The unappreciated ecology of landrace populations: Conservation consequences of soil seed banks in Cassava

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ABSTRACT

Failure to take into account the ecological complexity of landrace populations of crop plants limits our ability to conserve their genetic resources in situ. Soil seed banks are a central feature of the ecology of landrace populations of cassava; their existence has consequences for conservation. Seedlings recruited from seed banks are incorporated by farmers into their stocks of clones of this vegetatively propagated crop, transforming pure clonality into a mixed clonal/sexual reproductive system. Soil seed banks, and farmers' responses to them, play an important role in maintaining diversity in populations of cassava landraces. In a study combining genetic and ethnobiological approaches, we showed the following: (i) Recruitment from soil seed banks increased diversity of populations at the local scale. At the level of a field, the presence of plants issued from seeds resulted in significantly greater diversity of genotypes and phenotypes than if only individuals planted by farmers had been present. (ii) Farmers' use of seed banks has enabled indirect 'exchange' of locally adapted cassava germplasm between cultural groups, without requiring that groups actually encountered one another and engaged in social exchange of cultivars. (iii) Farmers have responded to catastrophic crop failure by using seed banks to regenerate stocks of clones. This use of seed banks should enable cassava populations to respond to disasters by an increase of genetic diversity, rather than by a narrowing of the genetic base, often feared in such situations.

1. Introduction

Local varieties of crop plants maintained by traditional farming systems (i.e., landraces) are important genetic resources. Their diversity helps farmers in low-input systems maintain relative stability of yield despite their limited ability to control environmental variation. They are also a source of genes for scientific plant breeding. Combating genetic erosion in landrace populations depends on understanding how their dynamic management by farmers affects demographic and genetic processes (Brush et al., 1995). However, the connection between farmer practices and crop ecology and evolution is still largely a 'black box' (Pujol et al., 2005). A deeper understanding of the natural and cultural ecology of crop populations is required for sound strategies of conservation of genetic diversity in landrace populations.

Soil seed banks constitute a reservoir of diversity, conserving—sometimes for decades—genes and genotypes "lost" from the above-ground compartment of the population (e.g., Jalili et al., 2003; Koch et al., 2003; Satterthwaite et al., 2007). As in wild plant populations (Degreef et al., 2002; Adams et al., 2005; Whigham et al., 2006), the presence of soil seed banks sometimes plays an important role in the dynamics of the diversity of landraces. Cassava (*Manihot esculenta* Crantz) is one of the best studied cases (Elias and McKey, 2000; Pujol et al., 2002).

In populations of landraces, the conservation implications of soil seed banks depend on how seed bank dynamics interact with farmers' practices. These interactions are virtually unstudied. We examine here how soil seed banks, and farmers' practices related to them, combine to influence the dynamics of diversity in cassava. Cassava is one of the principal tropical food crops, and is considered among the highest-priority candidate species for complete sequencing of the genome (Raven et al., 2006). Based on the cassava case, we then explore the implications for conservation of cassava and other crop plants whose natural history shows parallel features.

Cassava is propagated clonally by means of stem cuttings ('stakes'), but the dynamics of its diversity nevertheless rely also on sexual reproduction. Over the past 10 years, sexual reproduction has been recognized to play a major role in the maintenance of high levels of diversity in cassava populations managed by Amerindians (Salick et al., 1997; Emperaire et al., 1998; Elias et al., 2000a; McKey et al., 2001; Pujol et al., 2005). Sex is also involved in the dynamics of diversity in other 'vegetatively' propagated crops (Johns and Keen, 1986; Shigeta, 1996). For these crops, as for cassava, how the use of soil seed banks by farmers affects the temporal dimension of diversity remains obscure.

Under the shifting cultivation regimes typically employed by subsistence farmers of cassava, cohorts of seedlings, referred to here as 'volunteer' plants, are recruited from seeds produced during a previous cycle of cultivation that lay dormant throughout the intervening fallow period. Volunteer plants are observed by farmers, who incorporate some of them into their stocks of clonal propagules. Recombinant genotypes are thereby continually injected into populations (Elias et al., 2001b; Sambatti et al., 2001; Pujol et al., 2005).

The storage of viable seeds of cassava in soil over long periods, up to several decades (Salick et al., 1997; Elias and McKey, 2000; Pujol et al., 2002), may have numerous implications for the dynamics of diversity in this plant. Our interviews and discussions with Amerindian cassava farmers, and our experiments and observations carried out in their fields, suggested three testable hypotheses.

1. The presence of soil seed banks leads to increased genetic diversity of populations at local spatial scales, e.g., within a field. Seed banks generally increase effective population size, reducing drift and hence genetic erosion (Vitalis et al., 2004) because their genetic composition often differs from that of plants on the surface (e.g., Koch et al., 2003). In crop plants, farmer practices influence the composition of seed banks. In cassava, a farmer may plant different landraces, each constituted of one to many clones (Elias et al., 2000a; Emperaire et al., 1998) in the field from one cycle to the next, or the field may have been previously planted by another farmer possessing a different stock of landraces. At harvest time, farmers may therefore assimilate into their current pool of clones genetically different plants grown from seeds and thereby durably increase genetic and phenotypic variability in their fields.

