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Article

Ethnobotanical Diversity of Cassava (*Manihot esculenta* Crantz) in the Peruvian Amazon

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Abstract: Cassava is a key tropical crop that serves as a major source of nutrition throughout equatorial South America, Africa, and Asia. Genetic and paleoethnobotanical findings indicate that it was first domesticated on the southern margin of Amazonia ~10,000 years ago. However, anthropogenic processes underlying its subsequent diversification remain unclear. To shed light on them, we investigated agricultural practices and phenotypic variation in cassava on the upper Amazon River, in Loreto, Perú. We interviewed subsistence growers on five Amazon tributaries and collected data on the husbandry, morphology, and nutritional composition of their crops. We found 45 distinct cultivars. Many of their morphological features, such as stature and leaf dimensions, exhibited expected phenotype–phenotype associations. However, starch content showed no association with any other phenotype (mean $p = 0.57$), suggesting it has been under selective pressure exerted by growers. In addition, all cultivars' tubers had cyanide content under 25 ppm, a low level of toxicity by global standards. Evidence of sexual reproduction and cultivar hybridization was common and a probable source of new variation. However, cultivars from different rivers showed little evidence of differentiation, possibly as the result of human transport. Thus, human influences in the region simultaneously enhance and constrain variability in the crop.



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Keywords: Amazonia; cassava; manioc; yuca; *Manihot*; agriculture; Peru

1. Introduction

Cassava (*Manihot esculenta esculenta*, also commonly called manioc or yuca) is a key tropical crop [1,2]. Though little known in temperate regions, it is a nutritional staple throughout equatorial Central and South America, Africa, and Asia, and globally ranks among the top ten crops in production [2,3]. Cassava's success arises from its high yield and robustness [4]. Its below-ground tubers store large quantities of concentrated starch that can be efficiently extracted and refined, and it can be grown where water is scarce and soils are poor. It is also naturally pest-resistant, producing toxins that effectively deter predators but can be removed after harvesting [5]. Cassava's combination of productivity and durability is particularly attractive to smallholders, such as subsistence growers whose access to fertilizers and industrial pesticides is limited.

One of cassava's distinctive characteristics is its phenotypic variability. While it is grown primarily for a single purpose, starch production, cassava is represented by numerous closely related cultivars that vary in height, leaf color and shape, stem morphology, and other features [6–11]. Extensive variation is also found in its tubers, resulting in variable productivity [10]. Cassava's best known feature, the toxicity imparted by its cyanide-based defense system, is highly variable as well, with cyanide content in tubers ranging from near 0 to almost 800 ppm [12]. This diversity is an asset to both traditional growers, who can select cultivars fitting local environmental conditions, and industrial agriculture, which can manipulate specific traits to produce novel cultivars [13–17].

Cassava's variability raises questions about its origins, particularly the processes underlying its phenotypic diversification. Genetic evidence suggests that cassava was domesticated ~10,000 years ago by indigenous peoples on the southern margin of the Amazon basin, who derived it from a wild *Manihot esculenta* subspecies, *M. e. flabellifolia* [18]. Paleoethnobotanical findings indicate that it then spread rapidly through tropical South and Central America, reaching sites as far north as Panama by 8000 years ago [19]. The final stages of cassava's dispersal into Africa and Asia occurred only recently, when it was exported from Brazil in the 16th century [20,21]. Thus, cassava's diversity is anthropogenic, and largely the product of its agricultural history in the New World tropics.

Patterns of diversity on local scales emphasize the impact of human pressure on cassava's variability. Growers across Amazonia actively maintain phenotypically distinct cassava cultivars, which are typically imparted with traditional names [6,8–11,22,23]. Both the cultivars present and their nomenclature vary geographically [10,24–26]. Further, genetic evidence suggests that this is due to artificial selection imposed by growers, who often incorporate newly emerged types into their crops and cull plants with undesirable traits [14]. Thus, contemporary patterns of diversity reflect long term anthropogenic factors shaping the crop. In this study we assessed agricultural practices and phenotypic variation in cassava on five Amazon tributaries in Loreto, Perú, to explore human influences on diversity in the region.

2. Materials and Methods

2.1. Study Site

Our study was conducted on the Amazon River in northeastern Perú, 3500 km upstream of the river's mouth in Brazil (Figure 1). Ecologically, the region is dominated by lowland tropical rainforest and whitewater rivers, including numerous tributaries of the Amazon's main channel. Human populations in the area are concentrated in five municipalities including the city of Iquitos (population 470,000) and four towns (Indiana, Mazán, Nauta, and Pebas; populations ~10,000). However, the majority of the region is inhabited only by small, scattered communities of mestizo and indigenous smallholders (populations < 150). Our sampling focused on cassava agriculture in settlements on five rivers, namely, the Itaya, Nanay, Orosa, Pintuyacu, and Tahuayo. Four of the five are direct tributaries of the Amazon, and the Pintuyacu is a tributary of the Nanay. They enter the Amazon at varying distances from Iquitos, with the Itaya and Nanay entering at Iquitos, the Tahuayo entering 50 km upstream, and the Orosa 135 km downstream.

