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RESEARCH ARTICLE

Origins and genetic conservation of tropical trees in agroforestry systems: a case study from the Peruvian Amazon

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Abstract Hundreds of native tree species are currently found in extensive agroforestry ecosystems in the Peruvian Amazon, forming an important reservoir of biodiversity. To further promote conservation, farmers are encouraged to supplement intra-specific genetic diversity in these populations with seed collected from local forests. For some tree species, however, this approach may be inappropriate, as stands of these taxa already found on-farm may not be of local origin. Despite this issue being of importance for conservation, little information is available on the history of cultivated trees in the region, a situation that we here rectify for the important fruit tree *Inga edulis*. Based on nuclear SSR and chloroplast DHPLC analyses of closely geographically matched natural and planted stands at five sites, it appears that cultivated material of *I. edulis* is primarily of non-local origin, indicating that conservation based on new wide-scale infusions from local wild stands into farms may be inappropriate in the region. Although nuclear and chloroplast diversity were both lower in planted stands, values were still relatively high (~80 and 70% of natural stands, respectively), indicating that when farmers plant trees, good collection practice of seed from already cultivated *I. edulis* should be an effective means for ensuring long-term conservation on farms.

Keywords DHPLC · *Inga edulis* · On-farm tree conservation · Peruvian Amazon · SSR

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Introduction

Current land use practices in the tropics (FAO 2001) mean that the use and conservation of trees depends increasingly on their incorporation into managed agroforests (Clement and Villachica 1994; Simons et al. 2000; Kindt 2002; Reynel et al. 2003). These agroforestry systems have been established primarily for immediate human benefit, but they also serve as biodiversity refugia in landscapes subjected to heavy deforestation and degradation. Thus ''on farm'' populations established for human use can also potentially serve as a ''*circa situ*'' conservation resource. In the Peruvian Amazon, hundreds of native tree species occur in these agroforestry systems and provide a wide range of products and services to local inhabitants (Sotelo Montes and Weber 1997). Recognising the potential conservation importance of these systems for native tree species, conservation and development agencies encourage

local communities to undertake additional transfers of tree germplasm (and hence genetic diversity) from local natural stands into farmland populations (O'Neill et al. 2001; Weber et al. 2001). This is to maximise their value by ensuring they represent the diversity present in natural stands, by reducing the likelihood of inbreeding depression, and by providing the potential for adaptation to future environmental change. Whilst this strategy for an active influx of genetic diversity appears generally appropriate, it may be counterproductive if populations of tree species already cultivated on farms are predominantly of non-local origin—a theoretical possibility for those taxa that have long histories of cultivation in the Amazon (Lentz 2000; Miller and Nair 2006). In this case, an influx of local wild germplasm and subsequent inter-breeding with cultivated trees may result in productivity losses via a dilution of genotypes with favourable traits for farmers, and/or genetic incompatibilities between cultivated and wild genotypes. As the decision of farmers to cultivate trees depends ultimately on their productivity, the potential for on-farm conservation of these species may therefore be reduced. Despite the importance of the issue, the history of native tree species planted on farms is largely unknown in the tropics, and there is a lack of research on geographically matched natural and planted stands to provide insights into their origins (Hollingsworth et al. 2005).

Here, we assess the origin of planted tree stands in the Peruvian Amazon for the economically important fruit-tree species, *Inga edulis* Mart. (Fabaceae: Mimosoideae). Our primary objective was to determine whether or not planted stands were derived from local natural material. We wished to determine from this assessment whether large-scale infusions of genetic resources from local natural stands into cultivation are appropriate for *I. edulis* in the Peruvian Amazon.

Methods

Inga edulis as a model species

The genus *Inga* comprises ca. 300 species of neotropical rain forest trees that contribute significantly to the high α diversity of the Amazon (Pennington 1997). Archaeological records demonstrate that the genus has been important to humans over millennia, with direct evidence of ancient human-mediated transport of *I. feuillei* (Pennington 1997; León 1998). *Inga edulis* has a wide natural distribution across South America, is diploid and believed to be self-incompatible, the hermaphrodite flowers being pollinated by small birds, flying insects and possibly bats (Koptur 1984; Pennington 1997). Fruiting occurs after about 3 years, producing a long pod containing recalcitrant seeds covered by a fleshy sarcotesta that is eaten by humans (Pennington 1998). Natural dispersal is performed by mammals and possibly birds, which eat the sarcotesta and drop seeds nearby (Koptur 1984). Although the history of cultivation of this species is not well-documented, morphological studies suggest that humans have semi-domesticated *I. edulis* over a considerable time period (Clement 1989, 1999; Pennington 1997). Stands on farms in the Peruvian Amazon appear to contain some of the largest *I. edulis* pods observed anywhere for the species, with pods under cultivation generally much longer than those on wild trees (Pennington 1997).

Sampling of *Inga edulis*

In order to discriminate effectively between local vs. non-local origins of cultivated populations of *I. edulis*, we employed closely geographically matched sampling of natural and planted stands. In the case of a predominantly local origin, a planted stand should be more genetically similar to its paired wild stand than to other populations; in the case of a non-local origin, a planted stand is more likely to contain markers not found in the paired natural population. *Inga edulis* was therefore sampled from matched stands at each of five sites dispersed across lowland Amazonian Peru (Fig. 1). The same stands had been

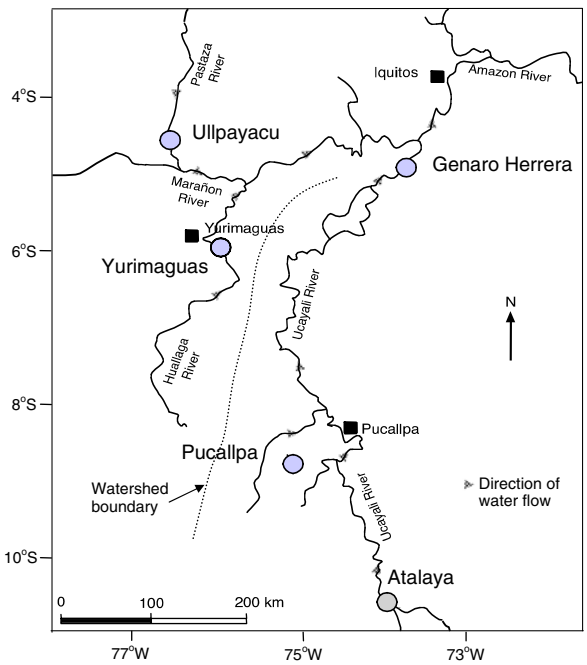

Fig. 1 Geographic locations of five sites in the Peruvian Amazon from which natural and planted stands of *Inga edulis* were sampled for assessment at the chloroplast *trnL-F* region and by five nuclear SSR loci (see also Hollingsworth et al. 2005). Sites are between ~150 and 500 km apart. The river drainage system and main cities of Iquitos, Yurimaguas and Pucallpa are indicated