2. Seed bank dynamics enable indirect 'exchange' of cassava germplasm between cultural groups that may have never even encountered each other. Amazonian cassava farmers frequently exchange stem cuttings, but exchanges between groups may be constrained by the social relations between them. Exchange is difficult if groups never mingle, e.g., because of geographical separation, avoidance, or conflictive relationships between groups. Storage of seeds in soil seed banks might provide a mechanism of indirect exchange. Farmers that open fields in old fallows previously cultivated by another group should encounter volunteer cassava seedlings, and may decide to incorporate this novel germplasm into their own stock of landraces.

3. Farmers' use of seed banks may enable cassava populations to respond to catastrophic crop failure by a regeneration of genetic diversity. Natural and manmade catastrophes often result in harvest loss and the loss of germplasm for planting the next crop. Shortage of planting material after disasters could result not only in prolonged famine, but also lead to a narrowing of the crop's genetic base (Richards and Ruivenkamp, 1997; Sperling, 1997). At first glance, vegetatively propagated crops such as cassava would appear to be particularly vulnerable to such loss, because bulky vegetative propagules are difficult to store in large numbers, and are more susceptible to rotting and desiccation, than are seeds. However, cassava farmers might use their knowledge of seed bank dynamics to regenerate their stocks.

We combined population-genetic and ethno-ecological approaches to investigate these three hypotheses in two Amerindian groups settled in northern South America, the Carib-speaking Makushi (Guyana) and the Arawakan-speaking Palikur (French Guiana), both of whom follow a typical Amerindian model of swidden agriculture of bitter cassava. Makushi culture and folk knowledge have been described (Forte, 1999), and their perceptions and practices regarding cassava reported (Elias et al., 2000b). The nature of Makushi landraces—genetically heterogeneous but phenotypically coherent entities—has been experimentally established and observed in fields (Elias et al., 2001a,b; Pujol et al., 2002). Palikur ethnohistory has been documented (Passes, 2004, and references therein), and their farming systems have been studied (Ouhoud-Renoux, 2000). We draw here on these publications and previously unpublished data. Together, they have established the background for the classification of landraces and the assignment of volunteer plants to landraces, as well as documentation of local history concerning the origin of different landraces, the occurrence of disasters, and the consequences of disasters for the management of cassava.

2. Materials and methods

2.1. Study sites

Surveys of landraces, studies of phenotypic diversity, and non-destructive sampling for genetic analysis were conducted in Amerindian cassava fields. For the Palikur, fields surveyed and sampled were located in the village of St. Georges de l'Oyapock (03°54′N, 51°48′W) in northeastern French Guiana. Makushi fields were studied in the villages of Rewa (4°02′N, 58°35′W) and Massara (03°54′N, 59°18′W) in Guyana. Ethnobiological studies were conducted with the indigenous peoples at these sites and among farmers in a second Palikur village, Kamuyene, located near Macouria (05°01′N, 52°28′W) in French Guiana.

2.2. Hypothesis 1: Impact of soil seed banks on diversity at local spatial scales

2.2.1. Turnover in a farmer's stock of landraces over time
Composition of landraces in Palikur fields was quantified on the basis of multiple transects. Each transect was conducted following the standard Point-Centered Quarter (PCQ) method (Cottam and Curtis, 1956). For a sample of four farmers in St. Georges whose fields were censused in both 1998 (Ouhoud-Renoux, 2000) and in 2002 (F. Renoux, unpublished data), we quantified the extent to which a given farmer's collection of landraces changed over time.

2.2.2. Density of volunteer plants in fields established in fallows of different ages
In 29 Palikur fields in St. Georges and Kamuyene, we estimated densities of volunteer plants originated from seeds by counting volunteer plants in a series of transects (the number varying proportionally with the size of the field) of 5×50 m (250 m^2). Non-linear models describing the reduction of density of volunteer plants in fields over time (Proc REG and GLM of the SAS software, SAS, 1996) were fitted to test for effects of both the length of the fallow period since the previous cycle of cultivation of the field (young fallows had lasted 3–5 years, whereas old fallows had lasted more than 15 years) and the age of the field on the density of volunteer plants.

2.2.3. Contribution of volunteer plants to diversity of Palikur landraces within fields
In 2002, landraces in swidden fields of 10 farmers in Kamuyene were censused using the PCQ method (Cottam and Curtis, 1956). In these fields we quantitatively compared the contributions to landrace diversity of two cohorts of plants, (i) established clones, i.e., those originating from stakes planted by farmers, and (ii) volunteer plants present in the same swidden field. Volunteer plants were uniformly classified into landraces by one informant acknowledged by all village members to be expert in landrace identification.

2.2.4. Contribution of volunteer plants to phenotypic diversity
For two newly opened Palikur swiddens at St. Georges (hereafter called Fields 1 and 2, as in Pujol et al. (2005) and Pujol and McKey (2006)), we scored cassava plants for three phenotypic markers: the coloration of petioles and of the principal veins of the leaf (green, or with little, medium, or intense red coloration), and leaf shape (narrow and linear lobes with parallel margins; elliptic lobes of medium width; broad lobes; narrow and pennatisect lobes). These are among the phenotypic traits often used by farmers to distinguish landraces in the field.