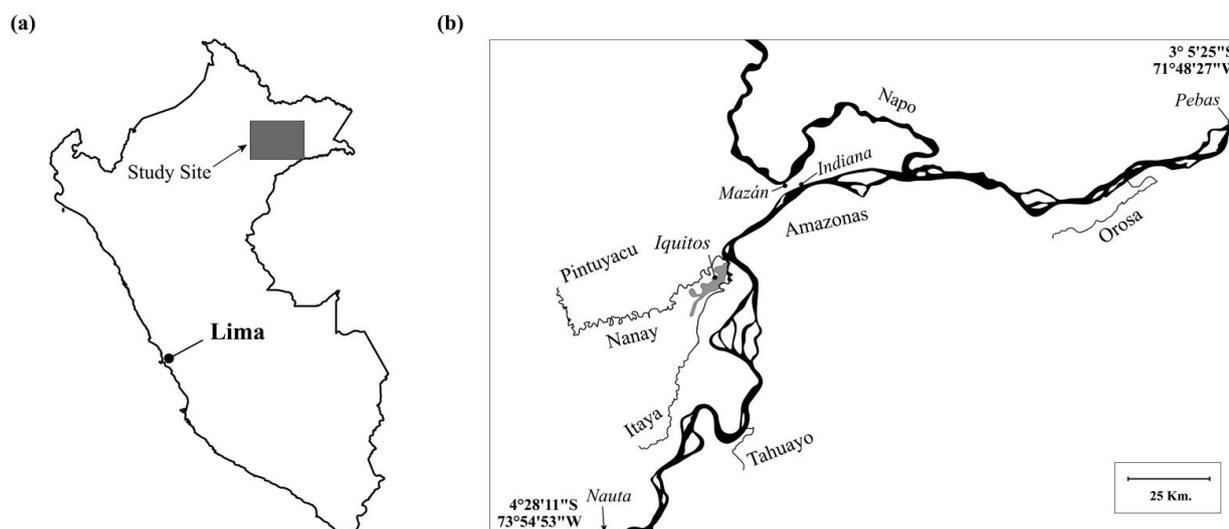


Figure 1. Map of the study site. (a) Site was centered on Amazon River in northeastern Perú, roughly 3500 km upstream of the Amazon's mouth on the Atlantic coast of Brazil. (b) Data were collected on five tributaries of the Amazon: the Itaya, Nanay, Orosa, Pintuyacu, and Tahuayo.

2.2. Collection Sites

Observations and samples were collected at riverfront communities, which we visited by boat. We identified collection sites opportunistically, focusing on villages with 50–100 residents. After landing, we talked informally with residents and asked about cassava cultivation in the area. We identified growers through these discussions and contacted them for interviews on aspects of local cassava culture and their own activities. Cassava is a key source of nutrition in the region and with a single exception all communities were maintaining cassava fields (chacras) within a 30 min walk of the village center.

A major goal in our study was to determine the breadth of cassava diversity in the region. Therefore, we sought to identify as many cultivars as possible, and conversations with growers were specifically aimed at finding all varieties grown locally. The discussions revealed that cultivars in the region nearly always have folk names, which we used as a guide. When growers reported cultivating a variety not yet encountered in our study, we offered to purchase a specimen. We then visited their chacras for observation, measurement, and collection.

2.3. Plant Phenotypes

Phenotypic measures were obtained from a typical mature plant in the chacra. The plant was measured with respect to as many as possible of 15 phenotypes describing the above- and below-ground parts of the plant, including two nutritional traits (Figure 2). Physical measures taken in the chacra included plant height, crown width, height of first branch point, stem diameter 10 cm above ground level, number of tubers, and total tuber mass. These were obtained using standard tools including a caliper, tape measure, and portable hanging scale. Leaves and a typical tuber from the plant were also collected for later analysis. Photographs of tubers and leaves included a calibration scale, allowing for later measures of lobe number, length of center lobe, maximum width of center lobe, leaf area, and petiole length using Adobe Illustrator software (Version 26.0.1).

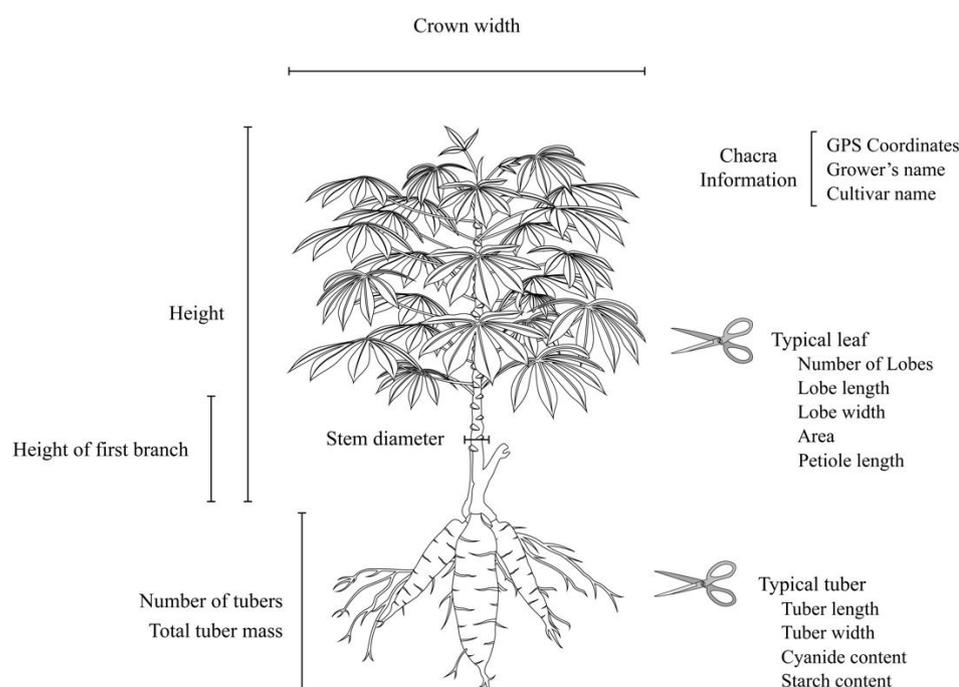


Figure 2. Plant phenotypes. Plants were measured with respect to 15 phenotypes. Measures taken in the field described structural aspects of the plant. Chemical assays of cyanide and starch content were performed in the laboratory, and physical dimensions of tubers and leaves were obtained from calibrated photographs. Location was recorded to allow for future visits to specific chacras.