analysed earlier in an assessment of genetic bottlenecks in *I. edulis* (Hollingsworth et al. 2005). At each site, material belonging to natural and planted categories was distinct and in close geographical proximity (normally within a few km). Natural trees were collected from old-growth primary forest that had a clear stratified structure with well-developed understorey, canopy, and emergent trees, and a lack of secondary growth of *Cecropia* and other trees that are often associated with disturbance by people. Planted individuals were sampled from farmland and confirmed as planted by farmers. It is not known how long *I. edulis* has been cultivated on particular farms, but it may extend over many years, several human, and numerous plant generations. Normally, only one or two trees were sampled from any particular farm, in order to ensure that collection was representative of the overall cultivated landscape at a site. Despite precautions to discriminate between categories, the long history of use of both *I. edulis* and slash-and-burn agriculture in the region means that it is not always easy to distinguish between natural and planted individuals. However, a consistent pattern between categories in our results (see following discussion) suggests that the measures we took to distinguish among stand types were adequate.

In order to allow proper comparison between categories at sites, natural and planted material was sampled from areas of similar size. The collection of each category normally extended across a ~20 by 20 km area at each location, but due to practical limitations some minor variation between stands at sites was unavoidable. For both natural and planted categories, leaf material was collected only from sexually mature trees, and preserved in silica gel. Sampled trees had a wide range of stem diameters. On average, the diameter of planted trees was lower than that of natural trees, indicating a process in which farmers are continually establishing (and protecting through weeding, etc.) new *I. edulis* individuals on their land. Total genomic DNA was extracted from dried leaves based on minor modifications to the protocol of Doyle and Doyle (1987).

SSR and chloroplast analysis

In a previous analysis, Hollingsworth et al. (2005) used five nuclear SSRs on 88 natural and 101 planted trees (i.e., ~18 and 20 individuals, respectively, by category, by site) to assess the allelic richness of the *I. edulis* stands considered also in the current study. Hollingsworth et al. (2005) concluded that planted stands were of lower allelic richness than neighbouring wild populations, indicative of genetic bottlenecks during cultivation. Hollingsworth et al. (2005) did not, however, consider the history of cultivated populations. Here, we take the same primary data set and extend analysis (see below) to a consideration of the origin of planted stands. In addition, to provide a complementary analysis to nuclear SSRs, we assembled new data based on an assessment of organellar variation. Since nuclear and organellar markers have different modes of inheritance, a combination of both can be particularly useful for insights into genetic structure and origin in plant populations (McCauley 1994; Petit et al. 2005), including in describing the role of humans in germplasm transfers (Dawson et al. 1996; McBreen and Cruzan 2004; Williams et al. 2005).

A previous DNA sequence-based phylogenetic analysis of *Inga* (Richardson et al. 2001) was used to develop two primer pairs to proximate parts of the chloroplast *trnL-F* region of *I. edulis* (primer pairs *Itrnlf0101-0368*, F 5′ GGC AAT CCT GAG CCA AAT CC, R 5′ CTG TCT GGT CTA ATC AAK TGA A; and *Itrnlf0759-1157*, F 5′ TCC ACT CAG ATC CGT TTG TGA, R 5′ CCT TTC GTG ACG CAT CAT CCT; expected product sizes of 259 and 319 bases, respectively). Primer pairs gave consistent single product amplifications across all *I. edulis* accessions, using standard PCR conditions (Hollingsworth et al. 2005) in 20 µl reactions, according to the following profile: 94°C 4 min; 94°C 1 min, 52°C 1 min, 72°C 2 min, 40 cycles; 72°C 5 min. Polymorphism in these regions was assessed via denaturing high-performance liquid chromatography analysis (DHPLC), a highly sensitive technique for detecting variation that has been applied primarily to human chromosomes, although also with some success to other organisms including plants (Oefner and Underhill 1998; Jin et al. 1999; Kota et al. 2001; Emmerson et al. 2003; Zhang and Hewitt 2003). As a reference for heteroduplex detection, a single *I. edulis* individual was chosen at random and amplified in large quantities. Before DHPLC, 5 µl of sample was mixed with 5 µl of reference, heated to 95°C for 3 min and then allowed to cool by standing at room temperature (15 min). About 5 µl of mixture was then loaded on an automated WAVE DHPLC detection instrument equipped with a DNA Sep column, using the WAVE MAKER program to determine the elution profile, according to the instructions of the column and instrument manufacturer (Transgenomic). The column temperature for optimum polymorphism detection was determined with the algorithm devised by the DNA Variation Group (Stanford University, http://insertion.stanford.edu/melt.html). Resultant DHPLC profiles were assessed manually to determine different haplotype conditions for each primer pair. Data from both products were then combined to provide overall haplotype states. In total, combined haplotypes were obtained for 77 natural and 86 planted trees (i.e., ~15 and 17 individuals, respectively, by category, by site).

Data analysis

Differentiation

Our primary objective in the current analysis was to test for genetic differentiation between *I. edulis* stands. To this end, we employed the ARLEQUIN 2.000 software package (Schneider et al. 2000) to generate F_{ST} values by stand category. Estimates were based on both SSR and chloroplast polymorphisms, and 10,000 permutations were used to assign significance values. In addition, pairwise F_{ST} values were calculated for all possible stand combinations and tested for significance based on 10,000 permutations and a stringent test ($P \leq 0.005$). To visualise relationships among populations, an unweighted pair-group method with arithmetic averaging (UPGMA) was used to undertake cluster analysis of Nei's (1978) genetic distances, using POPGENE 1.31 (Yeh et al. 1999).

Diversity

In an earlier study of the same stands, Hollingsworth et al. (2005) employed FSTAT 2.9.3.2 (Goudet 2002) to assess the difference in nuclear SSR allelic richness between natural and planted populations of *I. edulis*, using a rarefaction procedure to account for varying sample sizes. To allow a direct comparison of diversity levels between previous nuclear research and current chloroplast data, we employed the PAST 0.82 software package (Hammer et al. 2002) to undertake chloroplast rarefaction and a similar test of significance between stand categories. Chloroplast diversity was also calculated according to Nei's (1978) unbiased estimate (H).

