**INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA – INPA**
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# DINÂMICA EVOLUTIVA DE MANDIOCA (*Manihot esculenta* CRANTZ) EM TRÊS TIPOS DE SOLO MANEJADOS POR CABOCLOS NA REGIÃO DO MÉDIO RIO MADEIRA, AMAZONAS

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Manaus – Amazonas
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**Sinopse:**

Estudou-se a distribuição e a estrutura da diversidade genética de variedades de mandioca cultivadas tradicionalmente em três tipos de solo por comunidades de agricultores familiares no município de Manicoré, Amazonas. Foram conduzidos dois esquemas de amostragem, um com foco na distribuição da diversidade genética em escala local e outro com foco na diversidade genética intra-varietal.

**Palavras-chave:** Diversidade genética, estrutura genética, marcadores microssatélites, Terra Preta, Latossolos, Várzea

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*"Com efeito, o nosso conhecimento é limitado e a nossa profecia é imperfeita. Mas, quando vier o que é perfeito, desaparecerá o que é imperfeito."*
1Cor 12, 9-10

*"…o mal do século é a solidão, cada um de nós imerso em sua própria arrogância esperando por um pouco de afeição..."*
"Eperando por mim", Renato Russo

*"…we would share and listen and support and welcome*
*be propelled by passion not invest in outcomes*
*we would breath and be charmed and amused by difference*
*be gentle and make room for every emotion…"*
"*Utopia*", Alanis Morissette

**RESUMO**

A mandioca (*Manihot esculenta* Crantz) é o cultivo alimentício domesticado de origem amazônica mais importante no mundo. As variedades de mandiocas bravas são uma das principais fontes de energia para as populações tradicionais da Amazônia Central, e um crescente corpo de trabalhos tem aumentado o entendimento sobre a dinâmica evolutiva da mandioca sob cultivo tradicional. Entretanto a maioria destes estudos tem sido realizada em roças individuais ou em comunidades com acesso a um único tipo de solo, e em geral, no caso da região amazônica, nos Latossolos e Argissolos inférteis de terra firme, ignorando as observações de que as variedades de mandioca bravas também são cultivadas em solos de maior fertilidade. Recentes observações etnobotânicas ao longo da região do médio Rio Madeira constataram que várias comunidades de agricultores tradicionais plantam mandioca em ambientes de alta fertilidade, como os solos antropogênicos e solos de várzea, além dos Latossolos de terra firme. Nesta região foi observado que os agricultores manejam conjuntos específicos de variedades para cada tipo de solo, e que as variedades plantadas na várzea possuem características similares às variedades de solos antropogênicos. Estas observações geraram as hipóteses de que comunidades com acesso a mais tipos de solos devem manter maior diversidade genética do que comunidades com acesso a solos menos variados, e que a estrutura genética das variedades estaria relacionada ao tipo de solo em que são plantadas, sendo que as variedades de várzea seriam geneticamente mais relacionadas às variedades de solos antropogênicos. Para se testar tais hipóteses este estudo avaliou a diversidade genética, com base em 10 marcadores microssatélites, em variedades de mandioca bravas cultivadas tradicionalmente em diferentes tipos de solo (solos antropogênicos, Latossolos de terra firme e solos de várzea) ao longo do médio Rio Madeira. As variedades foram coletadas em dois esquemas de amostragem distintos, direcionados para a distribuição da diversidade genética em escala local e para a diversidade genética intra-varietal. No primeiro esquema observou-se que a variedades de várzea possuem maior diversidade genética ($\bar{A}$= 5,2; $H_O$= 0,606) que as variedades de solos antropogênicos ($\bar{A}$= 4,5; $H_O$= 0,538) e Latossolos de terra firme ($\bar{A}$= 4,2; $H_O$= 0,559). As variedades de várzea também são altamente diferenciadas das variedades de solos antropogênicos ($F_{ST}$ = 0,108) e Latossolos ($F_{ST}$ = 0,093), e estes dois últimos menos diferenciados entre si ($F_{ST}$ = 0,016). No segundo esquema observou-se que as variedades possuem uma alta variabilidade genética. Em geral as variedades são altamente diferenciadas entre si, com uma tendência de variedades com o mesmo nome, mas cultivadas em solos antropogênicos e na várzea, serem geneticamente diferenciadas. Adicionalmente, foi

detectado fluxo gênico entre algumas variedades, mesmo que a mandioca seja propagada vegetativamente. Os resultados destes dois esquemas, quando considerados em conjunto, revelam que os agricultores tradicionais da região do médio Rio Madeira mantêm alta diversidade genética entre e dentro das variedades de mandioca brava cultivadas em diferentes tipos de solo. Comunidades com acesso a tipos mais variados de solos não necessariamente mantém maior diversidade genética em suas variedades de mandiocas bravas, sendo isto observado nas comunidades em que o cultivo de mandioca é realizado em solos de várzea. A hipótese baseada nas observações etnobotânicas de estruturação genética relacionada aos tipos de solo parece ser parcialmente verdadeira, visto que existe estrutura genética entre variedades cultivadas em diferentes tipos de solo, mas ao contrário do esperado, as variedades de mandioca cultivadas na várzea parecem ser geneticamente distintas das variedades cultivadas em solos de terra firme (solos antropogênicos e Latossolos). Apesar disso, demonstrou-se que algumas variedades contribuem para a diversidade genética encontrada em outras, independentemente se são do mesmo tipo de solo ou não. Este estudo adiciona um novo componente à discussão da dinâmica evolutiva da mandioca, uma vez que esta é a primeira vez que se observa uma diferenciação genética entre diferentes ambientes de cultivo na Amazônia.

**ABSTRACT**

Manioc (*Manihot esculenta* Crantz) is the most important food crop worldwide that originated in Amazonia. Bitter manioc varieties are one of the most important food staples for traditional peoples in Central Amazonia, and a growing body of studies has increased our understanding of the evolutionary dynamics of the crop under traditional cultivation. However, most of these studies have been undertaken in single plots or in communities with access to a single soil type, and, in the case of Amazonia, generally Oxisols and Ultisols in non-flooded upland plateaus on the *terra firme*, despite the observations that bitter manioc cultivation is also practiced in highly fertile soils. Recently, ethnobotanical observations along middle Madeira River showed that numerous communities of smallholder farmers grow bitter manioc in the highly fertile soils of the floodplain and Amazonia dark earths (ADE), and in the clayey nutrient-poor Oxisols. It was observed that, in this region, farmers manage distinct sets of varieties for each soil type, and those varieties grown in the floodplain and ADE have similar characteristics. Such observations raised the hypotheses that communities in which farmers grow manioc in different soils maintain higher genetic diversity than communities in which manioc is grown on fewer soil types, and that the genetic structure of varieties would be related to soil types, with special emphasis on the relationship of varieties grown in the floodplain with those grown in ADE. To test these hypotheses, this study evaluated the genetic diversity, based on 10 microsatellite markers, of bitter manioc varieties traditionally cultivated in different soil types (namely ADE, Oxisols and floodplain) along the middle Madeira River region. Varieties were sampled in two distinct schemes to evaluate the distribution of genetic diversity on a local scale, as well as intra-varietal genetic diversity. For the first scheme, it was observed that floodplain varieties had greater genetic diversity ($\bar{A}$= 5.2; $H_O$= 0.606) than varieties grown on ADE ($\bar{A}$= 4.5; $H_O$= 0.538) and on Oxisols ($\bar{A}$= 4.2; $H_O$= 0.559). Floodplain varieties were also strongly differentiated from the varieties grown on ADE ($F_{ST}$ = 0.108) and Oxisols ($F_{ST}$ = 0.093), while these latter two soils were less differentiated ($F_{ST}$ = 0.016). For the second scheme, high intra-varietal genetic diversity was observed, along with significant differentiation among varieties, with a tendency for varieties having equivalent names, but grown on ADE and floodplain, to be genetically differentiated. Additionally, gene flow was detected among some of the varieties. When taken together, the results of these two sampling schemes reveal that along the middle Madeira River the traditional farmers maintain high levels of genetic diversity within and among the bitter manioc varieties grown in different soil types. Higher levels of genetic diversity are not

necessarily found in the communities in which farmers plant bitter manioc on more than one soil type: higher genetic diversity was observed for communities located in the floodplain. The hypothesis of closer relationships among varieties according to the soil types in which they are grown is partly true, since the varieties are genetically structured among different soil types, but, contrary to expectations, there seems to be an important genetic differentiation between varieties grown in the floodplain and varieties grown in upland soils (ADE and Oxisols). In spite of such differentiation, it was demonstrated that some varieties collaborate to the genetic diversity found within others, irrespective of whether they are from the same soil type or not. This study adds a new component to the discussion on manioc evolutionary dynamics, since it is the first time that differentiation of manioc varieties among environments of cultivation in Amazonia is examined with molecular data.

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## 1. INTRODUÇÃO GERAL

A expansão da sociedade urbana na Amazônia gera novas demandas para os geneticistas e melhoristas, tanto para melhorar organismos para atender demandas humanas, como para entender a dinâmica evolutiva das populações ainda não objeto de melhoramento, mas sujeitos ao impacto da sociedade moderna. Na Amazônia, a maioria das espécies usadas no setor agrícola é exótica. Num dos principais centros de mega-biodiversidade do planeta observa-se que as espécies nativas são menos visíveis no setor agrícola. Isto se deve em parte ao fato de que as espécies nativas raramente atendem as principais demandas dos mercados modernos: qualidade, uniformidade e preço.

A mandioca (*Manihot esculenta* ssp. *esculenta* Crantz) é o cultivo alimentício domesticado de origem amazônica que alcançou a maior importância no mundo e é uma das poucas exceções à tendência citada acima. É um arbusto perene, considerado bem adaptado a solos ácidos e de baixa fertilidade, sendo cultivado ao redor de toda a região tropical em agroecossistemas com estas características. Produz raízes que acumulam grandes quantidades de amido, sendo a principal fonte de carboidratos para mais de 800 milhões de pessoas e a terceira fonte mais importante de calorias nos trópicos (Lebot, 2009).

O gênero *Manihot* (Euphorbiaceae) compreende 98 espécies que são distribuídas na região Neotropical, ocorrendo desde a região central do México até o norte da Argentina (Rogers e Appan, 1973). As espécies deste gênero são perenes e variam de arbustos a árvores de pequeno porte, sendo que algumas desenvolvem raízes tuberosas, como a mandioca. Várias espécies têm sido apontadas como parentes silvestres da mandioca. Entretanto, hoje se sabe que ela foi domesticada a partir de populações de *Manihot esculenta* ssp. *flabellifolia* do sudoeste amazônico entre o oeste de Mato Grosso, Rondônia, leste do Acre e nordeste de Bolívia (Olsen e Schaal, 1999; Allem, 2002; Schaal *et al.*, 2006).

Após sua domesticação inicial, diferentes pressões seletivas deram origem aos dois grandes grupos de variedades, a mandioca mansa (macaxeira, mandioca doce ou aipim), e a mandioca brava (mandioca, mandioca amarga) (Mühlen *et al*., 2000; Elias *et al*., 2004). Esta dicotomia reflete a variação do conteúdo de glicosídeos cianogênicos (linamarina e lotaustralino), substâncias que, quando hidrolisadas a ácido cianídrico, se tornam altamente tóxicas. Variedades mansas possuem baixos teores destes componentes em suas raízes (<100 mg/Kg), e seu consumo é considerado seguro apenas com o processamento básico (descascamento e cozimento). Por outro lado, variedades bravas possuem teores elevados de tais componentes (>100 mg/Kg) e precisam passar por processamento de detoxificação antes

de serem consumidas. Embora esta classificação seja dicotômica, observa-se que a variação no teor de glicosídeos cianogênicos é contínua e que não há caracteres morfológicos associados especificamente às mandiocas mansas ou bravas (McKey *et al*., 2010a). Entretanto, a distinção entre estas duas classes é imediata entre agricultores tradicionais e é suportada por análises com dados moleculares (Mühlen *et al*., 2000; Elias *et al*., 2004; Peroni *et al*., 2007).

Recentemente Arroyo-Kalin (2010) formulou a hipótese de que a distinção de variedades bravas e mansas estaria relacionada às origens de civilizações sedentárias na Amazônia durante o período pré-Colombiano. Variedades mansas teriam surgido primeiro como fruto da seleção para menor toxidez realizada por horticultores incipientes em ambientes próximos às unidades familiares. Devido à natureza nômade das primeiras sociedades, as variedades mansas teriam sido dispersas amplamente na Amazônia. Com o início do período Formativo, em que houve o aumento do sedentarismo nas Américas, aumentou a necessidade por alimentos, que começaram a ser cultivados em ambientes mais afastados das unidades familiares. Tais ambientes são mais suscetíveis à pragas e doenças, o que favoreceria a seleção de variedades mais tóxicas e que tivessem maior produtividade. Desta forma, as variedades de mandioca bravas resultariam da intensificação da agricultura e do concomitante desenvolvimento de tecnologias que pudessem ser empregadas em sua detoxificação, garantindo a segurança alimentar das primeiras civilizações amazônicas sedentárias.

A mandioca tem sido a fonte de energia mais importante para as populações Amazônicas por milhares de anos (Heckenberger, 1998; Arroyo-Kalin, 2010; Fraser, 2010). Ao contrário do que se observa para a maioria das espécies domesticadas, parece existir uma forte seleção para a obtenção de variedades de mandioca com alta toxidez, especialmente na Amazônia onde a maioria das variedades cultivadas é brava (Clement *et al*., 2010). Alguns estudos identificaram que variedades bravas tendem a ser mais produtivas do que variedades mansas, o que pode ser devido à maior resistência a pragas e doenças por parte das variedades bravas (McKey e Beckerman, 1993; Wilson e Dufour, 2002; Wilson, 2003). A seleção de variedades entre os índios Tukano no noroeste da Amazônia parece estar relacionada às comidas que podem ser preparadas a partir delas, e as variedades bravas são utilizadas no preparo de um maior número de pratos (Wilson e Dufour, 2006).

Em geral, na região Amazônica, se observa que o cultivo de variedades bravas está associado às margens dos principais rios da bacia Amazônica e aos litorais oceânicos sul-americanos. Por sua vez o cultivo de variedades mansas é mais frequente nas cabeceiras

destes mesmos rios, na porção ocidental da Amazônia e é comumente cultivada em menor escala onde as variedades bravas são predominantes (McKey e Beckerman, 1993).

Uma parte considerável do cultivo de mandioca é realizada em áreas rurais pobres sem mecanização e insumos por meio da agricultura tradicional em sistemas agroflorestais de agricultura itinerante (*swidden-fallow cultivation*) (Emperaire, 2005). Nestes sistemas agrícolas, as roças são estabelecidas após a derrubada e queima de áreas de florestas secundárias (capoeiras) ou, menos frequentemente, florestas primárias. Uma vez delimitadas, as roças são utilizadas por períodos que variam de 1 a 4 anos, e depois são deixadas em pousio (os agricultores não cultivam mais a roça e uma nova vegetação secundária é deixada crescer) por um período de até 20 anos, para depois ter a vegetação novamente cortada e queimada e a roça novamente estabelecida (McKey e Beckerman, 1993; Elias *et al.*, 2000; Martins, 2001).

Embora tenha sido domesticada pela seleção de plantas que pudessem ser propagadas vegetativamente, a mandioca não perdeu a capacidade de reprodução sexual. A fertilidade é muito variável entre as variedades, e a produção de flores e sementes se dá de maneira irregular. As sementes são dispersas por dois mecanismos distintos: pela explosão dos frutos deiscentes (autocoria) e por formigas que se alimentam de parte das sementes (mirmecoria) (Elias e McKey, 2000). Além de ajudarem na dispersão, as formigas enterram as sementes que passam a ser componentes do banco de sementes do solo. Estas sementes apresentam dormência que é quebrada por temperaturas elevadas, que são atingidas quando a vegetação que recobria o solo é queimada no início de um novo ciclo de estabelecimento de roças (Peroni e Hanazaki, 2002; Pujol *et al.*, 2002). Entretanto uma elevação de temperatura mais discreta, causada simplesmente pela exposição do solo resultante do corte da vegetação, parece ser suficiente para quebrar a dormência de parte das sementes (Pujol *et al.*, 2002). Desta forma as sementes podem germinar e dar origem a plântulas voluntárias (produzidas por reprodução sexual), que se desenvolvem concomitantemente às variedades que foram propagadas vegetativamente (Martins, 2001; Pujol *et al.*, 2007; Duputié *et al.*, 2009). Na maioria dos casos, os agricultores conseguem distinguir entre as plântulas voluntárias e aquelas que foram plantadas. Os agricultores permitem, consciente ou inconscientemente, que as plântulas voluntárias se desenvolvam. Durante a época da colheita os agricultores podem avaliar se estas plantas devem ser selecionadas para propagação vegetativa (McKey e Beckerman, 1993; Peroni, 1998; Elias *et al.*, 2000; Rival e McKey, 2008). Uma vez que as plantas oriundas de sementes são selecionadas para uma nova propagação elas podem ser incluídas em variedades já estabelecidas e presentes nas roças, ou podem passar a formar uma

nova variedade (Peroni, 1998). Portanto, plântulas oriundas de sementes, germinadas espontaneamente, são essenciais para amplificar a diversidade genética intraespecífica (McKey e Beckerman, 1993; Elias *et al*., 2001; Martins, 2001; Sambatti *et al.*, 2001; Duputié *et al*., 2009). Ao amplificar a diversidade genética, a incorporação de plântulas voluntárias ajuda a manter o vigor vegetativo das variedades locais, já que existe correlação entre o tamanho, heterozigosidade e sobrevivência das plantas (Pujol *et al*., 2006), além de colaborar para a diminuição da susceptibilidade à pragas e doenças (Elias *et al*., 2004; Pujol *et al*., 2005).

Análises genético-moleculares têm corroborado a importância da prática de incorporação de plântulas voluntárias ao material genético ao detectarem valores de polimorfismo elevados em variedades locais (Elias *et al*., 2001; Elias *et al.* 2004; Peroni *et al*., 2007; Duputié *et al*., 2009), e ao demonstraram que as variedades locais são polifiléticas, com a preponderância de um clone e mais um conjunto de clones similares em termos morfológicos, mas diferentes em termos genéticos (Peroni, 1998; Peroni, 2004; Pujol *et al*., 2007). O estudo desta dinâmica evolutiva sob domesticação é essencial para se entender a cultura da mandioca e planejar sua conservação, pois diversos fatores de ordem cultural, social e econômica podem resultar em erosão genética severa da base genética local (Peroni e Hanazaki, 2002; Emperaire e Peroni, 2007).

