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Geographic differences in patterns of genetic differentiation among bitter and sweet manioc (*Manihot esculenta* subsp. *esculenta*; Euphorbiaceae)[1]

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- *Premise of the study:* Manioc (*Manihot esculenta* subsp. *esculenta*), one of the most important tropical food crops, is commonly divided according to cyanide content into two use-categories, "sweet" and "bitter." While bitter and sweet varieties are genetically differentiated at the local scale, whether this differentiation is consistent across continents is yet unknown.
- *Methods:* Using eight microsatellite loci, we genotyped 522 manioc samples (135 bitter and 387 sweet) from Ecuador, French Guiana, Cameroon, Gabon, Ghana, and Vanuatu. Genetic differentiation between use-categories was assessed using double principal coordinate analyses (DPCoA) with multivariate analysis of variance (MANOVA) and Jost's measure of estimated differentiation (D_{est}). Genetic structure was analyzed using Bayesian clustering analysis.
- *Key results:* Manioc neutral genetic diversity was high in all sampled regions. Sweet and bitter manioc landraces are differentiated in South America but not in Africa. Correspondingly, bitter and sweet manioc samples share a higher proportion of neutral alleles in Africa than in South America. We also found seven clones classified by some farmers as sweet and by others as bitter.
- *Conclusions:* Lack of differentiation in Africa is most likely due to postintroduction hybridization between bitter and sweet manioc. Inconsistent transfer from South America to Africa of ethnobotanical knowledge surrounding use-category management may contribute to increased hybridization in Africa. Investigating this issue requires more data on the variation in cyanogenesis in roots within and among manioc populations and how manioc diversity is managed on the farm.

Key words: cassava; crop management; crop migration; cyanogenesis; domestication; Euphorbiaceae; microsatellite loci; population structure; small-holder agriculture.

Manioc (*Manihot esculenta* subsp. *esculenta* Crantz), also known as cassava, mandioca, tapioca, and yuca, is one of the most important subsistence and economic crops of the tropics. Originally domesticated along the southwestern rim of Amazonia in South America (Allem, 1994; Olsen and Schaal, 1999, 2001), manioc is now grown throughout tropical regions. Manioc was first introduced into Africa from Brazil in the 16th century (Jones, 1959), with multiple subsequent introductions until the 1800s (Fregene et al., 2003). Its introduction into Oceania was much later; its presence in Vanuatu, for example, dates from around 1850 (Weightman, 1989; Sardos et al., 2008). Cultivated for its starchy roots, manioc is estimated to be the second most-harvested crop in least developed countries and the fourth most-harvested starch crop in the world (FAO STAT, 2010).

Manioc is also known for its extensive varietal diversity. Hershey (1994) estimated total manioc diversity to be over 7000 varieties, cultivars, and landraces. However, this figure should be considered an underestimate, since even though manioc is usually clonally propagated, plants are still capable of sexual reproduction, and the incorporation, conscious or accidental, of volunteer seedlings into clonally propagated stock is continuously generating new genotypes (Salick et al., 1997; Elias et al., 2000; Pujol et al., 2005, 2007). In this paper, we use the term landrace to refer to a set of clones identified by farmers under a single name. Landraces are separated into two primary categories based on traditional folk culinary use-categories: bitter (also called

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brava in Portuguese, amarga in Spanish) and sweet (aipim or macaxeira in Portuguese and dulce in Spanish).

Classification of manioc into use-categories is based on the taste of the uncooked roots, which depends largely on the levels of cyanogenic glucosides in the plant tissue (Dufour, 1988; McKey and Beckerman, 1993; Chiwona-Karltun et al., 2004). Tissue damage brings the cyanogenic glucosides into contact with the plant's endogenous glucosidases, releasing free hydrogen cyanide (HCN; de Bruijn, 1973; Hösel, 1981; Kakes, 1990). Generally, "bitter" manioc landraces produce over 100 mg/kg fresh weight (FW) free HCN when macerated (Dufour, 1988; McKey et al., 2010). Bitter manioc can be toxic to humans if chronically consumed without proper removal of the cyanide through a labor-intensive precooking process (Dufour, 1988; McKey and Beckerman, 1993). Some bitter manioc landraces can even cause acute toxicity, especially in children and populations with low dietary availability of sulfur-rich proteins (McKey et al., 2010). Landraces containing less than 100 mg/kg FW of cyanide are considered nontoxic and can be eaten without pretreatment (Lancaster et al., 1982; Dufour, 1988; Mowat, 1989).

However, evidence suggests that the bitter–sweet division is often a false dichotomy and that, in fact, HCN production in manioc can show continuous variation (Rogers, 1965). The primary method of landrace classification to a use-category is based on how the roots taste to the farmers, allowing for a large area of subjectivity in classification. Indeed, what tastes bitter to one farmer may not be bitter to another, based on cultural reasons (Chiwona-Karltun et al., 1998) and genetically based variation in taste perception (Soranzo et al., 2005). Because HCN content in manioc roots is highly influenced by environmental conditions (de Bruijn, 1973; Prinz, 1988; Bokanga et al., 1994), even the same genotype may taste bitter in some environments and sweet in others. Though the perception of bitterness by farmers affects a wide range of agricultural and culinary decisions, from where, when, and how to grow a landrace to how to prepare it for consumption, inconsistencies in use-category classification remain understudied.