Results

Differentiation between stands

DHPLC analysis revealed a total of seven ''combined'' chloroplast haplotypes in *I. edulis* stands (Fig. 2; see Appendix for raw data). Estimates of population differentiation based on these chloroplast haplotypes were similar for natural and planted stands (natural $F_{ST} = 0.201$; planted $F_{ST} = 0.184$; Table 1). F_{ST} estimates based on nuclear SSRs, however, revealed considerably less differentiation among natural stands compared to planted stands (natural $F_{ST} = 0.018$; planted $F_{ST} = 0.093$; Table 1; see Appendix for raw SSR data). This difference was statistically significant ($P = 0.031$ for between category comparison).

Pooling stands by category (natural vs. planted) revealed frequency differences (Δf) for chloroplast states, with

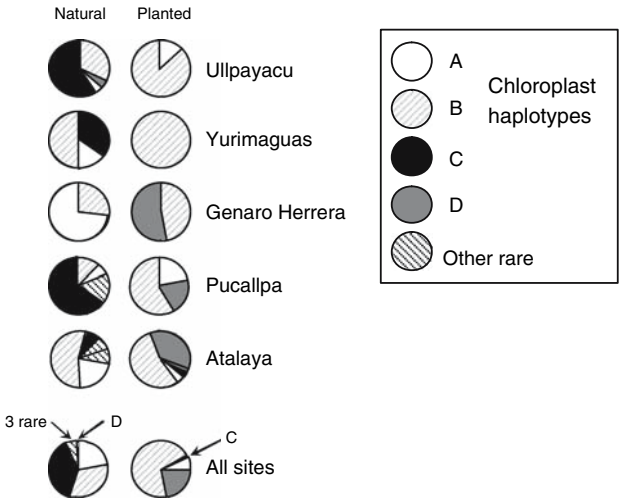

Fig. 2 Pie diagrams representing the different proportions of chloroplast DHPLC haplotype states in natural and planted stands of *Inga edulis* sampled from five sites in the Peruvian Amazon. Haplotype states were defined by combining data from an assessment of variation at two proximate amplification products within the chloroplast *trnL-F* region. Pucallpa and Atalaya natural stands contain rare alleles (haplotype F occurs only in the former population, on three occasions; haplotypes G and H are restricted to the latter stand, one unique occurrence each only)

$\Delta f > 0.1$ for four haplotypes (Appendix). Of particular note were haplotype C, which had 29 occurrences in natural material vs. one in planted, and haplotype D, which had one occurrence in natural material vs. 18 in planted ($N = 77$ and 86 for natural and planted stands, respectively; Fig. 2). For nuclear SSRs, large frequency differences between natural and planted categories were not expected to be common for individual alleles. This is because of the high levels of allelic richness observed at all loci (Hollingsworth et al. 2005; only 15 of 75 SSR alleles had an overall frequency of > 0.1 in tested material). However, $\Delta f \geq 0.1$ between natural and planted stands was observed for five alleles (from four loci: *Inga05* Allele01 and Allele03, *Inga08* Allele02, *Inga33* Allele01 and *Pel5* Allele04; see Appendix).

Table 1 Summary of overall differentiation (F_{ST} estimates) for chloroplast haplotype states and five nuclear SSR loci for natural and planted stands of *Inga edulis* sampled from five sites in the Peruvian Amazon

Populations	F_{ST} values	
	Chloroplast data	Nuclear data
Five natural stands	0.201	0.018
Five planted stands	0.184	0.093
All 10 stands	0.280	0.068

All values were significant ($P \leq 0.005$)

Comparisons between matched stands at sites

Considering individual sample sites, chloroplast haplotypes and nuclear SSR alleles both demonstrated frequency differences between matched stands. For chloroplast haplotypes, of particular note was the presence of haplotype D in planted stands, but absence in matched natural stands, at Atalaya, Genaro Herrera and Pucallpa (present in 7, 8 and 3 planted individuals, respectively; N = 19, 15 and 15, respectively, Fig. 2; all three sites in the Ucayali River watershed, Fig. 1). In addition, haplotypes A and C were present in > 50% of individuals at one (Genaro Herrera) and two natural stands (Pucallpa and Ullpayacu), respectively, but absent from matched planted stands (Fig. 2). Employing a stringent test ($P \leq 0.005$), pairwise F_{ST} values in matched comparisons were significant for chloroplast haplotypes in all cases except at Atalaya (Table 2). For SSRs, pairwise F_{ST} values were significant except at Ullpayacu and Yurimaguas. Considering both data sets, therefore, a significant difference for one or both genomes was found between natural and planted stands at all five sample sites.

Comparisons between sites

Considering planted stands only, pairwise F_{ST} values based on SSRs indicated a degree of structuring among sites, with Atalaya and Pucallpa in one group and Yurimaguas and Ullpayacu in a second (mean pairwise F_{ST} within groups = 0.043, between groups = 0.102; Table 2). Genaro Herrera occupied an intermediate position. These relationships were illustrated by a cluster analysis (Fig. 3). This pattern was also reflected by chloroplast haplotypes (mean pairwise F_{ST} within the same groups = 0.027, between groups = 0.215; Table 2).

Comparisons of diversity between natural and planted stands

Stand chloroplast haplotype diversity is represented visually in Fig. 2 (see Appendix for raw data). Three of seven haplotypes were rare: F occurred on only three occasions (all in the Pucallpa natural population), while G and H were present only once each in the entire study (both in the Atalaya natural stand). Unique haplotypes G and H do not appear to be artefacts from aberrant DHPLC column runs: in both cases, uniqueness was based on a specific unusual combination of rare but not unique types revealed during separate DHPLC analyses of the two individual chloroplast products tested (that is, no single-product DPHLC profile, for *Itrnlf0101-0368* or *Itrnlf0759-1157*, was unique).

