From Latin America to Oceania: The Historic Dispersal of Sweetpotato Re-examined Using AFLP

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Although originally domesticated in tropical America, the sweetpotato (*Ipomoea batatas* (L.) Lam.) has a long cultivation history in Oceania. While the post-Columbus dispersal of sweetpotato to Asia and Oceania is well documented, the hypothesis that there was prehistoric transfer by Peruvian or Polynesian voyagers from Peru to Oceania has long been a controversial issue. The objective of this study was to assess the genetic diversity and interrelationship of sweetpotato cultivars from Oceania and Latin America and to test the hypothesis of human transfer of this crop to the Pacific Islands in prehistoric time. Seventy-six sweetpotato cultivars from Peru-Ecuador, Mexico, the Philippines and eight Oceania countries were analyzed using amplified fragment length polymorphism (AFLP). Multidimensional scaling (MDS) and analysis of molecular variance (AMOVA) revealed wide genetic variation in the Oceania gene pool, greater than that of Peru-Ecuador. There was a significant sweetpotato "gene flow" from Mexico to Oceania. In contrast, there is little association between the Peru-Ecuador germplasm and that of Oceania. These results suggest that Peru-Ecuador may not be the source of the Oceania germplasm. Natural dispersal from Mesoamerica is an alternative explanation to the 'Kumara hypothesis' for the origin of the Oceania sweetpotato.

Sweetpotato (*Ipomoea batatas* (L.) Lam.) was originally domesticated in tropical America (Austin, 1988; Yen, 1982). The exact center of origin and domestication of the sweetpotato has not been well defined, neither has the wild ancestor of this species been found. Based on the numerical analysis of key morphological characters of sweetpotato and the wild Ipomoea species, Austin (1988) postulated that sweetpotato originated in the region between the Yucatán Peninsula of Mexico and the Orinoco River in Venezuela, within which the four major American taxa of the batata group are distributed. Recent reports using molecular markers to assess diversity have found the highest diversity in Central America, supporting the hypothesis that Central America is the primary center of diversity and most likely the center of origin of sweetpotato (Huang and Sun, 2000; Zhang et al., 2000).

The sweetpotato was one of the first plant introductions from the Americas into Europe from the voyage of Columbus in 1492. From Europe this crop was taken by the Portuguese explorers of the sixteenth century to Africa, India, Southeast Asia and the East Indies. This is the so-called '*batata* line' of dispersal. The *camote* line (from the Nahuatl word *camotli*) represents

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the direct transfer of Mexican sweetpotatoes by Spanish trading galleons between Mexico and the Philippines in the 16th century. In the Asia and Pacific region, there have been complex interchanges of sweetpotato after early introductions. (Yen, 1982).

There is a third line of prehistoric transmission: the introduction of the sweetpotato to the Pacific islands prior to the era of European exploration (Yen, 1982). Fossil carbonized storage roots of sweetpotato found in northern New Zealand have been dated back some 1000 years (Yen, 1991), which strongly supports the theory of prehistoric transfer. Here, the question is whether this was the chance spread of seeds by natural means or human transfer by Peruvian or Polynesian voyagers (Yen, 1982). The linguistic links between the Quechua and Polynesian names for sweetpotato, and their variations, support the Peruvian origin and human transfer of the Polynesian sweetpotato. The successful drift voyages of the Kon-Tiki expedition in 1947 (Heyerdahl, 1950), further put this sweetpotato issue within the context of the wider problem of the origin of the Polynesian people. Today, the 'Kumara' area includes many sweetpotato-producing countries in Oceania, including New Zealand, Tonga, Samoa, and the Cook Islands (Yen, 1974), where the sweetpotato is a widely used staple food.

The sweetpotato is an ancient crop in Peru (Yen, 1974; Austin, 1988). Examples unearthed at numerous archaeological sites representing both Inca and pre-Inca cultures suggest that the sweetpotato was one of the most important crops in this region (Urgent and Peterson, 1988). However, whether Peru was a major source of sweetpotato to Oceania is still unsettled. Our previous studies based on molecular markers showed that Peruvian sweetpotatoes are not closely related to those from Papua New Guinea (Zhang et al., 1998) and are also different from those of Mesoamerica (Zhang et al., 2000). Understanding the level of genetic diversity and geographic differentiation is critically important to germplasm conservation. Conservation strategies and methods depend largely on this kind of information from within a given gene pool.

In the present study, we assessed the genetic diversity of germplasm from Oceania and analyzed the relationship between Oceania sweetpotatoes and those of the Philippines, Mexico, and Peru-Ecuador. We show there is wide genetic variation in the Oceania gene pool, wider than that of Peru-Ecuador. We also show that the germplasm from Peru-Ecuador is not closely related to that of Asia and Oceania, whereas the 'gene flow' from Mexico to Asia and Oceania appears significant. We suggest that the Oceania sweetpotato probably came from Mesoamerica through non-human dispersal.

