

Traditional Amerindian cultivators combine directional and ideotypic selection for sustainable management of cassava genetic diversity

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Abstract

Plant domestication provides striking examples of rapid evolution. Yet, it involves more complex processes than plain directional selection. Understanding the dynamics of diversity in traditional agroecosystems is both a fundamental goal in evolutionary biology and a practical goal in conservation. We studied how Amerindian cultivators maintain dynamically evolving gene pools in cassava. Farmers purposely maintain diversity in the form of phenotypically distinct, clonally propagated landraces. Landrace gene pools are continuously renewed by incorporating seedlings issued from spontaneous sexual reproduction. This poses two problems: agronomic quality may decrease because some seedlings are inbred, and landrace identity may be progressively lost through the incorporation of unrelated seedlings. Using a large microsatellite dataset, we show that farmers solve these problems by applying two kinds of selection: directional selection against inbred genotypes, and counter-selection of off-type phenotypes, which maintains high intra-landrace relatedness. Thus, cultural elements such as ideotypes (a representation of the ideal phenotype of a landrace) can shape genetic diversity.

Introduction

While genetic diversity of crop plants has been extensively studied in traditional agroecosystems (e.g. Louette & Smale, 2000; Elias *et al.*, 2001; Zhang *et al.*, 2006; Barnaud *et al.*, 2007), the evolutionary processes leading to such diversity are still poorly understood. Notably, interactions between natural selection and artificial selection by farmers have rarely been documented for crop plants. Using the vegetatively propagated crop plant cassava as a model, we investigated how farmer practices interact with natural selection to shape the crop's genetic diversity.

Cassava (*Manihot esculenta* Crantz) is propagated through stem cuttings, traditionally under slash-and-burn cultivation systems. While one could expect farmers

to maintain only a low number of highly productive and resistant clones, large numbers of landraces have been recorded in all traditional cultivation systems studied so far (Boster, 1985; Salick *et al.*, 1997; Elias *et al.*, 2001; Sambatti *et al.*, 2001; Manu-Aduening *et al.*, 2005; Manusset, 2006). What we here call a landrace is what farmers recognize as a phenotypically distinct unit, giving it a distinct name. High diversity of cassava landraces is particularly marked in South America, where the crop was first domesticated (Olsen & Schaal, 1999).

Accidental or purposeful loss of some clones could lead to a continuous decrease in the number of cultivated landraces. Yet, new landraces also are created continuously: despite 8000 years of clonal propagation (Dickau *et al.*, 2007), most cassava clones have retained the capacity for sexual reproduction, and seedlings sometimes are incorporated into the stocks of stem cuttings (Elias *et al.*, 2001), as is common for several other vegetatively propagated crops (e.g. yam, Scarcelli *et al.*, 2006; taro, Caillon *et al.*, 2006). In cassava, new

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recombinant genotypes may be propagated as new landraces, or be incorporated into an existing landrace, and accordingly contribute to maintaining genetic diversity among and within landraces, respectively.

Some aspects of this process have already been studied by Pujol *et al.* (2005), in a Palikur Amerindian farming system. These authors show that the planting scheme imposed a cost to the creation of diversity through sexual reproduction, namely, a high probability of producing inbred genotypes, with reduced heterozygosity and agronomic performance. Pujol *et al.* (2005) show how farmers alleviate this cost, reinforcing natural selection through selective weeding of small, inbred seedlings. However, even after weeding, seedlings are still on average more inbred than the population of planted clones. Elias *et al.* (2001), working in a Makushi Amerindian village, highlight another problem arising from seedling incorporation: assignment of seedlings to existing landraces, performed on morphological grounds, tends to increase intra-landrace genetic diversity and to decrease inter-landrace differentiation. Incorporation of seedlings therefore may progressively alter the genetic identity of the landraces into which they are assimilated.