Measures of starch and cyanide content were obtained using chemical assays. The cyanide production of tubers was determined using a Sensafe Cyanide Complete Visual Kit (Sensafe part no. 484003), following the kit's instructions. Each sample was prepared by cutting a ~1.0 g piece from starchy flesh near the center of the freshly collected tuber, which was homogenized in distilled water using a Qiagen Tissue Ruptor II disruptor (Qiagen part no. 9002755). The resulting mixture was passed through 0.45 and 0.22 μm pore polyethersulfone (PES) filters (Biomed Scientific part nos. SFPES025045-100 and SFPES025022-100) using 5 mL disposable syringes (Medline part no. SYR105010Z). The filtrate was then analyzed following the kit's protocol. The Sensafe kit indicates cyanide content with a color indicator, which we measured using a Thermo Scientific Spectronic 200 spectrophotometer (Thermo Scientific part no. 840-281700) to detect absorbance at 500, 530, 606, and 650 nm wavelengths. Control curves were generated by carrying out the same process as for samples but with sequential dilutions of an Aqua Solutions 1000 ppm cyanide standard (Aqua Solutions part no. 2650-1L). From a strict standpoint, the HCN released by cassava is not actually present in the intact tuber, but is produced by a chemical reaction when the tuber is damaged [5,27]. However, for convenience, we referred to the result of the assays as cyanide content, toxicity, or cyanogenicity, which was given in parts per million (ppm). The starch content of tubers was determined using a BioAssay Systems EasyChrom Starch Assay Kit (Bioassay Systems part no. E2ST-100) following the kit's instructions for colorimetry. This kit carries out sequential enzymatic reactions of 7.5 mg of a dried, crushed sample with a color indicator, which we measured at 570 nm absorbance using the spectrophotometer per the kit instructions.

2.4. Statistical Analysis

Data were analyzed using the R version 4.0.3 software package [28]. Standard descriptive statistics, including means, variances, and distributions, were calculated using R's built-in functions. Linear regressions were performed and tested pairwise across all measured phenotypes using the R *lm* function. Principal components analyses were performed using the R built-in *prcomp* function [29].

Our pairwise testing procedure resulted in 91 tests, increasing the probability of spurious significant outcomes. Some corrections for multiple testing are available, such as the Bonferroni correction and false discovery rate correction [30,31]. However, these tend to be conservative. Moreover, many of the traits that we analyzed, such as the various leaf dimensions, were predicted to be associated a priori, complicating the interpretation of corrected values. Therefore, we treated *p*-values as indicators of confidence rather than as definitive statistical probabilities.

3. Results

In the course of ~330 km of travel on the five tributaries, we identified 45 traditionally named cassava varieties (Figure 3, Table 1). The number of discovered cultivars varied among tributaries, with the lowest number (6 cultivars) being on the Pintuyacu and Tahuayo, and the highest (18) on the Orosa.

The chacras that we observed were on terra firme sites established using slash-and-burn methods. Growers reported that chacras established this way were cultivated for 3–4 years after the burn, after which they were abandoned. Chacras varied greatly in size. Frequently a handful of plants (~5) would be grown in small gardens near homes, with larger numbers (>50) being grown in chacras farther away. Most chacras contained more than one cassava cultivar, and cultivars were usually randomly intermingled across the field.

The range of phenotypes we were able to obtain varied among chacras. In many cases, a complete set could be obtained through physical measurements in the field followed by photography and chemical analysis in the laboratory. However, field conditions limited measurements in some cases. For example, some growers declined to sacrifice a plant and measures were taken only with respect to parts above ground. Similarly, some plants were

deeply rooted, and one or more tubers broke off below ground too deeply to recover, preventing measurements of their weights and dimensions. In total, near-complete phenotypic measures were obtained from 27 and incomplete measures from 18.

Phenotypic differences among cultivars were evident even through casual observation. For instance, the overall stature of plants varied, ranging from short and broad to tall and thin (Figure 4). Similarly, leaves varied conspicuously in size, color, and shape (Figure 5). Statistical analysis confirmed our general observations. Variation was extensive with respect to both physical and nutritional traits, particularly those visually evident on visits to chacras (Table 2). For instance, leaf areas varied more than 8-fold (range = 44 cm² to 365 cm²), plant heights varied 3.5-fold (140 cm to 497 cm), and crown widths varied 5.5-fold (90 cm to 490 cm). Tuber mass also varied greatly, ranging from 0.5 kg to 5 kg (10-fold). The two nutritional measures, starch content and cyanide content, varied substantially as well, with starch ranging from 33% to 96% with a mean of 70.7% and cyanide content ranging from 1 to 25 ppm with a mean of 11.7 ppm (Figure 6).

Cultivar (base term)	River					Phenotyped
	Itaya	Nanay	Orosa	Pintuyacu	Tahuayo	
amarilla	●	○	○	○	○	●
andioca	○	○	○	●	○	●
añera	●	○	○	○	○	●
arpón	●	○	○	○	○	○
blanca	○	○	●	○	○	○
blanca (dark stem)	○	●	○	○	○	●
blanca (light stem)	○	●	○	○	○	●
brava (cultivated)	○	●	○	○	○	●
brava (fcral)	○	○	●	○	○	○
bufeo	○	○	○	●	○	●
cerveza	○	○	●	○	○	○
cogollo colorado	○	○	○	●	○	●
cogollo morado	○	○	●	●	○	○
colorada	○	○	○	○	○	●
crema	○	○	●	○	○	○
gallinazo	○	○	●	○	○	○
iguano	○	○	○	●	○	●
indianino	○	○	●	○	○	●
inviernino	○	○	●	○	○	○
lobera	○	○	●	○	○	●
lobera colorada	●	○	○	○	○	○
lobera negra	●	○	○	○	○	○
lupuna	○	○	●	○	○	○
lupunillo	○	○	●	○	○	●
mandioca amarilla	○	○	●	○	○	○
mano de tunche	○	○	●	○	○	○
morada	○	○	●	○	○	●
morada amarilla	○	○	○	○	●	●
motelillo	○	○	○	○	●	●
motelo	○	○	●	○	○	○
napino	○	○	●	○	○	●
palmera	○	○	●	○	○	○
palo blanco	○	○	●	○	○	○
palo negro Antonio (narrow leaf)	○	●	○	○	○	○
palo negro Antonio (wide leaf)	○	●	○	○	○	●
palo negro Arimuya	○	●	○	○	○	●
piririca	●	○	○	○	○	●
posheco enano	○	○	○	○	●	●
posheco gigante	○	○	○	○	●	●
señorita	●	○	○	○	○	●
umishina	○	○	○	○	●	●
ungurahui	○	●	○	○	○	●
vidrio	○	○	○	○	●	●
viejillo	○	○	○	●	○	○
virginia	○	●	○	○	○	●

Figure 3. Cassava cultivars identified across five Amazon tributaries. For a given cultivar, filled circles represent the source river and whether phenotypic data were obtained from it.

Table 1. Cultivar names and translations or interpretations.

