
Rio Negro	2	0.022	0.978
Rio Branco	1	0	1
Rio Solimões	1	0	1
São Paulo coast	1	0	1
Makushi	1	0	1
Total sweet	1	0	1
Total bitter	2	0.005	0.995
Total	2	0.005	0.995
Core of the core	2	0.013	0.987

d. GA16

Population	Number of alleles	Allelic frequencies, size in base pairs				
		104	112	114	118	124
Rio Negro	2	0.870	0	0.130	0	0
Rio Branco	2	0.667	0	0.333	0	0
Rio Solimões	2	0.813	0	0.188	0	0
São Paulo coast	2	0.600	0	0.400	0	0
Makushi	2	0.786	0	0.214	0	0
Total sweet	2	0.618	0	0.382	0	0
Total bitter	2	0.814	0	0.186	0	0

TABLE 2. CONTINUED

d. GA16

Population	Number of alleles	Allelic frequencies, size in base pairs				
		104	112	114	118	124
Core of the core	5	0.618	0.026	0.303	0.039	0.013

e. GA21

Population	Number of alleles	Allelic frequencies, size in base pairs								
		106	**108**	109	110	112	114	116	118	**120**
Rio Negro	4	0	0	0.022	0.065	0.174	0.739	0	0	0
Rio Branco	4	0.250	0	0	0	0.250	0.333	0.167	0	0
Rio Solimões	2	0	0	0	0	0.344	0.656	0	0	0
São Paulo coast	3	0	0	0	0	0.400	0.150	0.450	0	0
Makushi	6	0	0.016	0	0.024	0.306	0.621	0.024	0	0.008
Total sweet	3	0	0	0	0	0.565	0.239	0.196	0	0
Total bitter	8	0.016	0.011	0.005	0.032	0.223	0.681	0.027	0	0.005
Total	8	0.013	0.009	0.004	0.026	0.291	0.594	0.060	0	0.004
Core of the core	6	0	0	0.039	0.039	0.171	0.539	0.079	0.132	0

f. GA126

Population	Number of alleles	Allelic frequencies, size in base pairs						
		178	182	184	188	190	212	219
Rio Negro	5	0	0.500	0.043	0.152	0	0.261	0.043
Rio Branco	4	0	0.750	0.083	0.083	0	0	0.083
Rio Solimões	6	0	0.594	0.031	0.125	0.031	0.031	0.188
São Paulo coast	5	0	0.250	0.300	0.150	0	0.200	0.100
Makushi	7	0.164	0.402	0.025	0.287	0.016	0.082	0.025
Total sweet	6	0.022	0.239	0.283	0.087	0	0.196	0.174
Total bitter	6	0.102	0.505	0	0.247	0.016	0.097	0.032
Total	7	0.086	0.453	0.056	0.216	0.013	0.116	0.600
Core of the core	7	0.026	0.434	0.053	0.092	0.039	0.197	0.158

g. GA131

Population	Number of alleles	Allelic frequencies, size in base pairs								
		92	96	**100**	102	104	106	**112**	114	116
Rio Negro	7	0	0.065	0.022	0.130	0.065	0.043	0	0.326	0.348
Rio Branco	5	0	0.333	0	0	0	0.167	0.083	0.167	0.250
Rio Solimões	5	0	0.313	0	0.125	0	0.125	0	0.188	0.250
São Paulo coast	3	0	0.300	0	0	0	0.300	0	0	0.400
Makushi	7	0.016	0.033	0.16	0	0.115	0	0.041	0.295	0.484
Total sweet	5	0	0.283	0	0	0	0.261	0.022	0.196	0.239
Total bitter	9	0.011	0.075	0.016	0.054	0.091	0.011	0.027	0.269	0.446
Total	9	0.009	0.116	0.013	0.043	0.073	0.060	0.026	0.254	0.405
Core of the core	6	0	0.237	0	0.039	0.026	0.105	0	0.316	0.276

h. GA134

Population	Number of alleles	Allelic frequencies, size in base pairs						
		307	309	317	**319**	326	**328**	**332**
Rio Negro	3	0	0.326	0.609	0	0.065	0	0
Rio Branco	2	0	0.250	0.750	0	0	0	0
Rio Solimões	2	0	0.031	0.969	0	0	0	0
São Paulo coast	2	0	0.250	0.750	0	0	0	0
Makushi	7	0.081	0.105	0.605	0.121	0.040	0.008	0.040
Total sweet	2	0	0.217	0.783	0	0	0	0
Total bitter	7	0.053	0.144	0.649	0.080	0.043	0.005	0.027