This study aims at understanding how farmers in traditional cassava cultivation systems manage to take advantage of seedling incorporation, thereby maintaining the crop's genetic diversity, while avoiding the two associated pitfalls: incorporation of inbred seedlings and progressive loss of landrace identity. We tackle this issue in another Amerindian farming system, among the Wayãpi of southern French Guiana. The groups previously studied belong to the Carib (Makushi) and Arawakan (Palikur) linguistic families. The Wayãpi belong to a third linguistic family, Tupi-Guarani, and they are amongst the least acculturated Amerindian groups in French Guiana. In addition, large tracts of 'primary' forests are present in this region, so that long fallows can still be performed, as was the case over much of recent history. The Amerindian groups in which we have studied the dynamics of cassava management thus represent considerable diversity, both culturally and in the ecological context in which the agricultural system common to all, slash-and-burn cultivation, is conducted. We investigate (i) the genetic composition of landraces, (ii) the extent to which inbreeding is a factor in natural selection and in artificial selection by farmers, and (iii) how seedling incorporation affects landrace genetic identity.

Materials and methods

Study site

These issues were addressed in a very isolated Amerindian village, Trois Sauts, on the upper Oyapock river, in densely forested southern French Guiana. The nearest village, Camopi, is 150 km away, and can be reached only

by small motor boats. Trois Sauts consists of three settlements of Wayãpi people, totaling about 650 persons.

Wayãpi people have retained a traditional way of life, because of this isolation. They rely on hunting, fishing, gathering, and on small-scale cultivation of several crops, in a slash-and-burn system. Cassava is by far the most cultivated plant, but a number of other crops, among them yam, maize, sweet potato, and banana, are planted in small numbers (Grenand & Haxaire, 1977; Grenand & Grenand, 1996). Cassava cultivation is exclusively woman's work.

The cassava cultivation cycle

The cultivation cycle of cassava under a traditional Amerindian slash-and-burn farming system is presented in Fig. 1. Each year, each family clears and burns one to two fields. Women then plant cassava stem cuttings on small mounds (planted cuttings are termed 'C plants' throughout), usually in monovarietal patches. The fields are very lightly weeded, sometimes not at all. As mature cassava roots do not rot when left in the field, farmers harvest them according to their needs, from 6 months to 2 years after planting, then leave the field to fallow.

Wayãpi farmers prefer to perform long fallows (20 years or more). However, because of demographic explosion and settling around a school and a medical station, they are faced with limited availability of mature forest close to Trois Sauts (Grenand & Grenand, 1996). Farmers who do not own a motor boat thus have reduced the duration of the fallows in the past 30 years, and occasionally, old widows may totally suppress the fallow or reduce it to one or two years, in fields they cultivate very close to their houses.

The planting scheme, in monovarietal patches, as among the Palikur (Pujol *et al.*, 2005), leads to high probabilities of matings occurring between plants of the same landrace, which may be clonemates. Seeds produced during one cycle of cultivation only germinate during the following cycle (termed 'S plants', Fig. 1; Pujol *et al.*, 2002). Each seedling is assigned a name, generally of an existing landrace. Wayãpi farmers told us that every seedling they find is subsequently propagated clonally. They remember, for periods up to several years – which could correspond to trial periods – which individual plants they found as seedlings and chose to propagate clonally ('clones of seedlings' or 'CS plants').

Plant material

In January 2007, we worked with 10 woman farmers from Trois Sauts, accompanied by a local interpreter, and visited 21 fields. In each field, the farmer was asked to show us all of her landraces. Leaf material was collected for one plant of each landrace in each field. We also asked farmers which of their plants (if any) were clones of seedlings they had found in previous years, and