Materials and Methods

Plant materials

All plant materials were obtained from the sweetpotato germplasm collection at CIP. The 76 accessions were randomly selected for each country. For this study, the selection was made among land races or farmer's cultivars (Table 1).

Healthy young leaves were collected from accessions maintained in a screen house and in vitro. The leaf tissue was immediately immersed in liquid nitrogen and then transferred to –80°C, freeze-dried, and stored at room temperature until use.

DNA isolation and AFLP analysis

A modified DNA miniprep procedure, based on Doyle and Doyle (1990), was used to extract DNA. The amplified fragment length polymorphism (AFLP) protocol was from Vos et al. (1995). Commercial AFLP kits were purchased from Life Technologies (Gaithersburg, MD, USA), and the AFLP reaction was carried out according to the manufacturer's instructions. DNA restriction digestion was carried out using the enzyme combination

Table 1. CIP number and country of origin of 76 cultivars from Oceania, the Philippines, and Latin America.

CIP No.	Country	CIP No.	Country
CIP 441124	Solomon Islands	CIP 440399	New Caledonia
CIP 441126	Solomon Islands	CIP 440294	Cook Islands
CIP 441125	Solomon Islands	CIP 440447	Cook Islands
CIP 441119	Solomon Islands	CIP 440454	Fiji
CIP 441116	Solomon Islands	CIP 440456	Fiji
CIP 441120	Solomon Islands	CIP ARB 386	Peru
CIP 441117	Solomon Islands	ARB 234	Peru
CIP 441123	Solomon Islands	DLP 1900	Peru
CIP 441127	Solomon Islands	DLP 5314	Peru
CIP 441118	Solomon Islands	DLP1922	Peru
CIP 441221	Tonga	DLP 3824	Peru
CIP 440276	Tonga	DLP 206	Peru
CIP 441222	Tonga	DLP 2	Peru
CIP 440273	Tonga	DLP 1090	Peru
CIP 440274	Tonga	DLP 2344	Peru
CIP 440272	Tonga	EECH 18	Peru
CIP 440277	Tonga	DLP 1188	Ecuador
CIP 440693	Papua New Guinea	DLP 1161	Ecuador
CIP 440129	Papua New Guinea	DLP 1484	Ecuador
CIP 440706	Papua New Guinea	DLP 1192	Ecuador
CIP 440695	Papua New Guinea	DLP 1449	Ecuador
CIP 440696	Papua New Guinea	DLP 1447	Ecuador
CIP 440699	Papua New Guinea	DLP 1487	Ecuador
CIP 440297	Papua New Guinea	DLP 1156	Ecuador
CIP 440296	Papua New Guinea	DLP 1456	Ecuador
CIP 440130	Papua New Guinea	DLP 1403	Ecuador
CIP 440660	Philippines	DLP 1423	Ecuador
CIP 440657	Philippines	NIAR 221	Mexico
CIP 440290	Philippines	CTX 15	Mexico
CIP 440669	Philippines	CATIE 9360	Mexico
CIP 440683	Philippines	CATIE 9232	Mexico
CIP 440667	Philippines	CTX 29	Mexico
CIP 440671	Philippines	RCB-IF-30	Mexico
CIP 440666	Philippines	CATIE 9257	Mexico
CIP 440659	Philippines	CTX 33	Mexico
CIP 440665	Philippines	CTX 31	Mexico
CIP 440676	Philippines	IO1438	Mexico
CIP 440681	Philippines	CTX 32	Mexico

of EcoRI/MseI. After the ligation of the oligonucleotide adapters, the restriction-DNA fragments were amplified using a polymerase chain reaction (PCR). Primer annealing is targeted at the adapter and restriction-site sequence. Three-nucleotide extensions on both the EcoRI and MseI primers cause selective amplification of fragments. Primer combinations were chosen based on the numbers of polymorphic fragments in a set of 10 sweetpotato genotypes.

The ^{32}P-labelled PCR products were separated by electrophoresis on a 6% polyacrymide gel in a 1 x TBE buffer (Tris-Boric acid-EDTA) solution at 50 W for about 1.5 hours. The gel was dried and exposed to X-ray film overnight.

Gel scoring and data analysis

Different fragments produced with each primer were treated as unit characters and numbered sequentially on the X-ray film. Genotypes were scored for the presence

(1) or absence (0) of each fragment. Only those fragments with medium or high intensity were counted. Fragments with the same mobility on the gel, but with different intensities, were not distinguished from each other when cultivars were compared. Monomorphic fragments were not scored.