Patterns of traditional manioc cultivation throughout South America have resulted in some degree of isolation between the two categories. Many indigenous groups in areas of the Andean foothills of western Amazonia, including Peru (Salick et al., 1997) and Ecuador (Hinostroza, 1991), exclusively cultivate sweet manioc, while in the lower Amazon Basin farmers often grow bitter and sweet manioc with a predilection for the former (Renvoize, 1972). Large-scale geographical segregation of sweet and bitter landraces can be reinforced at the local level by small-scale segregation. When both bitter and sweet manioc are cultivated, farmers in South America often deliberately grow sweet and bitter varieties in separate fields or in distinct monovarietal patches within the same field (Elias et al., 2004; A. Duputié, personal observation).

In contrast, bitter and sweet manioc landraces are usually mixed in Africa, although a wide variation has been observed in manioc cultivation in Africa, ranging from bitter and sweet manioc being grown in complete sympatry (Jones, 1959; Chiwona-Karltun et al., 1998; Mkumbira et al., 2003; Delêtre, 2010), to sweet manioc being grown in home gardens and bitter manioc in the fields, mirroring practices of Amerindian farmers (Cock, 1985; McKey et al., 2010). In some parts of eastern Africa, farmers grow exclusively sweet manioc (Jones, 1959). In areas of the South Pacific, in particular in Vanuatu, only sweet manioc is grown (Weightman, 1989). Such geographical variation in on-farm management of bitter and sweet manioc raises many questions regarding the management of manioc genetic diversity in the different systems. Studies have shown that bitter and sweet varieties are differentiated for neutral genetic markers at the village scale in Guyana (Elias et al., 2004) and in Malawi (Mkumbira et al., 2003). The same pattern holds across Brazil (Mühlen et al., 2000, 2010); however, whether this pattern is consistent across larger geographical scales is unknown.

This study is an initial comparative examination of the differentiation between sweet and bitter manioc in parts of the crop's native range and in four countries in two areas of introduction. Manioc samples were collected in two South American countries, Ecuador and French Guiana; three central and western African countries, Cameroon, Gabon, and Ghana; and the South Pacific nation Vanuatu. Specifically, we asked: (1) Are manioc collections genetically structured by use-category, geography, or neither? (2) Are there consistent patterns in genetic differentiation among bitter and sweet manioc across multiple, geographically distant collections?

MATERIALS AND METHODS

***Sampling*—**We analyzed 522 manioc samples (135 from "bitter" landraces and 387 "sweet") from six countries: Cameroon, Ecuador, French Guiana, Gabon, Ghana, and Vanuatu (Table 1; for more detailed sampling information, see Appendix S1 in Supplemental Data with the online version of this article). Although African manioc samples were collected from central and western Africa only, for brevity, we refer to these samples as from "Africa" throughout the manuscript. Manioc was sampled by different investigators. Although sampling strategies varied, samples were generally collected with the aim of obtaining a representative sample of the diversity present at the local scale as perceived and managed by farmers (e.g., Duputié et al., 2007, 2009; Delêtre, 2010). For a more comprehensive description of sampling strategies, see Appendix S2 in the online Supplemental Data. To detect the underlying pattern of manioc diversity resulting from original dispersal and traditional management, we specifically asked for local landraces in the attempt to avoid "improved" cultivars disseminated by breeding programs. Determination of use-categories relied upon farmer categorization. This method of classification reflects whether farmers detoxify roots prior to consuming them, but does not rely upon quantification of HCN production.

***DNA extraction and microsatellite genotyping*—**DNA was extracted using Qiagen (Venlo, Netherlands) DNeasy Plant 96-well extraction kits. Each sample was genotyped using eight microsatellite loci (GA12, GA21, GAGG5, GA126, GA127, and GA134 [Chavarriaga-Aguirre et al., 1998] and SSR55 and

TABLE 1. Manioc (*Manihot esculenta* subsp. *esculenta*) sampling by country and use-category showing number of samples analyzed and number of genotypes obtained. Note that the Bitter and Sweet columns do not sum to equal the values in the Total column because duplicated genotypes across countries and use-categories were counted independently for each locality and use-category.

		Bitter	Sweet
Country	No. samples	No. samples, No. genotypes	No. samples, No. genotypes
Cameroon[1]	44	19, 7	25, 9
Ecuador[2]	24	0	24, 17
Gabon[3]	147	73, 34	74, 28
Ghana[4]	12	2, 2	10, 6
French Guiana[5]	64	41, 27	23, 8
Vanuatu[6]	231	0	231, 58
Total	522, 188	135, 70	387, 125

Notes: Samples provided by [1]Doyle McKey, [2]Alexandra Narváez Trujillo, [3]Marc Delêtre, [4]Joseph A. Manu-Aduening, [5]Anne Duputié, [6]Caroline Roullier.

SSR68 [Mba et al., 2001]). All loci were amplified jointly using the Qiagen Multiplex PCR Kit following the manufacturer's recommendations, in a final volume of 10 μL with 1 μL of undiluted DNA extraction product. Amplification was conducted after an initial denaturation phase of 15 min at 95°C; with 30 cycles of 30 s denaturation at 94°C, 30 s annealing at 57°C, and 60 s elongation at 72°C; followed by a final elongation phase of 30 min at 60°C. Genotyping was performed on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). Alleles were scored using the program GENEMAPPER 3.0 (Applied Biosystems) and visually confirmed. Two negative control wells containing 1 μL of ddH_2O in lieu of DNA were included in each 96-well reaction plate, all of which returned negative. Typing error rate was assessed by genotyping 100 samples two independent times, resulting in no genotype discrepancies.