We first compared combinations of the studied morphological characters (these combinations are hereafter called morphs) between all volunteer plants (N = 306 in Field 1 and N = 100 in Field 2) and a sample of established clones planted as stakes by farmers (30–33 individuals per landrace) in Fields 1 (five landraces which were also the five most frequent landraces in Palikur fields at St. Georges at that time; overall N = 155) and 2 (three landraces; overall N = 99). We used a Principal Component Analysis with the CORRESP procedure (SAS, 1996) to account for non-independence between morphological traits and generate independent synthetic variables representative of variability in morphs.

In this analysis, we also examined the contribution to diversity of the subset of volunteer plants that survived to the latest stage in the cultivation cycle (see Pujol and McKey, 2006). In Field 1, we sampled those plants that were harvested and assimilated by the farmer into existing landraces and included in the stock of clones for planting the next generation (N = 31 volunteer plants 23–24 months old; 10% of the original cohort). In Field 2, we sampled those plants still alive at the time of the last census of the field (N = 35 volunteer plants 15–18 months old), which was several months before harvest. In both fields, these plants had survived both human-caused mortality through manual weeding (Pujol et al., 2005) and many months of natural mortality (Pujol and McKey, 2006).

With multiple analysis of variance (MANOVA, SAS, 1996), we tested for differences in morphs (represented by the two principal components) between established clones and volunteer plants present in Fields 1 and 2 early in the cultivation cycle (soon after the fields were opened), and between established clones and those volunteer plants present during the last survey of each field. We also calculated Shannon's diversity index ($H' = -\sum \pi \log 2\pi$, Shannon, 1948) for phenotypic character states within Fields 1 and 2.

2.2.5. Contribution of volunteer plants to genetic diversity within fields
Among the Palikur, plants from Field 1 at St. Georges were characterized for six microsatellite loci, which had already been used to compare heterozygosity of established clones and volunteer plants in previous studies (Pujol et al., 2005; Pujol and McKey, 2006). To illustrate the distribution of genetic diversity in fields, we used principal component analysis (Guinand, 1996) applied to allelic data, as we did for morphological characteristics. Genetic differentiation between multilocus genotypes represented in established clones and volunteer plants was quantified by the Robertson–Hill corrected estimator RH' (Raufaste and Bonhomme, 2000). Significance was tested by a permutation test (50,000 permutations). We also calculated two pertinent estimators of within-landrace genetic diversity, their polyclonality (estimated by the number of different multilocus genotypes

divided by the total number of individuals) and the mean number of alleles per locus, and compared values of these two parameters for established clones, volunteer plants, and the overall pool after volunteer plants were assimilated into landraces. Genetix (Belkhir et al., 2004) was used to calculate all genetic characteristics except polyclonality.

2.3. Hypotheses 2 and 3: Indirect "exchange" of cassava germplasm between different cultural groups and recovery of germplasm from soil seed banks after crop loss

These hypotheses were examined using information obtained from observations, free discussions, and interviews of farmers conducted during field trips. Oral histories of different landraces of cassava were collected among both the Palikur and the Makushi, including landrace origin, landrace migration with human populations, and the role of cultivar exchange between different cultural groups.

3. Results

3.1. Hypothesis 1: Impact of soil seed banks on diversity at local spatial scales

3.1.1. Turnover in a farmer's stock of landraces over time
All four farmers from St. Georges whose fields were surveyed in 1998 (Ouhoud-Renoux, 2000) and in 2002 planted a different set of landraces, and in different proportions, in the two years (Table 1). In 2002, a total of seven different landraces planted in 1998 were dropped (one to five per farmer). Five of these landraces disappeared from the 2002 sample. Two farmers added landraces they had not planted in 1998; these included two already represented in at least one other sampled field and two others that were new. Landrace turnover also included some dramatic between-year shifts in the frequency of a landrace in a farmer's field. In the two extreme cases, the landrace 'inge' accounted for over 35 percent of all plants in the fourth farmer's field in 1998, but was absent from this person's field in 2002, while the landrace 'wauviye', absent from this farmer's field in 1998, accounted for over 45% of this individual's plants in 2002.

Table 2 – Effect of age of fields and length of the fallow period on the density of volunteer plants in non-linear quadratic and reciprocal models				
Model type	Effect	df	F	P
Quadratic	Age^2	1, 25	146.65	<0.0001
	Age	1, 25	186.95	<0.0001
	Fallow period	1, 25	3.65	0.0694
Reciprocal	1/Age	1, 26	178.87	<0.0001
	Fallow period	1, 26	3.35	0.0789

3.1.2. Seedling density in fields from fallows of different ages
In 29 Palikur swidden fields in St. Georges and Kamuyene, density of volunteer seedlings in newly opened fields was quite high (0.39 ± 0.10 plants m^{-2}) but decreased significantly with increasing age of the field (Table 2, Fig. 1). Length of the fallow period had no effect on density of volunteer plants. The decrease in density with age of fields showed the best fit ($R^2 = 0.92$) to a polynomial quadratic model ($D = 0.0077A^2 - 0.1537A + 0.6629$; D is density in plants per m^2
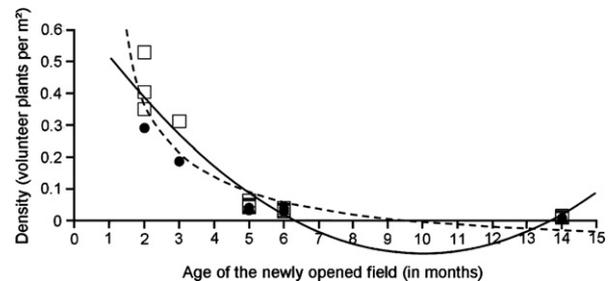

Fig. 1 – Decreasing density of volunteer plants with age in Palikur fields. White squares represent fields opened after a short fallow period whereas black discs represent fields opened after a long fallow period. The fitted non-linear models of density as a function of age of fields are represented by a plain (quadratic model) and a dashed line (reciprocal model).