Cultivar Base Term	Interpretation
amarilla	yellow
andioca	manioc
añera	year-old
arpón	harpoon
blanca	white
blanca (dark stem)	white with dark stem
blanca (light stem)	white with light stem
brava (cultivated)	“wild” cassava being cultivated
brava (feral)	“wild” cassava in abandoned chacra
bufeo	Amazon river dolphin (<i>Inia geoffrensis</i>)
cerveza	beer
cogollo colorado	red bud
cogollo morado	purple bud
colorada	red
crema	cream
gallinazo	vulture (black vulture, <i>Coragyps atratus</i> , is locally common)
iguano	iguano tree (<i>Dilodendron costaricense</i>)
indianino	from Indiana (a town in the area)
inviernino	winter type
lobera	sword of Saint Ferdinand
lobera colorada	red sword of Saint Ferdinand
lobera negra	black sword of Saint Ferdinand
lupuna	lupuna tree (<i>Ceiba pentandra</i>)
lupunillo	little lupuna tree (<i>Ceiba pentandra</i>)
mandioca amarilla	yellow manioc
mano de tunche	hand of tunche (a forest spirit)
morada	purple
morada amarilla	purple yellow
motelillo	little tortoise
motelo	tortoise
napino	from the Napo river (a large river in the area)
palmera	palm tree
palo blanco	white stem
palo negro Antonio (narrow leaf)	black stem from Antonio (a neighbor), narrow leaf morph
palo negro Antonio (wide leaf)	black stem from Antonio (a neighbor), wide leaf morph
palo negro Arimuya	black stem from Arimuya (a neighbor)
piririca	rough, like sandpaper
posheco enano	pale dwarf
posheco gigante	pale giant
señorita	lady
umishina	umisha-like (an umisha is a tree decorated for carnival)
ungurahui	ungurahui palm tree (<i>Oenocarpus bataua</i>)
vidrio	glass
viejillo	old man
virginia	Virginia is a nearby town

Pairwise regression tests revealed both anticipated and unanticipated associations (Figure 7). Particularly low p -values were found in associations between the structural components of the plant and between leaf parts. For instance, associations between height and lowest branch ($p = 0.002$) and between crown width and stem diameter ($p = 0.001$), were well-supported. Similarly low p -values were found in associations between leaf phenotypes such as petiole length and number of lobes ($p < 0.001$) and petiole length and lobe length ($p < 0.001$). In contrast, height and crown width showed no evidence of association ($p = 0.21$). Associations with nutritional measures also had high p -values. Starch

content showed no association with any phenotype, with an average p of 0.57 and a range from 0.06 (with leaf area) to 0.98 (with cyanide content). Similarly, the cyanide content of tubers was associated with tuber mass and number of tubers ($p = 0.013$ and 0.014), but no other phenotype (mean $p = 0.48$ for the remaining 12 tests).

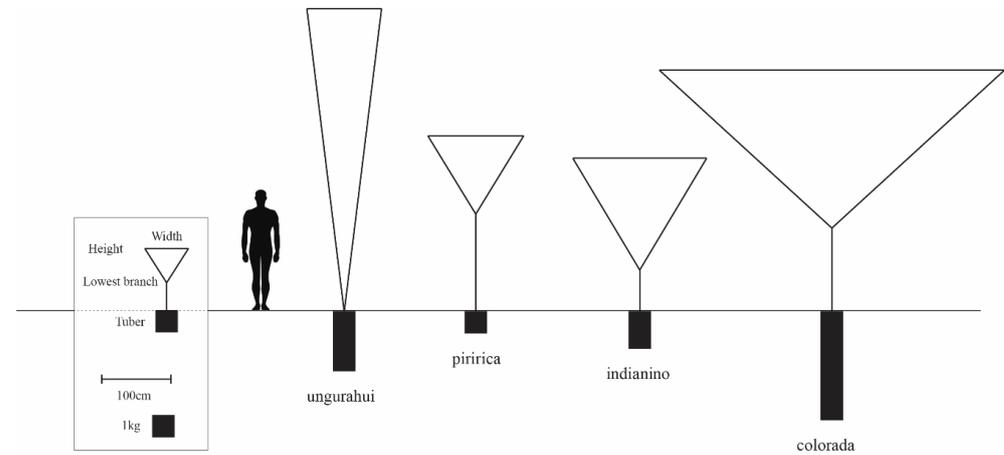


Figure 4. Plant dimensions. Cultivars' overall shapes were highly variable, ranging from short and broad to tall and thin. Regression analyses found no association between crown width and height.