***Statistical analysis*—**Duplicate clones were removed such that there was only one clone of each multilocus genotype per use-category per village before statistical analyses were conducted. Indeed, the lack of random sampling prevents extrapolation of "clonal frequency" from our data set, which was aimed instead at collecting the most diverse sample, i.e., only one or a few individuals per landrace per field or village. Samples were categorized into five sample groups: French Guiana bitter, South America sweet, Africa bitter, Africa sweet, and Vanuatu (sweet). Genetic differentiation among sample groups was assessed in two ways: (1) Jost's measure of estimated differentiation (D_{est}, Jost, 2008) with the program SMOGD 1.2.5 (Crawford, 2010); (2) double principal coordinate analysis (DPCoA), with significance of differentiation assessed by multivariate analysis of variance (MANOVA), using the program R 2.13 (R Development Core Team, 2011).

Worldwide genetic structure of the sample was explored using the model-based Bayesian clustering analysis implemented in the program STRUCTURE 2.2 (Pritchard et al., 2000). The program was run five times using the admixture model and assuming correlated allelic frequencies with 110 000 Markov chain Monte Carlo iterations (the first 10 000 were discarded as burn-in and were always sufficient to achieve convergence) and values of the number of clusters (K) ranging from $K = 1$ to 8, with no prior information regarding the geographic origin or toxicity levels of the landraces. The most likely number of clusters was determined as the number that maximized the second-order rate of change in posterior likelihood of the data given the model (Evanno et al., 2005).

Allele counts (A), number of private alleles (PA), and observed and expected heterozygosity (H_o and H_e) were computed using GENEPOP 3.4 (Raymond & Rousset, 1995). Rarified allelic richness (AR) and deficit of heterozygotes (f; Weir and Cockerham, 1984) were calculated using the program FSTAT 2.9.3.2 (Goudet, 2002). The significance of differences in AR was assessed with one-sided Wilcoxon signed-rank tests on locus-specific allelic richness using R.

RESULTS

***Allelic comparisons of collections on the global level*—**We identified 188 unique clones of manioc across all collections

TABLE 2. Summary of allelic data for eight microsatellite loci in five groups of manioc (*Manihot esculenta* subsp. *esculenta*). Sample groups are Africa bitter (bitter manioc from central and western Africa), Africa sweet (sweet manioc from central and western Africa), French Guiana bitter (bitter manioc from French Guiana), South America sweet (sweet manioc from French Guiana and Ecuador), and Vanuatu (sweet) (manioc from Vanuatu, all sweet). Number of genotypes (N), number of alleles per locus (A), expected (H_e) and observed (H_o) heterozygosity, deficit of heterozygotes (f), number of private alleles (PA), and rarefied allelic richness (AR) are listed for each collection and locus.

Sample group, N	Statistic	GA12[1]	GA21[1]	GA57[1]	GA126[1]	GA127[1]	GAGG5[1]	SSR168[2]	SSR55[2]	Total
Africa bitter, 43	A	3	5	3	7	4	2	8	5	37
	PA	0	0	0	2	0	0	0	1	3
	AR	3	4.22	3	6.57	3.99	2	7.21	4.86	4.36
	H_e	0.64	0.57	0.47	0.8	0.62	0.5	0.8	0.69	0.64
	H_o	0.76	0.5	0.6	0.93	0.69	0.57	0.86	0.79	0.71
	f	–0.17	0.11	–0.24	–0.14	–0.1	–0.11	–0.12	–0.09	–0.11
Africa sweet, 43	A	3	3	3	6	5	2	7	6	35
	PA	0	0	0	0	0	0	0	0	0
	AR	3	2.93	3	5.99	4.57	2	6.39	5.87	4.22
	H_e	0.46	0.53	0.58	0.8	0.64	0.49	0.73	0.64	0.61
	H_o	0.58	0.53	0.73	1	0.71	0.58	0.8	0.69	0.7
	f	–0.22	0.02	–0.22	–0.24	–0.1	–0.12	–0.09	–0.13	–0.14
Africa, total	AR	3	4.24	3	6.62	4.62	2	7.48	5.94	4.61
French Guiana bitter, 27	A	3	3	3	5	5	2	7	5	33
	PA	0	0	1	1	1	0	0	1	4
	AR	3	2.93	3	5	4.99	2	6.93	5	4.11
	H_e	0.65	0.51	0.43	0.74	0.66	0.5	0.78	0.74	0.63
	H_o	0.68	0.54	0.36	0.75	0.64	0.32	0.71	0.82	0.6
	f	–0.12	–0.1	0.18	–0.01	–0.04	0.37	–0.14	0.16	0.02
South America sweet, 25	A	3	4	3	6	4	2	8	6	36
	PA	0	0	0	1	0	0	0	1	2
	AR	3	4	3	6	4	2	8	6	4.5
	H_e	0.48	0.49	0.54	0.75	0.65	0.49	0.82	0.78	0.63
	H_o	0.46	0.54	0.65	0.81	0.73	0.38	0.65	0.77	0.62
	f	0.22	0.03	–0.3	–0.04	–0.04	0.15	0.05	0.14	0.02
South America, total	AR	3	5	4	7	6	2	10	7	5.5
Vanuatu (sweet), 58	A	2	3	3	5	5	2	5	6	31
	PA	0	0	0	0	0	0	0	0	0
	AR	2	2.91	3	4.91	4.45	2	4.99	5.36	3.7
	H_e	0.39	0.52	0.63	0.77	0.67	0.47	0.73	0.71	0.61
	H_o	0.53	0.55	0.67	0.81	0.72	0.47	0.83	0.71	0.66
	f	–0.36	–0.06	–0.06	–0.05	–0.07	0.03	0.01	–0.12	–0.07