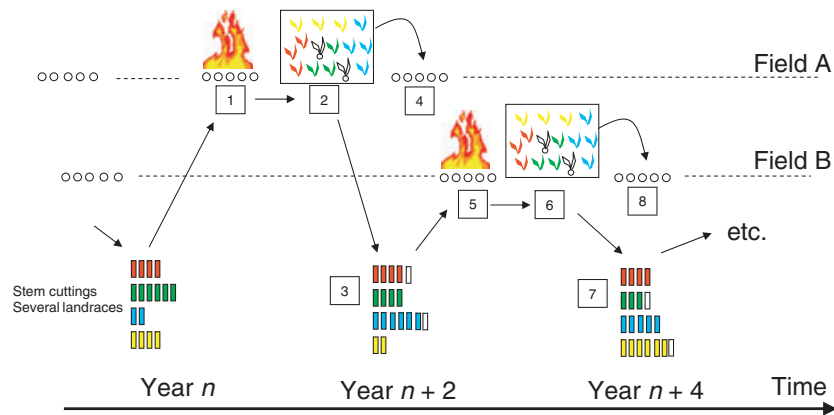


Fig. 1 Cassava cultivation in the Wayāpi Amerindian farming system. The pool of genetic resources managed by a farmer during year n is represented by a stock of stem cuttings classified in distinct landraces. (1) A new field is burnt. Fire triggers germination of buried seed bank (seeds are figured as open circles), produced during the last cultivation cycle. (2 and 6) Farmer plants stem cuttings in monovarietal patches (C plants, figured as coloured plants; each colour symbolizes a different landrace), intermingled with the volunteer seedlings (S plants, white outlined in black). (3 and 7) 1–2 years after planting, farmer harvests roots and reconstitutes her stock of stem cuttings, integrating volunteer plants into existing landraces (open sticks), based on her perception of the phenotype. (4 and 8) The field is left in fallow for some years (rarely, a few months, and up to 50 years or more). The seeds produced stay dormant in the soil. (5) Farmer opens a new field and plants her stem cuttings, often with different abundances than in the previous field. Farmers manage two fields at the same time – those planted in even years, such as pictured here, and those planted in odd years.

collected all of these plants. Finally, we collected material from all seedlings we could find. Extensive collection of seedlings was only possible in fields in the first year of cultivation, because the lack of weeding makes it difficult to systematically identify seed-issued plants in second-year fields.

Overall, 79 names were recorded, some of which were likely synonyms (P. Grenand, personal communication), leading to a total of 61 named landraces, with individual farmers cultivating 10–37 landraces (20.9 ± 7.7). A total of 269 C plants were collected, belonging to 54 landraces. Seven landraces were found only as products of recent events of sexual reproduction (i.e. as S, as CS or both). Analysis of saturation showed that no plateau was reached for the numbers of landraces: more names probably exist in this village (see Fig. S1).

Farmers indicated 38 plants as being CS in their fields (0–13 per farmer) and 129 seedlings were collected in the 13 fields in the first year of cultivation (0–49). In five of these fields, no seedlings were found. One field had been left in fallow for 25–30 years before renewed cultivation, and the four others had been left in fallow for so long that farmers did not recall their ever having been cultivated. Nevertheless, in all four fields, we found ceramic fragments testifying to past occupancy. Sampling is further detailed in Appendix S1.

Genotyping

All 436 plants were typed for 10 microsatellite loci [GA12, GA21, GA57, GA126, GA127, GA134, GAGG5 (Chavarriaga-Aguirre *et al.*, 1998); SSR55, SSR68,

SSR169 (Mba *et al.*, 2001)]. Extraction was conducted using Qiagen 96 Plant kit. All loci were amplified jointly using multiplex PCR Taq from Qiagen GmbH (Hilden, Germany), in a final volume of 10 μ L. Amplification was conducted on a PTC-100 thermocycler (MJ Research, Waltham, MA, USA) and genotyping was performed on an ABI 3130 sequencer (Applied Biosystems, Foster City, CA, USA). Genotypes were then eye-checked under GENE Mapper 3.0 software (Applied Biosystems). On each PCR plate, six wells were used for data control, from the extraction to the typing steps: one was empty, and the others contained replicates of individuals extracted on this or on other plates. The wells containing no individual never showed amplification, and all pairs of replicates were consistent. The locus with greatest allele length (GA134) could not be typed for 26 individuals because of weak amplification, and the dataset counted four additional missing data points.