TABLE 2. CONTINUED

h. GA134

Population	Number of alleles	Allelic frequencies, size in base pairs						
		307	309	317	**319**	326	**328**	**332**
Total	7	0.043	0.158	0.675	0.064	0.034	0.004	0.021
Core of the core	3	0	0.211	0.750	0	0.039	0	0

i. GA136

Population	Number of alleles	Allelic frequencies, size in base pairs					
		144	150	**152**	154	156	158
Rio Negro	5	0.065	0.217	0	0.109	0.239	0.370
Rio Branco	3	0	0.250	0	0.250	0	0.500
Rio Solimões	5	0.063	0.188	0.031	0.281	0	0.438
São Paulo coast	3	0	0.550	0	0.100	0	0.350
Makushi	6	0.153	0.210	0.008	0.185	0.032	0.411
Total sweet	3	0	0.543	0	0.109	0	0.348
Total bitter	6	0.128	0.165	0.011	0.197	0.080	0.420
Total	6	0.103	0.239	0.009	0.179	0.064	0.406
Core of the core	4	0	0.263	0	0.237	0.039	0.461

j. GA140

Population	Number of alleles	Allelic frequencies, size in base pairs							
		148	154	156	158	162	**166**	168	172
Rio Negro	5	0.043	0	0	0.239	0.174	0.174	0	0.370
Rio Branco	5	0.167	0	0	0.250	0.250	0.083	0.250	0
Rio Solimões	5	0	0	0	0.063	0.406	0.094	0.188	0.250
São Paulo coast	4	0.250	0	0	0.150	0.500	0	0.100	0
Makushi	6	0	0	0.033	0.295	0.049	0.115	0.041	0.467
Total sweet	5	0.152	0	0	0.239	0.413	0	0.152	0.043
Total bitter	7	0.011	0	0.022	0.237	0.113	0.140	0.048	0.430
Total	7	0.039	0	0.017	0.237	0.172	0.112	0.069	0.353
Core of the core	7	0.118	0.026	0.276	0.066	0.250	0	0.224	0.039

TABLE 3. ALLELES DETECTED IN *MANIHOT* ACCESSIONS FROM THE BRAZILIAN AND GUYANESE AMAZONIAS THAT WERE NOT PRESENT IN THE "CORE OF THE CORE," AND THEIR PRESENCE (×) AMONG WILD *MANIHOT* SPECIES OR SUBSPECIES.

Manihot species or subspecies	Locus and allele														
	GAGG5	GA12	GA21			GA131			GA134				GA136		G140
	119	143	106	108	120	92	100	112	307	319	328	332	144	152	166
M. brachyloba	×		×	×		×									
M. carthaginensis	×	×	×	×		×									
M. esculenta ssp. *flabellifolia*	×	×	×	×		×	×	×						×	×
M. peruviana	×	×		×			×	×						×	×
M. tristis														×	
New allele[a]					×				×	×	×	×	×		

[a] Present in cassava but absent in all wild *Manihot* species tested.
Source: Roa et al. (2000).

TABLE 4. MEAN NUMBER OF ALLELES PER LOCUS, AND PERCENTAGES OF HETEROZYGOSITY AS ASSESSED BY DIRECT COUNT AND BY THE NEI UNBIASED ESTIMATE.

Cassava population	Mean number of alleles per locus	Heterozygosity (direct count)	Nei unbiased estimate
Rio Negro	3.9 ± 0.5	0.561 ± 0.087	0.527 ± 0.076
Rio Branco	3.2 ± 0.4	0.517 ± 0.076	0.579 ± 0.082
Rio Solimões	3.4 ± 0.5	0.488 ± 0.111	0.485 ± 0.088
São Paulo coast	2.8 ± 0.4	0.740 ± 0.099	0.543 ± 0.071
Makushi	4.9 ± 0.7	0.450 ± 0.071	0.568 ± 0.074
Total sweet	3.3 ± 0.5	0.678 ± 0.075	0.558 ± 0.075
Total bitter	5.4 ± 0.8	0.465 ± 0.072	0.541 ± 0.072
Total	5.5 ± 0.8	0.506 ± 0.077	0.564 ± 0.074
Core of the core	4.5 ± 0.6	0.566 ± 0.074	0.545 ± 0.071

Source: Nei (1978).