Table 2 Pairwise F_{ST} values for natural and planted stands of *Inga edulis* sampled from five sites in the Peruvian Amazon

	Atalaya natural	Atalaya planted	Genaro Herrera natural	Genaro Herrera planted	Pucallpa natural	Pucallpa planted	Ullpayacu natural	Ullpayacu planted	Yurimaguas natural
Atalaya planted	**0.047** (0.070)								
Genaro Herrera natural	0.011 (0.171)	**0.063** (**0.345**)							
Genaro Herrera planted	**0.102** (0.184)	**0.067** (–0.029)	**0.062** (**0.440**)						
Pucallpa natural	0.008 (**0.301**)	**0.069** (**0.360**)	0.026 (**0.465**)	**0.100** (**0.432**)					
Pucallpa planted	**0.118** (–0.024)	**0.052** (–0.012)	**0.113** (0.243)	**0.110** (0.076)	**0.146** (**0.382**)				
Ullpayacu natural	0.036 (0.180)	**0.108** (**0.234**)	0.010 (**0.401**)	**0.089** (**0.320**)	**0.044** (0.005)	**0.128** (**0.253**)			
Ullpayacu planted	**0.060** (0.092)	**0.113** (**0.208**)	0.028 (**0.516**)	**0.119** (**0.368**)	**0.061** (**0.570**)	**0.142** (0.083)	0.018 (**0.445**)		
Yurimaguas natural	0.013 (–0.004)	**0.067** (0.113)	–0.004 (0.257)	**0.087** (**0.228**)	0.022 (0.166)	**0.102** (0.064)	–0.002 (0.040)	0.011 (0.191)	
Yurimaguas planted	0.027 (**0.271**)	**0.061** (**0.320**)	0.013 (**0.739**)	**0.105** (**0.500**)	**0.043** (**0.674**)	**0.090** (0.250)	0.015 (**0.561**)	**0.033** (0.065)	–0.008 (**0.327**)

Nuclear SSR values are given outside parentheses, chloroplast DHPLC values within. Significant differences based on 10,000 permutations and a stringent test ($P \leq 0.005$) are shown in bold

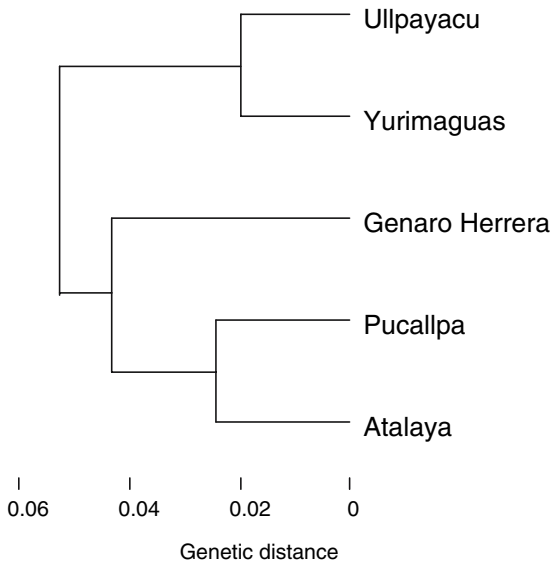

Fig. 3 Phenogram of nuclear SSR genetic distances among planted stands of *Inga edulis* sampled from five sites in the Peruvian Amazon

Table 3 indicates haplotype richness values by stand. Considering corrected estimates after rarefaction, to a sample equivalent to 11 individuals per population (n_{11}, based on the smallest number of individuals typed), natural stands from Atalaya and Pucallpa had the highest values (4.54 and 3.43, respectively), with planted material from Yurimaguas and Ullpayacu the lowest values (1.00 and 1.89, respectively). No planted stand had an n_{11} value higher than its neighbouring natural stand, although in one case, at Genaro Herrera, values were equal (2.00 for both). Atalaya, the site with the highest chloroplast diversity in natural material (n_{11} = 4.54), also had the highest diversity in planted material (n_{11} = 3.16). Mean n_{11} values for natural and planted categories were 3.24 and 2.21, respectively. Mean values for H were 0.583 (natural) and 0.399 (planted). Chloroplast data therefore corresponded with an earlier rarefaction of nuclear SSRs that revealed planted stands of *I. edulis* had lower allelic richness than natural populations (Hollingsworth et al. 2005; SSR allele numbers shown in Table 3 for comparison). However, similar to SSRs (where individual planted stands contained on average ~80% of the allelic richness found in natural material), haplotype diversity in cultivated stands was still quite high (~70% of that found in natural stands, based on both n_{11} and H values). Unlike nuclear SSR alleles (P = 0.009; Hollingsworth et al. 2005), the difference in haplotype richness between stand categories was not statistically significant (P = 0.117). At individual sites, differences in the level of variation revealed by each genome were observed, most notably at Atalaya, where stands contained the highest chloroplast diversity and the lowest SSR allelic richness values for both natural and planted categories.

Table 3 Chloroplast and nuclear diversity data for natural and planted stands of *Inga edulis* sampled from five sites in the Peruvian Amazon

Stand	Chloroplast data			Nuclear data
	N	n	n_{11}	A_{15}
Atalaya				
Natural	13	5	4.54	36.0
Planted	19	4	3.16	27.7
Genaro Herrera				
Natural	11	2	2.00	41.1
Planted	15	2	2.00	29.2
Pucallpa				
Natural	18	4	3.43	39.6
Planted	15	3	2.98	30.8
Ullpayacu				
Natural	17	4	3.29	41.1
Planted	22	2	1.89	34.4
Yurimaguas				
Natural	18	3	2.96	38.5
Planted	15	1	1.00	34.4
Across sites				
Natural	77	7	3.24[a]	39.3[a]
Planted	86	4	2.21[a]	31.3[a]
Total	163	7	2.73[a]	35.3[a]

[a] Arithmetic mean of individual stands

N denotes the number of individuals determined for chloroplast haplotypes employing DHPLC analysis, n the number of haplotypes revealed, and n_{11} estimates for haplotype richness corrected by rarefaction to account for varying stand sizes (values based on the lowest number of individuals typed from a stand, N = 11). A_{15} denotes allelic richness revealed at five nuclear SSR loci in an earlier study by Hollingsworth et al. (2005)

Discussion

Are planted stands of *Inga edulis* derived from local natural material?

Our data provide strong indications that planted stands of *I. edulis* at tested sites in the Peruvian Amazon have not originated primarily from closely neighbouring natural populations. At the five locations we tested, chloroplast data indicated significant differences between matched natural and planted populations at four sites, and nuclear SSRs indicated significant differentiation at three sites. Considering both genomes, data indicated significant differences at all five tested sites. Planted stands therefore appear to have relied on substantial germplasm infusions from beyond local natural populations, despite the recalcitrant nature of the seeds which limits the time period in which they remain viable. Archaeological studies from elsewhere in Peru also provide evidence for human

transport of *Inga* seed, such as transfer of *I. feuillei* from the Amazon basin to the Pacific coast (Pennington 1997; León 1998).