From these data, a matrix of Euclidean distances was calculated using RAPDistance v1.03 (Armstrong et al., 1995). The Euclidean distances between the 76 accessions were then presented in a two-dimensional scaling plot using the SAS multidimensional scaling (MDS) procedure (SAS, 1997) to recover the relative positions of the accessions in the plot. Based on the MDS pattern, the procedure for analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was applied to quantify the variance components for AFLP phenotypes. Individual variation was partitioned within and between regions. The components of interest were extracted and tested using nonparametric permutation procedures.

Variation between regions was then partitioned into pair-wise distances between regions to examine the regional contribution to total molecular diversity (Excoffier et al., 1994).

Results

Polymorphic bands and multidimensional scaling

The 10 primer combinations generated 210 polymorphic, clearly scorable fragments for the 76 cultivars. The number of polymorphic bands for all the Oceania countries were comparable to those found in the Mexican accessions and higher than those of Peru-Ecuador, except for Papua New Guinea. Moreover, there were 18 bands (9% of the total scored bands) that were present only in the Oceania and Mexican accessions.

The MDS plot (Figure 1) showed a weak relationship between accession and geographic origin, except for Peru-Ecuador, where the cultivars were clearly distinguishable from the rest. All of the 22

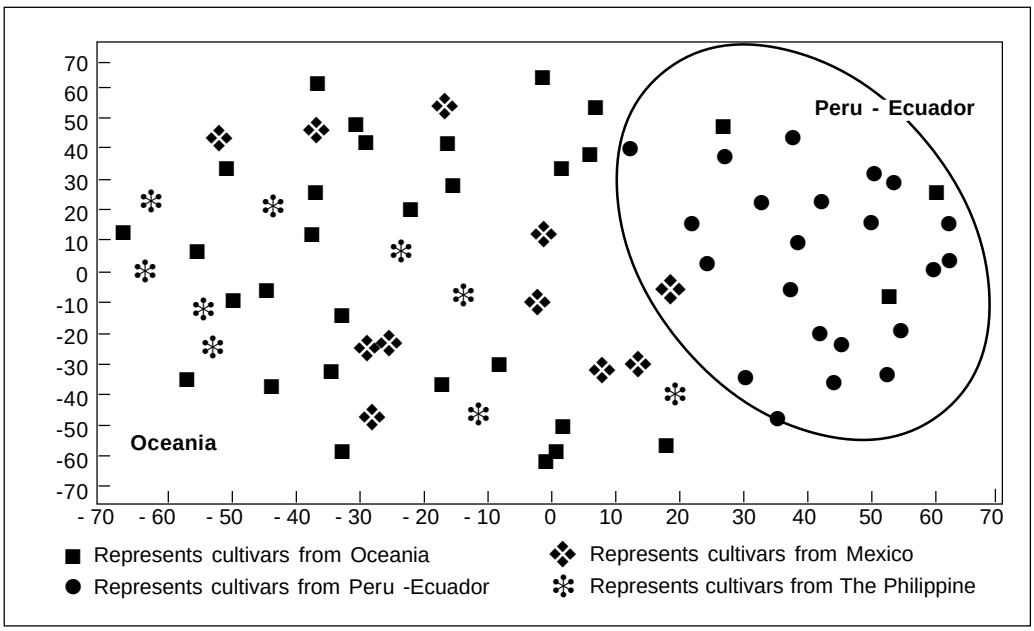

Figure 1. Interrelationship of sweetpotato cultivars from America and Oceania using multi-dimensional scaling (MDS). There is clear differentiation between Peru-Ecuador and the other countries. Mexican cultivars are closely associated with those of Oceania.

cultivars from Peru-Ecuador are closely grouped in one side of the plot.

Analysis of molecular variance

The AMOVA was conducted to quantify the diversity level and genetic relationship among regions by partitioning the variation within and among regions. There are various ways of grouping the four regions (or countries), but the current analysis assigned the four regions into two groups (Peru-Ecuador vs. the rest, see Table 2), based on the MDS plot.

The between-group variation accounts for 12.2% of the total molecular variance and is highly significant (Excoffier et al., 1994). Since the between-group variation actually quantifies the difference between Peru-Ecuador and the other regions (countries), it confirms the visual grouping observed in the MDS: there is statistically measurable divergence between the sweetpotato of Peru-Ecuador and the other regions (countries). The pair-wise distance among regions also demonstrates that Peru-Ecuador is significantly different from the other countries (Table 3).

The within-group (between-region) variation accounts for only 1.4% of the total molecular variance and is not statistically significant (Table 2). Since the within-group variation measures the differences between the regions (or countries) in Oceania, the Philippines, and Mexico, the non-significant result means that among these countries there is no detectable difference between the accessions of the 3 sources. Furthermore, the Mexican accessions are closely associated with the accessions from Oceania and the Philippines. The pair-wise distances between these regions (or countries) are close to zero, in sharp contrast to the long distances between these regions and Peru-Ecuador (Table 3).