[1] Chavarriaga-Aguirre et al., 1998
[2] Mba et al., 2001

TABLE 3. Allele counts in sample groups of manioc (*Manihiot esculenta* subsp. *esculenta*) samples. Numbers for each category represent the number of alleles across all eight loci that are present in each category. Total category count is found in the last column. Total count of alleles for each sample group is found along the bottom row of the table. All alleles sum to 48 total alleles identified across eight loci. The category "sweet-type alleles" refers to alleles appearing in African and South American sweet manioc but only one, not both, bitter groups. "Bitter-type alleles" are defined as those found in both bitter groups but only one sweet group. Bitter manioc samples from French Guiana are abbreviated "F. G. Bitter"; South American sweet manioc samples are abbreviated "S. Am. sweet"; samples from central and western Africa are abbreviated "Afr. bitter" and "Afr. sweet" for bitter and sweet manioc, respectively; and samples from Vanuatu, consisting entirely of sweet manioc, are abbreviated "Vanuatu (sweet)."

Number of alleles	F.G. Bitter	S. Am. Sweet	W. Af. Bitter	W. Af. sweet	Vanuatu (sweet)	Count
Present in all groups			21			21
Private to one group	4	2	3	0		9
In all groups except Vanuatu			2		0	2
Private to S. America		2	0			2
Private to Africa		0		1	0	1
Private to one use-category	1	0	1	0		1
Sweet-type	1	9	8	9	8	9
Bitter-type	1	0		1	0	1
With heterogeneous patterns	2		0	1		2
Total	34	11	14	11	9	48

(Table 1). The majority of these clones (98.3%) were only represented in a single collection locality. However, one well-traveled sweet clone was found in every sweet collection except Ecuador. Remarkably, seven genotypes (six from Africa, one from French Guiana) were inconsistently classified by farmers, with some individuals of the genotype classified as bitter and some individuals classified as sweet. We refer to these clones as double-classified clones.

Sweet manioc landraces showed higher allelic richness in South America than in both areas of introduction (Table 2; one-sided Wilcoxon signed rank tests on locus-specific rarefied allelic richness, South America vs. Vanuatu, $V = 0$, $N = 8$ loci, $P = 0.01$ and South America vs. Africa, $V = 0$, $N = 8$ loci, $P = 0.02$). This was not the case for bitter manioc ($V = 4$, $N = 8$ loci, $P = 0.86$).

The eight SSR loci returned 48 total alleles (Table 3). Of these, 21 alleles were present in all groups (Table 3). The remaining 27 alleles were absent in at least one group; we refer to these as informative. Nine of them (33%) were private to one group. Strikingly, nine others (33%) were shared by South American sweet manioc and African bitter and sweet manioc, but were not detected in South American bitter manioc. Similarly, bitter and sweet manioc shared a much greater proportion of alleles in Africa (70.6% of the 17 informative alleles present in Africa) than they did in South America (21.7% of the 23 informative alleles present in South America). For detailed presence/absence information for each allele and each sample group, see Appendix S3 in the online Supplemental Data.

***Differentiation between manioc groups*—**Overall, pairwise differentiation between groups is low (Table 4). Sweet and bitter manioc showed much stronger differentiation in South America ($D_{est} = 0.106$) than in Africa ($D_{est} = 0.003$). This pattern is further supported by the double principal coordinate analyses (DPCoA; Figs. 1, 2), which showed that the French Guiana bitter landraces were significantly differentiated not only from the South American sweet landraces ($F_{2,50} = 37.715$, $P < 0.001$; Fig. 2A) but also from all landraces, sweet and bitter combined, from western and central Africa and Vanuatu ($F_{4, 192} = 53.386$, $P < 0.001$; Fig. 1). Contrastingly, in Africa no significant differentiation was observed among any of the sample sets ($F_{25, 400} = 1.531$, $P = 0.12$; Fig. 2B).

TABLE 4. D_{est} (Jost, 2008) between pairs of manioc (*Manihot esculenta* subsp. *esculenta*) groups; values greater than 0.05 are in boldface. Samples from central and western Africa are denoted as "Africa."

Africa	Cameroon sweet	Gabon bitter	Gabon sweet	Ghana bitter	Ghana sweet
Cameroon bitter	0.0002	<0.0001	0.005	0.0002	0.0002
Cameroon sweet	—	**0.0605**	0.0136	<0.0001	0.0152
Gabon bitter	—	—	<0.0001	0.0032	0.0132
Gabon sweet	—	—	—	0.0031	0.0003
Ghana bitter	—	—	—	—	0.0225

South America	French Guiana bitter	French Guiana sweet
Ecuador	**0.1893**	**0.0547**
French Guiana bitter	—	0.0037

Global	Africa sweet	French Guiana bitter	South America sweet	Vanuatu
Africa bitter	0.003	**0.0572**	0.0348	0.0372
Africa sweet	—	**0.1175**	0.046	0.0253
French Guiana bitter	—	—	**0.1057**	**0.1289**
South America sweet	—	—	—	0.0346