Within- and between-landrace genetic diversity

We determined the number of different clones (i.e. multilocus genotypes) among C plants from each landrace, assessed Nei's diversity (Nei, 1987) for each locus for C plants, and estimated θ [Weir & Cockerham's (1984) estimator of F_{ST}] between all pairs of landraces (considering only C plants, and including only the 20 landraces with five plants or more) using FSTAT v.2.9.3.2 (Goudet, 1995). P -values were computed after 3800 permutations of genotypes among landraces and their significance was assessed using Benjamini & Hochberg's (2000) FDR test using R v.2.6.0 (R Development Core

Team, 2008). We also performed an analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) using the package ADE4 in R (Thioulouse *et al.*, 1997). Finally, the pairwise relatedness between plants was assessed using the methods of Wang (2002) and of Lynch & Ritland (1999), under KINGROUP v. 2 (Konovalov *et al.*, 2004).

Criteria used by farmers in seedling selection

We assessed whether seedlings were selected for incorporation on the basis of their level of inbreeding, and on the basis of their relatedness to the landrace to which they had been assigned. We determined individual multilocus heterozygosity (MLH) for each plant. The rate of selfing in the C, CS and S populations was assessed using the software RMES (David *et al.*, 2007), with the maximum likelihood method. The method implemented in RMES infers selfing rates from the multilocus structure (apparent heterozygosity), and not from the values of F_{IS} . Therefore, it avoids overestimating selfing rates owing to the presence of null alleles. Weir & Cockerham's (1984) estimator of F_{IS} was computed for each population, using FSTAT v.2.9.3.2.

We computed the relatedness of each S and CS plant to each C plant. We then determined the average and the maximum relatedness between the focal S or CS plant and C plants of each landrace (only landraces with at least three C plants were considered). We could therefore determine which landrace was most related to each S or CS plant, based either on average or maximum values. These calculations were made using R; all scripts used in this paper are provided in Appendix S1.

Results

Genetic composition of landraces and differentiation between them

Overall, landraces were genetically differentiated [$\theta = 0.20$ (0.18–0.22)]. Most pairwise differentiation tests between landraces were significant (Table S3). Forty per cent of the molecular variance occurred among landraces (AMOVA, $\Phi_{ST} = 0.40$, $P < 0.001$).

Despite this apparent differentiation, out of the 53 multilocus genotypes that were not unique, 36 genotypes were shared by two or more (up to seven) landraces. With the observed gene diversities, under random mating, and excluding linkage disequilibrium, the probability that sexual reproduction produces two identical multilocus genotypes is 7×10^{-7} . Among 269 C plants, 208 shared their multilocus genotype with one or more (up to 10) other plants, leading to a total of 481 pairs of identical multilocus genotypes. Therefore, even if it is not strictly impossible that some of these plants only appear to have the same genotype, but arise from different sexual recombination events, the huge majority of these pairs must represent true clones. Some clones are thus

shared between landraces, probably because of assignment errors occurring during the transmission of stem cuttings.

Of the 20 landraces with five or more C individuals, only one was monoclonal. All other landraces comprised several multilocus genotypes (up to nine). Most of these genotypes were unlikely to be issued from mutation of preexisting genotypes, as the clones belonging to a given landrace differed on average by 40% ($\pm 13\%$) of scored alleles. Such a wide genetic basis for each landrace rather suggests instead a high frequency of incorporation of new clones, issued either from sexual reproduction, or from assignment errors (i.e. 'migration' of clones from one landrace to another one because of misidentification).

Do farmers selectively incorporate seedlings into the stock of clones, and if so, on what criteria?