Farmer	Year	Landraces[1]											
		B1	B3	B5	B9	B11	B12	B13	J1	J2	J4	J5	J6
C1	1998	47.7	0.5	–	4.2	–	–	21.8	11.9	7.3	–	–	6.7
	2002	54.2	5.4	–	–	7.2	7.2	–	18.7	–	–	–	7.2
C2	1998	76.8	–	–	–	–	–	0.8	13.8	0.8	–	5.1	2.8
	2002	63.4	–	–	–	–	–	–	–	–	–	1.8	34.8
C3	1998	59.8	–	–	–	–	–	5.9	31.4	–	–	–	2.9
	2002	62.7	–	–	–	–	–	–	18.8	–	–	–	18.5
C4	1998	61.2	0.8	35.4	0.4	–	–	–	–	0.8	1.5	–	–
	2002	41.6	–	–	–	–	–	–	45.3	–	–	–	13.1

Table 1 – Comparison of the varietal composition of cassava populations in fields planted by the same farmers in St. Georges in 1998 and in 2002

1 Abundance of a landrace in each field is expressed as the percentage of all stakes planted by the farmer in that field. Palikur names of these landraces are as follows: B1: 'sansan'; B3: 'paikwene'; B5: 'inge'; B9: name unknown; B11: 'kutakwa'; B12: 'sansos'; B13: 'noswa'; J1: 'wauviye'; J2: 'kauki'; J4: 'kalichia'; J5: 'kanapa'; J6: 'burink'.

and A is age of the field in months). However, the fit was almost as good to a model based on reciprocal transformation of data ($R^2 = 0.88$, $D = 0.9245/A - 0.0827$). The latter type of model is biologically more realistic, since it forbids seedling density to increase over time, effectively modelling the case here, where seedlings germinate more or less synchronously (Pujol et al., 2002) and then undergo mortality over time (Pujol et al., 2005; Pujol and McKey, 2006).

3.1.3. Contribution of volunteer plants to the diversity of landraces

Volunteer plants were found in all nine Palikur fields surveyed at Kamuyene (Table 3). Two to seven landraces per field (mean = 4.66) were represented by volunteer plants, which was comparable to the three to six landraces per field represented by established clones (mean = 4.33). In each field, one to five landraces were present both as established clones and volunteer plants (mean = 3.11). As a consequence, volunteer plants increased the total number of landraces present in eight of the nine fields (mean increase = 1.56 landraces/field, or 26.4%).

For all nine fields combined, nine landraces were represented by established clones and nine by volunteer plants. While seven landraces were represented in common by established clones and volunteers, each of these compartments included two landraces that were not represented in the other. In addition, one volunteer plant encountered was considered by our informant not to fit into any known named landrace category.

3.1.4. Phenotypic diversity added to the field by the presence of volunteer plants

At St. Georges, where volunteer plants were exhaustively surveyed and established clones sampled in Fields 1 and 2, morphs of volunteer plants only partially matched those of the cohort of established clones, as revealed by the principal components analysis (Fig. 2a and b). In Fields 1 and 2 at an early stage (2–3 months after the field was opened), volunteer plants included, respectively 22 and 21 different morphs. Respectively in Field 1 and Field 2, eight and 16 of these morphs were not observed in established clones, which shared 14 and five morphs with volunteers and included two and three

Table 3 – Contribution of volunteer plants to landrace richness in nine fields censused in Kamuyene, 2002

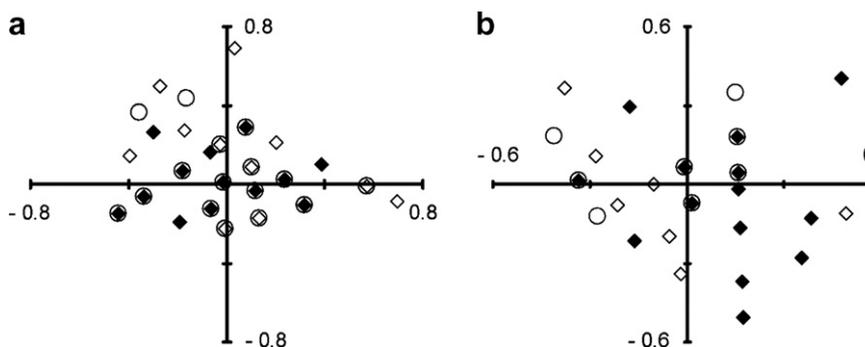| Farmer	Number of landraces represented by:				Percentage of landraces only represented by volunteer plants
	Established clones	Volunteer plants	In common	Total	
C5	3	4	2	5	40
C6	3	3	1	5	40
C7	5	6	5	6	16.7
C8	5	7	4	8	36.8
C9	3	5	3	5	40
C10	5	6	5	6	16.7
C11	4	4	3	5	20
C12	5	2	2	5	0
C13	6	5	3	8	25
Mean	4.33	4.66	3.11	5.88	26.4