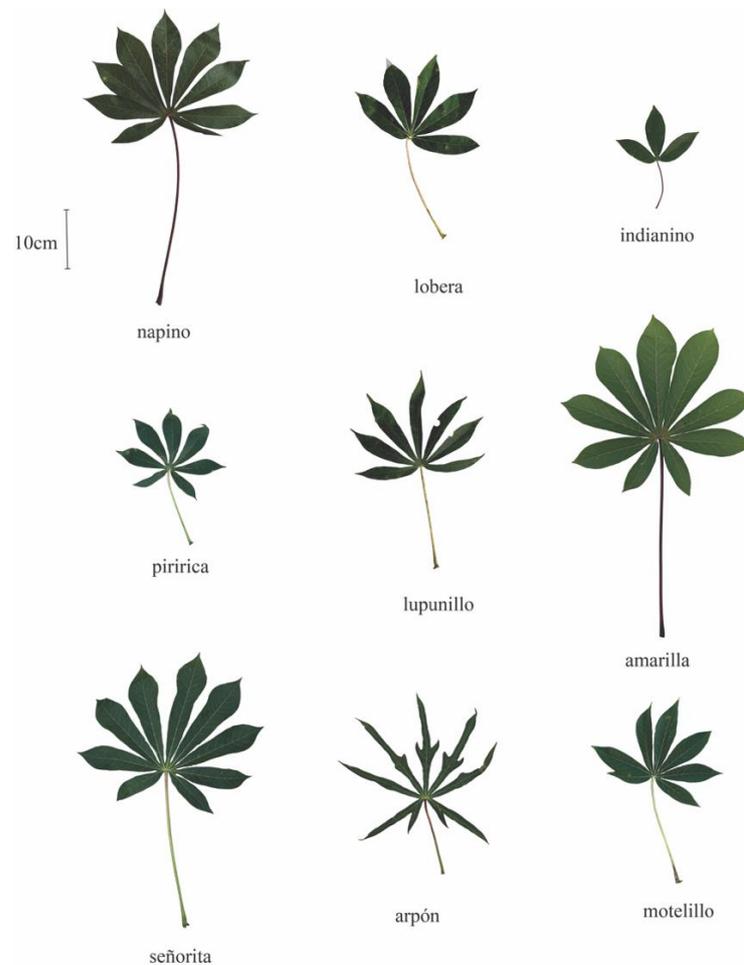


Figure 5. Exemplar specimens illustrating variation among cultivars. Number of leaf lobes ranged from 3 to 9, and areas ranged 8-fold (range = 44 to 365 cm²). Some cultivars had distinctive features, such as indianino, which had only three leaf lobes and arpón, which had arrow-shaped lobes.

Table 2. Summary of phenotypic variation.

Plant Part	Measure	Min.	Mean	Max.	Std. Dev.
Main structure	Height (cm)	140	311.8	497	90.9
	Crown width (cm)	90	235.8	490	114.2
	Height of lowest branch (cm)	30	148.8	435	81.4
	Stem diameter (mm)	17	25.8	39	6.1
Leaf	Number of lobes	3	6.0	9	2.0
	Lobe length (mm)	94	137.7	205	36.7
	Lobe width (mm)	17	37.6	62	14.1
	Area (cm ²)	44	156.8	365	87.2
Tuber	Number of tubers	3	8.6	14	2.97
	Total tuber mass (kg)	0.5	2.4	5.0	1.2
	Tuber length (mm)	120	292.5	462	97.3
	Tuber width (mm)	15	43.6	77	14.5
Nutrition	Cyanide content (ppm fresh mass)	1	11.7	25	7.6
	Starch (% dry mass)	33	70.7	96	16.2

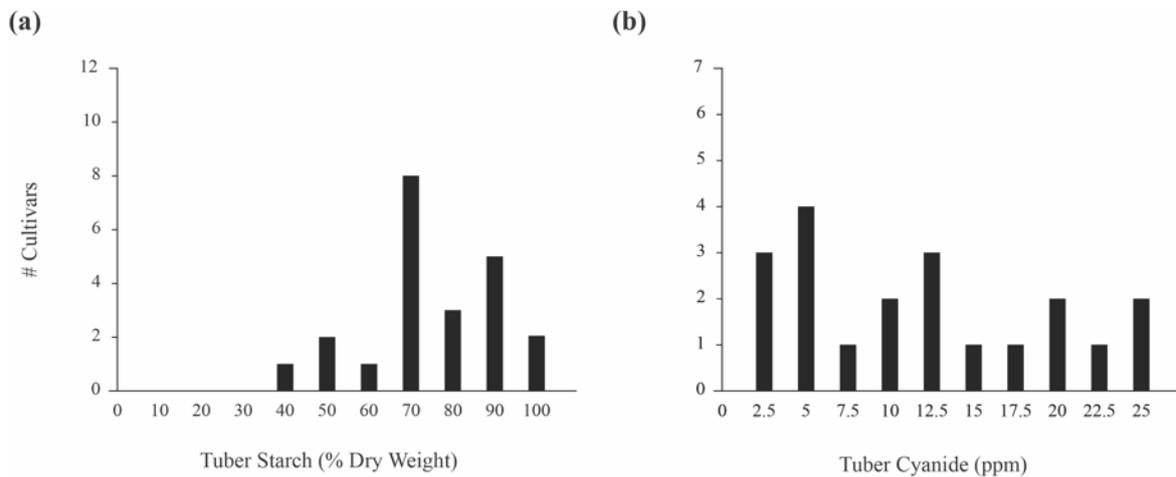


Figure 6. Frequency distributions of (a) starch and (b) cyanide content in tubers.

	Crown width	Lowest branch	Stem diameter	Num. lobes	Lobe length	Lobe width	Leaf area	Petiole length	Num. tubers	Total tuber mass	Tuber length	Tuber width	Cyanide ppm	Starch
Plant height	0.209	0.002	0.030	0.170	0.028	0.034	0.065	0.037	0.161	0.050	0.773	0.003	0.495	0.328
Crown width		0.065	0.001	0.023	0.118	0.059	0.555	0.060	0.740	0.022	0.511	0.381	0.321	0.326
Lowest branch			0.387	0.356	0.919	0.810	0.631	0.820	0.364	0.635	0.640	0.586	0.263	0.222
Stem diameter				0.689	0.551	0.381	0.311	0.770	0.195	0.015	0.417	0.389	0.131	0.446
Num. lobes					0.001	0.170	0.282	0.000	0.793	0.731	0.849	0.134	0.862	0.768
Lobe length						0.002	0.098	0.000	0.986	0.458	0.356	0.002	0.222	0.583
Lobe width							0.028	0.001	0.956	0.035	0.235	0.005	0.118	0.443
Leaf area								0.056	0.567	0.439	0.517	0.094	0.846	0.058
Petiole length									0.702	0.525	0.787	0.004	0.681	0.649
Num. tubers										0.003	0.722	0.185	0.014	0.798
Total tuber mass											0.073	0.667	0.013	0.831
Tuber length												0.498	0.344	0.702
Tuber width													0.484	0.880
Cyanide ppm														0.983

Figure 7. Results of pairwise regression analyses. Red cells indicate *p*-values below 0.05. Darkness of shading indicates closeness to *p* = 0, with light shading being near 0.05 and dark shading being near *p* = 0.

The first two principal components (PCs) in the PCA jointly accounted for 53% of the observed variance, with 38% on PC 1 and 15% on PC 2 (Figure 8). The accompanying loadings indicated that much of the variation in the first principal component was accounted for by the five leaf phenotypes (width, length, area, lobes, and lobe width). Substantial variance was also accounted for by tuber phenotypes including mass, number, length, and cyanide content, along with crown width and stem diameter. The inclusion of data on the river of origin in the PCA revealed that Itaya River was distinct from the Orosa with respect to 95% confidence intervals. However, when all rivers were considered, there was extensive overlap among all of them except for the Itaya, which was somewhat separate.