Wayāpi farmers value cassava seedlings they find in their fields, and all farmers told us that every seedling would subsequently be used for clonal propagation and incorporated into a landrace. However, this statement was inconsistent with the low number of CS plants they showed us, as compared with the high number of seedlings we found (only 38 plants were remembered as CS – some of which apparently dated back to 5 years or even more – as compared with 129 S found in a single year in these farmers' fields). We therefore tried to evaluate whether CS plants were a selected subset of S plants, by checking whether they presented distinctive genetic characteristics.

Heterozygosity

The C and CS plants did not present any heterozygote deficiency, but S plants were less heterozygous than expected under random mating (Table 1). Seedlings were partly issued from selfing (or cross-fertilization between clonemates) while neither CS nor C populations showed significant inbreeding (Table 1). This suggests that CS plants are not a random subset of S plants, but that outbred plants have been selected for.

Whereas mean MLH was not significantly increased in CS as compared with S, it was greater in C than in CS or S plants (Figs 2 and 3, *t*-tests: S vs. CS, $t = 0.076$, $P = 0.94$; C vs. CS, $t = 3.77$, $P = 0.001$; C vs. S, $t = 3.84$, $P < 0.001$, with $n_S = 129$, $n_{CS} = 38$ and $n_C = 269$).

Table 1 Assessment of inbreeding in the populations of seedlings (S), clones of seedlings (CS) and well-established clones (C).

Population	<i>n</i>	F_{IS}	<i>P</i> -value	S (95% CI)
S	129	0.108	<0.001	0.20 (0.11–0.29)
CS	38	0.029	0.53	<0.01 (0.00–0.10)
C	269	–0.033	0.30	0.01 (0.00–0.007)

n, population size; F_{IS} and one-tailed *P*-value for heterozygote deficiency; *s*, estimation of the proportion of plants issued from selfings and 95% confidence interval.

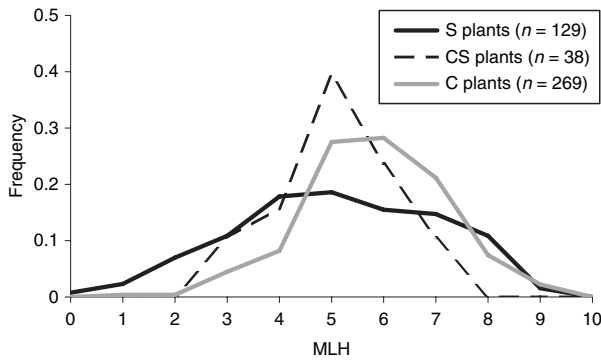


Fig. 2 Distribution of multilocus heterozygosity (MLH) (number of heterozygous loci, out of 10) in S, CS and C plants (black, dashed and grey lines, respectively).

Variance in MLH was lower in CS than in S plants (F -test, $F_{129,38} = 3.05$, $P < 0.001$). Consistently, expected heterozygosity was lower for CS plants (0.51 ± 0.22) than for S plants (0.57 ± 0.18 ; see Fig. 3). Hence, both the most homozygous and the most heterozygous seedlings appear not to be selected for clonal propagation.

Relatedness of S and CS plants to the landrace they most resemble

The S and CS plants further differed in their relatedness to the landrace to which they were assigned: CS plants were assigned more often than S plants to the genetically closest landrace: 12% of seedlings were assigned to the landrace containing their most related C plant (Wang’s coefficient), whereas 46% of CS plants were (pairwise comparison of proportions, 13/108 vs. 13/29, $P < 0.001$). Using Lynch & Ritland’s coefficient of relatedness did not modify these results (13/108 vs. 12/29, $P < 0.001$).

Average relatedness of seedlings to the landrace to which they were assigned was 0.05, as compared to 0.22 for CS plants (t -test, $t = 2.56$, $n_1 = 29$, $n_2 = 108$, $P = 0.016$), and to 0.37 for C plants (see Fig. 3). Note that, even for S plants, such a low average relatedness to

the landrace to which they are assigned is still greater than expected if landrace assignment were random (t -test, $t = 3.85$, $n_1 = 129$, $n_2 = 108$, $P < 0.001$).