Overall, the populations were genetically differentiated from each other, but the differentiation was low (average value of $F_{ST} = 0.0744 \pm 0.0155$; Table 5), the São Paulo population being the most genetically differentiated (Fig. 3). Sweet and bitter landraces were genetically differentiated (average value of $F_{ST} = 0.1054$; Table 6). Moreover, allele 184 of locus GA126 existed only in sweet landraces (Table 2f). In our sample, a few alleles were represented only in bitter landraces, but the reason could be the small sample size for sweet landraces, particularly when most of these alleles were rare among bitter landraces (Tables 2a–2j).

DISCUSSION

Significant variability, of the same order of magnitude as in a representative sample of a core collection, was found in local populations of cassava. Traditional cultivation practices may account for this high diversity (Boster 1984b, 1985, 1986; Elias, Rival, and McKey 2000; Emperaire, Pinton, and Second 1998; Peroni 1998; Sambatti, Martins, and Ando 2001). Local farmers enjoy diversity, and eagerly acquire new landraces. They rarely discard unproductive landraces, retaining them, even if at low frequencies, arguing that they can become productive under different climatic conditions.

However, landraces can be lost because of environmental pressures, such as severe climatic conditions, or attacks by herbivores. Such loss of landraces is compensated by extensive exchange of planting materials between farmers and even between distant villages, which allow farmers to recover lost landraces. Sexually produced seedlings are also incorporated into the harvest and planting material, thus adding to the genetic variability of local cassava populations. Recombination occurs, presenting possibly new arrangements of alleles, and thus producing new plants. Hence, local cultivation practices not only ensure the maintenance of local genetic diversity, but they also generate new diversity.

In this study, accessions collected from local populations had alleles that had previously been detected only in wild forms of cassava, or in other wild species of *Manihot.* Three hypotheses may account for the presence of these alleles. Either the alleles were initially present in the pool of cultivated cassava, and the sample designed for the core collection simply missed them, as it missed the six other new alleles; or they were not present, but have been acquired via mutation. The third hypothesis is that they may have been acquired via natural introgression of wild forms or wild species of *Manihot.* Indeed, many species of *Manihot* are interfertile with cassava (Bueno 1985; Jennings 1963; Nassar 1980; Nassar, da Silva, and Viera 1986). Sympatry between such wild species and cultivated cassava may allow formation of hybrids that can also exchange genes with cassava.

Given the high frequency of incorporation of volunteer seedlings into propagation material by local farmers, incorporation of introgressed forms is possible, and made even more likely by the probability that hybridization with wild species may confer resistance to pests or drought, as shown by improved commercial landraces produced by controlled hybridization. In a thorough review, Jarvis and Hodkgin (1999) pointed