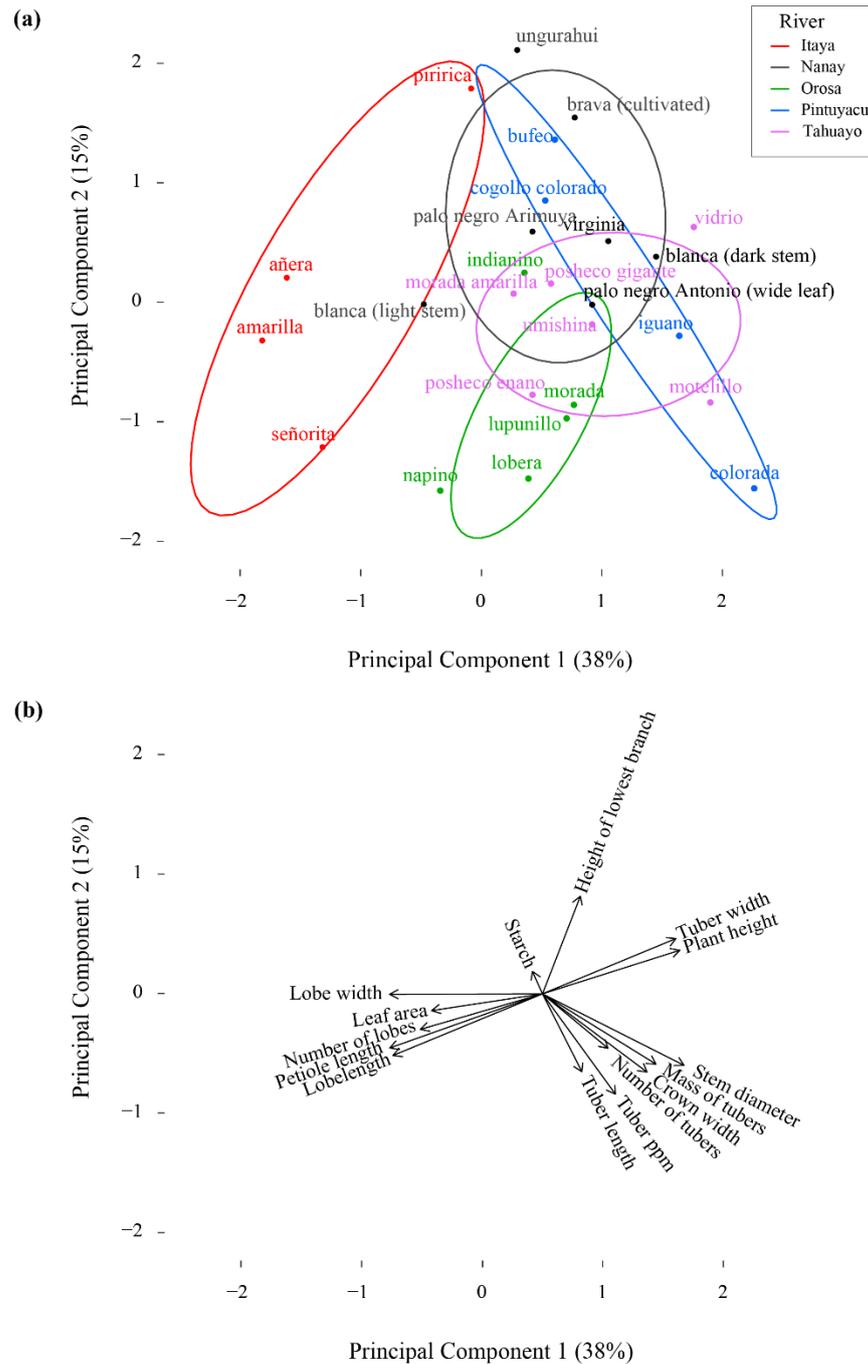


Figure 8. Principal components analysis. (a) Plot of cultivars on PCs 1 and 2, which jointly captured 53% of observed variance, with ovals indicating 95% confidence intervals for rivers. (b) Loading plot illustrating phenotype-specific contributions to variance.

4. Discussion

Cassava agriculture was ubiquitous and culturally embedded in our study area, dominating production by small communities in the region. Among the dozens of settlements we visited, all but one had residents maintaining chacras dedicated to cassava. Consistent with its pervasive presence, cassava was a cornerstone of residents' diets along with fish and plantains, and a focus of daily activity. As expected given cassava's nutritional and cultural significance, growers were highly practiced and knowledgeable about its management, attentive to the characteristics of cultivars, and selective about those grown in their fields. These patterns echo those in other regions of Amazonia, and support the longstanding hypothesis that humans are the impetus behind cassava's diversification throughout the Amazon basin [19].

4.1. Nomenclature

The most evident indicator of cassava diversity in our study was its nomenclature. In total we observed 45 cassava cultivars with traditional names and heard of no named variety that we could not examine directly. The names were highly variable and nearly always descriptive, and incorporated both Spanish and Yaguan (the prevailing indigenous language) terms (Table 1). Some referred specifically to the cultivar's traits, such as cogollo morado (whose uppermost leaf tufts are purple), amarilla (whose tubers' flesh is yellow), and palo blanco (whose main stem was nearly white). Others were reflected by their presumed place of origin, such as napino (from Napo River), indianino (from the town of Indiana), and palo negro Antonio (a palo negro variety discovered by a neighbor named Antonio). Many names were fanciful yet also descriptive. For instance, growers reported that bufeo (river dolphin) has tubers shaped like their namesake, long and thin. Similarly, vidrio (glass) was characterized by the fragility of its tubers. Other notable examples included arpón (harpoon), whose leaf lobes are arrow shaped, and motelo (tortoise), a short and broad variety.