A dinâmica evolutiva das variedades de mandioca cultivadas tradicionalmente tem sido examinada principalmente em roças individuais ou de comunidades locais de agricultores tradicionais e indígenas, sempre em um único tipo de solo e quase sempre em solos ácidos e pobres de terra firme (Elias *et al*., 2001; Pujol *et al.*, 2005). Uma dinâmica mais complexa existe onde as comunidades usam diferentes solos (Fraser e Clement, 2008). Ao longo do Rio Madeira, que liga o centro de domesticação da mandioca ao centro populacional indígena e moderno da Amazônia Central, numerosas comunidades plantam mandioca em ambientes de alta fertilidade, como nos solos antropogênicos (terra preta e terra mulata) e na várzea, e nos Latossolos e Argissolos de menor fertilidade da terra firme (Fraser e Clement, 2008; Fraser, 2010).

Os solos antropogênicos são encontrados nas áreas que foram ocupadas pelos assentamentos de grupos indígenas que habitavam a região amazônica a centenas ou mesmo milhares de anos, no período pré-Colombiano (Arroyo-Kalin, 2009; Woods e Denevan, 2009). Solos antropogênicos são caracterizados por possuírem fertilidade comparável ou até maior do que os solos da várzea, e muito maior que os Latossolos e Argissolos de terra firme. Estes tipos de solos são mais escuros devido a grande quantidade de carvão vegetal, são ricos

em matéria orgânica, fósforo, cálcio e alguns micronutrientes, e contém vestígios de cerâmica. Estes solos são cultivados intensivamente pelas populações tradicionais atuais, que fazem seu uso com maiores ciclos de cultivo e menores períodos de pousio (Falcão *et al.*, 2009; Fraser *et al.*, 2009).

A utilização de diferentes tipos de solos faz com que os agricultores selecionem diferentes características nas variedades de mandioca. Nas várzeas existe forte seleção antrópica e natural para ciclos curtos, uma vez que os períodos de cheia e vazante do rio comandam o ciclo agrícola, bem como seleção de variedades que cresçam bem em solos ricos em nutrientes. As variedades adaptadas à várzea são igualmente bem adaptadas aos solos antropogênicos, onde a riqueza de nutrientes permite a mesma velocidade de crescimento que na várzea (Fraser *et al.*, 2008; Fraser, 2010). Entretanto, variedades bem adaptadas à várzea, que são mais exigentes em fertilidade, não produzem bem em solos da terra firme, pois os Latossolos e Argissolos naturais da área são mais ácidos e pobres em nutrientes. Os solos de terra firme, em geral, são cultivados por maiores períodos, bem como deixados em maiores períodos de pousio.

Durante estudos etnobotânicos realizados em comunidades de caboclos ribeirinhos ao longo do médio Rio Madeira, Fraser e Clement (2008) e Fraser (2010) detectaram uma tendência ao manejo de variedades de mandioca específicas que são preferencialmente cultivadas em um determinado tipo de solo. Os caboclos que praticam agricultura itinerante nestas comunidades desenvolveram variedades de mandioca selecionadas para diferentes tipos de solo (várzea, solos antropogênicos e solos de terra firme) e diferentes durações de ciclos de plantio (menor para várzeas e solos antropogênicos, e maior para terra firme) (Fraser *et al.*, 2008, Fraser, 2010). Variedades com desenvolvimento mais rápido são denominadas pelos caboclos como “mandiocas fracas”, enquanto as variedades que possuem desenvolvimento mais lento são denominadas “mandiocas fortes”. De acordo com os caboclos, variedades “fracas” são mais produtivas em “solos fracos” e variedades “fortes” são mais produtivas em “solos fortes”. A classificação dos solos não está diretamente relacionada com sua fertilidade, mas sim com a estrutura da vegetação secundária nas áreas capoeiras. “Solos fortes” possuem uma vegetação secundária mais desenvolvida e “solos fracos” possuem uma vegetação secundária menos desenvolvida. Dessa forma solos antropogênicos são considerados “fracos” uma vez que seu cultivo é mais intenso, com menores períodos de pousio (havendo menos tempo para o desenvolvimento de capoeiras), ao passo que solos de terra firme são considerados “fortes”, já que são deixados em maiores períodos de pousio (havendo mais tempo para o crescimento de capoeiras). Entretanto, caso uma área de roçado em solo

antropogênico seja deixada por mais tempo em pousio ela poderá ser considerada como uma área de solo “forte”, o contrário podendo ocorrer caso roçados em áreas de terra firme com Latossolos ou Argissolos sejam usados mais intensivamente. Desta forma, a maioria dos agricultores acaba por experimentar variedades adaptadas a um determinado tipo de solo em outros tipos de solos (Fraser e Clement, 2008). Assim, existe a possibilidade de que sementes de plantas mais bem adaptadas a um tipo de solo sejam adicionadas ao banco de sementes de outro tipo de solo e que, ao germinarem, dividam espaço com variedades diferentes e melhor adaptadas, criando a possibilidade de fluxo gênico entre variedades adaptadas a solos diferentes. Essa dinâmica entre diferentes tipos de solo deveria ajudar a manter mais diversidade genética em comunidades que utilizam mais de um tipo de solo do que em comunidades que utilizam apenas um solo. Fraser e Clement (2008) e Fraser (2010) também observaram que as variedades de várzea e de solos antropogênicos possuem características similares (como maturação rápida e menor quantidade de amido) e diferentes das variedades plantadas nos solos inférteis da terra firme (maturação mais lenta e maiores quantidades de amido). Desta forma, estes autores também sugeriram que as variedades de várzea seriam geneticamente mais similares às variedades de solos antropogênicos e estas duas seriam diferenciadas das variedades dos solos inférteis de terra firme.

Estas hipóteses são facilmente testadas com a aplicação de marcadores moleculares no estudo da organização e distribuição da variação genética de variedades de mandioca cultivadas tradicionalmente nas comunidades envolvidas. Os marcadores moleculares são quaisquer características das cadeias polipeptídicas ou das sequências de DNA que se prestam à diferenciação de dois ou mais indivíduos em uma população e que são herdadas geneticamente (Milach, 1998). Os marcadores moleculares microssatélites são atualmente os mais utilizados para estudos genéticos de populações naturais ou cultivadas. Microssatélites são sequências curtas de DNA com 1 a 6 nucleotídeos repetidos em tandem, que se distribuem aleatoriamente por todo o genoma, são codominantes e apresentam alto grau de polimorfismo (Powell *et al.*, 1996; Goldstein e Pollock, 1997). Estas propriedades fazem com que os microssatélites sejam adequados para análises que envolvam grupos de indivíduos com divergência recente, como populações ou variedades de uma mesma espécie.

Atualmente existem 186 locos microssatélites para mandioca descritos na literatura (Chavariaga-Aguirre *et al*., 1998; Mba *et al.*, 2001). Estes marcadores microssatélites vêm sendo utilizados para quantificar a diversidade genética presente nas amostras de bancos de germoplasma e coleções nucleares (Chavariaga-Aguirre *et al*., 1998; Fregene *et al*., 2003; Elias *et al.*, 2004; Siqueira *et al.*, 2009), no auxílio à determinação do centro de origem do

cultivo (Olsen e Schaal, 2001; Olsen, 2004), para a validação da taxonomia popular (*folk taxonomy*) feita pelos agricultores (Peroni *et al*., 2007), detecção de zonas de hibridização entre a mandioca e seus parentes silvestres (Duputié *et al.*, 2007), além de estimar a diversidade genética presente nas variedades de mandioca cultivadas e manejadas tradicionalmente e suas implicações para a dinâmica evolutiva do cultivo (Mühlen *et al*., 2000; Elias *et al.*, 2001; Peroni, 2004; Pujol *et al.*, 2005; Duputié *et al*., 2009).

Esta dissertação é formada por dois capítulos que se referem ao teste de hipóteses genéticas que foram levantadas a partir de observações etnobotânicas na região do Médio Rio Madeira, como mencionado acima, com base em diferentes esquemas de amostragem. No capítulo 1 foi avaliada a distribuição da diversidade genética apresentada por variedades de mandioca bravas em escala local. Os níveis de diversidade genética para o conjunto de variedades cultivadas em diferentes tipos de solo em diferentes comunidades foi avaliado para testar as hipóteses de que comunidades com acesso a um maior número de solos manteriam maior diversidade genética do que comunidades com acesso a um menor número de solos, e de que os tipos de solo estão relacionados à estruturação genética das variedades. No capítulo 2 foi avaliada a diversidade genética intra-varietal das variedades mais comumente cultivadas nos solos antropogênicos, solos de várzea e solos inférteis da terra firme na região do médio Rio Madeira. Foram testadas as hipóteses de que variedades cultivadas em várzea seriam geneticamente mais relacionadas às variedades de solos antropogênicos, e se variedades colaboram para a diversidade genética encontrada em outras variedades distintas.

Este trabalho busca colaborar para um maior entendimento de um manejo aparentemente mais complexo da diversidade genética local e de como esta diversidade genética está estruturada entre as variedades de mandioca cultivadas em diferentes tipos de solo na região do médio Rio Madeira, adicionando informações novas à discussão da dinâmica evolutiva sob domesticação da mandioca sob cultivo tradicional em diferentes ambientes amazônicos.

# 2. OBJETIVOS

## 2.1. OBJETIVO GERAL

- Avaliar a dinâmica evolutiva de variedades de mandioca (*Manihot esculenta* Crantz) cultivadas tradicionalmente em três tipos de solo (várzea, solos antropogênicos e solos inférteis de terra firme) e manejadas por caboclos da região do médio Rio Madeira, Manicoré, Amazonas, Brasil.

## 2.2. OBJETIVOS ESPECÍFICOS

- Estimar, com marcadores microssatélites, a diversidade genética apresentada por variedades bravas de mandioca cultivadas em comunidades com acesso a diferentes tipos de solo;

- Avaliar como a variabilidade genética de conjuntos de variedades presentes nas roças está estruturada entre os diferentes tipos de solos manejados por diferentes comunidades de caboclos;

- Estimar a diversidade genética dentro das variedades de mandioca mais comumente cultivadas nos solos antropogênicos, solos de várzea e solos inférteis da terra firme na região do médio Rio Madeira;

- Avaliar, por meio dos padrões de fluxo gênico, se as variedades cultivadas em solos antropogêncos e de várzea são geneticamente mais relacionadas entre si do que com as variedades cultivadas em solos inférteis da terra firme.

## Capítulo 1

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**Genetic structure of traditional varieties of bitter manioc in three soils in Central Amazonia**

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Running title: Genetic structure of manioc in three Amazonian soils

## Abstract

Manioc is the most important food crop that originated in Amazonia and many studies have increased our understanding of its evolutionary dynamics under cultivation. However, most of these studies focused on manioc cultivation in environments of low soil fertility, generally Oxisols. Recent ethnobotanical observations showed that bitter manioc performs well in high fertility soils, such as Amazonian dark earths (ADE) and the floodplain. We used 10 microsatellite loci to investigate the genetic diversity and structure of bitter manioc varieties grown in different soil types in communities of smallholder farmers along the middle Madeira River in Central Amazonia. Bitter manioc varieties from the floodplain showed higher levels

of genetic diversity ($\bar{A}$= 5.2, $H_O$= 0.606) than ADE ($\bar{A}$= 4.5, $H_O$= 0.538) and Oxisols ($\bar{A}$= 4.2, $H_O$= 0.559). The varieties grown in the floodplain were strongly differentiated from the varieties grown in Oxisols ($F_{ST}$ = 0.093) and ADE ($F_{ST}$ = 0.108), suggesting an important genetic structure among varieties grown in the floodplain and upland soils (ADE and Oxisols). This is the first time that genetic divergence of bitter manioc in cultivation in different Amazonian soils in a small geographic area is reported.

**Introduction**

Manioc (*Manihot esculenta* Crantz ssp. *esculenta*) was domesticated from wild populations of *M. esculenta* subsp. *flabellifolia* in southwestern Amazonia, in what are now Rondônia and northwestern Mato Grosso states in Brazil (Allem 1994; Olsen & Schaal 1999, 2001; Olsen 2004). Today manioc is the sixth major food crop produced globally and the primary staple for more than 800 million people in the tropics (Lebot 2009).

After its initial domestication, different selective pressures originated two major groups of varieties. Sweet manioc has low contents of cyanogenic glycosides (<100 ppm fresh weight), while bitter manioc has larger amounts of cyanogenic glycosides (>100 ppm fresh weight) (Mühlen *et al*. 2000; Elias *et al*. 2004; McKey *et al*. 2010a). Although this classification is dichotomist, continuous variation in the content of cyanogenic glycosides is observed among varieties and there are no morphological characters that differentiate these two groups of varieties (McKey *et al*. 2010a). However, the separation of bitter and sweet manioc is genetically supported (Mühlen *et al*. 2000; Peroni *et al*. 2007) and it is recognized by farmers (McKey & Beckerman 1993; Elias *et al*. 2000; Peroni *et al*. 2007).

In Amazonia, most manioc cultivation is done by smallholder farmers with low inputs in traditional systems of swidden-fallow cultivation (Emperaire 2005). The swiddens are established in areas of secondary vegetation that are cleared and burnt (slash and burn), used for 1 to 4 cycles of cultivation and then fallowed for a variable period of time before a new swidden is established (McKey & Beckerman 1993; Martins 2001).

Although manioc is vegetatively propagated, sexual reproduction plays an important role in the evolutionary dynamics of the crop. The seeds produced become part of the soil seed bank and may sprout among the vegetatively propagated varieties in the plot (Martins, 2001; Pujol *et al*. 2007; Duputié *et al*. 2009). Smallholders, consciously or unconsciously, may let the volunteer seedlings (originated from sexual reproduction) grow and by the time of

harvest they decide if they will use these plants for the next cycle of vegetative propagation (Elias *et al.* 2000; Rival & McKey 2008). If the plants from seedlings are used for clonal propagation, the farmers may either incorporate the seedlings into an existing variety or create a new variety (Elias *et al*. 2000; Martins 2001; Duputié *et al.* 2009).

Genetic analysis demonstrated the importance of the incorporation of seedlings for maintaining high polymorphism and showed that local varieties are polyclonal, with one predominant clone and a set of individuals with similar phenotypes, but different genotypes (Elias *et al*. 2001; Peroni *et al*. 2007; Duputié 2009). These analyses of the evolutionary dynamics under cultivation are essential for planning manioc conservation, since many factors may cause severe genetic erosion of the genetic resources of manioc (Peroni & Hanazaki 2002; Emperaire & Peroni 2007). However, such studies have focused mainly on single swiddens or single local communities of smallholder or indigenous farmers, generally located in upland areas of low soil fertility, partially because manioc is thought to be the ideal crop for low input traditional systems on nutrient poor soils (McKey *et al*. 2010a).

The evolutionary dynamics may be more complex where manioc is cultivated in different soil types. Recently, Fraser & Clement (2008) and Fraser (2010), working in communities along the middle Madeira River, in central Brazilian Amazonia, observed that bitter manioc also performs well in high fertility soils, such as Amazonian Dark Earths (ADE) and Fluvent Entisols of the floodplains. ADE are fertile anthropogenic soils found in areas occupied by Amerindians in the pre-Columbian period (Arroyo-Kalin 2009; Woods & Denevan 2009). These soils have large amounts of organic matter, phosphorous, calcium, other micronutrients and pieces of ceramics, and are dark in color due to large amounts of charcoal. The Fluvent Entisols found in the floodplains of Amazonian whitewater rivers result from the deposition of nutrient rich sediments. Both are much more fertile than the highly weathered clayey Oxisols of the non-flooded upland plateaus and have historically produced nearly a third of the manioc flour consumed in Manaus, the capital of Amazonas (Gutjahr 2000).

Fraser & Clement (2008) and Fraser (2010) argued that communities of smallholder farmers along the middle Madeira River manage different configurations of bitter varieties for the different soil types in which manioc is cultivated. They hypothesized that higher genetic diversity would be expected for communities in which manioc is cultivated in more than one soil type. Additionally, these authors observed similar agronomic characteristics among the varieties grown in ADE and in the floodplain, such as fast maturation and low starch content,

distinct from those observed for the varieties grown in Oxisols, which mature more slowly and have higher starch content. Their interviews elicited information that farmers plant floodplain varieties more often in ADE than in Oxisols, suggesting a close relationship between these soils.

We used a set of 10 microsatellite loci to evaluate the genetic diversity and the genetic structure of bitter manioc varieties systematically sampled in different soil types in communities of traditional smallholder farmers along the middle Madeira River in order to explore two of the main questions raised by ethnobotanical observation. Do communities that cultivate bitter manioc in different soil types present higher genetic diversity? Is the genetic diversity of bitter manioc varieties structured according to the soil types, with a closer relationship of varieties grown in ADE and in the floodplain?

## Materials and Methods

### *Field sites and authorizations*

The manioc varieties sampled in this study are cultivated in the municipality of Manicoré (5°18'S; 61°18'W), Amazonas state, Brazil, along the middle Madeira River's bluff plateaus and floodplain. Manicoré is an essentially agricultural municipality, with numerous communities of traditional smallholder farmers that produce vegetables and fruit for sale to the major urban center of Manaus, the state capital. Manioc is one of the most important crops. Manioc cultivation is practiced in traditional swidden-fallow agriculture systems on *terra firme* (non-flooding plateaus) Oxisols and Amazonian Dark Earths, as well as on floodplain Entisols (Fraser & Clement 2008).

Varieties grown in Oxisols and ADE soils were sampled at the communities of Água Azul, Barreira do Capanã and Barro Alto. Varieties grown in floodplain soils were sampled at Fortaleza, Pau Queimado, Verdum, Água Azul and Barreira do Capanã communities (Figure 1). At each community, objectives of the study were explained to obtain prior informed consent and no proprietary traditional knowledge was accessed, which allowed us to meet Resolution 21 requirements for basic research that does not require authorization from Brazil's Council for Genetic Patrimony (CGEN in the Brazilian acronym), which was consulted before field work. Our study was authorized by the *Instituto Nacional de Pesquisas da Amazônia*'s Committee for Research Ethics (protocol 235/09).