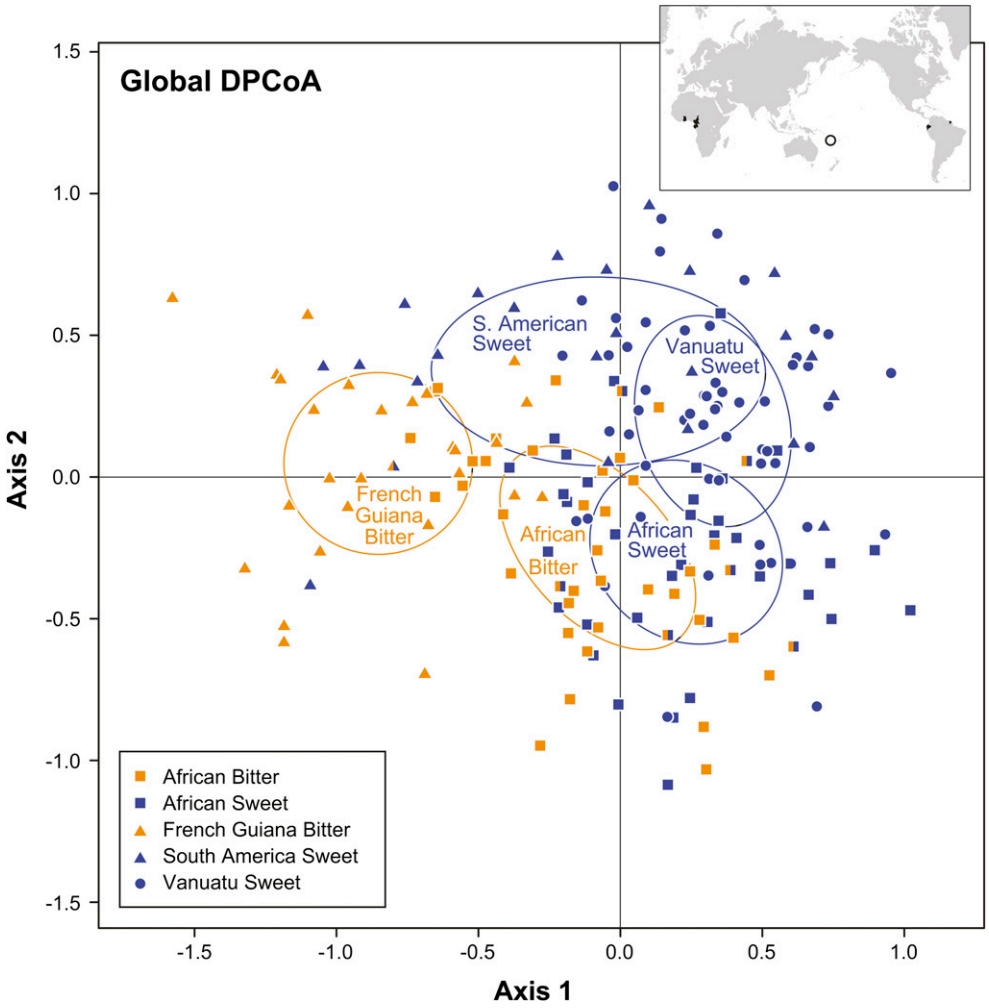

Fig. 1. Double principal coordinate analysis of genetic differentiation among all manioc (*Manihot esculenta* subsp. *esculenta*) samples. Groups are coded at the continental scale, bitter samples in yellow, sweet samples in blue. Double-classified clones are coded split yellow-blue. Axes were determined so as to best represent genetic distance between samples as a measure of number of allelic differences. Ellipses around manioc groups represent 66% of sample set diversity. Sweet and bitter samples from central and western Africa are denoted as African sweet and African bitter. Bitter samples from French Guiana are significantly differentiated from all other groups of samples (MANOVA: $F_{4,192} = 53.386$, $P = 2.2 \times 10^{-6}$).

These results are further consistent with genetic structuring inferred by STRUCTURE (Fig. 3). The most likely clustering was obtained for $K = 3$, with one cluster formed of French Guiana bitter landraces, and two clusters gathering all other landraces (i.e., African bitter plus all sweet landraces). Forcing STRUCTURE to form $K = 2$ clusters did not result in distinct global "bitter" and "sweet" clusters, but rather maintained French Guianan bitter landraces as distinct from all others, including bitter landraces from west and central Africa. Graphs of log posterior likelihood and delta K as a function of K are shown in online Appendix S4.

DISCUSSION

Do manioc samples exhibit differentiation based on use-category, geography, or both, and do we see consistent patterns across sampling locations?—Genetic differentiation between bitter and sweet manioc does not show consistent patterns across continents. In South America, sweet and bitter landraces are strongly differentiated, which is consistent with the previously observed patterns at the local and regional levels in Amazonia (Mühlen et al., 2000, 2010; Elias et al., 2004; Peroni et al., 2007). Although it could be argued that bitter–sweet differentiation might be a small-scale pattern nested within larger geographical differentiation, we postulate that hybridization between bitter and sweet manioc in South America is limited by farmers' distinct management of the two types separately in regions where both are grown and by the persistence of regions where only one of the two types is grown (e.g., sweet manioc in Peru and Ecuador).