The largest source of diversity comes from the within-region variation, which accounts for 86.4% of the total variance. This large within-region variation is mostly from the wide variation between individual cultivars in the same region (or country). The samples from the Philippines contain the highest internal diversity (37.0), comparable to that of Oceania (36.0), whereas the internal diversity in

Table 3. Genetic distances among sweetpotato cultivars from four regions.

Regions	Peru-Ecuador	Mexico	Philippines
Mexico	0.151 ***		
Philippines	0.149 ***	0.021^{NS}	
Oceania	0.188 ***	0.043^{NS}	0.000^{NS}

Notes: *** Significant at .001 level; ^{NS} = Non-significant.

Table 2. Analysis of molecular variance for the extraction of components of AFLP variation among groups, regions, and among individuals within regions.

Source of variation	DF	SSD[1]	MSD[2]	Variance component	% Total[3]	P value[4]
Among groups (Peru-Ecuador vs. the other regions)	1	201.2	201.2	4.97	12.2	<.001
Regions within group (among Mexico, the Philippines, and Oceania)	2	84.1	42.0	0.547	1.4	NS
Individuals within regions (or countries)	72	2486.5	35.0	35.0	86.4	<.001
Peru-Ecuador	21	661.8	31.5			
Mexico	10	337.3	33.7			
Philippines	11	407.1	37.0			
Oceania	30	276.9	36.0			
Total	75					

[1] Sum of squared deviations.

[2] Mean squared deviations.

[3] Percent of total molecular variance.

[4] Probability of obtaining a larger component estimate. Number of permutations = 1000.

Peru-Ecuador is the lowest (31.5). The partitioning of the variation into each region (or country) confirms the visual pattern in the MDS plot and supports the conclusion that there is a higher level of internal diversity in Oceania than in Peru-Ecuador.

Discussion

The high level of genetic diversity and the low within-region geographic proximity in Oceania agrees with the previous findings of Jarret and Austin (1994), who found higher diversity in accessions from Oceania than in those from Peru, based on RAPD analysis. The high diversity in Asia and Oceania was also found by Huang (2001). This high diversity and the low geographic proximity within Oceania, as well as between the Oceania countries and the Philippines and Mexico, are compatible with the history of the introduction and cultivation of the sweetpotato in Oceania and Asia. While the original spread of sweetpotato within Oceania was largely due to the Maori, who carried it with them on their voyages, there were multiple introductions from different sources after the initial transmission. For example, there were at least three notable introductions to New Zealand. There are three Maori words used to describe sweetpotatoes, which reflect the introduction of the sweetpotato to New Zealand: *kumara* (which, as mentioned above, has parallels to the Quechua word *kuma*), *merikana* (meaning *American*), and *waina* (meaning *vine*) (Yen, 1974). In the Philippines, there were introductions via the trade route between Mexico and Manila in the 17th century (O'Brien, 1972; Yen, 1982) in addition to the introduction from China by the end of the 16th century. Botanical evidence also suggests introductions from Indonesia and west New Guinea (Yen, 1991). These repeated introductions from different sources have contributed to the high diversity in Asia and Oceania, but they tend to confound any regional differentiation between these countries.

As a crop of tropical American origin, the means of pre-historic dispersal of sweetpotato to the west has long been a controversial issue. Besides the lack of morphological similarities between Peruvian sweetpotatoes and those of Oceania (Yen, 1974), the 'Kumara' hypothesis also suffers from the lack of evidence that such human contact ever occurred (Emory, 1968). The results of the present study demonstrate that Oceania sweetpotatoes have a weak genetic association with those of Peru-Ecuador. Moreover, the level of genetic diversity in Peru-Ecuador is lower than that of the Oceania countries and the Philippines, which suggests that the Oceania and Asian sweetpotato may not be of Peruvian origin. A similar result was found by Gichuki (2001). The possibility of alternative mechanisms, such as the transfer of botanical seeds by birds, could explain the pre-European transfer of sweetpotato into Polynesia. The accidental introductions explain the pre-historic arrival of the plant and also account for the absence of other cultural traits which might have been expected to occur if human contact had taken place (O'Brien, 1972). Coconut and gourd are two examples of non-human dispersal of cultivated pantropic species (Ridley, 1930). If this applies to the sweetpotato, the source of seeds could be from a tropical American region where this crop goes through a reproductive stage and produces seeds. The close relationship between the Mexican accessions and those from Oceania suggest that the sweetpotato in Oceania may be of Mesoamerican origin.

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