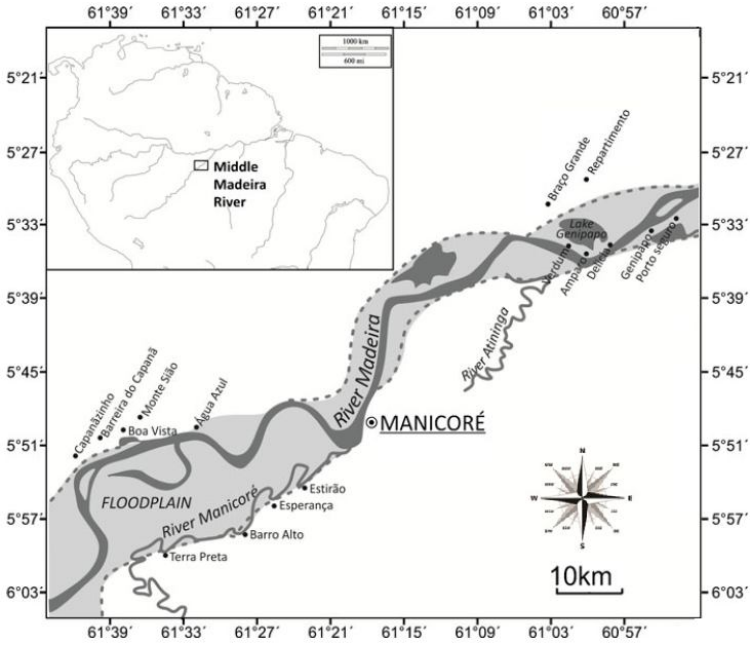


Figure 1. Map showing the study site near the municipality of Manicoré in the middle Madeira River region. (Map by Victoria Frausin)

***Variety sampling***

Adult individuals of each variety were collected in swiddens, following a hierarchical sampling design in which the swiddens represent the household unit *inside* soil types, *inside* communities (Peroni *et al*. 2007). We visited 48 swiddens (17 in ADE soils, 14 in floodplain soils and 17 in Oxisols) and, with the consent of its owner, leaves of 1 plant of each variety of bitter manioc recognized by the farmer in the plot were sampled. Seedlings and sweet manioc varieties in each swidden were also opportunistically sampled. Additionally, seedlings in two recently burnt but not yet planted fields were also sampled (1 field in Oxisol and 1 in the floodplain), with 16 and 34 seedlings sampled, respectively. A total of 367 individuals were sampled (184 of bitter manioc, 21 of sweet manioc and 162 seedlings), with an average of 7.3 individuals/swidden, representing 53 varieties named by the farmers (43 bitter and 10 sweet). Each swidden had 1-9 varieties, with 0-17 seedlings. When sampling by soil types is considered, ADE had 110 individuals (46 seedlings), floodplain 111 (42 seedlings) and Oxisols 146 (74 seedlings). Leaves were dried in silica gel, and later stored at -20°C in freezers at the laboratory.

***DNA extraction and microsatellite genotyping***

Genomic DNA was extracted from 50 mg of powdered leaf tissues following the CTAB protocol (Doyle & Doyle 1987), with a minor modification (β-mercaptoethanol was not used). DNA was quantified by comparison with known concentrations of standard DNA (lambda DNA – Fermentas) in electrophoresis agarose gels (0.9% w/v) stained with GelRed (Biotium).

In order to provide a basis for comparisons with previous studies, 10 microsatellite markers were chosen. Seven of them (GA21, GA126, GA131, GA134, GA136, GA140, GAGG5) were described by Chavarriaga-Aguirre *et al.* (1998), and 3 (SSRY13, SSRY89, SSRY 164) by Mba *et al.* (2001). PCR reactions were carried out in a final volume of 10 µL with 20 ng of genomic DNA, 1X buffer ($Mg^+$ free), 2.5 ng of BSA, 2.5 mM of $MgCl_2$, 250 µM of each dNTP, 2.5 pmols of each forward and reverse primer, and 1 U *Taq* DNA polymerase. Amplifications were carried out in a Verirti thermocycler (Applied Biosystems) as follows: an initial denaturation step of 94°C for 2 min followed by 30 cycles at 94°C for 1min; 56°C for 1 min; 72°C for 2 min, and a final step of extension at 72°C for 25 min. Quality and non-ambiguous amplification were checked by electrophoresis in agarose gels (2% w/v) stained with GelRed (Biotium). Each forward primer sequence was labeled with fluorescence (either FAM, 6-HEX or NED), which allowed genotyping in multiplexed systems in the DNA sequencer ABI3130xl (Applied Biosystems). GeneScan™ -500 ROX™-Size Standard (Applied Biosystems) was used to size the alleles, and data collection and analysis were performed using the GeneMapper software v.4.0 (Applied Biosystems).

***Data analysis***

*Genetic diversity*

Genetic diversity parameters including total (A) and mean ($\bar{A}$) number of alleles, observed ($H_O$) and expected ($H_E$) heterozigosities, number of private alleles (Ap) and the inbreeding coefficients (*f*, Weir & Cockerham 1984) were estimated with GenAlEx (Peakall & Smouse 2006) for each locus and for each group of varieties.

The recognition of identical and distinct multilocus genotypes (MLGs) was performed with GenClone v.2.0 (Arnaud-Haond & Belkhir 2007) in order to investigate the congruence in farmers' identification of individuals from the same and different varieties. The analysis was performed for the 195 adult individuals with no missing data.

*Parentage analysis*

To evaluate which varieties were possible genitors, assignment of parents for the seedlings was performed with Cervus v.3.0 (Marshal *et al*. 1998; Kalinowski *et al*. 2007). The candidate parents were the 205 adults collected and the 162 seedlings were considered the offspring. Parentage analysis was performed to identify the most likely parents of seedlings after a simulation conducted with the default number of offspring (10,000) and proportion of loci genotyped (0.992). Number of candidate parents and the minimum number of genotyped loci were set to 6 and 8. The proportion of candidate parents sampled and misgenotyped loci were set to 0.9 and 0.05. Delta parameter was chosen at relaxed and strict levels of confidence of 80% and 95%.

*PCoAs and relationships among swiddens*

The dispersion of genetic diversity among varieties and swiddens was evaluated by Principal Coordinate Analysis (PCoA), carried out with GenAlEx (Peakall & Smouse 2006). The dispersion of swiddens, the basic evolutionary unit in this study, in PCoAs was cross referenced to their community of origin and soil type. Because sampling was opportunistic, the sweet varieties were only grouped according to soil type.

Based on $D_A$ genetic distances (Nei *et al*. 1983) among the swiddens a dendrogram was constructed with the Neighbor-Joining method (Saitou & Nei 1987), with 1,000 bootstrap replicates, using PHYLIP v.3.6 (Felsenstein 2005). The final tree was formatted with TreeDyn (Chevenet *et al*. 2006).

*Genetic structure*

Pairwise fixation indices ($F_{ST}$) and estimates of gene flow ($Nm$) [assuming $Nm = (1/F_{ST}-1)/4$ and $M = 2Nm$] were obtained with Arlequin v.3.5 (Excoffier & Lischer 2010), in order to investigate genetic differentiation among groups of varieties for soils and communities. Significance tests were carried out with 10,000 permutations.

Mantel tests (Smouse *et al*. 1986) were performed among matrices of genetic distance, linearized $F_{ST}$ (Slatkin 1995) and Nei genetic distances ($D_A$, Nei *et al*. 1983), and matrices of geographic distances, matrices of soil types and matrices of communities. These latter two were coded as binary matrices where zero was given to swiddens that were in the same soil type or community and one otherwise, following the example of Vigouroux *et al*. (2008). Calculations were performed with Arlequin v.3.5 (Excoffier *et al*. 2005). Significance tests

were carried out with 100,000 permutations. $D_A$s were calculated with MSA v.4.05 (Dieringer & Schlötterer 2003).

To investigate the distribution of genetic variation Analysis of Molecular Variance (AMOVA) was conducted with Arlequin v.3.5 (Excoffier & Lischer 2010). The hierarquical levels considered were: bitter or sweet varieties, swiddens within communities, swiddens within soil types, groups of swiddens within soil types within communities, swiddens within upland soils (ADE and Oxisols) and floodplain, swiddens within ADE and Oxisols. Statistical significance was assessed based upon 10,000 permutations.

## Results

*Genetic diversity*

The adult individuals (bitter and sweet varieties, N=205) and the set of seedlings (N=162) had an average of 5.6 and 5.3 alleles per locus, respectively, and the number of alleles varied from 2 to 9 (Table 1). Although similar values were found for the set of seedlings, adults had higher observed heterozygosity, and 3 loci with negative inbreeding coefficients (excess of heterozygotes), while seedlings had none.

Table 1. Characteristics of microsatellite loci: size range in base pairs (bp), number of alleles (*A*), observed ($H_O$) and expected ($H_E$) heterozygosities and inbreeding coefficients (*f*) estimated from 205 individuals of sweet and bitter manioc (adults) and from 162 seedlings.

| | Size range | adults | | | | seedlings | | | |
|---|---|---|---|---|---|---|---|---|---|
| locus | (bp) | *A* | $H_O$ | $H_E$ | *f* | *A* | $H_O$ | $H_E$ | *f* |
| GA21 | 106-118 | 4 | 0.522 | 0.553 | 0.054 | 5 | 0.451 | 0.495 | 0.087 |
| GA126 | 180-220 | 8 | 0.816 | 0.722 | -0.133 | 7 | 0.463 | 0.755 | 0.385 |
| GA131 | 94-114 | 6 | 0.743 | 0.768 | 0.03 | 6 | 0.469 | 0.617 | 0.237 |
| GA134 | 310-322 | 4 | 0.247 | 0.651 | 0.619 | 4 | 0.263 | 0.578 | 0.544 |
| GA136 | 142-156 | 6 | 0.701 | 0.716 | 0.018 | 5 | 0.648 | 0.689 | 0.056 |
| GA140 | 156-172 | 6 | 0.647 | 0.749 | 0.134 | 6 | 0.42 | 0.725 | 0.419 |
| GAGG5 | 115-125 | 4 | 0.581 | 0.582 | -0.002 | 4 | 0.256 | 0.364 | 0.293 |
| SSRY13 | 196-242 | 9 | 0.799 | 0.791 | -0.012 | 9 | 0.571 | 0.682 | 0.159 |
| SSRY89 | 104-118 | 2 | 0.093 | 0.098 | 0.046 | 2 | 0.105 | 0.111 | 0.05 |
| SSRY164 | 154-186 | 7 | 0.608 | 0.684 | 0.109 | 5 | 0.568 | 0.706 | 0.193 |
| | Mean | 5.6 | 0.576 | 0.631 | 0.086 | 5.3 | 0.421 | 0.572 | 0.242 |

Within adult individuals, bitter varieties had an average of 5.6 alleles per locus while sweet varieties had 3.5 (Table 2). Interestingly, apart from the difference in sampling numbers, sweet varieties had a larger observed heterozygosity and a negative inbreeding coefficient. Bitter varieties had 21 private alleles, while the sweet varieties had none. Seedlings had a smaller observed heterozygosity and an inbreeding coefficient about three times greater than adult individuals. Seedlings from the Oxisols and ADE soils showed smaller heterozigosities and larger inbreeding coefficients than the seedlings from the floodplain, which showed an excess of heterozygotes.

Table 2. Number of individuals (N), mean number of alleles ($\bar{A}$), number of private alleles (Ap), observed ($H_O$) and expected ($H_E$) heterozygosities and inbreeding coefficients ($f$) for various groupings of manioc varieties and seedlings from Manicoré, based on 10 microsatellite loci. Level 1 – Bitter and sweet varieties as defined by farmers, adults and seedlings. Level 2 – Seedlings grouped by soil types. Level 3 – Bitter varieties grouped by soil types. Level 4 – Bitter varieties grouped by communities.

| Level 1 | N | $\bar{A}$ | Ap | $H_O$ | $H_E$ | $f$ |
|---|---|---|---|---|---|---|
| Bitter varieties | 184 | 5.6 | 21 | 0.567 | 0.615 | 0.072 |
| Sweet varieties | 21 | 3.5 | 0 | 0.654 | 0.574 | -0.141 |
| Adults | 205 | 5.6 | 5 | 0.576 | 0.631 | 0.086 |
| Seedlings | 162 | 5.3 | 2 | 0.421 | 0.572 | 0.242 |
| Level 2 | | | | | | |
| Seedlings ADE | 46 | 4.1 | 3 | 0.398 | 0.564 | 0.260 |
| Seedlings Floodplain | 8 | 2.9 | 2 | 0.588 | 0.488 | -0.207 |
| Seedling Oxisols | 58 | 3.7 | 4 | 0.394 | 0.469 | 0.184 |
| Floodplain field | 34 | 3.5 | 1 | 0.497 | 0.487 | -0.014 |
| Oxisol field | 16 | 2.6 | 0 | 0.344 | 0.469 | 0.262 |
| Level 3 | | | | | | |
| ADE | 59 | 4.5 | 1 | 0.538 | 0.568 | 0.047 |
| Floodplain | 56 | 5.2 | 10 | 0.606 | 0.595 | -0.015 |
| Oxisols | 69 | 4.2 | 1 | 0.559 | 0.582 | 0.043 |
| Level 4 | | | | | | |
| Água Azul | 45 | 3.9 | 2 | 0.534 | 0.592 | 0.097 |
| Barreira do Capanã | 46 | 4.3 | 1 | 0.572 | 0.595 | 0.043 |
| Barro Alto | 43 | 3.7 | 0 | 0.543 | 0.511 | -0.052 |
| Pau Queimado | 21 | 4.5 | 2 | 0.600 | 0.525 | -0.094 |
| Fortaleza | 12 | 4.2 | 1 | 0.588 | 0.560 | -0.039 |
| Verdum | 17 | 3.8 | 2 | 0.641 | 0.561 | -0.113 |

In general, the floodplain varieties had higher levels of genetic diversity, and less inbreeding than the ADE and Oxisols sets of varieties (Table 2). The floodplain is also the environment with the highest number of private alleles. Most of the private alleles are at low frequencies, but, interestingly, only the floodplain varieties showed private alleles with frequencies higher than 0.05.

The communities showed an average of 4.1 alleles per locus for their bitter varieties (Table 2). Communities with swiddens in the floodplain showed slightly higher heterozigosities and also had greater numbers of private alleles than the others. In turn, negative inbreeding coefficients were found for the floodplain communities, and also for Barro Alto, where swiddens were sampled in ADE and Oxisols.

A total of 195 adult individuals representing 50 (40 bitter, 10 sweet) different varieties of manioc showed 82 different MLGs. Unique combinations of alleles were found for 52 individuals; 15 were from ADE, 24 from the floodplain and 13 from Oxisols. The other 143 individuals presented 30 different MLGs; 3 of them were shared by individuals from ADE, 8 from floodplain, 4 from Oxisols, and 15 were shared by individuals of varieties from more than one soil type.

Farmers did not assign a varietal name to 8 individuals. Unique MLGs were found for 5 of these individuals. A non-designated individual in Pau Queimado had the same MLG as *Macaxeira Pão*, a sweet variety sampled in the same community, but at a different swidden. Another non-designated individual sampled at a floodplain swidden in Verdum had the same MLG as *Macaxeira Roxa*, a sweet variety that was sampled at an ADE swidden in Barro Alto. The third non-designated individual was sampled at an ADE swidden in Barro Alto, and shared the same MLG with 11 other individuals from 7 different varieties sampled either in ADE, Oxisols or floodplain swiddens in 3 communities (Barreira do Capanã, Barro Alto and Fortaleza).

Thirteen varieties were collected only once and 6 (3 from ADE and 3 from floodplain) of them had unique MLGs. The varieties *Roxona* and *Flecha* found only once in ADE had the same MLG as other varieties found in different soil types. The variety *Poré* (from an Oxisol) was equal to one of the 3 MLGs of the variety *Coxa Branca*. The variety *Guia Roxa* (from the floodplain) shared the same MLG with 3 other floodplain varieties. The varieties *Mané Velho* and *Jabuti Amarelo* shared the same MLG with individuals of *Faianca* and *Roxinha*. Additionally, a variety identified as a different form of *Tartaruga*, sampled in Barro Alto at an ADE swidden, had the same MLG as an individual of *Arroz*.

All of the 11 more common varieties (sampled in 5 or more swiddens) had at least 3 different MLGs. Except for *Arroz*, *Olho Roxo* and *Juvenal*, the other varieties were found in all soil types, and had at least one MLG shared with at least one different variety of at least one different soil type. *Olho Roxo* and *Juvenal* were exclusively from the floodplain, and shared MLGs with other varieties from floodplain and, in the case of *Juvenal*, with other varieties from other soil types.

*Parentage analysis*

For the 162 seedlings used in the parentage analysis, 55 individuals (34%) were assigned to the two most likely parents with the relaxed confidence level of 80%. Within the strict confidence level of 95% only 2 individuals (1.2%) were assigned to possible parents. The first was a seedling collected in Água Azul at an ADE swidden which had individuals of varieties *Jabuti* and *De maniva*, from another ADE swidden, as the most likely parents. The other seedling was collected in Barreira do Capanã at an ADE swidden and had an individual of the variety *Roxinha*, from another ADE swidden, and a non-designated individual, from an Oxisol swidden, as the most likely parents. In both cases, the swiddens were in the same community in which the seedlings were sampled.

At the 80% level, possible parents had the same MLG for only one individual, and most likely parents of equal varieties were assigned to 5 individuals (in two cases the varieties were not at the swidden). The other 50 seedlings were assigned as result of inter-varietal crosses. Four seedlings had both of the most likely parents at the swiddens in which they were present and 23 had at least one of the most likely parents at the same swidden. Another 22 individuals had their most likely parents in different swiddens, and half of those had at least one of the parents belonging to varieties present at same swiddens in which the seedlings were sampled. Interestingly, 41 seedlings (80.4% of those assigned) had both of their most likely parents from varieties that occurred within swiddens of the same community, while 9 seedlings (16.3% of those assigned) had at least one of their most likely parents from varieties collected in different soil types.

*PCoAs and relationships among swiddens*

The first two principal coordinates of the analysis of genetic variability among individuals explained 54.2% of the total variation and showed that the sweet varieties tend to form a separate group from the bitter varieties, which are also more dispersed (Figure 2A).