Fig. 2 – (a, b) Distribution of cassava plants plotted along the first (X-axis) and second (Y-axis) principal components based on leaf and petiole colours and leaf shape (morph), in Field 1 (a) and in Field 2 (b). Each point represents one combination. Diamonds represent volunteer seedlings present just after the field was opened. Among them, filled diamonds represent volunteer plants that were incorporated in the stock of vegetative propagules in Field 1 (a) and volunteer plants present during the last survey before the harvest and preparation of clonal propagules in Field 2 (b). Circles represent established clones. A diamond included in a circle means that the corresponding morph was shared by established clones and volunteer plants.

morphs not represented in volunteers. At the latest stage studied in the cultivation cycle, volunteer plants in Field 1 comprised 13 different morphs, four of them not represented among established clones (Fig. 2a). In Field 2, nine of the original 16 morphs present in volunteers but not in established clones were still represented (Fig. 2b). MANOVA results (Table 4) showed highly significant differences, in both Fields 1 and 2, between morphs of volunteer plants and of established clones, both in young fields and mature fields. As a result of the increase in the number of morphs and their redistribution, diversity of morphs was increased by the presence of volunteer plants. For the overall cassava population (i.e., established clones and volunteers), diversity in young fields ($H' = 4.67$ and $H' = 5.17$ in Fields 1 and 2, respectively) or mature fields (Field 1 $H' = 4.75$; Field 2 $H' = 4.95$) was always higher than for established clones alone ($H' = 4.61$ and $H' = 4.04$ in Fields 1 and 2, respectively).

However, in contrast to the nine fields censused in Kamuyene, in neither of these two fields were any of the volunteer plants classified by the field owner as belonging to a landrace that was not already represented by established clones in the same field.

3.1.5. Contribution of volunteer plants to genetic diversity of landraces
In these same two Palikur swidden fields, the principal components analysis of allelic frequencies (Fig. 3), in which the first four axes accounted for 88% of the variation, revealed that several allelic combinations (multilocus genotypes) present in volunteer plants classified as belonging to a landrace were absent in established clones of the same landrace. Furthermore, permutation tests showed significant genetic differences between established clones and volunteer plants assigned to the same landrace (RH' = 0.17, P < 0.001).

Average polyclonality in landraces increased from 39% to 42% when stakes prepared from volunteer plants by the owner of Field 1 were assimilated into this farmer's set of clones (Table 5). However, not all landraces had increased polyclonality after assimilation. Mean number of alleles per locus increased in all four landraces in which volunteer plants were assimilated (average increase by 23.5 ± 15.4%, see Table 5 for details).

3.2. Hypothesis 2: Indirect "exchange" of germplasm between different cultural groups

The beginning of Palikur settlement near Macouria dates to the late 1960s, when a few individuals from the Rio Urucauá area (Amapá state, Brazil), after a brief sojourn in St. Georges, came to Macouria. Not until the early 1970s, however, did they begin to practice swidden agriculture on a substantial scale in the area, slashing and burning fields in areas of secondary and mature forest near the present village of Kamuyene. They planted stakes of landraces brought with them from St.

Table 4 – Differences in morphology (summarized by principal components) between volunteer plants and established clones in the two surveyed fields at St Georges tested by MANOVA

Field	Groups compared	N	Wilks' lambda	F	df	P
Field 1	VS/Ce	461	0.79	62.54	2	<0.0001
	VPI/Ce	186	0.84	17.82	2	<0.0001
Field 2	VS/Ce	199	0.84	18.70	2	<0.0001
	VPF/Ce	134	0.80	16.06	2	<0.0001

VS, volunteer seedlings present in the field just after it was open. VPI, volunteer plants assimilated into the stock of clonal propagules. VPF, volunteer plants present in Field 2 during the last survey before field harvest and preparation of clonal propagules. Ce, established clones.
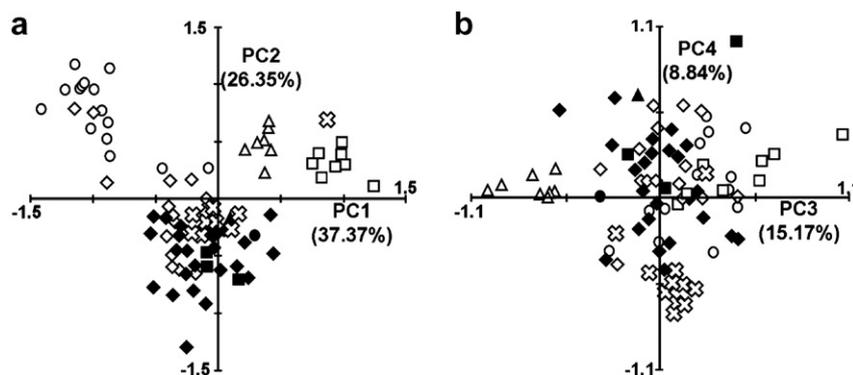

Fig. 3 – (a, b) Principal component analysis of genetic diversity at six microsatellite loci for established clones (empty symbols) and volunteer plants (filled symbols) for each of the five landraces present in Field 1. The first two dimensions of the PCA (first and second principal components) are represented in (a). (b) Shows the third and the fourth dimensions. The percentage of total genetic variability accounted for by every principal component is given under the identity of the principal component (between parentheses). Different symbols represent different landraces: circles, 'burink'; triangles, 'kutakwa'; crosses, 'noswa'; squares, 'sansan'; lozenges, 'wauviye'.