Conclusions on origin must be made cautiously in studies involving farm stands, as stochastic processes connected with biased sampling (collection of consanguineous individuals planted within individual farms) could theoretically influence results by artificially inflating forest-farm pairwise F_{ST} values. However, we minimised any such bias by sampling across a wide range of farm locations at each of our five chosen collection sites. Furthermore, chloroplast data appear to be particularly relevant in indicating a largely non-local origin of planted germplasm: at three sites, planted stands contained a relatively high frequency haplotype that was completely absent in matched natural material, an observation difficult to explain by an alternative hypothesis for the origin of cultivated material that involves stochastic sorting and haplotype elimination from local natural stands. While data suggested a largely non-local origin for cultivated material, it was, however, interesting to note that some correlation in diversity levels between matched stands was observed, possibly due to limited present-day seed transfer from forest to farmland [as demonstrated by Brodie et al. (1997) in community surveys].

Our approach of assessing the origin of planted stands through close geographical matching with natural populations across a range of well-separated sites has been undertaken occasionally for annual crops (e.g., for barley, see Jana and Pietzrak 1988). However, we are not aware of any directly comparable research for tropical trees. Nevertheless, a small number of molecular studies on other indigenous trees in the Americas also indicate that use of non-local germplasm has contributed to the development of tree cultivation within the native range of a species. In particular, *Bactris gasipaes*, a palm tree with a long history of human use in the Amazon region and elsewhere in the Americas, appears to have undergone wide-scale dissemination through cultivation from one or a few primary sources rather than local domestication at many separate sites within the distribution of the wild progenitor (Clement et al. 1997; Mora-Urpí et al. 1997; Adin et al. 2004; Rodrigues et al. 2004). Recent work on traditional crops also indicates non-local sources in establishing cultivated populations within the native ranges of their progenitors within the Amazon region. For example, *Manihot esculenta* was apparently domesticated in just a single geographic area on the edge of the southern Amazon Basin (Olsen and Schaal 2001; Olsen 2004). A somewhat contrasting example is provided by the jocote fruit tree (*Spondias purpurea*) in Mesoamerica, where a recent chloroplast phylogeographic study showed some local correspondence between the geographical distribution of haplotypes in wild and cultivated populations (Miller and Schaal 2005; see also Miller and Schaal 2006).

Do planted stands of *Inga edulis* have a common origin?

While it appears probable that planted stands of *I. edulis* in the Peruvian Amazon are derived substantially from non-local material, the question of the actual source (or sources) of cultivated germplasm is a more difficult one that would require extensive further sampling to address fully. Nevertheless, our data do suggest that cultivated *I. edulis* is not a homogenous entity: there is differentiation between planted material from Atalaya and Pucallpa compared to Yurimaguas and Ullpayacu for both SSR and cpDNA data. As these two pairs of populations are in different watersheds of the Amazon River system, this could be an indication of the importance of boat transport in mediating seed exchange, and thus in determining the genetic composition of cultivated *I. edulis* within watersheds. Testing this hypothesis would require further detailed sampling within and among watersheds.

Human activity and diversity within planted stands of *Inga edulis*

The lower nuclear and organellar variation observed in planted *I. edulis* indicate that human activity is impacting on levels of population genetic diversity in cultivated stands for both genomes (see also Hollingsworth et al. 2005). Despite some genetic narrowing, however, planted *I. edulis* stands remain relatively diverse, containing on average ~80% (nuclear) and 70% (organellar) of the variation found in natural stands. Planted populations of *I. edulis* do not therefore appear to have experienced extreme genetic bottlenecks, even though they appear to have originated substantially from non-local germplasm sources, a process that could have significantly narrowed their genetic base due to genetic bottlenecks associated with sampling and subsequent losses during transit (Brodie et al. 1997; Weber et al. 1997; Kindt and Lengkeek 1999; Lengkeek et al. 2005). For *I. edulis*, therefore, concerns about loss of adaptability and potential inbreeding depression caused by genetic bottlenecks, which are important considerations for many cultivated out-breeding tree species in agroforestry systems (Simons et al. 1994; Boshier 2000; Lengkeek 2003), do not appear to be serious at this time. Inbreeding depression is a particular concern for fruit trees, as farmers depend directly on non-related matings for fruit production (abortion problems lead to reduced or possibly no fruit

production; inbreeding depression thus impacts immediately on farmer use).

Implications for conservation

To promote the conservation and sustainable use of tree species in the Peruvian Amazon, farmers are currently encouraged to undertake additional transfers of germplasm from natural stands into local cultivation (O'Neill et al. 2001; Weber et al. 2001). Observations of relatively high genetic differentiation between matched natural and planted stands of *I. edulis*, however, suggest that in this instance such transfer may not be the best means to promote on-farm conservation, because of possible productivity losses through interbreeding (out-breeding depression) and genetic dilution (Jamnadass et al. 2005). Instead, effort may be better focused on a strategy that involves maintaining the rather high level of genetic diversity already found within current planted stands. Practically, this would involve farmer-training exercises to maintain good collection practices of currently cultivated *I. edulis* germplasm as farmers ''regenerate'' diversity in subsequent rounds of on-farm propagation. In addition, coordinated exchange of *I. edulis* seed through improved network development among neighbouring farms may be appropriate, assuming that neighbouring farms obtained their material from similar sources. However, any strategy must be dynamic and also recognise that farmers have been introducing, and will continue to introduce, selected germplasm from other sources, notably from nearby markets (Brodie et al. 1997). Whether a similar strategy is more appropriate than encouraging introduction from local forests for the conservation of other Amazonian trees requires further testing of their origin and cultivation history, but *Bactris gasipaes* (discussed earlier) appears to be another species that may fall within the same category as *I. edulis* in having a predominantly non-local origin of cultivated material. In any case, the example of *I. edulis* is cautionary for a generic tree conservation policy in the region based on extensive germplasm transfer from local natural forest to farmland, in particular for species with long histories of human cultivation and selection.