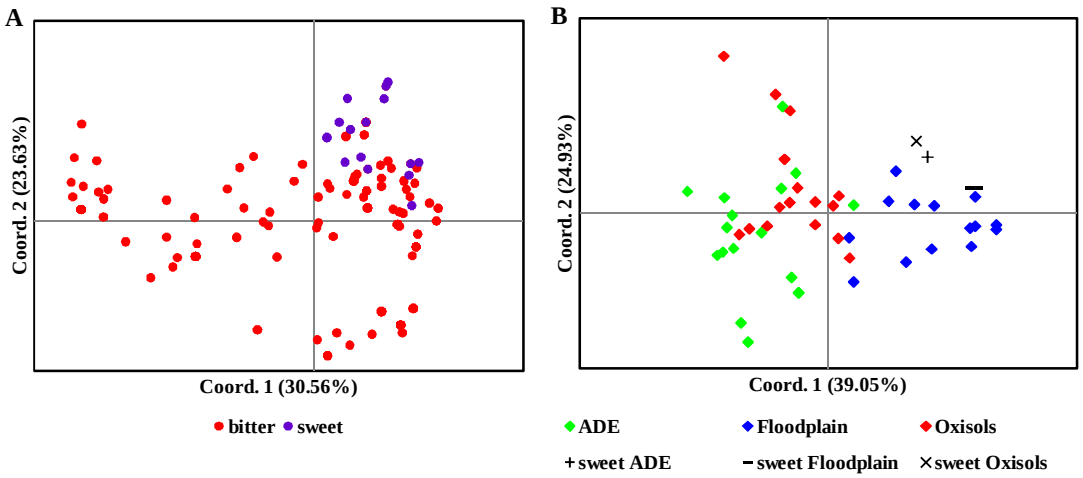


Figure 2. Principal coordinates analysis based on diversity in 10 microsatellites. A – Dispersion of individuals of bitter and sweet varieties of manioc. B – Dispersion of bitter manioc swiddens and sweet varieties of manioc from the three different soil types in Manicoré.

When individuals were grouped according to swiddens on different soil types, the first two axes of the PCoA explained 64% of the variation. ADE and Oxisol swiddens were mixed in a diffuse group, while the floodplain swiddens formed a nearly distinct group, closer to sweet varieties (Figure 2B). There was no clear tendency of swiddens from the same community to form groups (data not shown).

Relationships among swiddens observed with cluster analysis based on $D_A$ (Nei *et al*. 1983) revealed the same pattern observed in the PCoAs (Figure 3). All floodplain swiddens, except the one from Água Azul, were grouped close to each other, while swiddens from ADE and Oxisols formed a distinct group, with three main subgroups which are not related to soil types or communities. Sweet varieties formed a consistent group in which ADE is closer to Oxisol, and were located within the floodplain group. Most of the branches lack support, but bootstraps tended to be higher for swiddens that are geographically close to each other.

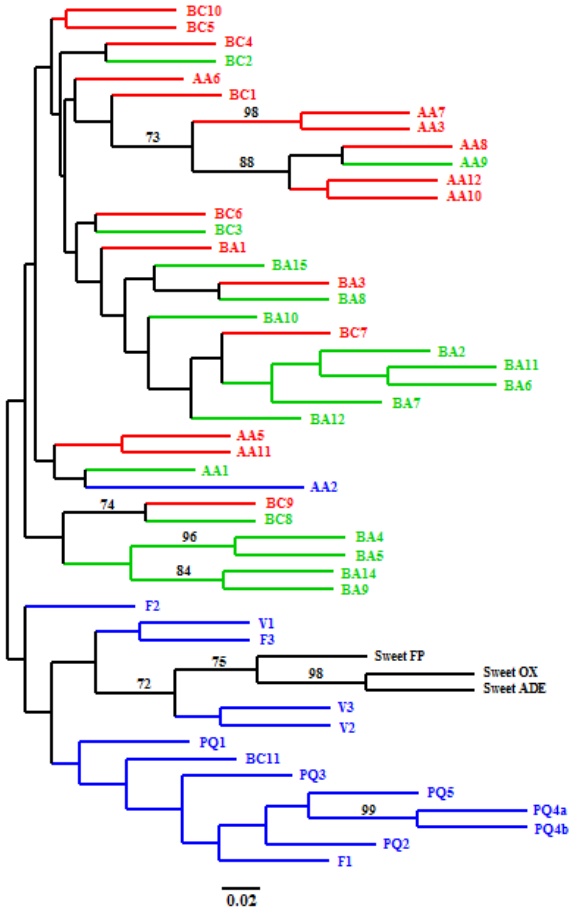


Figure 3. Neighbor-Joining dendrogram based on Nei *et al.*'s (1983) genetic distance ($D_A$), showing the relationships among bitter manioc swiddens and sweet varieties of manioc from the three different soil types in Manicoré. Colored branches represent the soil types (green=Amazonian dark earths, red=Oxisols, blue=Floodplain). Branch labels indicate the community (AA=Água Azul, BC=Barreira do Capanã, BA=Barro Alto, F=Fortaleza, PQ=Pau Queimado and V=Verdum) and the number of swiddens. Sweet varieties were grouped according to the soils. Bootstrap values greater than 70% are indicated.

*Genetic Structure*

Pairwise $F_{ST}$ between floodplain/ADE and floodplain/Oxisols were about six times higher than between ADE/Oxisols; conversely, the number of migrants was more than six times higher for the latter pair (Table 3). Mean pairwise $F_{ST}$ among communities (0.097) was higher than among soil types (0.073).

Table 3. Genetic differentiation ($F_{ST}$ – below diagonal) and gene flow ($Nm$ – above diagonal) among bitter varieties of manioc sampled from three soil types and six communities in Manicoré. Significant values $^{*}$($P$<0.05) and $^{**}$($P$<0.01) are indicated.

| | ADE | Floodplain | Oxisols | | | |
|---|---|---|---|---|---|---|
| ADE (N=59) | - | 2.05 | 15.29 | | | |
| Floodplain (N=56) | 0.108$^{**}$ | - | 2.45 | | | |
| Oxisols (N=69) | 0.016$^{**}$ | 0.093$^{**}$ | - | | | |
| | Água Azul | Barreira do Capanã | Barro Alto | Pau Queimado | Fortaleza | Verdum |
| Água Azul (N=45) | - | 21.85 | 3.34 | 1.42 | 3.3 | 1.97 |
| Barreira do Capanã (N=46) | 0.011 | - | 6.38 | 1.51 | 4.68 | 2.32 |
| Barro Alto (N=43) | 0.069$^{**}$ | 0.038$^{**}$ | - | 0.87 | 2.03 | 1.11 |
| Pau Queimado (N=21) | 0.151$^{**}$ | 0.141$^{**}$ | 0.221$^{**}$ | - | 4.8 | 1.77 |
| Fortaleza (N=12) | 0.068$^{**}$ | 0.048$^{**}$ | 0.106$^{**}$ | 0.046$^{*}$ | - | 6.21 |
| Verdum (N=17) | 0.114$^{**}$ | 0.098$^{**}$ | 0.184$^{**}$ | 0.121$^{**}$ | 0.035$^{*}$ | - |

Although the geographic extent of this study spans only about 75 km, Verdum was the community most isolated from the others (mean distance of 13.3 Km from each other), and showed high levels of genetic differentiation and low numbers of migrants. Mantel tests showed no significant correlation between differentiation coefficients or $D_A$ and geographic distances (R=0.15, $P$=0.39 and R=0.34, $P$=0.11, respectively). Additional Mantel tests showed a correlation between linearized $F_{ST}$ and soil types (R=0.31, $P$=0.02). This correlation was almost two times greater when ADE and Oxisols swiddens were considered to be from the same soil type (R=0.59, $P$<0.01). $D_A$ (Nei *et al.* 1983) showed no correlation with soil types. No significant correlation was detected when the matrices of $D_A$ or linearized $F_{ST}$ were compared with the matrix of communities.

The AMOVA between sweet and bitter varieties indicated that most of the variance is found within these groups, but there is a reasonable percentage of variation between groups (Table 4). Although the corresponding value of $F_{ST}$ was high (0.133), the number of migrants was not low ($Nm$=3.2). Variation found among communities is slightly greater than variation among soil types, but the corresponding $F_{ST}$s were similar (0.081 and 0.085). AMOVA for groups of swiddens from the same soil type within a community showed that most of variation was found within soil types in the communities, followed by the variation found among communities and then by the variation found among soil types within a community. The corresponding $F_{ST}$ was 0.098 in this latter AMOVA.

Table 4. Analysis of molecular variance (AMOVA) for six hierarchical groupings of manioc varieties in Manicoré. Level 1 – Bitter and sweet varieties. Level 2 – Swiddens within communities. Level 3 – Swiddens within soil types. Level 4 – Soils within communities. Level 5 – Swiddens within upland and floodplain soils. Level 6 – Swiddens within upland soils. d.f. = degrees of freedom

| Source of variation | d.f. | Sum of squares | Variation components | Percentage of variation |
|---|---|---|---|---|
| Between bitter and sweet varieties | 1 | 37.81 | 0.46 | 13.34 |
| Within groups of varieties | 408 | 1223.40 | 3.00 | 86.66 |
| Total | 409 | 1261.21 | 3.46 | |
| Among communities | 5 | 95.86 | 0.28 | 9.29 |
| Among swiddens within communities | 42 | 107.14 | -0.03 | -1.17 |
| Within swiddens | 320 | 900.98 | 2.81 | 91.88 |
| Total | 367 | 1103.98 | 3.06 | |
| Among soil types | 2 | 59.76 | 0.22 | 7.08 |
| Among swiddens within soil types | 45 | 141.81 | 0.04 | 1.43 |
| Within swiddens | 320 | 902.42 | 2.82 | 91.5 |
| Total | 367 | 1103.99 | 3.08 | |
| Among communities | 5 | 95.86 | 0.23 | 7.47 |
| Among soils within communities | 5 | 22.65 | 0.07 | 2.41 |
| Within soils of communities | 357 | 985.48 | 2.76 | 90.12 |
| Total | 367 | 1103.99 | 3.06 | |
| Between ADE/Oxisols and Floodplain | 1 | 51.05 | 0.30 | 9.6 |
| Among swiddens within groups of soils | 46 | 150.52 | 0.06 | 1.87 |
| Within swiddens | 320 | 902.42 | 2.82 | 88.53 |
| Total | 367 | 1103.99 | 3.18 | |
| Between ADE and Oxisols | 1 | 8.71 | 0.04 | 1.58 |
| Among swiddens within ADE and Oxisols | 32 | 92.86 | 0.01 | 0.39 |
| Within swiddens on ADE and Oxisols | 222 | 625.82 | 2.82 | 98.03 |
| Total | 255 | 727.39 | 2.87 | |

As suggested by PCoAs and cluster analysis, floodplain varieties are genetically differentiated from upland varieties (ADE and Oxisols). When these latter two groups are compared, the variation found between them is greater than that found among communities or among each soil type, as well as its corresponding $F_{ST}$ (0.114). Indeed, when AMOVA is performed among swiddens within ADE and Oxisols, there is little variation among varieties of these soils. The corresponding $F_{ST}$ is by far the smallest among all hierarchical levels tested and the only one which was not significant ($F_{ST}$=0.019, $P$=0.344).

## Discussion

*Genetic diversity of manioc varieties*

We observed high levels of genetic diversity in manioc (mean of 5.6 alleles per locus, mean $H_O$=0.57), which were similar to previous studies. Analyzing 115 varieties of bitter and sweet manioc, with nine of the loci used in this study, Peroni *et al*. (2007) found 4.5 alleles per locus and a mean $H_O$ of 0.67. Elias *et al*. (2004), with eight of the loci used here, analyzed 117 accessions of bitter and sweet manioc, found 5.5 alleles per locus and a mean $H_O$ of 0.51. Using seven of the loci used here for 55 sweet and bitter varieties, Mühlen *et al*. (2000) found 4.4 alleles per locus and mean $H_O$ of 0.56.

The 82 MLGs found for the 50 varieties included in the analysis agree with previous studies, which detected that manioc varieties are formed of a major set of genetically identical individuals plus morphologically similar but genetically different individuals (Peroni 2004; Pujol *et al*. 2007). Interestingly, the floodplain varieties had the greater number of unique MGLs, which certainly contributed to their distinction from upland soils.

For some varieties all individuals, collected in different swiddens, did have identical MLGs, but amongst the most frequent varieties (collected in more than 5 swiddens), all had at least three different MLGs that were not associated to different soil types or to different communities. Incorporation of seedlings into the varieties (Martins 2001; Pujol *et al*. 2005; Duputié *et al*. 2009) and the occurrence of somatic mutations in plants used to prepare stems cuttings might also be possible sources of new MLGs within varieties (McKey *et al*. 2010b), especially given the high mutation rates of microsatellites (Powell *et al*. 1996).

We also found individuals assigned to different varietal names with identical MLGs, which suggests that the designation of varieties may not be uniform among the smallholder farmers in communities along the middle Madeira River. Salick *et al*. (1997), in Peruvian Amazonia, detected that over 50% of the names given to manioc varieties were unique to particular familiar groups cultivating the variety, often resulting in the same phenotype having distinct names in different families. A parallel line can be traced with our study, which found the same genotype in individuals assigned to distinct varieties. For instance, the two individuals of the variety *Manicoré* had the same MLG as individuals of the variety *Manaus* collected in the same community, but in swiddens of different families. Another possibility is that genotype-environment interactions may cause unique expressions of the same genotype to be identified as new varieties (Emperaire *et al*. 1998). A possible example is the variety

*Roxa*, collected only once at an ADE swidden, which had the same MLG as some individuals of the variety *Roxinha*, collected in Oxisol swiddens. Variation in designations of the varieties among farmers might play an important role in maintaining the diversity within the crop, since it opens the possibility of keeping sets of individuals with unique genetic features, at different locals, within a variety.

*Seedlings*

It was not surprising that the set of seedlings showed lower heterozygosity than adults and a deficiency of heterozygotes. Elias *et al*. (2001) genotyped two populations originated from volunteer seedlings and found lower observed heterozygosity than for the 29 varieties sampled in Guyana. Pujol *et al*. (2005) also found highly inbreed populations of seedlings in two swiddens of Palikur Amerindians in French Guyana. Although manioc is thought to be preferentially allogamous (Martins 2001), there may be high rates of self-fertilization since, given the grouped distribution of plants of the same variety frequently observed in swiddens, there is a good chance that surrounding individuals are clones (Pujol *et al*. 2005). This is quite probably the explanation of the high inbreeding coefficient and the lower number of private alleles observed in the seedlings. The high proportion of inter-varietal crosses in parentage analysis (at the 80% level, 50 of the 55 seedlings had different varieties as the two most likely parents) may be contrasting to lower heterozygosities. However, most of the seedlings that were collected in the same swidden and were assigned in this analysis had one (half-sibs) or both (full-sibs) of their most likely parents in common. This may have also contributed to the lower heterozygosities and higher inbreeding coefficient of seedlings when compared to adults.

Low proportions of assignments at the strict and even at the relaxed levels of confidence in parentage analysis may be due to different reasons. Parentage analysis in Cervus is more powerful when sexes of candidate parents are known and the proportion of parents assigned may be different from the expected after simulations if there are close relatives of the true parents among the candidate parents. MLG analysis showed that, of the 205 parent candidates, 143 individuals were distributed in 30 MLGs. Cervus probably interpreted individuals with the same MLG as very close relatives or even the same individual, which dramatically reduces the number of parental candidates – from 143 to 30.

Most of the seedlings assigned had both of their most likely parents from varieties that occurred within the same community. According to the AMOVA, the sets of varieties in swiddens within each community are very similar, thus, it is more likely that parental varieties are in the same communities as the seedlings, either in the same swidden or not. This may be explained by more intensive exchange of varieties within than among the communities. Indeed, exchange of varieties has been demonstrated as being part of the social networks where manioc varieties are traditionally grown (Boster 1986; Peroni & Hanazaki 2002; Emperaire & Peroni 2007; Oliveira 2008). In such social networks the exchange of varieties tends to be more intensive between farmers with closer kinship relations, which we may expect to be more frequent in the same community. Few seedlings' most-likely parents were varieties collected in different soil types, which may be because it is unlikely that pollination occurs between plants located in distant swiddens in different soil types, since manioc is pollinated by wind and small insects (Lebot 2009), or that the seed dispersal reaches great distances, since manioc seeds are dispersed by authocory (explosion of dehiscent fruit) followed by myrmechory (dispersal by ants), although some birds may have occasional roles in dispersal (Elias & McKey 2000). One fifth of the seedlings had individuals of varieties that did not occur at the same swidden as possible parents. This suggests that different varieties than the ones present at the time of sampling might have been grown in the same or in close by swiddens in prior cycles of cultivation, which is a common occurrence in traditional communities that practice swidden-fallow systems (Pujol *et al*. 2007).

*Bitter and sweet varieties*

Although the number of bitter varieties sampled was much larger, heterozygosity was higher for the sweet varieties ($H_O$= 0.56 versus 0.65, respectively). Also, while bitter varieties showed a deficit of about 7% of heterozygotes, sweet varieties had an excess of observed heterozigosity ($f$= -0.14). Previous studies (Mühlen *et al*. 2000; Elias *et al*. 2004; Peroni *et al*. 2007) also found higher observed heterozigosities in sweet varieties (only Peroni *et al*. sampled more sweet than bitter individuals). The higher number of private alleles in bitter varieties found in this study is probably explained by the difference in sampling numbers, and certainly contributes to the genetic difference among the two groups of varieties. Elias *et al*. (2001) found 22 private alleles in bitter varieties and only one in sweet varieties, while Peroni *et al*. (2007) found three private alleles in sweet varieties and none in bitter varieties.