Table 5 – Contribution to clonal diversity of landraces of volunteer plants assimilated by the field owner into his stock of propagules, Field 1, St. Georges

	Before assimilation			After assimilation		
	N	Poly	MNA	N	Poly	MNA
'burink'	29	0.55	2.17	32	0.53	2.83
'kutakwa'	30	0.23	2.00	32	0.25	2.50
'noswa'	35	0.31	2.67	35	0.31	2.67
'sansan'	28	0.29	2.00	32	0.34	2.83
'wauviye'	31	0.55	3.33	80	0.51	4.00
Pool of landraces	153	0.39	3.83	211	0.42	4.17
Volunteer Plants	31	1	3.33	–	–	–

N, sample size; Poly, polyclonality (estimated by the number of different multilocus genotypes/N); MNA, mean number of alleles per locus.

Georges, such as 'sansan' and 'burink'. The secondary forest was in areas most recently exploited by Creole farmers, who had grown for subsistence both bitter and sweet cassava, along with bananas and plantains.

Numerous farmers reported to us that after they first burned plots in this area of secondary forest, in the late 1960s, scattered volunteer seedlings appeared. Following the usual practice, these seedlings were allowed to grow and some of them were clonally multiplied. They were one of the sources, along with direct exchange, of three phenotypically distinct landraces that were new to the Palikur and were thus given new names as landraces. Two of these new landraces—'seinegakat' ('white stem') and 'priyegakat' ('black stem')—were assigned to the category of landraces having roots with white flesh ('seinevui'); the third, 'kotmaye' (etymology unclear), was placed in the group of landraces with yellow-fleshed roots ('wauviyevui'). Palikur farmers stated that these volunteer plants originated from cassava planted by the previous Creole occupants and that these landraces were particularly well suited to the poor, sandy soils frequent along the coast. Thus, the incorporation of volunteer plants enriched the initial stock of landraces and genotypes brought by new migrants to this area. Because they perform well under local conditions, these landraces are now among the most frequent in the village's farms. Thus, while these farmers and some of their landraces migrated over large areas, the existence of the seed bank enabled the newly arrived Palikur to acquire local landraces, independently of the existence and nature of social relations with other occupants, contemporary or past, of the area into which they moved.

When Makushi farmers choose to prepare fields in sites previously farmed by someone else, they often experiment with volunteer seedlings recruited from the seed bank, allowing them to grow, observing their performance, preparing stakes from the most vigorous ones, and thereby augmenting their personal stock of landraces. One Makushi farmer from Rewa opened a farm in an area three hours by canoe from the village, in a location that, according to oral tradition, was last occupied by a group of Carib-speakers 50 years previously. He found a volunteer seedling in the new farm, and decided to multiply this novel morphotype, naming it 'kari'na pîye' ('Carib stick' in English) (see Elias et al., 2000b).

3.3. Hypothesis 3: Recovery of cassava germplasm from soil seed banks after crop loss

Amerindian cassava farmers who have lost their stock of landraces due to natural (e.g., drought) or man-made disasters (e.g., war) recover them by clearing and burning old fallows, then massively incorporating volunteer plants. Oral history recorded both among the Palikur and the Makushi shows that they have responded to catastrophic loss of cassava crops by exploiting their knowledge of cassava seed bank dynamics, slashing and burning fallows, then encouraging the growth of volunteer plants and, upon harvest (root yield of volunteers is lower than that from stakes, but not negligible), using their stems to prepare stakes, thereby regenerating their stock of clones.

Several Palikur interviewed at Kamuyene stated that many Palikur left their settlements in Amapá state to take refuge in French Guiana following the outbreak of the First World War, pre-emptively placing themselves under the protection of their French allies. Returning to their homeland after the war, they recovered many of their cassava landraces by clearing old fallows and multiplying plants issued from volunteer seedlings. It seems certain that the genetic base of locally adapted germplasm would have been much narrower following this upheaval had it not been possible to regenerate germplasm from soil seed banks. Similar rounds of Palikur migration between Amapá and French Guiana appear to have occurred repeatedly, as a result of disease epidemics and warfare (Passes, 2004).

Makushi farmers have also had to respond to catastrophes. When a severe El Niño-induced drought destroyed cassava crops in the North Rupununi area of Guyana in the 1950s, leading to the virtually complete loss of stakes for planting, Makushi farmers slashed and burned old fallows, recovering local varieties by multiplying plants issued from volunteer seedlings (Rival, 1998). Drought is common and constitutes a much more serious environmental disaster than flooding, for during droughts Makushi farmers regularly lose everything, stakes as well as the harvest of tuberous roots. Drought creates real shortages. Accidental fire is also recurrent, but is a less general catastrophe, affecting only few farmers simultaneously. After drought or fire, farmers recover most of the stock of landraces they lost through the soil seed bank.