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Appendix

Frequencies of alleles at five nuclear SSR loci, and ''combined'' haplotype states at the chloroplast *trnL-F* region, for natural and planted stands of *Inga edulis* sampled from five sites in the Peruvian Amazon

Locus	Atalaya natural	Atalaya planted	Genaro Herrera natural	Genaro Herrera planted	Pucallpa natural	Pucallpa planted	Ullpayacu natural	Ullpayacu planted	Yurimaguas natural	Yurimaguas planted	All natural	All planted	Total
Nuclear variation													
Inga03	*N* = 15	*N* = 18	*N* = 17	*N* = 22	*N* = 24	*N* = 21	*N* = 16	*N* = 22	*N* = 16	*N* = 18	*N* = 88	*N* = 101	*N* = 189
Allele01	0.133	0.056	0.118	0.068	0.125	0.071	0.344	0.182	0.188	0.222	0.176	0.119	0.146
Allele02		0.028	0.059						0.031		0.017	0.005	0.011
Allele03			0.029	0.023			0.031		0.031		0.017	0.005	0.011
Allele04	0.133	0.139	0.324	0.227	0.042	0.238	0.219	0.205	0.094	0.194	0.153	0.203	0.180
Allele05	0.333	0.194	0.088	0.023	0.167	0.333	0.063	0.068	0.156	0.167	0.159	0.153	0.156
Allele06	0.067	0.056	0.059		0.063	0.024	0.031		0.063		0.057	0.015	0.034
Allele07	0.167	0.389	0.088	0.364	0.313	0.143	0.031	0.091	0.063		0.148	0.198	0.175
Allele08		0.056	0.059	0.046	0.042	0.071	0.031			0.056	0.028	0.045	0.037
Allele09	0.033	0.028	0.029		0.063		0.031	0.023		0.111	0.034	0.030	0.032
Allele10			0.059	0.046	0.042	0.024	0.125	0.182	0.156	0.056	0.074	0.064	0.069
Allele11	0.033		0.059	0.023			0.063	0.046	0.031	0.056	0.034	0.025	0.029
Allele12	0.033		0.029	0.046		0.048	0.031	0.046	0.063		0.028	0.030	0.029

Appendix continued

Locus	Atalaya natural	Atalaya planted	Genaro Herrera natural	Genaro Herrera planted	Pucallpa natural	Pucallpa planted	Ullpayacu natural	Ullpayacu planted	Yurimaguas natural	Yurimaguas planted	All natural	All planted	Total
Allele13				0.136								0.030	0.016
Allele14					0.042			0.068			0.011	0.015	0.013
Allele15								0.023				0.005	0.003
Allele16								0.068		0.083		0.030	0.016
Allele17	0.033	0.056			0.021	0.048			0.063		0.023	0.020	0.021
Allele18					0.021				0.063	0.028	0.017	0.005	0.011
Allele19	0.033				0.063					0.028	0.023	0.005	0.013
Inga05	*N* = 15	*N* = 18	*N* = 17	*N* = 22	*N* = 24	*N* = 21	*N* = 16	*N* = 22	*N* = 16	*N* = 18	*N* = 88	*N* = 101	*N* = 189
Allele01	0.933	0.583	0.794	0.523	0.854	0.452	0.844	0.818	0.844	0.778	0.852	0.629	0.733
Allele02		0.028	0.059	0.114	0.021	0.071	0.094	0.114	0.063	0.111	0.045	0.089	0.069
Allele03	0.067	0.389	0.059	0.364	0.104	0.452	0.063	0.068	0.094	0.111	0.080	0.277	0.185
Allele04			0.029								0.006		0.003
Allele05			0.029								0.006		0.003
Allele06			0.029								0.006		0.003
Allele07					0.021	0.024					0.006	0.005	0.005
Inga08	*N* = 15	*N* = 18	*N* = 17	*N* = 22	*N* = 24	*N* = 21	*N* = 16	*N* = 22	*N* = 16	*N* = 18	*N* = 88	*N* = 101	*N* = 189
Allele01	0.033	0.028	0.118	0.046	0.063		0.281	0.364	0.281	0.278	0.148	0.144	0.146
Allele02	0.833	0.722	0.765	0.636	0.896	0.286	0.563	0.546	0.656	0.667	0.756	0.564	0.653
Allele03		0.083	0.029	0.182	0.021	0.191		0.023	0.031	0.028	0.017	0.104	0.064
Allele04			0.059	0.046		0.143			0.031		0.017	0.040	0.029
Allele05			0.029								0.006		0.003
Allele06	0.067			0.023	0.021	0.167	0.063				0.028	0.040	0.034
Allele07				0.023								0.005	0.003
Allele08							0.063	0.068		0.028	0.011	0.020	0.016
Allele09	0.067	0.083		0.023		0.071					0.011	0.035	0.024
Allele10				0.023								0.005	0.003
Allele11							0.031				0.006		0.003
Allele12		0.056				0.095						0.030	0.016
Allele13		0.028				0.048						0.015	0.008
Inga33	*N* = 15	*N* = 18	*N* = 17	*N* = 22	*N* = 24	*N* = 21	*N* = 16	*N* = 22	*N* = 16	*N* = 18	*N* = 88	*N* = 101	*N* = 189
Allele01	0.400	0.694	0.265	0.250	0.250	0.714	0.188	0.182	0.344	0.528	0.284	0.460	0.378
Allele02	0.067		0.147	0.386	0.104	0.048	0.281	0.023	0.125	0.083	0.142	0.114	0.127
Allele03	0.100	0.083	0.177	0.091	0.063	0.191	0.094	0.046	0.094	0.111	0.102	0.104	0.103
Allele04	0.233	0.222	0.147	0.223	0.125	0.024	0.125	0.159	0.063	0.028	0.136	0.134	0.135
Allele05			0.059		0.167		0.031	0.068	0.094	0.139	0.080	0.040	0.058
Allele06	0.033		0.088		0.083		0.031	0.386	0.094	0.056	0.068	0.094	0.082
Allele07			0.029						0.031		0.011		0.005
Allele08	0.033		0.029	0.023	0.021		0.031		0.063	0.056	0.034	0.015	0.024