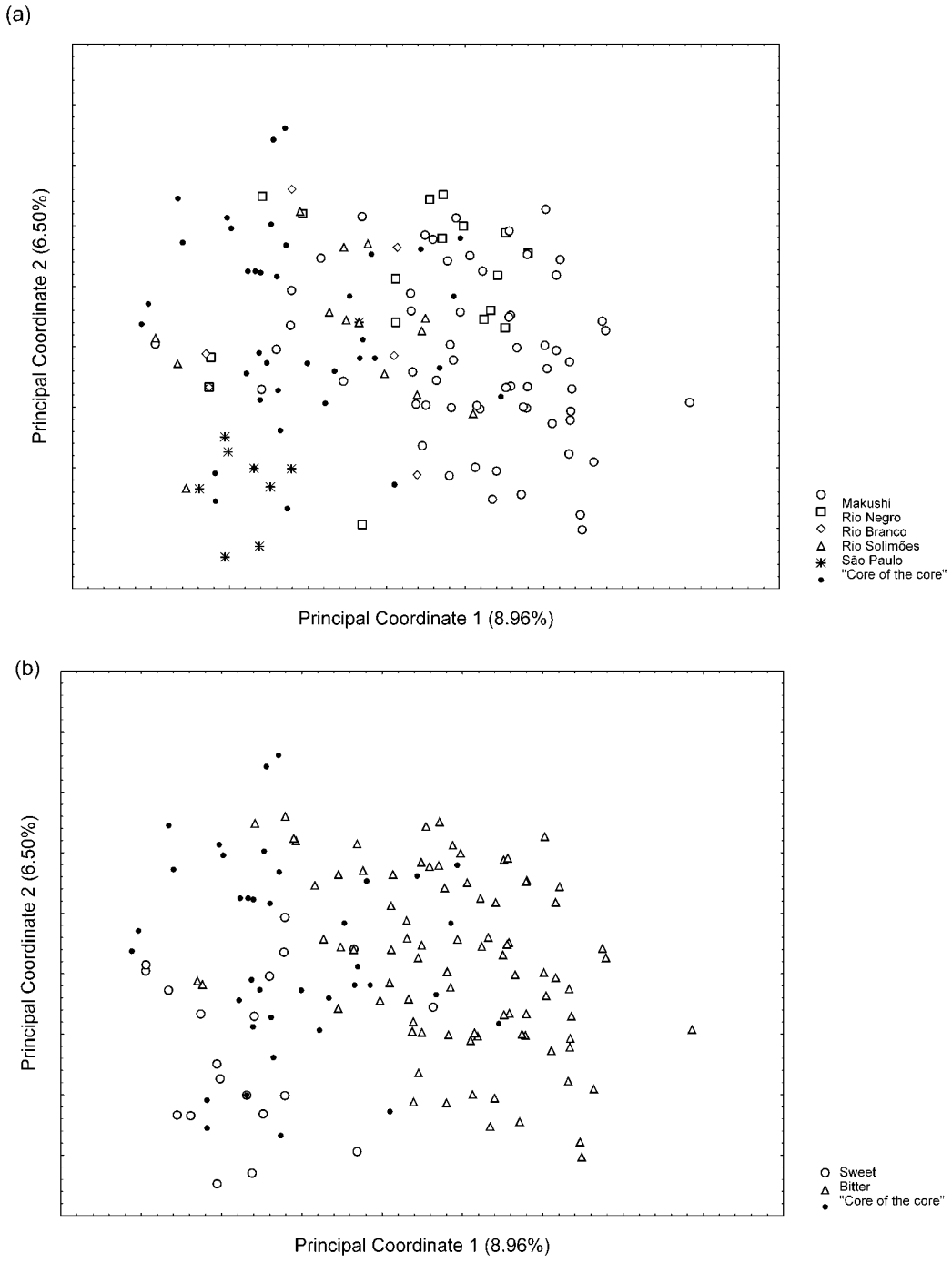

Fig. 2. A principal coordinate analysis was performed on all individuals of this study on cassava genetic diversity, including the ''core of the core.'' Principal components 1 and 2 in (a) the different populations and the ''core of the core,'' and (b) the sets of sweet cassava, bitter cassava, and the ''core of the core.''

TABLE 5. VALUES OF F_{IS}, F_{ST}, AND F_{IT} BETWEEN THE FIVE POPULATIONS OF CASSAVA LANDRACES ACCORDING TO MICROSATELLITE LOCUS.

Microsatellite locus	F_{IS}	F_{ST}	F_{IT}
GAGG5	0.1397	0.0263	0.1624
GA12	0.185	0.1618	0.3169
GA13	0.0008	−0.0012	−0.0004
GA16	−0.1308	0.0244	−0.1031
GA21	0.2321	0.0998	0.3088
GA126	−0.082	0.0627	−0.0141
GA131	0.0972	0.0659	0.1567
GA134	0.2883	0.0636	0.3336
GA136	−0.0916	0.0278	−0.0612
GA140	−0.0746	0.1118	0.0456
Mean	0.0559	0.0744	0.1262
SD (X)	0.0501	0.0155	0.0524