4. Discussion

Our findings confirm the importance of soil seed banks in the maintenance of phenotypic and genotypic diversity in local populations of cassava. Because different farmers have different stocks of clonal propagules, and because there is a turnover of landraces and genotypes in an individual farmer's stock over time, the landraces and genotypes planted in a field are often different from those that were planted in the same field in the previous cycle of cultivation. Soil seed banks constitute a "genetic memory", combining the genetic diversity of successive generations. Soil seed banks also permit the indirect exchange of locally adapted germplasm between successive occupants of an area, even if they belong to different cultural groups between which no direct contact has occurred. Finally, soil seed banks enable farmers rapidly to regenerate genetic diversity "lost" after natural or man-made catastrophes. Taken together, these are dramatic effects with important implications for conservation strategies, not only for cassava, but also for other crop plants in which seed banks play a role in population dynamics.

4.1. Impact of soil seed banks on local diversity

This study is the first to quantify the consequences of volunteer plants for diversity at the level of the field: their presence increases phenotypic and genetic diversity of the local population, and their incorporation and clonal propagation by farmers increases the variability, at both these levels, of the landraces into which farmers assimilate them. Clonal propagation involves the use of sub-samples of the diversity present in one generation to start the next one. This practice is expected to steadily diminish diversity. However, exceptionally high phenotypic and genotypic diversity has been documented in landraces of cassava in many traditional settings, such as Amerindian Amazonia (Boster, 1984; Salick et al., 1997; Emperaire et al., 1998; Elias et al., 2000a). Our results reported here for the Palikur mirror and extend those published earlier for the Makushi, in whose swiddens volunteer plants are also abundant (density: 0.13 ± 0.15 seedlings m^{-2}, on average 86.6 seedlings per field Elias and McKey, 2000) and play an important role for the dynamics of diversity in cassava. Our findings show that soil seed banks regularly contribute new recombinant genotypes from various origins that are incorporated by farmers into their stocks of clonal propagules. The multiplication of volunteer plants introduces new genotypes into landraces, increasing their allelic and morphologic diversity. This use of soil seed banks therefore acts against the fixation of a few genotypes and explains why landraces remain polyclonal (Elias et al., 2000a, 2001b; Pujol et al., 2005).

Such increases in diversity have two causes. Firstly, whereas stocks of clonal propagules may be dominated by a few genotypes, each member of a cohort of volunteer plants is genetically unique. Secondly, the plants grown in the current cycle of cultivation may be different in their genetic composition from those planted in the previous cycle (the parents of volunteer plants). This is strongly suggested by the presence in Palikur fields of landraces represented only by volunteer plants, and not among the field's cohort of established clones. We have documented two reasons for such differences: (i) the

same field is sometimes cultivated in successive cycles by different farmers with different stocks of landraces; and (ii) individual farmers modify their stocks of landraces over time. Turnover in Palikur farmers' stocks of landraces is rapid; we documented cases of dramatic change in landrace composition over four years. Rapid turnover in stocks of landraces has also been documented among the Yanesha, sweet cassava farmers in Peru (Hamlin and Salick, 2003). Over 15 years, Yanesha farmers replaced most of the landraces they were cultivating. They also modified their cultivation practices, favoring a lower diversity of more productive landraces in fields. The presence of soil seed banks means that such decreases in diversity are not necessarily irreversible.

Cassava seeds can remain viable in soil over a surprisingly long period of time. Anecdotal information from the Makushi suggests periods of up to 50 years (see also the "gift of the ancestors" in Salick et al., 1997; Elias and McKey, 2000). The absence of an effect of duration of fallow on the density of volunteer seedlings in Palikur fields also suggests that seeds survive long periods in the soil. As many seedlings appeared in fields that had lain fallow for more than 15 years as in fields that had lain fallow for only 3–5 years. The time span of the genetic memory conferred by the soil seed bank is thus in the range of several decades.

4.2. Indirect "exchange" of germplasm between different cultural groups

Beyond these effects at the local scale, the assimilation of volunteer plants opens the door to indirect "exchange" of cassava germplasm among cultural groups via soil seed banks. We have documented here for two groups how soil seed banks are consciously exploited by new migrants to an area to regenerate a part of the pool of locally adapted landraces grown by its previous occupants. Considering the long and active history of human migrations in Amazonia (Denevan, 1976; Hill and Santos-Granero, 2002, and references therein), such indirect exchange of cassava germplasm could continue over very long periods. At Kamuyene, clay bricks, porcelain fragments, and other artefacts abundant in Palikur cassava fields and fallows provide evidence of occupation during the colonial period. Historical records (essentially maps) show that this area has been continuously occupied by two groups, Europeans and their African slaves (later Creoles), since 1750. Throughout this period, cassava has been a major subsistence crop, and indirect exchange mediated by soil seed banks, as documented here, could have been continuous throughout this period. In consequence, the structure of diversity in cassava is expected to reflect such indirect exchanges, with geographical differentiation being perhaps more marked than is differentiation along cultural/linguistic boundaries, such as has been observed in maize (*Zea mays*, Perales et al., 2005).