In contrast, African manioc landraces showed no genetic differentiation based either on use-category or geography. STRUCTURE analyses (Fig. 3) and allele presence/absence tracking (Table 3) suggest that lack of differentiation in Africa is due primarily to allele sharing among African bitter manioc and global sweet manioc. Our results contrast to those of Mkumbira and colleagues (2003), who did find bitter–sweet differentiation

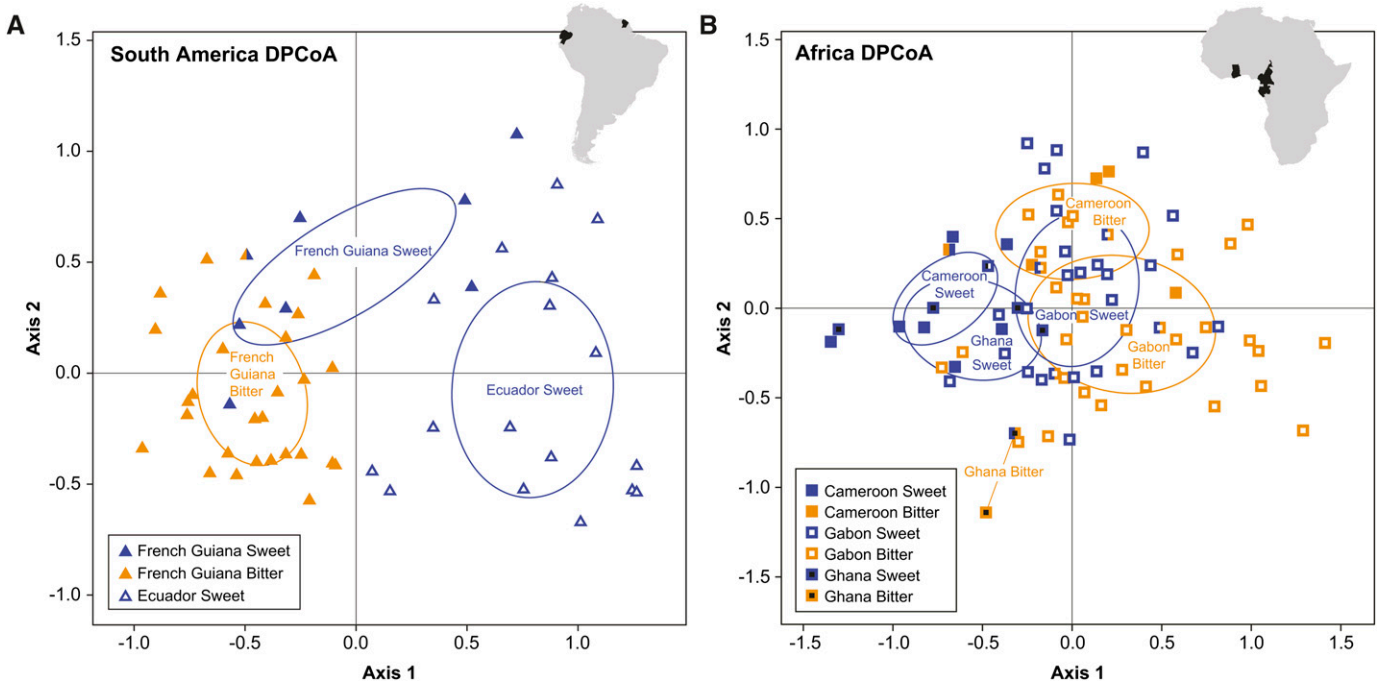

Fig. 2. Double principal coordinate analysis of genetic differentiation among bitter and sweet manioc (*Manihot esculenta* subsp. *esculenta*) in (A) South America, (B) central and western Africa (denoted as "Africa"). Groups are coded at the national scale, bitter samples in yellow, sweet samples in blue. Legend and axes as in Fig. 1. In South America, bitter samples from French Guiana are significantly differentiated from all sweet samples (MANOVA: $F_{2,50}$ =37.715, P = 0.0002). All samples from French Guiana are significantly differentiated from Ecuadorian samples (MANOVA: $F_{2,50}$ = 53.214, $P = 2.6 \times 10^{-5}$). In Africa, no significant differentiation is observed (MANOVA: $F_{25,400}$ = 1.531, P = 0.12).

among 10 landraces in Malawi. This contrast could be due to the differences in geographical origin of our samples because manioc was introduced independently in western and eastern Africa (Jones, 1959). Further sampling from other regions in Africa along these two main waves of introduction would help test this hypothesis. Many possible scenarios could have resulted in the observed lack of differentiation between sweet and bitter manioc in western and central Africa (e.g., loss via genetic drift in African bitter varieties of alleles characteristic of South American bitter manioc, secondary selection for bitter landraces from within an original sweet collection, with or without previous introgression of alleles influencing cyanogenic potential in sweet clones in Africa, or hybridization in Africa between sweet and bitter manioc). We begin our discussion with the third, most plausible, scenario of hybridization between use-categories in Africa. The improbability of the other two scenarios is discussed under the section "*Alternative hypotheses*."

***Manioc introduction into Africa and subsequent crop management*—**Manioc was first brought to Africa in the 1550s by Portuguese traders who valued manioc flour as a provision for slave ships (Jones, 1959; Ross, 1975; Carter et al., 1992; Hillocks et al., 2002). Manioc flour is almost always produced from bitter manioc, suggesting that the first manioc landraces introduced in western Africa would mostly have been Brazilian bitter landraces (Jones, 1957; Nweke, 1994; Chiwona-Karltun et al., 1998; H. Ceballos, CIAT, personal communication). Though our sampling did not include Brazilian manioc landraces, Elias and colleagues (2004) found little genetic differentiation between (primarily bitter) manioc from Brazil and from the Guianas. Furthermore, the lack of differentiation observed in our African data contrasts sharply with the results obtained by Mühlen and colleagues (2010), who demonstrated bitter–sweet differentiation among 263 bitter and 302 sweet landraces from throughout Brazil.