Another linguistic aspect of cassava culture in our study region was that traditional cultivar names incorporated two general terms for the plant, yuca or rumo, with yuca being a borrowed Spanish term and rumo being the Yagua term. Both terms were common on all five of the sampled rivers. They were used interchangeably with the base names of the cultivar (e.g., iguano or añera), regardless of whether it was Spanish or Yaguan. However, while the term yuca was used as both a prefix or a suffix in names, rumo was used exclusively as a suffix. For instance, the amarilla type was interchangeably referred to as yuca amarilla or amarilla yuca, while gallinazo was referred to only as gallinazo rumo. Other examples included andioca (yuca andioca or andioca yuca), añera (yuca añera or añera yuca), ungurahui (only ungurahui rumo), and arpón (only arpón rumo). However, there was no clear pattern in the terms' usage, which seemed to be a matter of convention and convenience.

The naming conventions that we observed were similar to those reported in previous studies but differed somewhat. The number of traditionally named cultivars we found (45) is similar to that found by Boster [22], who reported 50 cultivars grown by the Aguaruna in the Upper Peruvian Amazon, Elias et al. [32], who reported 76 grown by the Makushi in Guyana, Fraser [8], who reported 45 on the middle Madeira River, and Salick et al. [9], who reported ~150 grown by the Amuesha (also in the Upper Peruvian Amazon). While not perfectly comparable due to differences in the populations under study, these findings suggest that growers likely have access to 50–150 named cassava cultivars in their general regions. In addition, the naming categories that we observed closely agree with those identified by Boster [22], who noted that cassava type names among the Aguaruna derive from four major sources: folk genera of plants and animals, descriptions of characteristics of the cultivar, place of presumed origin, and name of the person who introduced it. Similar patterns were observed by Salick et al. [9], and Elias et al. [32]. However, while the cassava cultivars studied by Boster, Salick et al., and Elias et al. were named using indigenous

languages, cassava types in our study region were named in Spanish, or occasionally a combination of Spanish and Yaguan.

A further layer of complexity in cultivar naming was that different growers at a location usually used the same name for a given cultivar, but not always. Further, when names did differ, they were sometimes related. For instance, in one case, three growers had a friendly dispute in our presence about the name of a single specimen, with one calling it arpón (harpoon cassava), one calling it lobera negra (black sword cassava), and a third calling it flecha (arrow cassava). Thus, the names differed, but all three reflected the shape of the plant's leaf lobes, which have an unusual pointed shape reminiscent of all three terms (Figure 5). Together these findings indicate that while names within localities are generally consistent, they are not perfectly so. In addition, different localities may have different names for the same cultivar, or give the same name to different cultivars. Thus, cultivar names are accurate identifiers but they are not perfect and should not be viewed as diagnostic outside local areas.

4.2. Chacra Organization

Of the 45 cassava cultivars we identified, all were found in chacras on terra firme, high land rarely flooded by changes in river level, rather than várzea, which is periodically inundated. The distinction is important because the two landscapes differ profoundly in growing conditions [8]. Because várzea is periodically flooded, which removes low vegetation and deposits sediment, it provides naturally open areas with nutrient rich substrates, allowing rapid planting and growing (6–9 months). However, cassava grown in várzea must be planted as soon as river levels subside and must mature quickly or the crop is ruined by new floods. In contrast, chacras on terra firme require major preparation including clearing of forest followed by burning to enrich the soils prior to planting—An enormous investment of time and energy. As a result, chacras on terra firme allow longer growing times (12–18 months) because they are invulnerable to floods. However, they are not naturally replenished with nutrients and must be moved every few years. The availability of two distinct land types provides cassava growers with the opportunity to make strategic decisions about which terrain type to plant and when, optimizing productivity.

We found that while the presence of different terrain types offers the opportunity to make decisions about growing conditions, in practice growers preferred to plant their crops on high ground. They offered two reasons for this. First, they often stated that they prefer chacras in terra firme simply because it is what they are used to, opting to grow in várzea only when they needed to plant and harvest quickly for some reason, such as in the case of a lost crop. However, they also cited a specific advantage to chacras on terra firme, which related to cassava being a subsistence crop rather than a cash crop on the tributaries we visited. Subsistence growers often prefer to harvest the crop slowly as needed rather than all at once, periodically uprooting a limited number of tubers for a series of meals or to make masato (cassava beer), and occasionally larger numbers to make fariña (cassava meal). One way this was accomplished was by planting a combination of cultivars with differing maturation times, allowing continuous harvest for an extended period. One grower reported strategically planting cultivars with 6-, 12-, and 18-month maturation times simultaneously, a very large time and energy investment allowing gradual harvest for nearly two years. Another approach to extended harvesting took advantage of cultivars' differing durability underground. In this case, less durable varieties were harvested first and more durable types harvested later.

A key observation was that fruiting cassava plants were often present in chacras. Fruiting in cassava chacras has been reported at many sites in the Amazon and growers are known to selectively incorporate the resulting hybrids into their crops [33]. The extent of fruiting we observed varied with the level of chacras' maintenance. New chacras, which were usually well maintained and clear of vegetation except for the crop plants, tended to include few if any fruiting plants, and then only at their forest boundaries. Older chacras, which were not as well maintained, often contained some fruiting plants among

the crop with more at their peripheries. Fruiting was common in abandoned chacras. These observations, together with evidence that different cultivars are interplanted, confirm that sexual reproduction occurs to varying extents in chacras and likely includes cultivar hybridization. Thus, while we did not find evidence that sexual reproduction in chacras was actively fostered by humans, it was a common result of agricultural practices and chacra organization.

Studies in other regions of Amazonia found that growers selectively incorporate the offspring of sexual reproduction into their crops [14,25,32–34]. This is a potentially important source of new genetic and phenotypic variation. We did not directly observe growers importing novel offspring, but we believe it does occur. Sexual reproduction was common in chacras and offspring were available for growers to propagate should they find one. Thus, growers had frequent opportunities to do so. In addition, because the reproduction was occurring in or adjacent to active chacras, incorporation might occasionally occur incidentally, as well.

4.3. Phenotypic Variation

Growers were adept at identifying cultivars on the basis of their morphology. When queried about the cues used, they typically stated that they paid simultaneous attention to the overall size and shape of the plant, its branching tendencies, its stem, the shape of its leaves, and its tubers. The loading plot from our PCA revealed trends consistent with this. It indicated that a major fraction of variance among cultivars was accounted for by shared contributions from leaf traits including lobe width, leaf area, number of lobes, petiole length, and lobe length. A similar cluster of phenotypes with shared contributions on the loading plot included tuber length, tuber ppm, number of tubers, mass of tubers, crown width, and stem diameter.