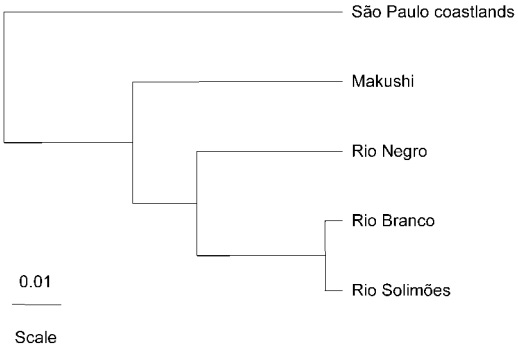

Fig. 3. A UPGMA dendrogram illustrates the genetic distances between populations of cassava landraces, as assessed by Nei (1978) genetic distances between each pair of populations.

out that interspecific hybridization of crop plants with their wild relatives is poorly documented, despite its importance for the dynamics of crop diversity. Cassava is a typical example for which data are needed.

Our results suggest that the core collection maintained at CIAT, represented here by the ''core of the core,'' does not give an accurate representation of diversity in cassava. While cassava was certainly domesticated in Amazonia (Olsen and Schaal 1999), traditional landraces are often neglected by cassava breeders, who are not aware of the diversity managed by Amerindians or other local people. Moreover, mainly sweet landraces are grown in nontraditional agrosystems because of commercial needs. Sampling efforts for the core collection have therefore been biased toward sweet landraces, leading to the underrepresentation of a large proportion of cassava diversity. Strategies to conserve crop plant diversity should therefore focus not only on static ex situ collections, but should also take into account evolutionary processes acting on the crop, such as those exerted by local farmers in a context of traditional agriculture (Brush 1995).

The five populations studied here were not strongly differentiated in geographical terms, probably because of extensive exchange of planting materials. However, a slight genetic differentiation was observed between sets of bitter and sweet landraces of cassava. In a phylogeographic study involving several *Manihot* species and cultivated cassava, Olsen and Schaal (1999) obtained evidence that cassava was domesticated either once or a limited number of times in a restricted area. This, together with the pattern of genetic diversity detected in this study, indeed supports the hypothesis of domestication of both forms of cassava from the same set of wild ancestors (however, see Perry [2002]).

An early differentiation between sweet and bitter forms may have followed, because of different uses and different constraints in different environments. Differentiation may have been facilitated by geographic separation between populations selected for toxicity and those selected for lack of toxicity. Indeed, McKey and Beckerman (1993) suggested that it may be advan-

TABLE 6. GENETIC DIFFERENTIATION BETWEEN SWEET AND BITTER LANDRACES OF CASSAVA ACCORDING TO MICROSATELLITE LOCUS.

Microsatellite locus	F_{IS}	F_{ST}	F_{IT}
GAGG5	0.1413	0.0477	0.1822
GA12	0.2695	0.0305	0.2918
GA13	0.0037	−0.0145	−0.0107
GA16	−0.1414	0.0919	−0.0365
GA21	0.2115	0.2420	0.4023
GA126	−0.0832	0.1239	0.0510
GA131	0.1100	0.0899	0.1901
GA134	0.3152	0.0162	0.3263
GA136	−0.1105	0.1027	0.0035
GA140	−0.0512	0.1569	0.1137
Mean	0.0700	0.1054	0.1680
SD (X)	0.0554	0.0225	0.0470

tageous in terms of production to grow bitter cassava in the poor soils of the Amazonian lowlands, whereas, in the rich Andean soils, there is no point growing bitter landraces that require time to be detoxified. Even when farmers grow both types of landraces, the bitter and sweet landraces are usually separated. In these groups, bitter cassava is usually grown in the fields as a main crop, and sweet cassava is planted near the house (Balée and Gély 1989; Elias, Rival, and McKey 2000; McKey and Beckerman 1993). Consequently, because of geographical separation at several levels, reinforced by disruptive selection, gene flow between sweet and bitter landraces may have been much less frequent than gene flow within each group of landraces, leading to the genetic differentiation observed.

In the present study, one allele was specific to sweet forms. Although the samples studied were not initially defined to study this question, one can wonder about the possible distinction of two subspecies of cultivated cassava, defined on the basis of the genetic specificities of sweet and bitter landraces. The genetic differentiation detected concerns presumably neutral markers, and is thus unlikely to be explained by any hypothesis of parallel or convergent evolution. More data, based on a much broader, more appropriate sample, are needed to test the speculative scenario proposed.

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