Though no precise date of introduction is known for sweet manioc, it is believed to have reached Africa much later than bitter manioc (Jones, 1959). Though it may have reached the coastal plains of Ghana in the 18th century (Manu-Aduening et al., 2005), it did not spread inland in Ghana until as late as the early 1980s (Korang-Amoako et al., 1987). Similarly, historical and genetic analyses of manioc in Gabon suggest a smaller and more recent introduction of sweet manioc (Delêtre, 2010).

The patchy and inconsistent pattern of manioc introduction to Africa is matched by a similarly inconsistent traditional knowledge associated with manioc cultivation and detoxification (McKey et al., 2010; Delêtre, 2010). The lack of consistent transfer of traditional knowledge, coupled with environmental and sociopolitical constraints to traditional lifestyles, has led to numerous outbreaks of cyanide-related diseases in Africa over the last three decades (Ministry of Health Mozambique, 1984; Cliff et al., 1997; Nhassico et al., 2008), whereas similar problems have not been reported in South America (for a comprehensive discussion, see: McKey et al., 2010).

Although in some parts of eastern Africa only sweet manioc is grown (Jones, 1959), both use-categories are cultivated in the western and central African countries we studied, with no consistent pattern of small-scale segregation (Carter et al., 1992; Delêtre, 2010). Communities relying upon manioc for subsistence generally prefer bitter manioc (Wilson and Dufour, 2002), though not exclusively (Chiwona-Karltun et al., 1998, 2004). In Gabon, Delêtre (2010) observed considerable variation in crop management, including bitter and sweet landraces planted together in the same field, in distinct monovarietal

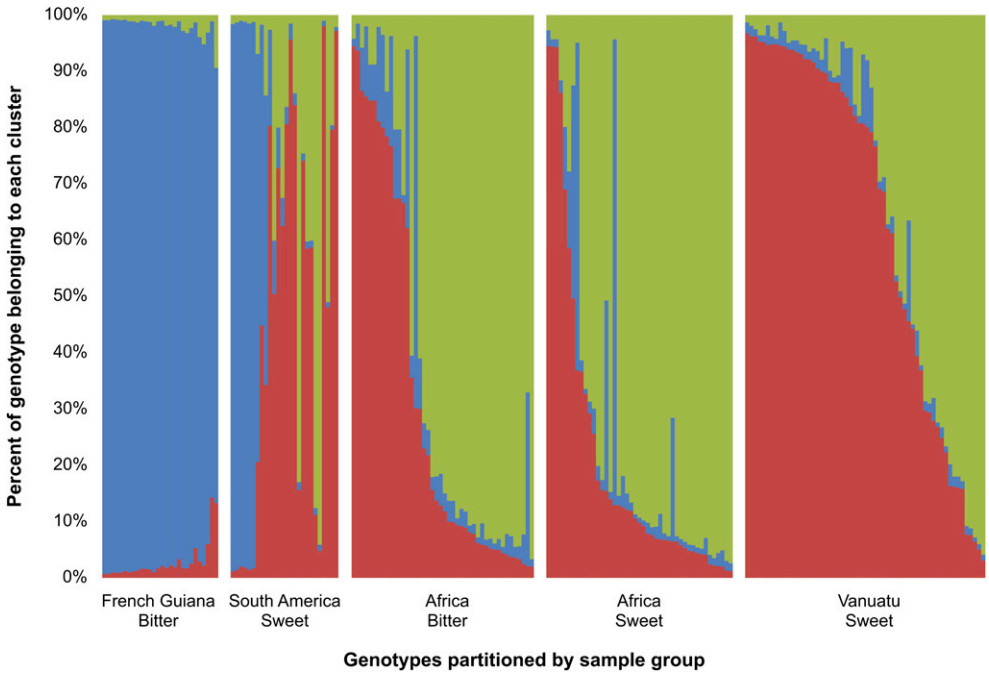

Fig. 3. STRUCTURE output of manioc (*Manihot esculenta* subsp. *esculenta*) sample groups sorted by use-category and geography for most likely number of clusters $K = 3$. Each genotype is represented by a vertical bar colored proportionately to amount of genotype belonging to each cluster. Genotypes are arranged by sample group. Samples from central and western Africa are denoted as "Africa." This clustering shows a fairly strong French Guiana bitter cluster (blue), with two admixed clusters grouping all sweet samples and bitter African samples (red and green).

stands, and separated, with bitter and sweet in distinct locations, similar to a pattern frequent in South America (McKey and Beckerman, 1993; Wilson, 2002). Mixed fields of bitter and sweet manioc were observed in Ghana, although 76% of farmers cultivated only one or two landraces, effectively creating patchworks of monovarietal stands (Manu-Aduening et al., 2005). In Cameroon, various planting strategies are employed that are largely driven by pest control (Poubom et al., 2005). Poubom and colleagues (2005) observed completely mixed farming stands (usually defended from predating monkeys by wind-powered noise-making contraptions), monovarietal stands of bitter manioc, and fields with sweet landraces surrounded by a wide swath of bitter landraces to provide protection for the sweet landraces from monkeys and other herbivores, a pattern that has also been reported in South America (McKey and Beckerman, 1993).