The distinction of bitter and sweet varieties is traditionally (McKey & Beckerman, 1993; Elias *et al*. 2000) and genetically (Mühlen *et al*. 2000, Peroni *et al*. 2007) well documented. With a more systematic sampling of sweet varieties, probably the individuals would form two distinct groups in PCoA representing the two major groups of varieties, although some overlap would be expected given the continuum of cyanogenic glycoside levels. Additional interesting patterns of relationships of bitter and sweet varieties were detected in PCoA (Figure 2) and the swiddens' dendrogram (Figure 3). Sweet varieties were closely related to the bitter varieties of floodplain. These interesting relationships may be due to the origins of sweet and bitter manioc, as suggested by Arroyo-Kalin (2010), although our sampling strategy was not designed to examine this. This author hypothesized that during the process of manioc domestication sweet varieties arose first, probably in dump heaps of non-sedentary peoples. Because sweet manioc matures faster and does not demand technologies for detoxification, it would be more appropriated for nomadic peoples (Baleé 1992). Dump heaps latter developed into homegardens (Lathrap 1977), where sweet varieties are often found today (McKey *et al*. 2010; Elias *et al*. 2000). When floodplains filled enough for horticulture, the homegarden varieties adapted to rich soils would have been "pre-adapted" to the rich soils of the floodplains, where cultivation could be intensified somewhat. Bitter varieties would have arisen when sedentary societies appeared and manioc started to be grown in larger swiddens in more intensive systems beyond the homegardens (Arroyo-Kalin 2010), generally on the *terra firme* upland soils, simply because there is more space and no flood threat on the *terra firme*. Arroyo-Kalin's hypothesis will be better tested genetically with the use of chloroplast DNA polymorphisms with a large-scale sampling of both bitter and sweet South American manioc varieties traditionally cultivated either by indigenous or non-indigenous peoples.

*Genetic differentiation among environments of cultivation*

Because different environments of manioc cultivation were never emphasized in previous molecular genetic studies it is difficult to compare our results with others in different regions. The floodplain varieties presented higher genetic diversity than ADE and Oxisols varieties, and this may result from farmers' selection for variable characteristics due to the unstable nature of the floodplain. It was observed that natural and farmers' selection lead to high genetic diversity in barley landraces traditionally cultivated in adverse environments, and that these populations may be useful for understanding the mechanisms that enhance stability

in stressful environments and the adaptive role of individual traits (Ceccarelli & Grando 2000). Therefore, bitter manioc varieties from the floodplain may be used as a model for understanding adaptive mechanisms of cultivation that occur in this environment.

Based on ethnobotanical observations, Fraser & Clement (2008) and Fraser (2010) suggested that the communities along the middle Madeira River developed distinct varieties of bitter manioc adapted to different soil types. As a consequence, those communities with access to different soil types would maintain higher levels of genetic diversity. This seems not to be necessarily true, since communities in which bitter manioc is grown in the three soil types (Água Azul and Barreira do Capanã) had lower genetic diversity than communities in which bitter manioc is cultivated only in floodplain soils (Pau Queimado, Fortaleza and Verdum). Another hypothesis of Fraser (2010) is that the genetic structure of varieties would be related to the different cultivation environments. This second hypothesis is partly true, since a higher level of genetic differentiation for bitter varieties was found between the floodplain and upland soils (ADE and Oxisols), as suggested by $F_{ST}$ and AMOVA (Tables 3 and 4).

Fraser & Clement (2008) and Fraser (2010) also observed that bitter manioc varieties grown in the floodplain and ADE appeared to have similar ecological adaptations (such as fast maturation and low starch content), possibly due to farmers' selection and the consequent development of genetic distinctions attributable to these adaptations. Contrary to the expectation based on ethnobotanical observations, bitter varieties of ADE were more related to bitter varieties of Oxisols, rather than to those in the floodplain, as shown by the Mantel test, PCoA (Figure 2), and the dendrogram (Figure 3). Our data seems to refute the ethnobotanical hypothesis and poses new questions: are the similar ecological adaptations observed for ADE and floodplain varieties outcomes of adaptive convergence of traits directed by farmer's selection? Is this selection conscious or unconscious?

As expected, most of the variation detected by AMOVA is found within the swiddens, since they are represented by a set of different varieties. The AMOVA for groups of swiddens of the same soil type within communities suggested that the exchange of varieties may be more intensive within the communities than among the communities. Indeed, exchange of varieties may be expected to be more frequent within communities due to the social networks discussed above.

Overall, our results strongly suggest a genetic differentiation among bitter manioc varieties cultivated in different environments. Previous differentiation among environments of

cultivation was also suggested by Salick *et al*. (1997). These authors worked in indigenous communities in Peruvian Upper Amazonia and concluded that morphological characteristics in manioc vary with soil types, but less extensively than variation with elevation and topography. However, as highlighted by the authors, the genetic basis involved in the correlations was unknown. Agronomic characterization of the Brazilian germplasm collection also revealed variation among ecogeographic regions of Brazil; however, this collection was not yet been fully characterized with molecular markers (Cordeiro & Abadie 2007).

**Conclusions**

This is the first time that the genetic structure and gene flow dynamics of numerous manioc varieties on 3 different soils, 2 of which are very nutrient rich and not well represented in the manioc literature, have been examined at a fine geographic scale. Ethnobotanical observation suggested that in the middle Madeira River region communities that cultivate bitter manioc in more than one soil type would maintain higher genetic diversity, and that the genetic structure of bitter varieties would be related to the soil type in which they are grown, with an expected closer relationship between varieties grown in the floodplain and ADE. Based on levels of genetic variability estimated with 10 microsatellite markers for bitter manioc varieties grown in different localities, floodplain communities maintain more genetic diversity than communities on other soils, thus higher genetic diversity is not necessarily found within communities in which farmers have access to different soil types. There was little evidence that the varieties from the floodplain are genetically closer to the varieties from ADE, rather, there is significant genetic differentiation between varieties grown in the floodplain and those grown in upland soils.

The genetic data used in this study showed a divergent evolutionary trajectory of manioc in cultivation in different Amazonian environments. Interesting new questions, which have many genetic, evolutionary and ecological implications, may be raised: what are the selective forces that drive the genetic differentiation of the floodplain varieties from the upland soils varieties? While this may seem trivial, periodic extreme flood events may eliminate enormous numbers of floodplain varieties and require significant farmer action to recreate them. What are the differences in the traditional farmer's management that may explain such differentiation? To what extent do the patterns of distribution of the genetic diversity of bitter manioc varieties among different environments observed in this study apply to the rest of Amazonia? May these patterns also be found in sweet manioc? We have just

added one new component to the discussion of the evolutionary dynamics of manioc under cultivation, and much remains to be done. We also hope that studies like this encourage the adoption of social policies that raise the awareness of the value of traditional practices of smallholder farmers, who are the current curators of the *on-farm in situ* conservation of manioc genetic resources.

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## Capítulo 2

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**High intra-varietal genetic diversity in bitter manioc cultivated in different soil types in Central Amazonia**

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Running title: Intra-varietal genetic diversity in manioc

## Abstract

Bitter manioc is one of the most important food crops in Central Amazonia and is considered to be well adapted to the clayey, acid, nutrient poor Oxisols of this region. However, ethnobotanical observations showed that bitter manioc is also frequently cultivated in the highly fertile soils of the floodplains and Amazonian dark earths (ADE) in communities of smallholder farmers along the middle Madeira River. Intra-varietal genetic diversity of the varieties most commonly cultivated in three soil types (ADE, floodplain and Oxisols) in this region was evaluated with 10 microsatellite markers. Although manioc is clonally propagated, these varieties had high levels of genetic diversity, corroborating previous studies carried out in areas of low soil fertility in other regions of Amazonia. Ethnobotanical observations

predicted close relationships among varieties from the floodplain and ADE, but our data suggests a closer relationship among varieties of upland soils (ADE and Oxisols). Generally, varieties were highly genetically differentiated from each other. However, some of the varieties contribute to the variability found within other varieties, whether they are grown in the same soil type or not, which also contributes to the maintenance and amplification of genetic diversity within the varieties cultivated in communities along the middle Madeira River.

**Introduction**

Manioc (*Manihot esculenta* Crantz ssp. *esculenta*) was domesticated in southwestern Amazonia at least 7,000 years ago (Allem 1994; Olsen & Schaal, 1999) and today it is cultivated in all tropical countries (Lebot 2009). Manioc is grown for its tuberous starchy roots, which are the primary source of carbohydrates for more than 800 million people (Lebot 2009).

Cultivated manioc can be classified into two major groups of varieties. The sweet varieties have low amounts of cyanogenic glycosides (<100 ppm fresh weight) and may be consumed after simple processing (peeling and boiling) or even raw. On the other hand, bitter varieties have high amounts of these toxic substances (>100 ppm fresh weight) and need to be detoxified before consumption (McKey *et al*. 2010a). Although there is a continuum in the levels of cyanogenic glycosides among varieties, genetic differentiation between sweet and bitter varieties was evidenced by molecular markers (Mühlen *et al*. 2000; Peroni *et al*. 2007). Traditional farmers are able to distinguish bitter and sweet varieties (Elias *et al*. 2000; Rival and McKey 2008), although there are no morphological characters associated to these two groups.

In Amazonia, manioc is generally cultivated in low-input swidden-fallow systems practiced by communities of traditional farmers (Emperaire 2005). Areas of secondary vegetation are cleared and burnt, and the swiddens are used for one to three cycles of cultivation and then are fallowed. Secondary vegetation is left to grow for a variable period of time before the area is again used for the establishment of a new swidden (Elias *et al*. 2000; Martins 2001).

Although manioc is vegetatively propagated by farmers, sexual reproduction is still possible and may be very common. The seeds produced are included in the soil seed banks,

and volunteer seedlings (plants originated from sexual reproduction) grow at the same time as plants that were vegetatively propagated (Pujol *et al.* 2007). The farmers may let the seedlings grow and by harvesting time they decide if these plants are going to be used for vegetative propagation (Rival & McKey 2008). If so, farmers may either incorporate them into an existing variety or assign a new varietal name (Martins 2001; Rival & McKey 2008). The process of incorporation of seedlings is essential to maintain genetic diversity within the crop (Elias *et al.* 2001; Pujol *et al*. 2007; Duputié 2009).

Despite the existence of sweet varieties, there is strong selection for manioc varieties with high toxicity, especially in Amazonia where the majority of varieties are bitter (Clement *et al*. 2010). Bitter manioc cultivation is dominant along the major rivers in Central Amazonia (McKey *et al*. 2010a). Most of the bitter manioc is grown on the nutrient-poor Oxisols of non-flooded upland plateaus, in which manioc cultivation is practiced less intensively, with longer cropping periods and longer periods of fallowing (Fraser & Clement 2008). However, about 30% of manioc flour sold in Manaus (capital of Amazonas state) comes from manioc grown in the highly fertile Fluvent Entisols found in the floodplains of Amazonian whitewater rivers (Gutjahr 2000). Despite the importance of manioc cultivation on high-fertility soils, most research on manioc genetic diversity, especially in Amazonia, was undertaken in environments of low soil fertility.

Recently, Fraser & Clement (2008) and Fraser (2010) showed that bitter manioc is a widespread crop in the highly fertile Amazonian dark earths (ADE), in communities of smallholder farmers along the middle Madeira River. ADE are anthropogenic soils, located in upland plateaus, associated with Amerindian settlements from the pre-Columbian period (Arroyo-Kalin 2009; Woods & Denevan 2009) and as fertile as the floodplain soils. Manioc cultivation in ADE is more intensive, with shorter cropping periods and shorter periods of fallowing (Fraser & Clement 2008). Based on ethnobotanical observation, Fraser & Clement (2008) and Fraser (2010) suggested that traditional farmers from the communities along the middle Madeira River manage distinct sets of varieties according to their suitability for the different environments of cultivation, which are characterized principally by the soils type (ADE and Oxisols on the uplands versus Entisols in the floodplain). These authors observed that local varieties are termed “weak” (low starch, fast maturing) or “strong” (high starch, slow maturing) and that this classification reflects their suitability for cultivation in different soil types. “Weak” varieties are associated to “weak” soils that support short cropping cycles and require short fallowing periods, and thus are more suitable to cultivation in ADE soils. On

the other hand, “strong” varieties are associated with “strong” soils that support long cropping cycles and require the long fallowing periods practiced in Oxisols. Because the seasonality of flood events, varieties cultivated in the floodplain have fast maturation. The authors hypothesized that the “weak” varieties have origins traceable to the floodplain soils, and it would be expected that the varieties grown in ADE are genetically related to the varieties grown in the floodplain. Additionally, it was observed that if cultivation in Oxisols becomes more intensive, i.e., with shorter fallowing periods, these soils would be classified as weak; the opposite occurs if ADE soils are less intensively cultivated. The farmers then adjust their selection of varieties in a certain soil type to varieties adapted to other soil types (Fraser & Clement 2008). This variation in the perception of the suitability of varieties for different soil types opens the possibility that gene flow (through sexual reproduction and incorporation of seedlings) occurs among varieties adapted to different soil types.

The objective of this study was to test two hypotheses raised by ethnobotanical observation: (I) if the varieties from ADE and the floodplain are genetically closer and more distantly related to the varieties from Oxisols, and (II) if different varieties contribute to the genetic diversity present in other varieties. We evaluated these hypotheses based on the genetic diversity and structure detected with a set of 10 microsatellite markers for some of the most frequent bitter manioc varieties cultivated in different soil types in communities of traditional farmers along the middle Madeira River.

## Materials and Methods

### *Field sites and authorizations*

Manicoré (5°18’S; 61°18’W) is an essentially agricultural municipality located in Amazonas state, Brazil, in the middle Madeira River region (Figure 1). Numerous communities surrounding the city are composed of smallholder farmers who grow manioc in traditional swidden-fallow agriculture systems in upland ADE and Oxisols, as well as in floodplain soils.

The varieties included in this study are amongst the most frequently grown in each soil type in the region (Fraser 2010), and were sampled in the riverside non-indigenous communities of Água Azul, Barreira do Capanã, Barro Alto, Pau Queimado and Verdum. No proprietary traditional knowledge was accessed, which allowed us to meet Resolution 21 requirements for basic research that does not require authorization from Brazil’s Council for

Genetic Patrimony (CGEN, Brazilian acronym). Our study was authorized by the Instituto Nacional de Pesquisas da Amazônia's Committee for Research Ethics (protocol 235/09).

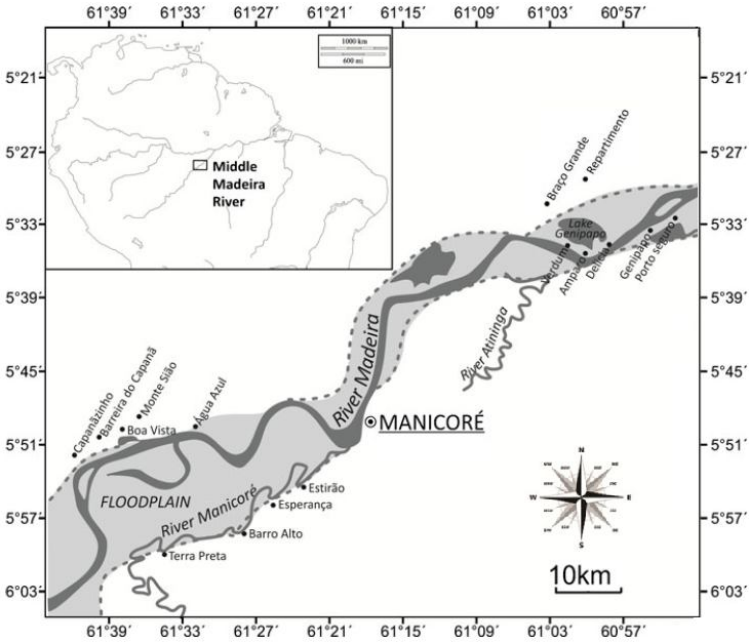


Figure 1. Map of the middle Madeira River showing the communities near the municipality of Manicoré, Amazonas, Brazil, in which manioc is cultivated on the floodplain, Amazonian dark earths and on Oxisols. (Map by Victoria Frausin)

### *Sampling*

Four varieties were selected in ADE, five in floodplain and five in Oxisols. One variety was collected in the three soil types (*Tartaruga*), one in ADE and Oxisols (*Arroz*), two in ADE and floodplain (*Pirarucu Branco* and *Pirarucu Amarelo*), two in floodplain only (*Mãe Joana* and *Olho Roxo*), and three in Oxisols only (*Aruari*, *Jabuti* and *Roxinha*). This sampling strategy was designed to test the ethnobotanical hypotheses, along with a sampling of important varieties from only one soil. With the consent of farmers, leaves from 20 to 30 individuals of each variety (identified by the farmer) were collected. Leaves were dried in silica gel, and later stored at -20°C in freezers at the laboratory.

***DNA extraction and microsatellite genotyping***

Genomic DNA was extracted from 50 mg of powdered leaf tissues with the CTAB2% method described by Doyle & Doyle (1987), with a minor modification: β-mercaptoethanol was not used. DNA was quantified by comparison with known concentrations of standard DNA (lambda DNA – Fermentas) in electrophoresis agarose gels (0.9% w/v) stained with GelRed (Biotium).

Ten microsatellite markers, 7 (GA21, GA126, GA131, GA134, GA136, GA140, GAGG5) described by Chavarriaga-Aguirre *et al*. (1998), and 3 (SSRY13, SSRY89, SSRY164) described by Mba *et al*. (2001) were chosen. PCR reactions were carried out in a final volume of 10 μL with 20 ng of genomic DNA, 1X buffer ($Mg^+$ free), 2.5 ng of BSA, 2.5 mM of $MgCl_2$, 250 μM of each dNTP, 2.5 pmols of each forward and reverse primer, and 1 U *Taq* DNA polymerase. Amplifications were carried out in a Verirti thermocycler (Applied Biosystems) as follows: an initial denaturation step of 94°C for 2 min followed by 30 cycles at 94°C for 1min; 56°C for 1 min; 72°C for 2 min, and a final step of extension at 72°C for 25 min. Quality and non-ambiguous amplification were checked by electrophoresis in agarose gels (2% w/v) stained with GelRed (Biotium). Forward sequences of primers were labeled with fluorescence (either FAM, 6-HEX or NED) and genotyping was performed in multiplexed systems in the DNA sequencer ABI3130xl (Applied Biosystems). GeneScan™ - 500 ROX™- Size Standard (Applied Biosystems) was used to size the alleles, and data collection and analysis were performed using GeneMapper v.4.0 (Applied Biosystems).

***Data analysis***

*Intra-varietal genetic diversity*

Genetic diversity parameters of total ($A$) and mean ($\bar{A}$) number of alleles, observed ($H_O$) and expected ($H_E$) heterozigosities, number of private alleles (Ap) and the inbreeding coefficient ($f$) were estimated with GenAlEx (Peakall & Smouse 2006) for each locus, for each variety and for the groups of varieties of each soil type.

The recognition of identical and distinct multilocus genotypes (MGLs) was performed with GenClone v.2.0 (Arnaud-Haond & Belkhir 2007) in order to investigate the presence of common MLGs among different varieties. Individuals with missing data were excluded from this analysis.