Appendix continued

Locus	Atalaya natural	Atalaya planted	Genaro Herrera natural	Genaro Herrera planted	Pucallpa natural	Pucallpa planted	Ullpayacu natural	Ullpayacu planted	Yurimaguas natural	Yurimaguas planted	All natural	All planted	Total
Allele09	0.067		0.059	0.023	0.042	0.024	0.063		0.063		0.057	0.010	0.032
Allele10							0.063				0.011		0.005
Allele11	0.033				0.063		0.063				0.034		0.016
Allele12							0.031				0.006		0.003
Allele13								0.091				0.020	0.011
Allele14								0.046				0.010	0.005
Allele15	0.033				0.042				0.031		0.023		0.011
Allele16					0.042						0.011		0.005
Pel5	*N* = 15	*N* = 18	*N* = 17	*N* = 22	*N* = 24	*N* = 21	*N* = 16	*N* = 22	*N* = 16	*N* = 18	*N* = 88	*N* = 101	*N* = 189
Allele01			0.059	0.091	0.042	0.119			0.094		0.040	0.045	0.042
Allele02	0.167	0.167	0.118	0.046	0.083	0.071	0.188	0.205	0.125	0.139	0.131	0.124	0.127
Allele03	0.200	0.333	0.265	0.227	0.125	0.262	0.156	0.205	0.250	0.222	0.193	0.248	0.222
Allele04	0.100	0.194	0.147	0.523	0.021	0.071	0.063	0.046	0.063	0.056	0.074	0.183	0.132
Allele05			0.029		0.063		0.031	0.023	0.094	0.167	0.045	0.035	0.040
Allele06	0.067	0.083	0.206	0.023	0.083		0.063	0.136	0.125	0.056	0.108	0.059	0.082
Allele07	0.033	0.028	0.029		0.104	0.024	0.125		0.031	0.083	0.068	0.025	0.045
Allele08	0.067		0.059		0.021		0.063	0.114	0.094	0.083	0.057	0.040	0.048
Allele09	0.133	0.111	0.029		0.229	0.071	0.125	0.114	0.063	0.056	0.125	0.069	0.095
Allele10			0.029								0.006		0.003
Allele11	0.067	0.028	0.029		0.063		0.063		0.063	0.028	0.057	0.010	0.032
Allele12				0.091	0.083	0.119					0.023	0.045	0.034
Allele13	0.033				0.021		0.063	0.023		0.028	0.023	0.010	0.016
Allele14	0.100				0.021		0.031				0.028		0.013
Allele15	0.033	0.056			0.021	0.262	0.031	0.068			0.017	0.079	0.050
Allele16								0.023		0.056		0.015	0.008
Allele17								0.023				0.005	0.003
Allele18										0.028		0.005	0.003
Allele19								0.023				0.005	0.003
Allele20					0.021						0.006		0.003
Chloroplast variation													
trnL-F	*N* = 13	*N* = 19	*N* = 11	*N* = 15	*N* = 18	*N* = 15	*N* = 17	*N* = 22	*N* = 18	*N* = 15	*N* = 77	*N* = 86	*N* = 163
HaploA	0.231	0.053	0.727		0.055	0.200	0.059	0.136	0.167		0.208	0.081	0.141
HaploB	0.538	0.526	0.273	0.467	0.111	0.600	0.294	0.864	0.500	1.000	0.338	0.698	0.527
HaploC	0.077	0.053			0.667		0.588		0.333		0.376	0.012	0.184
HaploD		0.368		0.533		0.200	0.059				0.013	0.209	0.117
HaploF					0.167						0.039		0.018
HaploG	0.077										0.013		0.006
HaploH	0.077										0.013		0.006

N denotes the number of individuals typed for a marker

References

Adin A, Weber JC, Sotelo Montes C, Vidaurre H, Vosman B, Smulders MJM (2004) Genetic differentiation and trade among populations of peach palm (*Bactris gasipaes* Kunth) in the Peruvian Amazon – implications for genetic resource management. Theor Appl Genet 108:1564–1573

Boshier DH (2000) Mating systems. In: Young A, Boshier D, Boyle T (eds) Forest conservation genetics: principles and practice. CSIRO Publishing, Melbourne, Australia, pp 63–79

Brodie AW, Labarta-Chávarri RA, Weber JC (1997) Tree germplasm management and use on-farm in the Peruvian Amazon: a case study from the Ucayali Region, Peru. Research report. Overseas Development Institute, London, United Kingdom and International Centre for Research in Agroforestry, Nairobi, Kenya

Clement CR (1989) A center of crop genetic diversity in western Amazonia. BioScience 39:624–631

Clement CR (1999) 1492 and the loss of Amazonian crop genetic resources. I. The relation between domestication and human population decline. Econ Bot 53:188–202

Clement CR, Villachica H (1994) Amazonian fruits and nuts: potential for domestication in various agroecosystems. In: Leakey RRB, Newton AC (eds) Tropical trees: the potential for domestication and the rebuilding of forest resources. HMSO, London, UK, pp 230–238

Clement CR, Aradhya MK, Manshardt RM (1997) Allozyme variation in spineless pejibaye (*Bactris gasipaes* Palmae). Econ Bot 51:149–157

Dawson IK, Simons AJ, Waugh R, Powell W (1996) Detection and pattern of interspecific hybridisation between *Gliricidia sepium* and *G. maculata* in Meso-America revealed by PCR-based assays. Mol Ecol 5:89–98

Doyle JJ, Doyle JL (1987) CTAB DNA extraction in plants. Phytochem Bull 19:11–15

Emmerson P, Maynard J, Jones S, Butler R, Sampson JR, Cheadle JP (2003) Characterizing mutations in samples with low-level mosaicism by collection and analysis of DHPLC fractionated heteroduplexes. Hum Mutat 21:112–115

FAO (2001) Global forest resource assessment 2000. Main report. FAO Forestry Paper 140. Food and Agriculture Organization, Rome, Italy

Goudet J (2002) FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.3.2. Available at: http://www2.unil.ch/izea/softwares/fstat.html

Hammer O, Harper DAT, Ryan PD (2002) PAST – palaeontological statistics, version 0.82. Palaeontological Museum, University of Oslo, Oslo, Norway

Hollingsworth PM, Dawson IK, Goodall-Copestake WP, Richardson JE, Weber JC, Sotelo Montes C, Pennington RT (2005) Do farmers reduce genetic diversity when they domesticate tropical trees? A case study from Amazonia. Mol Ecol 14:497–501

Jamnadass R, Hanson J, Poole J, Hanotte O, Simons AJ, Dawson IK (2005) High differentiation among populations of the woody legume *Sesbania sesban* in sub-Saharan Africa: implications for conservation and cultivation during germplasm introduction into agroforestry systems. For Ecol Manage 210:225–238

Jana S, Pietzrak LN (1988) Comparative assessment of genetic diversity in wild and primitive cultivated barley in a centre of diversity. Genetics 119:981–990

Jin L, Underhill PA, Doctor V, Davis RW, Shen P, Cavalli-Sforza LL, Oefner PJ (1999) Distribution of haplotypes from a chromosome 21 region distinguishes multiple prehistoric human migrations. Proc Natl Acad Sci USA 96:3796–3800

Kindt R (2002) Methodology for tree species diversification planning in African agro-ecosystems. PhD Thesis, University of Gent, Gent, Belgium

Kindt R, Lengkeek AG (1999) Tree diversity on farm – use it or lose it. In: Agricultural biodiversity conservation. Intermediate Technology Development Group East Africa, Nairobi, Kenya, pp 75–85

Koptur S (1984) Outcrossing and pollinator limitation on fruit set: breeding systems of neotropical *Inga* trees (Fabaceae: Mimosoideae). Evolution 38:1130–1143