No correlation was found between the number of seedlings assigned to a landrace and the polyclonality of the landrace (number of clones/number of C plants), nor with its average MLH (Figs S2 and S3). Average relatedness of C plants to other C plants belonging to the same landrace was 0.37 (SD 0.28; only landraces with three or more C plants were considered).

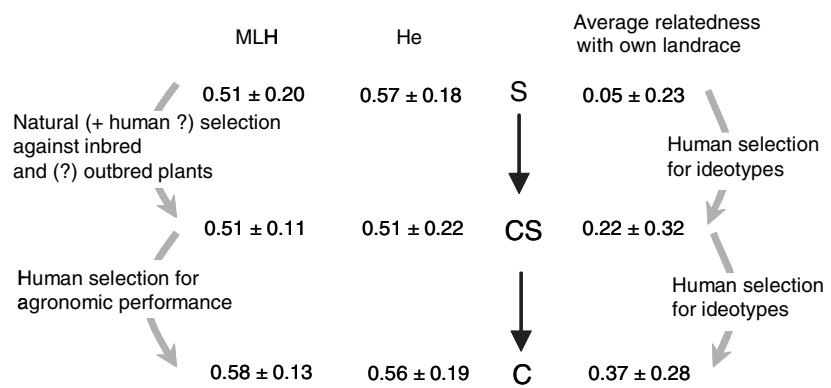
Discussion

Differentiation among landraces

Even though several clones were shared among landraces, we found evidence for differentiation between landraces. Sharing of clones between landraces is probably due to misidentification of some landraces by some farmers, either because the landrace was transmitted to them under an ‘incorrect’ name – this is all the more plausible since farmers readily exchange clones (Elias *et al.*, 2001; Pujol *et al.*, 2007) – or because of weak morphological differentiation of landraces. In any event, such assignment leads to increased intra-landrace diversity.

As compared to other traditional cassava cultivation systems, the number of named landraces found was in the high part of the range. Elias *et al.* (2001) observed 76 landraces in a Makushi village; Manusset (2006) found fewer than 40 landraces in most communities in French Guiana, except among the Wayana Amerindians, who cultivate about 100 landraces. As already observed in the Makushi and in the Palikur farming systems (Elias *et al.*, 2000; Pujol *et al.*, 2005), most landraces were polyclonal. In most cases, the different genotypes composing a landrace could not have issued from somatic mutations (data not shown). Some of these genotypes may come from other villages, as Wayāpi farmers like to exchange cuttings, a common feature among traditional cultivators of cassava (Elias *et al.*, 2000), as well as other plants, and

Fig. 3 Evolution of average observed MLH (\pm SD), expected heterozygosity (He), and average relatedness with the landrace to which the plants have been assigned (Wang’s coefficient; average \pm SD), from seedlings (S) through recently incorporated seedlings (CS) to well-established clones (C). The different selection pressures are indicated against the grey arrows.



one that sometimes strongly contributes to shaping crop diversity (Allinne *et al.*, 2008). Yet, the Wayāpi are very isolated, so that the observed pattern of diversity mainly reveals a complex process of seedling incorporation.

The two phases of landrace construction

Our observations suggest that the new genotypes eventually selected for incorporation into the stock of stem cuttings (landraces) are subject to selection on at least two stages (see Fig. 3). Furthermore, two kinds of selective pressures co-occur: directional selection (e.g. for agronomic performance) and ideotypic selection, that is, a selection for plants that fall within a given range of phenotypic variability, and are thus recognized by farmers as belonging to the same landrace. In addition to this continuous incorporation of new genotypes within landraces, established clones are continuously selected or inadvertently lost, shaping the dynamics of the collections of landraces and of genotypes within landraces.