*Genetic structure*

The genetic structure among varieties was investigated with classic pairwise fixation indexes ($F_{ST}$) and estimates of gene flow ($Nm$) among the varieties [assuming $Nm = (1/F_{ST} - 1)/4$ and $M = 2Nm$] were estimated with Arlequin v.3.5 (Excoffier & Lischer 2010). Significance tests were carried out with 10,000 permutations.

Genetic structure among the varieties was further investigated with Structure v.2.3.3 (Pritchard *et al*. 2000; Hubisz *et al*. 2009). Structure implements Bayesian approaches to infer the number of clusters ($K$) that are best explained by the genotypic data, and in which Hardy-Weinberg and linkage disequilibrium are minimized. The optimal $K$ was chosen based on values of $\Delta K$ (Evanno *et al*. 2005), after 5 simulations for each $K$, with $K$ varying from 1 to 16. Simulations were performed with no prior population information under the no admixture model for independent allele frequencies (see Discussion) with 500,000 iterations of the Markov chain Monte Carlo after a burn-in period of 100,000. A graphical bar plot to represent the membership coefficients of each individual within the varieties was generated using Distruct (Rosenberg 2004).

To evaluate the distribution of genetic variation among the varieties, Analysis of Molecular Variance (AMOVA) was conducted with Arlequin v.3.5 (Excoffier & Lischer 2010). Statistical significance for the data were assessed based upon 10,000 permutations.

Nei *et al*. (1983) genetic distances ($D_A$) were estimated among the swiddens with MSA v.4.05 (Dieringer & Schlötterer 2003), and a dendrogram was constructed with the Neighbor-Joining method (Saitou & Nei 1987) with PHYLIP v.3.6 (Felsenstein 2005), using 1,000 bootstrap replicates to estimate significance. The final tree was formatted with TreeDyn (Chevenet *et al*. 2006).

To further investigate the contribution of some varieties to the genetic variability of others, Bayesian analysis of migrants was also carried out in Structure v.2.3.3 (Pritchard *et al*. 2000; Hubisz *et al*. 2003). The varieties were grouped based on the number of clusters previously inferred ($K$) and the software was run to determine posterior probabilities of each individual to be a migrant or to have migrant ancestry. The run was performed with prior population information: the parameter GENSBACK was set to 3 (the number of prior generations to be tested), and the 0.05 default value of MIGPRIOR (rate of migration events) was used. Additionally, the no admixture model for independent allele frequencies was used, with 500,000 iterations of the Markov chain Monte Carlo after a burn-in period of 100,000.

## Results

*Intra-varietal genetic diversity*

The whole set of individuals (N=390) presented from 2 to 8 alleles per locus and a mean of 4.5 (data not shown). All 14 varieties had higher observed heterozigosities than expected by random mating and the excess of heterozygotes was reflected in negative values of inbreeding (Table 1). Observed heterozygosities were higher than expected heterozygosities also for all soil types; the floodplain varieties had a higher mean observed heterozygosity than varieties from ADE and Oxisols, which had similar mean values of $H_O$. The varieties *Tartaruga* and *Olho Roxo* from the floodplain, and *Aruari* and *Jabuti* from Oxisols had private alleles. Interestingly, three of the varieties had some private alleles with high frequencies, and most of the private alleles found per soil type had frequencies greater than 0.05 (Table 2).

Table 1. Number of individuals (N), mean number of alleles ($\bar{A}$), number of private alleles (Ap), observed ($H_O$) and expected ($H_E$) heterozygosities and inbreeding coefficients ($f$) for 14 bitter manioc varieties grown in 3 different soil types in Manicoré, Amazonas, Brazil, and for the set of varieties in each soil type, based on 10 microsatellite loci. Soil types in which varieties were grown are coded as ADE (Amazonia dark earths), FP (floodplain) and OX (Oxisols).

| | N | $\bar{A}$ | Ap | $H_O$ | $H_E$ | $f$ |
|---|---|---|---|---|---|---|
| *Pirarucu Branco* ADE | 20 | 1.5 | - | 0.495 | 0.250 | -0.981 |
| *Pirarucu Branco* FP | 20 | 1.6 | - | 0.505 | 0.255 | -0.838 |
| *Pirarucu Amarelo* ADE | 30 | 1.7 | - | 0.607 | 0.306 | -0.862 |
| *Pirarucu Amarelo* FP | 30 | 1.8 | - | 0.607 | 0.307 | -0.754 |
| *Arroz* ADE | 20 | 2.4 | - | 0.495 | 0.460 | -0.076 |
| *Arroz* OX | 30 | 1.7 | - | 0.503 | 0.257 | -0.752 |
| *Tartaruga* ADE | 30 | 2.2 | - | 0.503 | 0.268 | -0.530 |
| *Tartaruga* FP | 30 | 2 | 3 | 0.707 | 0.361 | -0.770 |
| *Tartaruga* OX | 30 | 2.4 | - | 0.507 | 0.276 | -0.445 |
| *Mãe Joana* FP | 30 | 2.5 | - | 0.593 | 0.363 | -0.451 |
| *Olho Roxo* FP | 30 | 2 | 2 | 0.510 | 0.266 | -0.695 |
| *Aruari* OX | 30 | 2.4 | 3 | 0.583 | 0.329 | -0.357 |
| *Jabuti* OX | 30 | 2.6 | 1 | 0.567 | 0.445 | -0.157 |
| *Roxinha* OX | 30 | 1.5 | - | 0.500 | 0.250 | -1.000 |
| ADE | 100 | 3.3 | 1 | 0.532 | 0.505 | -0.033 |
| Floodplain | 140 | 3.6 | 6 | 0.590 | 0.510 | -0.078 |
| Oxisols | 150 | 3.6 | 5 | 0.531 | 0.526 | -0.009 |

Table 2. Private alleles (with frequencies >0.05) within bitter manioc varieties and within soil types in Manicoré, Amazonas, Brazil. Soil types in which varieties were grown are coded as ADE (Amazonian dark earths), FP (floodplain) and OX (Oxisols).

| | Locus | Allele | Frequency |
|---|---|---|---|
| *Tartaruga* FP | GA140 | 168 | 0.500 |
| *Tartaruga* FP | SSRY89 | 118 | 0.500 |
| *Olho Roxo* FP | GA126 | 188 | 0.500 |
| *Olho Roxo* FP | SSRY13 | 212 | 0.483 |
| *Aruari* OX | SSRY13 | 222 | 0.483 |
| ADE | SSRY164 | 154 | 0.130 |
| Floodplain | GA126 | 188 | 0.107 |
| Floodplain | GA140 | 168 | 0.108 |
| Floodplain | SSRY89 | 118 | 0.107 |
| Floodplain | SSRY13 | 212 | 0.104 |
| Floodplain | SSRY13 | 238 | 0.196 |
| Oxisols | GA131 | 114 | 0.173 |
| Oxisols | SSRY13 | 222 | 0.095 |

High variation within and among varieties is evident when comparing the multilocus genotypes (MLGs) that are shared and unique to each variety (Figure 2). The 384 individuals included in this analysis presented 35 different MLGs, 21 of them were unique and were distributed within 8 varieties. The other 363 individuals were distributed among 14 MLGs, which were shared by 2 to 73 individuals. *Pirarucu Amarelo* from the floodplain and *Roxinha* from Oxisols were the only two varieties with a single MLG. The other varieties showed at least two different MLGs, and the Oxisol varieties *Tartaruga* and *Jabuti* presented four different MLGs. The varieties *Pirarucu Amarelo* (ADE), *Tartaruga* (floodplain), *Tartaruga* (Oxisol) and *Jabuti* presented more than one individual with MLGs different from the predominant MLG within the variety.

*Genetic structure among varieties*

Measures of genetic differentiation ($F_{ST}$) and gene flow ($Nm$) estimated among varieties of each soil type, revealed very high values for pairwise $F_{ST}$ and also restricted numbers of migrants between varieties that were assigned different names by farmers (Table 3). Some varieties with equivalent names but grown in different soil types (for example, between *Tartaruga* of Oxisols and *Tartaruga* of the floodplain) also presented significant differentiation values.

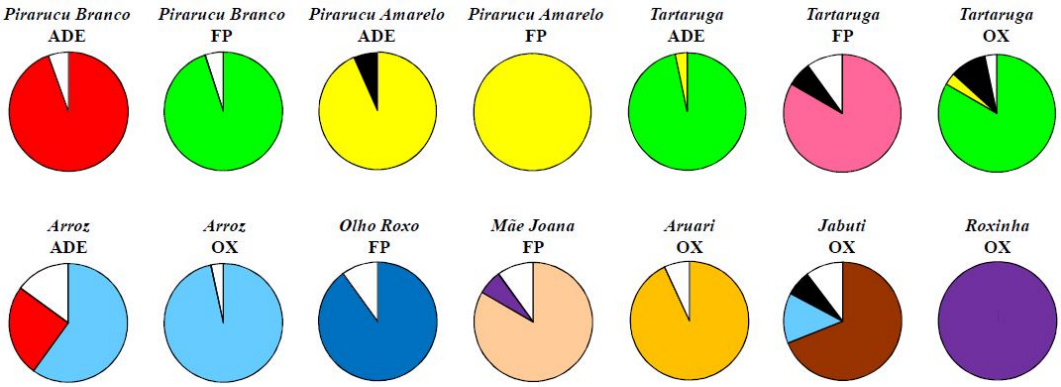


Figure 2. Proportions of multilocus genotypes (MLGs) for the 14 bitter varieties cultivated in three soil types in communities in Manicoré, Amazonas, Brazil. Different colors represent distinct MLGs. Black represents individuals that shared MLGs different from the dominant MLGs of their varieties. White represents unique MLGs for the whole set of varieties. Soil types in which varieties were grown are coded as ADE (Amazonian dark earths), FP (floodplain) and OX (Oxisols).

Table 3. Genetic differentiation ($F_{ST}$ – below diagonal) and gene flow (*Nm* – above diagonal) among the 14 bitter manioc varieties grown in 3 different soil types in Manicoré, Amazonas, Brazil, estimated with 10 microsatellite loci. The varieties are presented in the same order as in Table 1, with the two first letters indicating the name and the other two or three letters indicating the soil in which they were grown. Boldfaced values indicate comparisons between varieties with the same name, but grown in different soil types. Significant values ($P$ <0.001) are indicated in italics. Values of *Nm* coded as *Pan* arise from $F_{ST} \leq 0$ and indicate panmitic pairs of varieties.

| | PB ADE | PB FP | PA ADE | PA FP | AR ADE | AR OX | TA ADE | TA FP | TA OX | MJ FP | OR FP | AU OX | JA OX | RO OX |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PB ADE | | **0.16** | 0.45 | 0.44 | 0.40 | 0.13 | 0.17 | 0.25 | 0.17 | 0.29 | 0.22 | 0.19 | 0.59 | 0.24 |
| PB FP | ***0.61*** | | 0.30 | 0.30 | 0.36 | 0.19 | *Pan* | 0.38 | *Pan* | 0.23 | 0.20 | 0.41 | 0.43 | 0.38 |
| PA ADE | *0.36* | *0.45* | | ***Pan*** | 0.40 | 0.17 | 0.32 | 0.40 | 0.32 | 0.36 | 0.62 | 0.31 | 1.08 | 0.49 |
| PA FP | *0.36* | *0.45* | **-0.01** | | 0.41 | 0.17 | 0.32 | 0.40 | 0.33 | 0.36 | 0.61 | 0.31 | 1.07 | 0.49 |
| AR ADE | *0.39* | *0.41* | *0.38* | *0.38* | | **2.42** | 0.35 | 0.44 | 0.37 | 0.39 | 0.27 | 0.28 | 1.06 | 0.30 |
| AR OX | *0.65* | *0.57* | *0.59* | *0.59* | ***0.09*** | | 0.20 | 0.23 | 0.20 | 0.19 | 0.14 | 0.16 | 0.38 | 0.15 |
| TA ADE | *0.60* | -0.02 | *0.44* | *0.44* | *0.41* | *0.56* | | **0.38** | ***Pan*** | 0.23 | 0.21 | 0.41 | 0.44 | 0.41 |
| TA FP | *0.50* | *0.40* | *0.38* | *0.38* | *0.36* | *0.52* | ***0.40*** | | **0.39** | 0.36 | 0.31 | 0.25 | 0.56 | 0.21 |
| TA OX | *0.59* | -0.02 | *0.44* | *0.43* | *0.41* | *0.55* | **-0.01** | ***0.39*** | | 0.24 | 0.21 | 0.42 | 0.45 | 0.42 |
| MJ FP | *0.46* | *0.52* | *0.41* | *0.41* | *0.39* | *0.56* | *0.52* | *0.41* | *0.51* | | 0.33 | 0.30 | 0.54 | 0.28 |
| OR FP | *0.53* | *0.56* | *0.29* | *0.29* | *0.48* | *0.65* | *0.55* | *0.45* | *0.54* | *0.43* | | 0.27 | 0.44 | 0.25 |
| AU OX | *0.57* | *0.38* | *0.44* | *0.44* | *0.47* | *0.62* | *0.38* | *0.50* | *0.37* | *0.46* | *0.48* | | 0.37 | 0.40 |
| JA OX | *0.30* | *0.37* | *0.19* | *0.19* | *0.19* | *0.40* | *0.36* | *0.31* | *0.36* | *0.32* | *0.36* | *0.40* | | 0.51 |
| RO OX | *0.51* | *0.40* | *0.34* | *0.34* | *0.45* | *0.63* | *0.38* | *0.54* | *0.37* | *0.47* | *0.50* | *0.39* | *0.33* | |

Bayesian analysis of genetic structure among varieties, suggested that the optimal number of clusters was 12 ($\Delta K$, Evanno *et al*., 2005), which matched neither the number of varieties (14) nor the number of different varietal names (9). Generally, varieties with the same name were in the same clusters at $K$=12, except for the *Tartaruga* from the floodplain, which was in a different cluster from the *Tartaruga* found in ADE and Oxisols. These latter two varieties were grouped in the same cluster as the variety *Pirarucu Branco* from the floodplain (Figure 3). Thus, a total of 10 genetic groups were recovered in the Structure analysis, seven of them containing only one variety and the others containing groupings of the varieties *Pirarucu Amarelo* (from the floodplain and ADE), *Arroz* (from ADE and Oxisol), and a conspicuous cluster grouping the varieties *Tartaruga* (from ADE and Oxisols) plus *Pirarucu Branco* (from the floodplain). The varieties which were assigned to the same cluster by Structure showed the same MLGs, and the other varieties, each one assigned to distinct clusters, had distinct predominant MLGs.

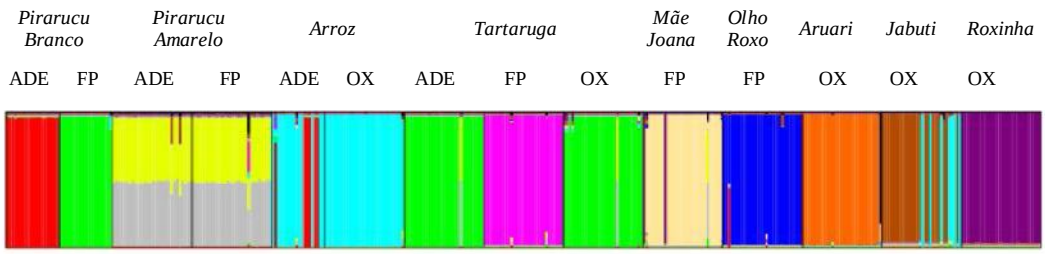


Figure 3. Best clustering result ($K$=12) of bitter manioc varieties grown in different soil types in Manicoré, Amazonas Brazil, using 10 microsatellite markers and the software Structure. Each individual is represented as a vertical line partitioned into colored segments, the length of which is proportional to the individual's estimated $K$ cluster membership coefficients. Soil types in which varieties were grown are coded as ADE (Amazonian dark earths), FP (floodplain) and OX (Oxisols).

Relationships among the varieties based on $D_A$ (Nei *et al*. 1983) genetic distance using the Neighbor-Joining algorithm (Figure 4) corroborated the interpretations of the Structure results. Varieties which were grouped in the same cluster by Structure were also closer to each other in the dendrogram, and had high bootstrap support. Reasonable bootstrap support was also found between *Jabuti* and *Arroz*. Soil types did not influence grouping of varieties.

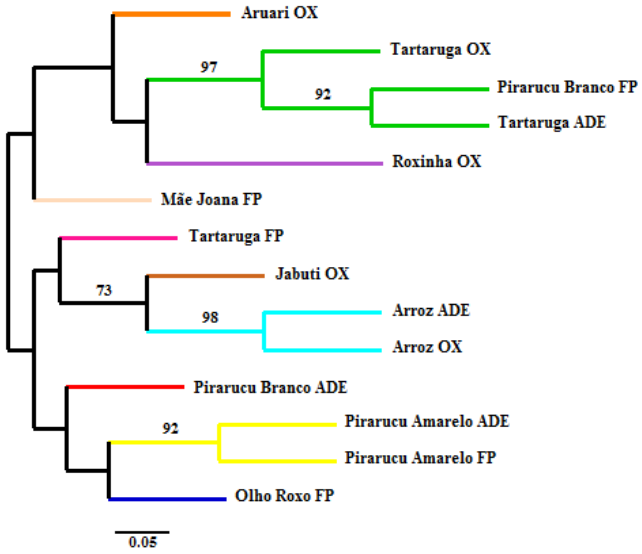


Figure 4. Neighbor-Joining dendrogram based on Nei *et al.*'s (1983) genetic distance ($D_A$) showing the relationships among 14 varieties grown in different soil types in Manicoré, Amazonas, Brazil. Soil types in which varieties were grown are coded as ADE (Amazonian dark earths), FP (floodplain) and OX (Oxisols). Bootstrap values greater than 70% are indicated.

Analysis of molecular variance revealed that most genetic variation is found within varieties, but a large proportion of variation is also found among them (Table 4), which is not unexpected given the sampling strategy. Additional AMOVAs showed that no variation was found among soil types and little was found among communities, which is another result of the sampling strategy.