Kota R, Wolf M, Michalek W, Graner A (2001) Application of denaturing high-performance liquid chromatography for mapping of single nucleotide polymorphisms in barley (*Hordeum vulgare* L). Genome 44:523–528

Lengkeek AG (2003) Diversity makes a difference: farmers managing inter- and intra-specific tree species diversity in Meru Kenya. PhD Thesis, Wageningen University, Wageningen, The Netherlands

Lengkeek AG, Dawson IK, Jaenicke H (2005) Genetic bottlenecks in agroforestry systems: results of tree nursery surveys in East Africa. Agroforest Syst 63:149–155

Lentz DL (2000) Anthropocentric food webs in the Precolumbian Americas. In: Lentz DL (ed) Imperfect balance: landscape transformations in the pre-Columbian Americas. Historical ecology series. Columbia University Press, New York, USA, pp 89–119

León J (1998) History of the utilization of *Inga* as fruit trees in Mesoamerica and Peru. In: Pennington TD, Fernandes ECM (eds) The genus *Inga*: utilization. The Royal Botanic Gardens, Kew, London, United Kingdom, pp 5–13

McBreen K, Cruzan MB (2004) A case of recent long-distance dispersal in the *Piriqueta caroliniana* complex. J Hered 95: 356–361

McCauley DE (1994) Contrasting the contribution of chloroplast DNA and allozyme polymorphism among local populations of *Silene alba*: implications for studies of gene flow in plants. Proc Natl Acad Sci USA 91:8127–8131

Miller RP, Nair PKR (2006) Indigenous agroforestry systems in Amazonia: from prehistory to today. Agroforest Syst 66: 151–164

Miller A, Schaal B (2005) Domestication of a Mesoamerican cultivated fruit tree, *Spondias purpurea*. Proc Natl Acad Sci USA 102:12801–12806

Miller AJ, Schaal BA (2006) Domestication and the distribution of genetic variation in wild and cultivated populations of the Mesoamerican fruit tree *Spondias purpurea* L. (Anacardiaceae). Mol Ecol 15:1467–1480

Mora-Urpí J, Weber JC, Clement CR (1997) Peach palm. *Bactris gasipaes* Kunth. Promoting the conservation and use of underutilized and neglected crops, No. 20. Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany and International Plant Genetic Resources Institute, Rome, Italy

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590

Oefner PJ, Underhill PA (1998) DNA mutation detection using denaturing high-performance liquid chromatography (DHPLC). In: Dracopoli NC, Haines JL, Korf BR et al (eds) Current protocols in human genetics, supplement 19. Wiley and Sons, New York, USA, pp 7.10.1–7.10.2

Olsen KM (2004) SNPs, SSRs and inferences on cassava's origin. Plant Mol Biol 56:517–526

Olsen KM, Schaal BA (2001) Microsatellite variation in cassava (*Manihot esculenta*, Euphorbiaceae) and its wild relatives: further evidence for a southern Amazonian origin of domestication. Am J Bot 88:131–142

O'Neill GA, Dawson IK, Sotelo-Montes C, Guarino L, Guariguata M, Current D, Weber JC (2001) Strategies for genetic conservation of trees in the Peruvian Amazon. Biodiver Conserv 10:837–850

Pennington TD (1997) The genus *Inga*. The Royal Botanic Gardens, Kew, London, United Kingdom

Pennington TD (1998) *Inga* management. In: Pennington TD, Fernandes ECM (eds) The genus *Inga*: utilization. The Royal Botanic Gardens, Kew, London, United Kingdom, pp 159–167

Petit RJ, Duminil J, Fineschi S, Hampe A, Salvini D, Vendramin GG (2005) Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. Mol Ecol 14:689–701

Reynel C, Pennington RT, Pennington TD, Flores C, Daza A (2003) Arboles útiles de la Amazonía Peruana: un manual con apuntes de identificación, ecología y propagación de las especies. Universidad Nacional Agraria La Molina, Lima, Perú

Richardson JE, Pennington RT, Pennington TD, Hollingsworth PM (2001) Rapid diversification of a species-rich genus of neotropical rain forest trees. Science 293:2242–2245

Rodrigues DP, Astolfi Filho S, Clement CR (2004) Molecular marker-mediated validation of morphologically defined landraces of Pejibaye (*Bactris gasipaes*) and their phylogenetic relationships. Genet Res Crop Evol 51:871–882

Schneider S, Roessli D, Excoffier L (2000) Arlequin, version 2.000: a software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland

Simons AJ, MacQueen DJ, Stewart JL (1994) Strategic concepts in the domestication of non-industrial trees. In: Leakey RRB, Newton AC (eds) Tropical trees: the potential for domestication and the rebuilding of forest resources. HMSO, London, United Kingdom, pp 91–102

Simons AJ, Jaenicke H, Tchoundjeu Z, Dawson I, Kindt R, Oginosako Z, Lengkeek A, De Grande A (2000) The future of trees is on farm: tree domestication in Africa. In: Krishnapillay B, Soepadmo E, Lotfy Arshad N et al (eds) Forests and society: the role of research, sub-plenary sessions, 1. Proceedings of the XXIst IUFRO World Congress. IUFRO Secretariat, Vienna, Austria and the Forest Research Institute Malaysia, Kuala Lumpur, Malaysia, pp 752–760

Sotelo Montes C, Weber JC (1997) Priorización de especies arbóreas para sistemas agroforestales en la selva baja del Perú. Agroforest Amér 4:12–17

Weber JC, Labarta Chávarri RL, Sotelo Montes C, Brodie AW, Cromwell E, Schreckenberg K, Simons AJ (1997) Farmers' use and management of tree germplasm: case studies from the Peruvian Amazon Basin. In: Simons AJ, Kindt R, Place F (eds) Policy aspects of tree germplasm demand and supply, proceedings of an international workshop. International Centre for Research in Agroforestry, Nairobi, Kenya, pp 57–63

Weber JC, Sotelo-Montes C, Vidaurre H, Dawson IK, Simons AJ (2001) Participatory domestication of agroforestry trees: an example from the Peruvian Amazon. Dev Prac 11:425–433

Williams DA, Overholt WA, Cuda JP, Hughes CR (2005) Chloroplast and microsatellite DNA diversities reveal the introduction history of Brazilian peppertree (*Schinus terebinthifolius*) in Florida. Mol Ecol 14:3643–3656

Yeh FC, Yang R-C, Boyle T (1999) POPGENE, version 1.31. University of Alberta, Alberta, Canada

Zhang D-X, Hewitt GM (2003) Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. Mol Ecolgy 12:563–584