The first stage of incorporation is the selection for seedlings that will be propagated for at least one clonal generation (CS plants). We showed that inbred plants were counter-selected during this phase (compare S and CS plants in Fig. 3). In the Wayāpi system, selfing rate was estimated at 20%, a figure comparable to that found among the Palikur (18%; David *et al.*, 2007), but was 0 among clonally propagated plants. This selection can be achieved both through natural and artificial selection by farmers. Indeed, weeding (Pujol *et al.*, 2005) and intra-specific competition (Pujol & McKey, 2006) have been shown to be partly responsible for the counter-selection of inbred seedlings in the Palikur system. Intraspecific competition probably also plays a role in the Wayāpi system, but weeding is not commonly practiced. However, the most inbred seedlings may be too small to supply suitable stem cuttings at the time of harvest.

Artificial selection of plants issued from seeds to be used for clonal propagation also leads to higher average relatedness to the landrace to which the plants are assigned (Fig. 3). This means that the plants used for incorporation in a given landrace are more prone to be descendants of plants of this landrace, and may therefore also partly share the phenotype defining this landrace. This pattern therefore suggests that selection, during this first phase, acts to preserve the ideotype of each landrace. Boster (1985) pointed how perceptual distinctiveness (i.e. ideotypic divergent selection) is important to Aguaruna farmers. This mechanism probably holds true in the Wayāpi system too.

Finally, during this first stage of selection, outbred individuals are also counter-selected (Fig. 3), again suggesting ideotypic selection. Very heterozygous individuals may indeed be issued from crosses between very different landraces, leading to atypical phenotypes which do not correspond to existing ideotypes. Counter-selec-

tion of off-type seeds or seedlings has indeed often been described in traditional farming systems (e.g. Louette & Smale, 2000; Alvarez *et al.*, 2005), although, to our knowledge, never on genetic bases [even though it was suspected by Barnaud *et al.* (2008) for sorghum].

The second phase of selection corresponds to the few years during which the farmers keep propagating CS plants, still remembering their sexual origin. During this phase, both average MLH and relatedness with the landrace to which the plant has been assigned increase (compare CS and C plants on Fig. 3). This means that, while the status of 'former seedling' is progressively forgotten, only CS plants most related to the landrace they phenotypically most resemble are selected as a source of cuttings. In the meantime, only the most heterozygous of these plants are used for clonal propagation. This suggests that farmers continuously select CS plants, reinforcing ideotypic selection and increasing average MLH, probably through the selection of the CS plants with the highest agronomic performance. This selection probably also applies to already established clones (C plants), and is still in interaction with natural selection, counter-selecting plants that are not resistant to biotic or abiotic stresses.

Selection of the products of sexual recombination at late stages (i.e. at the CS stage) accounts for the discrepancy between our results and those of Elias *et al.* (2001). Those authors, working at the seedling stage only, found that seedling incorporation increased intra-landrace diversity, while lowering inter-landrace diversity. The selection mechanisms we document here, occurring at the late seedling stage and during the period when incorporated plants are tested, result in a lower increase of intra-landrace diversity than if all seedlings were incorporated, and thus help maintain landrace differentiation.

Is the selection regime stationary?

One could ask why CS plants do not reach the level of heterozygosity of C plants. Landraces to which numerous seedlings were assigned (and which therefore may absorb numerous new clones) were not more polyclonal, nor more homozygous, than were landraces to which no or few seedlings were assigned (Fig. S3). These observations can be accounted for in two ways: either only the most heterozygous CS plants will eventually be incorporated, after some years of field trial, or there has been a recent change in the selection regime. In other words, the C plants we now observe may stem from CS (and S) populations that were more heterozygous than the populations we observed.