The analysis of genetic structure and MLGs among varieties clearly showed that individuals with the same MLGs were present in different varieties. An additional Structure analysis was done to further explore the relationships of ancestry among these individuals. Analysis of migrants found 21 individuals with significant putative ancestry in clusters other than those to which they were assigned by farmers (Table 5). Only one individual of *Pirarucu Amarelo* from the floodplain had possible ancestry in two varieties, with considerably higher probabilities of being a descendent of *Tartaruga* from the floodplain. The likelihoods of this individual having had parents, grandparents or great-grandparents from the variety *Tartaruga* were nearly the same. One individual of the floodplain variety *Olho Roxo* showed high probability of having migrant parents from the ADE variety *Pirarucu Branco*. The other 19 individuals were detected as being migrants from other varieties.

Table 4. Analysis of molecular variance (AMOVA) for hierarchical groupings of 14 bitter manioc varieties grown in ADE, Oxisols and floodplain soils and communities in Manicoré, Amazonas, Brazil. The levels are: among the varieties; among varieties within soil types; among varieties within communities. d.f. = degrees of freedom.

| Source of variation | d.f. | Sum of squares | Components of variation | Percentage of variation |
|---|---|---|---|---|
| Among varieties | 13 | 906.67 | 1.22 | 43.67 |
| Within varieties | 766 | 1210.99 | 1.58 | 56.33 |
| Total | 779 | 2117.66 | 2.80 | |
| Among soil types | 2 | 125.11 | -0.03 | -1.29 |
| Among varieties within soil types | 11 | 781.55 | 1.25 | 44.74 |
| Within varieties | 766 | 1210.99 | 1.58 | 56.54 |
| Total | 779 | 2117.65 | 2.80 | |
| Among communities | 4 | 313.05 | 0.07 | 2.47 |
| Among varieties within communities | 9 | 593.62 | 1.17 | 41.44 |
| Within varieties | 766 | 1210.99 | 1.58 | 56.09 |
| Total | 779 | 2117.66 | 2.82 | |

Table 5. Individuals of bitter manioc grown in different soil types in Manicoré, Amazonas, Brazil, with significant probabilities of being migrants or having recent migrant ancestors according to Bayesian analysis based on 10 microsatellites performed with Structure. Soil types in which varieties were grown are coded as ADE (Amazonian dark earths), FP (floodplain) and OX (Oxisols).

| Sample ID | Farmer assignment | Putative ancestry | No migrant ancestry | Migrant | Migration event at prior generations | | |
|---|---|---|---|---|---|---|---|
| | | | | | 1st | 2nd | 3rd |
| 62 | *Tartaruga* ADE | *Pirarucu Amarelo* | 0 | 1 | 0 | 0 | 0 |
| 91 | *Tartaruga* OX | *Pirarucu Amarelo* | 0 | 1 | 0 | 0 | 0 |
| 152 | *Pirarucu Amarelo* FP | *Arroz* | 0.359 | 0 | 0.001 | 0.083 | 0.09 |
| | | *Tartaruga* FP | | 0 | 0.126 | 0.153 | 0.116 |
| 162 | *Arroz* ADE | *Pirarucu Branco* ADE | 0 | 1 | 0 | 0 | 0 |
| 173 | *Arroz* ADE | *Pirarucu Branco* ADE | 0 | 1 | 0 | 0 | 0 |
| 174 | *Arroz* ADE | *Pirarucu Branco* ADE | 0 | 1 | 0 | 0 | 0 |
| 175 | *Arroz* ADE | *Pirarucu Branco* ADE | 0 | 1 | 0 | 0 | 0 |
| 177 | *Arroz* ADE | *Pirarucu Branco* ADE | 0 | 1 | 0 | 0 | 0 |
| 178 | *Arroz* ADE | *Pirarucu Branco* ADE | 0 | 1 | 0 | 0 | 0 |
| 241 | *Mãe Joana* FP | *Roxinha* OX | 0 | 1 | 0 | 0 | 0 |
| 249 | *Mãe Joana* FP | *Roxinha* OX | 0 | 1 | 0 | 0 | 0 |
| 265 | *Mãe Joana* FP | *Pirarucu Amarelo* | 0 | 0.998 | 0.001 | 0 | 0 |
| 273 | *Olho Roxo* FP | *Pirarucu Branco* ADE | 0.021 | 0 | 0.743 | 0.185 | 0.043 |
| 330 | *Aruari* OX | *Mãe Joana* FP | 0 | 1 | 0 | 0 | 0 |
| 346 | *Jabuti* OX | *Arroz* | 0 | 1 | 0 | 0 | 0 |
| 349 | *Jabuti* OX | *Arroz* | 0 | 1 | 0 | 0 | 0 |
| 353 | *Jabuti* OX | *Arroz* | 0 | 1 | 0 | 0 | 0 |
| 356 | *Jabuti* OX | *Arroz* | 0 | 1 | 0 | 0 | 0 |
| 357 | *Jabuti* OX | *Arroz* | 0 | 1 | 0 | 0 | 0 |
| 358 | *Jabuti* OX | *Arroz* | 0 | 1 | 0 | 0 | 0 |
| 360 | *Jabuti* OX | *Arroz* | 0 | 1 | 0 | 0 | 0 |

## Discussion

*The maintenance of high genetic diversity within bitter manioc varieties*

High genetic diversity and also great excess of heterozygotes were found in all varieties included in this study. The set of 390 individuals representing 14 varieties had a mean number of alleles per locus of 4.5, which was proportionally lower than the mean of 3.8 alleles per locus found for 153 individuals of five manioc varieties from Palikur Amerindians in French Guyana (Pujol *et al*. 2007). Observed heterozygosities much higher than expected heterozigosities were also found for the same set of Palikur varieties (Pujol *et al*. 2005). These authors also demonstrated that inadvertent human selection for heterozygosity results in high intra-varietal diversity in manioc because the Palikur selectively retain heterozygous volunteer seedlings in their swiddens. Pujol & McKey (2006) demonstrated that the size of seedlings was correlated with their multilocus heterozygosities and with their survival. This occurs because during the weeding of swiddens small seedlings are removed and larger ones are retained, and these more heterozygous seedlings become candidates for clonal propagation. Such a process allows maintenance of heterozygosity (through clonal propagation) and genetic diversity (through incorporation of seedlings) in manioc varieties (Pujol *et al*. 2005).

The importance of the incorporation of seedlings for increasing the genetic diversity found within varieties was also evidenced with the results of MLG analysis. These results agree with previous studies that showed that manioc varieties are polyclonal, with a preponderance of one clone and mixture of other different genotypes (Peroni 2004; Pujol *et al*. 2007). The Palikur varieties had from six to 16 different MLGs, and at least one new MLG was found within four of the varieties after the incorporation of seedlings (Pujol *et al*. 2005, 2007). For 12 varieties of the Makushi Amerindians, in French Guyana, Elias *et al*. (2001) found from one to six MLGs before seedling incorporation, and from two to 15 different MLGs within varieties after the incorporation. Interestingly, in two communities along the middle Madeira River, Fraser & Clement (2008) observed that about one third of the farmers intentionally used seedlings for clonal propagation. Such farmers made stem cuttings from those seedlings they considered to be most attractive or healthy, and planted them separately for evaluation. Most of these farmers believe that the seedlings always yield clones of an existing variety (Fraser & Clement 2008). These authors also observed farmers who unintentionally incorporate seedlings into existing varieties. Such farmers did not remove seedlings (some or all of them) during weeding, harvested them and did not separate stem

cuttings of the seedlings from the rest of the varieties. Therefore, the incorporation of seedlings may play an important role on the amplification of genetic diversity within varieties cultivated along the middle Madeira River, just as occurs among the Makushi and Palikur.

The fact that three varieties had at least one private allele with high frequency may be related to farmers' selection of specific traits in the varieties *Tartaruga* and *Olho Roxo* from the floodplain and *Aruari* from Oxisol. It is also possible that such alleles resulted from somatic mutations that occurred in plants used to prepare stem cuttings in previous cycles of cultivation (McKey *et al*. 2010b). Eight of the 12 private alleles found for the soil types had frequencies greater than 0.05, and each soil type had one private allele with high frequency different from those found for the varieties. Since 45 alleles were found, it is unlikely that only these three alleles are related with differential features in the sets of varieties of each soil type, and they may be related to the different genetic composition of varieties sampled in each soil. Microsatellites in expressed sequence tags (EST-SSRs) may be more useful to unravel the associations of microsatellite alleles with specific traits (Li *et al.* 2004).

*Genetic structure among bitter manioc varieties grown in Central Amazonia*

As suggested by the high pairwise $F_{ST}$ and very low numbers of migrants, the varieties are greatly differentiated from each other. Similar results were found by Pujol *et al.* (2005) with Palikur varieties, although pairwise $F_{ST}$ found by these authors were somewhat lower. Higher $F_{ST}$ among the varieties sampled here may mean that incorporation of seedlings into existing varieties occurs less frequently in communities along the middle Madeira River than in Palikur communities.

Incorporation of seedlings has been proved to increase intra-varietal diversity (Elias *et al.* 2001; Sambatti *et al.* 2001; Pujol *et al*. 2007). If the seedlings incorporated into a variety resulted from crosses with another variety (or even between two other varieties) this process may be seen as gene flow events. However, manioc is exclusively propagated by stem cuttings, which means that the ancestry of all individuals of a variety (within a swidden during a given cycle of cultivation) is restricted to a small number of individuals. This is why we chose the no admixture model of ancestry, which assumes that each individual comes purely from one of the *K* populations, for further analysis of genetic structure in Structure. Initial simulations were performed under the correlated allele frequencies model (allele frequencies in different populations are likely to be similar), but as indicated by very high

pairwise values of $F_{ST}$ this is probably not the case. Simulations were redone under the no admixture model with independent allele frequencies (allele frequencies in different populations are expected to be reasonably different). Both allele frequency models rendered similar results, but we adopted the independent allele frequencies for the reason specified above.

The associations of varieties into Structure groups agreed with the amount of genetic differentiation previously found: all pairs of varieties with low pairwise $F_{ST}$ were grouped in the same cluster, while the other varieties were distinct from each other. However, the Bayesian approach supplied additional information on the relations of ancestry among all varieties. For instance, it is possible to verify that within the variety *Jabuti* there are some individuals that appear to come from the variety *Arroz*, which, in turn, has individuals that genetically resemble individuals of the variety *Pirarucu Branco*.

Fraser & Clement (2008) and Fraser (2010) suggested that traditional farmers in communities along the middle Madeira River developed distinct sets of varieties for the different soil types in which manioc is grown. These authors observed similar adaptations in varieties from ADE and the floodplain, and suggested that the varieties of these two soil types would be genetically closer and more divergent from the varieties grown in Oxisols. However, the varieties *Tartaruga* and *Pirarucu Branco* from the floodplain formed distinct clusters from the corresponding varieties in ADE. Additionally, the varieties *Tartaruga* and *Arroz* from ADE were grouped with the corresponding varieties in Oxisols, contrary to the expectations of Fraser & Clement (2008) and Fraser (2010). The varieties *Pirarucu Amarelo* from the floodplain and ADE were the exception and were grouped together in Structure. This may be due to the fact that these were the only two varieties with the same name sampled in different soils of the same community. The fact that varieties with equivalent names, but sampled in different soils of different communities were, in general, grouped in the same cluster of Structure, reinforces the finding that the greatest genetic divergence is found among the varieties cultivated in the middle Madeira River, rather than among soils. Additionally, a certain pattern may be inferred based on the varieties sampled in more than one soil type, especially for *Tartaruga*: there seems to be a tendency for varieties from the floodplain to be genetically differentiated from corresponding varieties in upland soils, with *Pirarucu Amarelo* being an exception. Therefore, the hypothesis derived from ethnobotanical observations (Fraser & Clement 2008; Fraser 2010) may need to be modified: There seem to be sets of

manioc varieties that are genetically differentiated between the floodplain and upland soils (ADE and Oxisols) along the middle Madeira River, rather than between ADE and Oxisols.

The genetic structure found with Structure was corroborated by the relationships among the varieties based on the Neighbor-Joining dendrogram. No clear patterns of associations of varieties from the same soil type or from the same community were observed, although the sampling strategy certainly contributed to this also. The lack of bootstrap support for most of the branches may be due to the fact that, as detected by $F_{ST}$ and Structure, the varieties sampled are so distinct from each other that it may be difficult to establish genetic relationships among them without many more microsatellite loci. The reasonable support found for the relationship between *Arroz* and *Jabuti* may be explained by the great proportion of individuals within *Jabuti* assigned to *Arroz* by Structure. However, the variety *Arroz* from ADE also had a great proportion of individuals assigned to the variety *Pirarucu Branco* from ADE, but they were not closely related. This may be the result of higher similarity in allele frequencies, or even in allele composition, between the varieties *Arroz* and *Jabuti*, as suggested by the lower $F_{ST}$ found between Arroz (ADE)/Jabuti (Oxisol) than between Arroz (ADE)/Pirarucu Branco (Floodplain) (0.19 and 0.41, respectively) and by the composition of MLGs in these varieties.

The lack of variation among soil types and the low variation among communities found in AMOVA was rather surprising since the floodplain communities had a generally different set of varieties when compared to the upland communities (ADE and Oxisols) (Alves-Pereira *et al*. 2011 – in preparation), although this is also partially an artifact of the sampling strategy. Nevertheless, the results of AMOVA also corroborate both the finding that intra-varietal genetic diversity is high, as suggested by indices of diversity, and the finding that the varieties included in this study are, in general, very distinct from each other, as suggested by coefficients of differentiation and Structure analysis.

An intriguing result was that the variety *Pirarucu Branco* from the floodplain has been shown to be almost identical to the varieties *Tartaruga* from ADE and Oxisol. These three varieties had the same predominant MLGs, even though *Pirarucu Branco* was collected in a different community from the others. The naming done by farmers is based on perceptual distinctiveness among the varieties, i.e., farmers recognize varieties as a group of individuals that share a determined set of morphological characteristics that are different from other varieties (Boster 1985; Elias *et al*. 2000). The morphological characteristics are influenced by genotype-environment interactions that may cause different phenotypic expressions of the

same genotype, and create morphotypes that are identified as distinct varieties (Emperaire *et al.* 1998). Therefore, it is possible that when the variety *Tartaruga* was moved from the uplands (ADE or Oxixols) to the floodplain (or vice versa) it suffered a name change due to differential phenotypic expression of the same genotype in different environments.

Although distinct varieties have different sets of morphological characteristics, there also may be a certain range of variation in the morphological characteristics that identifies each variety (Boster 1985; Elias *et al*. 2000; Duputié *et al*. 2009). Therefore, it may be assumed that within the variety *Pirarucu Branco* the individuals from the floodplain and from ADE, in spite of having different MLGs, present a set of morphological characteristics which are sufficiently similar to be grouped into the same range of phenotypic variation, which encourages farmers to give them the same varietal name. The same argument may be valid for the individuals of the variety *Tartaruga* from the floodplain, which were genetically distinct from the individuals of upland soils (ADE and Oxisol).

It was also shown that within the varieties those individuals assigned to other clusters by Structure had MLGs distinct from the most common MLG of that cluster, but equal to the predominant MLG of a different variety (for example, between *Arroz* and *Pirarucu Branco* from ADE). These results may be due to farmer confusion when assigning individuals to varieties. The term confusion is relative because genotype-environment interactions may influence the naming of varieties (Emperaire *et al.* 1998), and because the criteria for distinguishing varieties may vary among farmers (Salick *et al*. 2001; Sambatti *et al*. 2001; Emperaire & Peroni 2007; Heckler & Zent 2008).

The general pattern observed from the migration analysis in Structure followed the tendencies identified in the MLG analysis, with migrations occurring between varieties irrespective of the communities or the soil type. There may be different reasonable interpretations for migrations here, such as the transfer of stem cuttings between different swiddens or communities (Emperaire & Peroni 2007), farmers' confusion in identifying an adult individual (Sambatti *et al*. 2001), incorporation of seedlings into existing varieties (Martins 2001; Pujol *et al*. 2007) or ambiguous identification of the same material by different farmers (Oliveira, 2008).

The varieties in which the migrant individuals have putative ancestry are not definitive results, because it is possible that the "real" ancestors of genetically divergent individuals are in other varieties that were not sampled in this study. Moreover, the real migration rates may be higher or lower than the ones assumed in this analysis, and they may be variable across

space. Indeed, the exchange of material among farmers, the capacity for identifying varieties, rates of incorporation of seedlings and the naming of varieties may vary greatly among and even within different regions (Sambatti *et al*. 2001; Peroni & Hanazaki 2002; Fraser & Clement, 2008; Oliveira 2008). Thus, the most meaningful results of migrant analysis are that different varieties contribute to the genetic diversity of other varieties, and that such a process is not related to the soil types or to the communities in which the varieties are grown. Moreover, whatever may be the explanations of given migration events, all of them ultimately contribute to the maintenance of high genetic diversity within the varieties traditionally cultivated in the communities along the middle Madeira River.

**Conclusions**

This study was the first to report on the genetic diversity and the genetic structure of varieties traditionally grown in different soil types in a small geographic region. Microsatellite variation revealed that traditional farmers of different communities along the middle Madeira River maintain high levels of genetic diversity within some of the most frequently cultivated varieties in the region. Although there is large intra-varietal genetic diversity, varieties have distinct genetic features that, in general, differentiate one from the other. The genetic structure is primarily related to the varieties *per se* and there was no clear tendency of more similarity between varieties from the floodplain and ADE, as predicted by ethnobotanical observation. Rather, there seems to be an interesting and important genetic structure between varieties grown in the floodplain soils and the respective varieties grown in upland soils (ADE and Oxisols). While indirect estimates pointed to restricted gene flow between the varieties, the Bayesian analysis revealed that some of the varieties contribute to the variability found within other varieties, whether they are from the same community or are grown in the same soil type or not, which also contributes to maintain and amplify genetic diversity within the varieties cultivated in communities along the middle Madeira River. We think it is worthwhile to invest in social policies that value the practices of smallholders, as they maintain high levels of genetic diversity within their cultivated varieties of bitter manioc. These farmers are natural collaborators for the *on-farm in-situ* conservation of the genetic resources of manioc.