4.3. Seed banks and the genetic response of cassava populations to catastrophic crop failure

Local seed systems often have greater resilience in the face of disaster than is usually thought, with loss of genetic diversity often being less than expected (ODI, 1996; Hodgkin and Anishetty, 1998; Longley and Richards, 1999; De Barbentane

Nagoda and Fowler, 2003). The causes of this resilience are probably various, ranging from secret seed stocks and other consciously adaptive strategies to changes in evolutionary processes under conditions produced by crises (Longley and Richards, 1999), but few cases have been explored in detail. Our study illustrates a potentially widespread mechanism leading to such resilience in cassava: recourse to soil seed banks as a source of new planting material leads to a large increase in the role of sexual reproduction relative to clonal propagation, with long-lasting effects on genotypic diversity of local populations. In the aftermath of regeneration, all plants will be of different recombinant genotypes. Instantaneously, all varieties will become strongly polyclonal. It will take time for each polyclonal variety to lose genotypes through drift. Peruvian Amerindian potato farmers also respond to loss of planting material by greater dependence on plants grown from seeds (Johns and Keen, 1986). Crop failure might thus lead to a post-catastrophe increase in genetic diversity, offering the opportunity to select a new pool of preferred phenotypes. Cassava thus joins the "...handful of cases to suggest that the changes brought about by a crisis may ... promote local diversity" rather than decreasing it (Longley and Richards, 1999).

Warfare and other upheavals, common throughout Amazonian history (Balée, 1984; Fausto, 2001, and references therein) have probably often led to temporary abandonment of a group's territory, suggesting that recovery of genetic diversity by exploiting soil seed banks has been an important phenomenon in the functioning of cassava populations.

The resilience conferred to local seed systems by such mechanisms reinforces the need for precaution in responses to crop failures, to avoid actions that may do more harm than good (De Barbentane Nagoda and Fowler, 2003). Failure to recognize such mechanisms of recovery may lead well-meaning institutions to make questionable decisions in advising and helping farmers.

An example from Africa suggests another phenomenon thus far undocumented, to our knowledge, in Amazonia. Faced with catastrophically high incidence of viral disease in their cassava fields, and having observed that volunteer seedlings were usually disease-free, farmers in Kenya abandoned their fields, then slashed and burned fallows to favor the growth of volunteer seedlings, later harvesting their roots and using their stems to provide a new stock of cassava stakes (Jennings, 1963). Incorporation of volunteer seedlings could reduce disease load by two mechanisms. First, systemic viral pathogens of cassava are not transmitted to seeds (Lozano, 1989). Secondly, if viral pathogens are adapted to frequent host genotypes, volunteer plants may enjoy rare-genotype advantages.

We must consider that farmers are not recovering sensus stricto their stock of clones by means of the seed bank, they are regenerating it. A parallel can be drawn with the regeneration of 'ex situ' collections in laboratory seed banks. The aim of 'ex situ' collections is to regenerate old material, some of which may no longer be locally adapted. The parallel with soil seed banks in cassava is that in both cases, genotypic diversity in the population should be higher after regeneration of the stock of varieties than before, allowing people to choose among a wide diversity of phenotypes the ones they will further propagate in fields (or conserve in the ex situ collection). However, the time spent to fit a new pool of adapted clones may also constrain recovery from a catastrophe.

5. Conclusion

The temporal dynamics of soil seed banks are integrated into cassava cultivation by Amerindian farmers of northern Amazonia, creating strategies well adapted to managing diversity over time. The storage of dormant seeds in soil seed banks conserves cassava diversity at both long and short time scales. Not only does the seed bank facilitate the regeneration of existing diversity, it also has the potential of adding new clonable genotypes adapted to current local conditions. Use of seed banks is thus an important component of the dynamic in situ conservation practiced by farmers. Reports of the incorporation of volunteer plants by African cassava farmers (e.g., Jennings, 1963; e.g., de Waal et al., 1997; Chiwona-Karltun et al., 1998) suggest that use of seed banks is very widespread. Agencies charged with assuring sustainable agriculture of cassava and other vegetatively propagated crops should be more aware of such interactions between the dynamics of unmanaged sexual reproduction, farmers' knowledge about such facets of their crops' biology, and local practices of biodiversity management driven by this knowledge. Taking such interactions into account would result in more effective conservation policies.

Cassava and wild relatives with which it is interfertile share the same underlying sexual reproductive ecology and hybridize in nature (A. Duputié, unpublished data). The presence in both wild and domesticated compartments of soil seed banks (Pujol et al., 2002) can increase the probability of encounter of the two compartments in space and time, with effects that depend on the directionality of gene flow and its ecological context. Gene flow from wild populations, facilitated by seed banks, could add to the adaptive diversity of domesticated populations (Jarvis and Hodgkin, 1999), but could also create weedy races; gene flow in the other direction can contribute to "genetic swamping" of populations of wild relatives (Ellstrand et al., 1999). Seed banks also add a new dimension to discussions of biotechnology and the risks associated with introduction of transgenic varieties of cassava. As in *Beta* (Viard et al., 2004), cassava seed banks might facilitate the transfer of transgenes to non-target organisms, whether landraces or wild relatives of crops. The use of sexual reproduction by farmers of vegetatively propagated crops such as cassava thus has implications not only for these crops, but also for conservation of the biodiversity of their wild relatives.

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