Admixture after introduction to Africa?—The farming practices documented on African manioc farms offer opportunities for substantial hybridization between sweet and bitter varieties that, when followed by incorporation, exchange, and diffusion of hybrid volunteer seedlings, could have obscured genetic differentiation between sweet and bitter varieties. However, gene mixing between bitter and sweet categories is seemingly asymmetrical, with greater flow from sweet to bitter manioc: while eight SSR alleles typical of sweet manioc (what we call "sweet-type" alleles) were found in bitter manioc from western and central Africa, only one neutral allele typical of bitter manioc ("bitter-type" allele) was present in sweet manioc from the same region (Table 3). Furthermore, Bayesian clustering analyses formed one cluster comprising French Guiana bitter landraces and two clusters grouping all other landraces, including African bitter and all sweet manioc (Fig. 3). These last two clusters were unrelated to either taste or geography and were present even in areas where bitter manioc is not grown, suggesting that the subdivision of sweet manioc into two distinct clusters is not due to hybridization between sweet and bitter landraces. Interestingly, Mühlen and colleagues (2010) identified two groups of sweet manioc in Brazil. In conjunction with our data, future research comparing this subdivision within sweet manioc in Africa and Brazil is certainly warranted. Additionally, the larger number of double-classified clones in Africa (six) compared with South America (one) suggests that there may be clones in Africa that have levels of cyanogenesis intermediate between bitter and sweet and thus may be considered alternately bitter or sweet depending on environmental influences and differences in individual taste. These clones may be the product of bitter–sweet crosses.

Although our sampling was not designed to assess the relative frequency of sexual reproduction in African and South American systems, our results suggest that there may be higher rates of cross mating between bitter and sweet manioc in Africa than in South America. Indeed, given the pattern of allele sharing in Africa and the impact that bitter–sweet mixing could have on reliable detoxification of manioc landraces, future research assessing frequency of bitter–sweet crosses in African farming systems could be critical to our understanding of when and how often farmers incorporate volunteer manioc seedlings into their stock of propagules, as well as how they classify these new clones as bitter or sweet.

Determinism of cyanogenesis in manioc—Evidence suggests, however, that toxicity in manioc roots is highly labile and ranges along a biochemical continuum (Rogers, 1965; de

Bruijn, 1973; Nye, 1991; Bokanga et al., 1994). Cyanogenesis in manioc seems to be primarily controlled by additive effects of a complex of recessive minor QTLs (Hahn et al., 1973; Mahungu, 1994; Sayre et al., 2011; Whankaew et al., 2011), with broad-sense heritability around 43% (Kizito et al., 2007), leading to cases where offspring of sweet-by-sweet crosses can produce sufficient HCN to be classified as bitter, or vice-versa (Valle et al., 2004; Kawuki et al., 2011).

Additionally, cyanogenic potential of a particular genotype is highly dependent upon environmental factors. Drought and elevated soil nitrogen concentrations (de Bruijn, 1973; Bokanga et al., 1994), as well as the plant's increasing age (Prinz, 1988), tend to increase the content of cyanogenic glucosides. Thus, the combination of inheritance mechanisms of cyanogenesis in manioc, environmental effects on HCN production, and the broad range of cyanogenesis levels encompassed by the category bitter, could easily allow for a scenario whereby most offspring of bitter-by-sweet crosses carry sufficient cyanogenesis-conferring alleles to be categorized by farmers as bitter regardless of their neutral genotype. This may explain why we did not find any sweet manioc showing "bitter-type" alleles: hybrids of parents from the two categories are more likely to be classified as bitter than as sweet.

***Alternative hypotheses*—**The weak founder effect indicated by our rarified allelic richness assessments makes genetic drift unlikely as a single explanation for the patterns we observed, though drift may have compounded the effect of admixture by causing the loss of bitter-type alleles in African bitter manioc. A scenario involving a secondary selection of bitter manioc from within a sweet collection (introgressed with additional cyanogenesis-conferring alleles or otherwise) is less parsimonious than the admixture scenario, given the widely accepted inference of the early presence of bitter manioc in Africa (Jones, 1957; Nweke, 1994; Chiwona-Karltun et al., 1998; H. Ceballos, CIAT, personal communication).

***Conclusions*—**The complicated history of manioc introduction into and dispersal within Africa, along with the dramatic shift in cultivation techniques from South American Amerindians to African smallholders, likely combined to create a situation favorable to hybridization between sweet and bitter manioc. Our data support this hypothesis, revealing significantly less differentiation between bitter and sweet manioc in our central and western African samples. Additionally, our results suggest that use-categories are less well defined in Africa, both genetically (in terms of differentiation) and ethnobotanically (classification by farmers). Our data align with public health data noting the persistence of cyanide-related diseases in Africa, which could partially result from incorrect classification of bitter manioc by farmers. Systematic ethnographic studies of manioc farming systems in Africa focusing on management of bitter and sweet manioc, processing techniques, and management of manioc volunteer seedlings, will be necessary to test whether variation in crop management strategies and use-category classification contributes to bitter–sweet hybridization in Africa. Our results also stress the need to quantify the natural distribution of cyanogenesis in manioc use-categories across native and introduced regions. These investigations would improve our understanding of manioc genetic diversity and the biological and cultural bases of manioc toxicity.

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