The cyanide levels that we observed indicated that the cultivars in our study region were relatively nontoxic. In a survey of variability in Africa, Chiwona-Karltun et al. [12] found a range from 5 to 800 ppm, and Yeoh et al. [35] found a range from 2 to 390 ppm in Colombia. Globally, cassava cultivars are frequently divided by growers into “bitter” and “sweet/cool” types [10,25,26]. The two differ in taste as a result of cassava’s cyanide generating mechanisms, with toxicity positively associating with bitterness [12,36]. There is no formally recognized threshold distinguishing bitter and sweet/cool cultivars. However, Chiwona-Karltun et al. [12] found that types described by growers as bitter had cyanide contents of 114 ppm or greater and cultivars described as sweet/cool had contents of 31 ppm and lower. All of our samples were predicted to fall into the sweet/cool category under this criterion. This finding agrees with the observation of Bradbury et al. [26] that bitter cassava cultivars on the Amazon tend to be restricted to eastern regions, far downriver of our study site.

As expected given their anatomical relationships, we found associations between phenotypes relating to the above ground structure of the plant generally had low p -values. For instance, low p -values found between plant height and lowest branch, and between crown and width stem diameter ($p = 0.002$ and 0.001 , respectively) were anticipated because they contribute jointly to the plant’s stature and are anatomically connected. The low p -values of associations between leaf phenotypes such as petiole length and number of lobes ($p = 0.001$), lobe width and lobe width ($p = 0.002$), and number of lobes and lobe length ($p = 0.001$), were similarly anticipated due to their anatomical relationships. However, unexpected patterns were also present. For example, the lack of association between plant height and crown width ($p = 0.21$) was not anticipated because they would seem to both be a function of the overall size of the plant.

The lack of association between starch content and other phenotypes was particularly noteworthy. Because cassava is grown nearly exclusively for its starch production, relationships between starch content and other phenotypes could provide important cues for growers aiming to maximize productivity. However, we found no association between starch content and any other measure ($p > 0.2$ for 13 of 14 tests and $p = 0.06$ for the 14th, leaf

area). In addition, the vector of starch's contribution in the PCA loading plot was short on both PC 1 and PC 2, suggesting starch content accounts for little systematic variance among cultivars (Figure 8). We propose an explanation for both results. Because starch content is a critical criterion for crop value it has likely been under purifying selection exerted by growers aiming to keep it high. In addition, the fact that the starch content does not appear to associate with other phenotypes suggests that it has been evolving independently. Thus, the lack of association between starch content and other phenotypes is consistent with its longstanding importance to Amazonian cassava growers.

Like starch content, cyanide content is an explicitly important consideration for cassava growers in Amazonia. It is essential because it determines the pest resistance of the crop, affecting its productivity and the processing effort required to render it edible [10]. Thus, as with starch content, associations between cyanide content and other phenotypes could be a useful cue for growers aiming to optimize production. In this case, the results of our regression analyses revealed potentially important associations. Consistent with Nye's findings [24], most phenotypes showed no sign of association with toxicity ($p > 0.12$ for 12 of 14 tests). However, associations of cyanide content with number and mass of tubers both had low p -values ($p < 0.015$). This suggests that tuber morphology may be a predictor of cyanide content that could guide decisions about processing. Because this finding was unexpected and discovered after we had left the field site, we have not yet had the opportunity to ask growers about whether they recognize the relationship.

The extensive overlap between rivers of origin in the PCA suggested that the phenotypic differences between them are small and lie on a continuum (Figure 8). This may be explained by our anecdotal observation that growers often traverse the region to visit family, hunt, purchase supplies, and other activities, and might carry cultivars with them as they travel. Evidence of cultivar exchanges potentially resulting in migration was documented in Africa by Delêtre et al. [37], and in Amazonia by Heckler and Zent [38], Boster [7], and others. Such migration can have potent diluting effects on population structure, and we hypothesize that it accounts for the lack of divergence between the rivers that we studied [39].

5. Conclusions

Our findings confirm many previous reports on cassava-based agriculture in the Amazon. We found that, as in most of Amazonia, cassava is the primary crop in our study region and a focal point in agriculture and nutrition. As in other regions, cassava growers in the area cultivate numerous distinct varieties, which are maintained for their differing attributes and often named after them. As reported previously, we observed high levels of phenotypic diversity among cultivars, and growers were skilled at recognizing them on the basis of appearance. In addition, fruiting plants were common in and around chacras, confirming that sexual reproduction is likely a source of novel genetic variation growers incorporate into their crops.

Our findings also shed new light on human influences. Our principal component analyses provide the first evidence independent of growers' self-reports that, while cultivars vary in many phenotypes useful for recognition, leaf phenotypes are likely a particularly informative cue. Our findings also suggest for the first time that starch content, arguably the most important of cassava's phenotypes, is not associated with other important aspects of morphology. This is evidence that starch content has been under selective pressure from growers independent of other traits, testifying to its particular importance among Amazonian peoples. Further, cultivars' toxicity was uniformly low at our study site, distinguishing it from Lower Amazonia, where high toxicity varieties are common. Lastly, we found little or no phenotypic differentiation among cultivars from different rivers, and cultivar names were indicative of having nonlocal origins. We hypothesize that this may have been due to growers' travel and transport of cultivars across the region.

A powerful tool for further investigating the basis of diversity among cultivars in the Upper Amazon is genetic analysis, which can reveal the mechanistic basis of form,

function, and evolutionary history. Genetic studies of phylogenetic relationships among cassava cultivars in other regions have uncovered key aspects of cassava’s geographical origins and dispersal, particularly in Lower Amazonia [40,41]. Applying these methods to our Upper Amazonian sample places diversity there in the broader context of cassava’s emergence and diffusion. Evidence of hybridization in cassava also continues to emerge, and genetic approaches have the potential to quantify and characterize the impact of the sexual reproduction we observed in many chacras [33]. Lastly, the advent of whole genome sequencing is yielding new opportunities to detect the effects of natural selection, including pressures on specific genes [16,41]. This likely brings forward strides in answering questions about the evolution of traits specifically favored and disfavored by growers throughout the history of the crop.

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