Such a change could have occurred, as the Wayāpi Amerindians recently switched towards shorter fallows (Grenand & Grenand, 1996). The longer the preceding fallow, the fewer seedlings found in the field (Fig. S4). In a long-fallow system, mortality is likely to be severe

before germination (e.g. during the long period of storage in the soil seed bank) or just after, and this mortality could be selective. Inbreeding depression can be strong in early life stages, such as germination (Husband & Schemske, 1996; McCue & Holtsford, 1998; Koch *et al.*, 2003). We are not aware of studies documenting that seed viability in the seed bank decreases faster over time for inbred than for outbred seeds, but such a mechanism could explain our results. Under this hypothesis, farmers cultivating on long fallow cycles would have to choose seedlings to incorporate from an already very heterozygous pool of surviving seedlings. Average MLH of seedlings would thus be higher than what we observed in this shorter-fallow system – and perhaps, close to the level observed in C plants. Ideotypic selection and counter-selection of inbred plants would therefore lead to high average MLH of plants selected for clonal propagation. Under shorter fallows, a higher number of seedlings, among which a larger proportion would be inbred, would germinate, and ideotypic selection by farmers would be less efficient in keeping only the very heterozygous plants.

The mismatch between farmers' statements (i.e. that they incorporate every seedling into their stock of clones) and their actions (i.e. selection of a small number of seedlings for incorporation), is consistent with such a hypothesis. In the past, under long fallows, farmers would actually have incorporated all the (few, and already very heterozygous) seedlings they would have found in their fields. Under today's shorter fallows, current farmers, taught by their mothers and grandmothers to keep all seedlings, would be faced with huge numbers of seedlings (which, not having been subject to natural selection, have low average MLH). New selection criteria may be developing, but do not yet enable selection of seedlings with the same level of heterozygosity as the established clones.

This hypothesis of a recent change in selection regime has no effect on the interpretation of our other results. Except for the increase in MLH between CS and C plants (Fig. 3, lower-left arrow), all of our results would also be expected under the postulated former selection regime.

Intercultural comparison of cassava cultivation systems

Seedling incorporation has been documented in a large number of cassava farming systems, among Amerindians (Salick *et al.*, 1997; Elias *et al.*, 2001; Pujol *et al.*, 2005) but also in Africa, outside the plant's area of origin, where this practice appears to be more occasional (Manu-Aduening *et al.*, 2005; M. Delêtre, personal communication). Among the Amuesha of central Peru, landrace management is the shaman's prerogative (Salick *et al.*, 1997), but in the Guianas, three groups belonging to different language families (Carib, Arawak and Tupi-Guarani) share a common taste for diversity, with all

farmers consciously incorporating seedlings into their stocks of clones. This results in most landraces being polyclonal.

The restricted geographic frame of the Guianas nevertheless shows variations in the practices concerning seedling incorporation. While Makushi and Wayãpi Amerindians show a strong interest in seedlings, and keep a memory of the recently incorporated plants for some years (this study; M. Elias, personal communication), Palikur Amerindians tend to express less interest in seedlings (Pujol *et al.*, 2005).

A feature common to all these agroecosystems is the shortening of fallows, because of demographic pressures and low land availability. Among the Wayãpi, fallows are still longer than in both other systems (M. Elias, personal communication), but are shortening dramatically. This phenomenon might lead to a modification of the seedling selection system, and to a progressive loss of the interest expressed in seedlings, as observed among the Palikur.

To conclude, we showed that human selection contributes to the elimination of inbred seedlings, and increases landrace genetic diversity in a canalized fashion, keeping cassava plants assigned to a given landrace within a predefined range of phenotypic and genetic diversity (Fig. 3). Through weeding and through selection of stems for clonal propagation, Palikur and Wayãpi farmers thus affect the microevolutionary dynamics of cassava landraces, but in somewhat different ways. Better knowledge of interactions between natural selection and artificial selection by farmers will be useful for *in situ* conservation of crop genetic resources.

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Supporting information

Additional supporting information may be found in the online version of this article:

Appendix S1 Supplementary Methods and Results.

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