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## 3. SÍNTESE

### *3.1. Alta variabilidade genética dentro e entre variedades*

Em ambos os capítulos as estimativas dos parâmetros básicos de diversidade genética e as análises de genótipos multilocos (GMLs) evidenciam alta variabilidade genética nas variedades de mandiocas bravas cultivadas tradicionalmente na região do médio Rio Madeira. Os índices de diversidade encontrados no capítulo 1 para o conjunto de variedades foram elevados ($\bar{A}$ = 5,6; $H_O$ = 0,576) e similares a estudos realizados em outras regiões (Mühlen *et al.*, 2000; Elias *et al*., 2004; Peroni *et al*., 2007). Quando a diversidade genética intra-varietal foi investigada no capítulo 2 todas as 14 variedades apresentaram heterozigosidades observadas maiores que as esperadas (com $H_O$ variando de 0,495 a 0,707). A análise de GMLs mostraram que todas as variedades amostradas em mais de 5 roças no capítulo 1, e a maioria das variedades incluídas no capítulo 2 corroboram estudos posteriores que mostraram que as variedades locais de mandioca são polifiléticas, sendo compostas por um clone preponderante mais um conjunto de indivíduos com genótipo diferente, mas similares em termos morfológicos (Peroni, 2004; Pujol *et al*., 2007). A grande variação genética entre variedades pode ser percebida pelo fato das AMOVAs do capítulo 1 apontarem que a maior parte da variação genética é encontrada dentro das roças, que são representadas por indivíduos de variedades distintas, e é evidente quando demonstrado no capítulo 2 que variedades distintas tendem a ter conjuntos de GMLs diferentes, pelos valores muito elevados de $F_{ST}$ par a par encontrados na maioria dos casos e pela grande proporção de variação encontrada entre variedades pelas AMOVAs.

A incorporação de plântulas voluntárias, praticada consciente ou inconscientemente, tem sido apontada como o principal processo que permite a amplificação da diversidade genética dentro do cultivo (Elias *et al*., 2001; Martins, 2001; Peroni, 2004; Pujol *et al*., 2007; Duputié *et al*., 2009). Enquanto tal processo garante a amplificação da diversidade genética, a propagação vegetativa proporciona a manutenção de indivíduos heterozigotos (Pujol *et al*., 2005). De fato Fraser e Clement (2008), em suas observações etnobotânicas em duas comunidades na região do médio Rio Madeira, constataram que uma grande parte dos agricultores incorpora, de forma consciente ou inconsciente, plântulas ao conjunto de variedades que manejam em suas roças. É interessante observar que foram detectados 82 GMLs para o conjunto de 50 variedades de mandiocas bravas e mansas incluídas na análise do capítulo 1. Como observado para outras regiões, a incorporação de plântulas parece ter um papel importante para amplificação da diversidade genética também na região do médio Rio

Madeira. Entretanto outros processos que podem colaborar para a amplificação da diversidade genética nas variedades de mandioca não devem ser negligenciados. É possível que mutações somáticas que ocorreram nas plantas utilizadas para produzir estacas para propagação vegetativa também contribuam para a geração de diversidade genética (McKey *et al*., 2010b). Apesar de mutações não ocorrerem com tanta freqüência, mesmo nas regiões de microssatélites que possuem taxas de mutação mais elevadas que porções mais conservadas do genoma, elas podem ser fixadas em uma variedade através da propagação clonal após um único ciclo de cultivo. Além disso, os critérios para a designação de nomes de variedades podem ser variáveis entre diferentes comunidades ou mesmo entre diferentes agricultores (Salick *et al.*, 1997; Oliveira, 2008).

### *3.2. Elevados valores de endogamia para o conjunto de plântulas*

O conjunto de plântulas e o conjunto de variedades apresentaram números médios de alelos por loco similares ($\bar{A}$ = 5,3 *versus* 5,6), entretanto o coeficiente de endogamia foi cerca de três vezes maior para o conjunto de plântulas (*f*=0,242 *versus* 0,086). O fato das plântulas apresentarem um grande déficit de heterozigotos enquanto as variedades apresentam um grande excesso de heterozigotos parece contradizer a importância da incorporação de plântulas para a manutenção de alta diversidade genética dentro das variedades. Entretanto esta contradição é desfeita ao se considerar que nem todas as plântulas são incorporadas. Pujol e McKey (2006) demonstraram que o tamanho das plântulas voluntárias é relacionado com suas heterozigosidades e sobrevivência. Estes autores demonstraram que índios Palikur, durante a capina das roças, removem, consciente ou inconscientemente, as plântulas de menor tamanho, possibilitando que as plântulas maiores e mais heterozigotas se desenvolvam e que sejam avaliadas durante a época de colheita. Caso decidam pela incorporação, a chance de adicionarem plantas heterozigotas ao conjunto de variedades existente nas roças é maior. É possível que tal processo também possa ocorrer entre agricultores na região do médio Rio Madeira.

Apesar da baixa eficiência, a análise de parentesco forneceu resultados interessantes. Dentre eles o fato de que um quinto dos indivíduos teve como ambos mais prováveis pais variedades que não ocorriam na mesma roça e a própria baixa eficiência da análise podem ser um indicativo de que foram utilizadas em ciclos de cultivo anteriores variedades geneticamente distintas das utilizadas no ciclo de cultivo à época em que a amostragem foi realizada.

### *3.3. Divergência entre variedades manas e bravas*

Embora esta questão não faça parte dos objetivos principais deste trabalho, a coleta de variedades mansas encontradas nas roças em meio às variedades bravas no capítulo 1 permitiu constatar, como seria esperado, a divergência genética entre mandiocas mansas e bravas. A diferença no número amostral (21 indivíduos de variedades mansas e 184 de variedades bravas) reflete a observação de que as mandiocas mansas são cultivadas em menor escala onde o cultivo de mandiocas bravas é predominante (McKey e Beckerman, 1993; McKey *et al.*, 2010a). Trabalhos formulados especialmente para avaliar esta questão encontraram uma considerável divergência genética entre as variedades mansas e bravas (Mühlen *et al*., 2000; Peroni *et al*., 2007), confirmando uma base genética para a distinção entre estes dois grupos de variedades observada por agricultores tradicionais (McKey e Beckerman, 1993; Elias *et al.*, 2000).

O fato das variedades mansas estarem mais proximamente relacionadas às variedades bravas cultivadas na várzea pode ter alguma relação com a hipótese sobre a divergência das variedades mansas e bravas sugerida por Arroyo-Kalin (2010). Neste contexto, as variedades mansas que surgiram primeiro sob um cultivo ainda incipiente, em um ambiente fértil ao redor das unidades familiares, teriam sido experimentadas nos solos férteis das várzeas quando o cultivo da mandioca começou a ser mais intensivo. Conforme a intensificação aumentava como resposta à demanda crescente por alimentos para as primeiras civilizações sedentárias na Amazônia, o cultivo pode ter sido levado para ambientes de terra firme mais estáveis do que às várzeas. Embora tenham interpretações meramente especulativas, estes resultados levantam novas questões sobre a divergência entre variedades mansas e bravas em diferentes ambientes de cultivo na Amazônia: o relacionamento mais próximo entre variedades mansas e variedades bravas da várzea continuará sendo observado com amostragens mais sistemáticas em outras regiões da Amazônia? Quais os fatores genético-evolutivos, ecológicos e antropológicos contribuem para tal padrão de relacionamento?

### *3.4. Estrutura genética entre variedades cultivadas em ambientes diferentes*

Embora os capítulos deste trabalho foram desenhados para se testar hipóteses um tanto diferentes, quando tomados em conjunto fornecem evidências complementares sobre esta diferenciação entre ambientes de cultivo, e os resultados mais marcantes deste trabalho são os que se referem a esta questão.

No capítulo 1 a diferenciação genética entre o conjunto de variedades cultivadas na várzea e o conjunto de variedades cultivadas nos solos inférteis de terra firme foi cerca de seis

vezes maior do que a diferenciação genética encontrada entre os solos de terra firme. Além disso, a variação genética encontrada entre o conjunto de roças da várzea e o conjunto de roças dos solos de terra firme pela AMOVA foi menor apenas do que a encontrada entre as variedades mansas e bravas. Evidências desta diferenciação também podem ser detectadas nas análises de coordenadas principais e na análise de agrupamento com o método *Neighbor Joining*. No capítulo 2, apesar das variedades não estarem estruturadas de acordo com o tipo de solo, pode ser observada um a tendência de que variedades com nomes iguais, mas cultivadas em solos antropogênicos e na várzea, sejam geneticamente distintas, ao passo que as variedades de nomes iguais cultivadas em solos antropogênicos e nos solos inférteis da terra firme são geneticamente mais semelhantes entre si.

Como sugerido pelos trabalhos baseados em observações etnobotânicas (Fraser e Clement, 2008; Fraser, 2010), os diferentes tipos de solo estão relacionados à estruturação genética das variedades de mandiocas bravas que são cultivadas na região do médio Rio Madeira. Entretanto, ao contrário do esperado por estes autores, as variedades de várzea não são geneticamente mais relacionadas às variedades de solos antropogênicos. Parece existir uma forte diferenciação genética entre as variedades cultivadas na várzea e as variedades cultivadas em solos de terra firme (solos antropogênicos e Latossolos). A diferenciação de variedades de mandioca cultivadas em diferentes ambientes da Amazônia peruana já tinha sido previamente sugerida com base na variação morfológica (Salick *et al*, 1997), entretanto a base genética desta diferenciação é desconhecida. Também deve existir uma diferenciação entre ambientes em uma escala geográfica maior: Corderiro e Abadie (2007) relatam que a caracterização parcial da coleção brasileira de germoplasma de mandioca aponta para diferenciação entre as regiões ecogeográficas do Brasil. Apesar deste trabalho contradizer grande parte das hipóteses formuladas a partir observações etnobotânicas, os trabalhos de Fraser e Clement (2008) e Fraser (2010) tiveram o mérito de atentar que a variação em ambientes de cultivo estava sendo negligenciada pelos trabalhos anteriores que avaliavam questões sobre a dinâmica evolutiva da mandioca. Este trabalho é o primeiro a demonstrar uma diferenciação com base em dados moleculares entre variedades de mandiocas bravas relacionadas com diferentes ambientes de cultivo na Amazônia.

Apesar da grande diferenciação encontrada entre as variedades e da estruturação genética relacionada aos diferentes ambientes de cultivo, análises Bayesianas sugeriram que algumas variedades colaboram com a diversidade genética encontrada dentro de outras variedades, independentemente de serem cultivadas no mesmo tipo de solo ou não. O deslocamento de variedades entre diferentes roças ou comunidades, a troca de variedades

entre agricultores, as interações genótipo-ambiente que podem interferir na designação do nome de variedades, os critérios adotados para a designação de variedades são alguns dos vários fatores de ordem genética, ecológica e social que podem contribuir para que isto seja observado. Independentemente de quais fatores estejam envolvidos, todos eles exercem um papel importante na manutenção e amplificação da diversidade genética dentro do cultivo. Ao demonstrar-se que as variedades locais de mandioca possuem uma ampla diversidade genética demonstra-se o papel fundamental que os agricultores tradicionais da região do médio Rio Madeira exercem no auxílio à conservação e amplificação dos recursos genéticos de mandioca.

### *3.5. Conclusões Gerais*

Com base nos resultados e discussões expostos ao longo dos dois capítulos desta dissertação, as principais conclusões deste trabalho são:

- As comunidades de agricultores tradicionais manejam alta diversidade genética entre e dentro das variedades de mandiocas bravas cultivadas em três tipos de solos ao longo da região do médio Rio Madeira;
- Comunidades com acesso a tipos de solo mais variados não necessariamente manejam mais diversidade genética em suas variedades do que comunidades em que agricultores têm acesso a menos tipos de solos. Os agricultores das comunidades estabelecidas na várzea manejam variedades com maior diversidade genética.
- Parece existir uma importante estruturação genética entre variedades de mandiocas bravas relacionada ao tipo de solo em que são cultivadas, pois as variedades cultivadas na várzea são geneticamente diferenciadas das variedades cultivadas nos solos de terra firme.
- Apesar de existir uma forte diferenciação genética entre as variedades e entre ambientes de cultivo, e de estimativas indiretas apontarem fluxo gênico restrito entre as variedades, análises Bayesianas mostraram que algumas das variedades contribuem com a diversidade genética encontrada em outras variedades, independentemente de serem cultivadas no mesmo tipo de solo ou não.

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## APÊNDICES

Apêndice 1 – Listagem das variedades tradicionais amostradas no primeiro esquema de coleta e sua ocorrência nos tipos de solo (SA = solos antropogênicos; LS = latossolos; VA = várzea) nas comunidades do interior do município de Manicoré. Os nomes das variedades e o tipo (brava ou mansa) foram designados pelos agricultores. N= número total de indivíduos amostrados por variedade.

| Variedade | Solo | | | Comunidades (n° de indivíduos) | N |
|---|---|---|---|---|---|
| | SA | LS | VA | | |
| **Bravas** | | | | | |
| **Açaízinho** | | | X | Verdum (2) | 2 |
| **Amarelinha** | | X | X | Água Azul (2), Fortaleza (1) | 3 |
| **Arara** | X | | X | Barro Alto (1), Verdum (1) | 2 |
| **Arroz** | X | X | | Água Azul (9), Barreira do Capanã (5), Barro Alto (4) | 18 |
| **Aruari** | X | X | X | Água Azul (6), Barreira do Capanã (6), Barro Alto (3) | 15 |
| **Azulão** | X | | | Água Azul | 1 |
| **Boliviana** | | | X | Pau Queimado | 1 |
| **Coxa Branca** | X | X | X | Barreira do Capanã | 4 |
| **Curuçá** | | | X | Verdum | 3 |
| **De Maniva** | X | | | Água Azul | 1 |
| **Faianca** | | X | | Água Azul | 2 |
| **Flecha** | X | | | Barreira do Capanã | 1 |
| **Glaí** | X | X | | Barreira do Capanã | 4 |
| **Grelo Roxo** | | | X | Fortaleza | 2 |
| **Guia Roxa** | | | X | Fortaleza | 1 |
| **Jabuti** | X | X | X | Água Azul (8), Barreira do Capanã (7) | 15 |
| **Jabuti Amarelo** | | X | | Água Azul | 1 |
| **Jabuti-Arroz** | | X | | Água Azul | 1 |
| **Jiju** | X | X | X | Barreira do Capanã (1), Barro Alto (4), Pau Queimado (2), Fortaleza (1) | 8 |
| **Juvenal** | | | X | Barreira do Capanã (1), Pau Queimado (6), Fortaleza (2) | 9 |
| **Mãe Joana** | | | X | Água Azul (1), Fortaleza (3) | 4 |
| **Manaus** | X | X | X | Barro Alto (6), Pau Queimado (1) | 7 |
| **Mané Velho** | | X | | Água Azul | 1 |
| **Manicoré** | X | | | Barro Alto | 2 |
| **Maniva de Veado** | X | | | Barro Alto | 1 |
| **Mucurão** | | | X | Pau Queimado (1), Fortaleza (1) | 2 |
| **Olho Roxo** | | | X | Pau Queimado (6), Fortaleza (1) | 7 |
| **Piraíba** | | X | | Barreira do Capanã | 2 |
| **Pirarucu Amarelo** | X | X | X | Águ Azul (6), Barreira do Capanã (1), Verdum (2) | 9 |
| **Pirarucu Branco** | X | X | X | Barreira do Capanã (4), Pau Queimado (1) | 5 |
| **Poré** | | X | | Barreira do Capanã | 1 |
| **Roxa** | | X | | Barreira do Capanã | 1 |
| **Roxinha** | X | X | X | Água Azul (3), Barreira do Capanã (6), Barro Alto (5), Verdum (1) | 15 |
| **Roxona** | X | | | Barreira do Capanã | 1 |
| **Saranzal** | X | X | | Barro Alto | 2 |
| **Sem Nome** | X | X | X | Águ Azul (2), Barreira do Capanã (1), Barro Alto (1), Pau Queimado (3), Verdum (1) | 8 |
| **Sempre Serve** | | | X | Verdum | 3 |
| **Tartaruga** | X | X | X | Barro Alto (13), Verdum (1) | 14 |
| **Tartaruga B** | X | | | Barro Alto | 1 |
| **Tartaruga Baixinha** | | | X | Verdum | 1 |

| | | | | | |
|---|---|---|---|---|---|
| **Tartaruga Branca** | | | X | Verdum | 1 |
| **Tartaruga Casca Roxa** | | | X | Verdum | 1 |
| **Tico Baco** | X | | | Água Azul | 1 |
| **Mansas** | | | | | |
| **Macaxeira** | | X | X | Barro Alto (2), Fortaleza (1) | 3 |
| **Macaxeira Bolacha** | | X | X | Barro Alto (1), Fortaleza (1) | 2 |
| **Macaxeira Branca** | X | | X | Barro Alto (1), Verdum (1) | 2 |
| **Macaxeira Casca Roxa** | | | X | Pau Queimado | 1 |
| **Macaxeira Casca Vermelha** | X | | | Barro Alto | 1 |
| **Macaxeira da Pele Roxa** | | | X | Barreira do Capanã | 1 |
| **Macaxeira Pão** | X | | X | Barreira do Capanã (1), Pau Queimado (2), Verdum (2) | 5 |
| **Macaxeira Roxa** | X | | X | Barro Alto (1), Pau Queimado (1) | 2 |
| **Macaxeira Roxinha** | X | | | Barro Alto | 1 |
| **Macaxeira sem nome** | | | X | Pau Queimado (2), Verdum (1) | 3 |

Apêndice 2 – Listagem das variedades tradicionais amostradas nos diferentes tipos de solo no segundo esquema de coleta e sua ocorrência nas comunidades do interior do município de Manicoré. Os nomes das variedades foram designados pelos agricultores. N= número de indivíduos amostrados por variedade.

| **Variedade** | **N** | **Comunidade** |
|---|---|---|
| **Solos Antropogênicos** | | |
| **Pirarucu Branco** | 20 | Barreira do Capanã |
| **Pirarucu Amarelo** | 30 | Água Azul |
| **Arroz** | 20 | Barreira do Capanã |
| **Tartaruga** | 30 | Barro Alto |
| **Latossolos** | | |
| **Arroz** | 30 | Água Azul |
| **Tartaruga** | 30 | Barro Alto |
| **Aruari** | 30 | Barreira do Capanã |
| **Jabuti** | 30 | Barreira do Capanã |
| **Roxinha** | 30 | Barro Alto |
| **Várzea** | | |
| **Pirarucu Branco** | 20 | Pau Queimado |
| **Pirarucu Amarelo** | 30 | Água Azul |
| **Tartaruga** | 30 | Verdum |
| **Mãe Joana** | 30 | Água Azul |
| **Olho Roxo** | 30 | Pau Queimado |