Genet Resour Crop Evol (2009) 56:257–262
DOI 10.1007/s10722-008-9362-6

RESEARCH ARTICLE

Coconut (*Cocos nucifera* L.) DNA studies support the hypothesis of an ancient Austronesian migration from Southeast Asia to America

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Received: 7 March 2008 / Accepted: 1 July 2008 / Published online: 26 July 2008

Abstract The centre of origin of coconut extends from Southwest Asia to Melanesia. Nevertheless, its pre-Columbian existence on the Pacific coast of America is attested. This raises questions about how, when and from where coconut reached America. Our molecular marker study relates the pre-Columbian coconuts to coconuts from the Philippines rather than to those of any other Pacific region, especially Polynesia. Such an origin rules out the possibility of natural dissemination by the sea currents. Our findings corroborate the interpretation of a complex of artefacts found in the Bahía de Caraquez (Ecuador) as related to South-East Asian cultures. Coconut thus appears to have been brought by Austronesian seafarers from the Philippines to Ecuador about 2,250 years BP. We discuss the implications of molecular evidence for assessing the possible contribution of early trans-pacific travels to and from America to the dissemination of domesticated plants and animals.

Keywords Coconut · *Cocos nucifera* · Early trans-Pacific travels · Lethal yellowing disease · Microsatellite markers

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Introduction

Most coconut specialists presently agree that coconut didn't originate in America, but in Malesia, a biogeographical region that includes the Malay Peninsula, Indonesia, the Philippines and New Guinea (Harries 1995). However, its pre-Columbian presence in large numbers on the Pacific coast of Latin America is established by an account by Oviedo who accompanied Balboa when crossing the Isthmus of Panama in 1513. It is attested from Punta de Burico at the Costa Rican border to Cabo Corrientes in Colombia (Zizumbo Villarreal and Quero 1998). Its presence in Peru has also been claimed (De Bisschop 1963), but remains debated (Patiño 1963).

Descendents of this pre-Columbian population still exist in that region and some of them are untouched by genetic contamination: the genetic structure of the Panama Tall cultivar (formerly known in Jamaica as "San Blas") has been extensively studied using RFLP (Lebrun et al. 1998) and microsatellites (Baudouin et al. 2008; Lebrun et al. 2005) markers. Its distinctive low genetic diversity can be interpreted as the result of a founder effect: a small number of individuals would have reached America and their descendants would have spread on a large area without genetic contamination from other populations. Due to the small number of founders, the genetic features of this variety would be a highly biased sub-sample of the genes that existed in the population of origin. Coconuts populations exhibiting

this unique molecular pattern are found today from on the Pacific coast from Costa Rica to Peru. Their fruit characteristics are quite similar to those of the San Ramon and Tagnanan varieties from the Philippines (Vargas and Blanco 2000).

But this variety has long been popular for its fruit characteristics (Cook 1910) and was introduced in the Caribbean. In Jamaica, its good behaviour, when confronted to the epidemic Lethal Yellowing Disease was noted (Been 1981) and lead to large scale planting of hybrids involving the Panama Tall. However, at the end of last century, a new outbreak of the disease caused massive losses in the Panama Tall as well as in the hybrids. It seems that the low genetic diversity in the Panama Tall was directly or indirectly the cause of this apparent resistance breakdown (Baudouin et al. 2008; Broschat et al. 2002). Identifying the origin of the Panama Tall could help plant breeders to identify varieties with a broader spectrum of resistance factors. This could lead to improve the sustainability of coconut cultivation in the affected areas.

Efforts to characterize coconut genetic diversity at a global level have lead to the construction of a microsatellite database of coconut genetic diversity, including the Panama Tall and its potential parent populations. This was an opportunity to reconsider questions that have been debated for a long time, without reaching conclusive answers (Ward and Brookfield 1992): how did coconut reach the continent, when and where did they land and where did they come from? Microsatellite markers helped us answering the last question. Interestingly, this answer indirectly suggests plausible answers to the others.

Material and methods

The coconut genotypes we analyzed here belong to a set of 1,215 individuals that were analyzed using 30 microsatellite markers. In this set, 104 individuals represent the Panama Tall cultivar. We selected 54 of them, based on the absence of genetic contamination from exotic germplasm (see Baudouin et al. 2004, 2008 for details). Eighty other Tall coconut populations from the Pacific Ocean were represented by 754 individuals and we grouped them into 11 regions (Table 1). This grouping reflects both geographical proximity and genetic similarities between populations. The Mexican Pacific populations are placed close to the Filipino populations because their ancestors were imported from the Philippines in the seventeenth century. They are thus difficult to distinguish based on molecular data only.

The 30 markers we used comprise 13 of the 14 marker set, developed as parts of a "microsatellite kit for coconut cultivar identification" (Baudouin and Lebrun 2002; Lebrun et al. 2005). The remaining 17 markers were developed in the framework of the

Table 1 Classification of the Pacific cultivars used in the present study

Code	Region		Number of individuals	Number of populations
A3	South-East Asia			
	A3a	Continent	66	9
	A3b	Indonesia	25	5
	A3c (Ph)	Philippines	43	4
	A3c (Me)	Mexico	46	6
A4	Melanesia			
	A4a	North New Guinea	38	8
	A4b	South New Guinea	34	8
	A4c	PNG: New Britain and other Islands	48	10
	A4d	Markham Valley	21	3
	A4e	Vanuatu, Solomons and New Caledonia	360	10
A5	Micronesia		43	11
A6	Polynesia		30	6
Total			754	80

Although the Filipino and Mexican cultivars form a single group from a genetic point of view, we separated them for this study

Generation Challenge Programme "Molecular characterization of tier 2 (orphan) crops".

Identifying the origin of the Panama Tall is similar to the population assignment problems, such as those treated by software such as Geneclass 2 (Piry et al. 2004) or Structure (Falush et al. 2003). It is however different, because the concerned populations may have evolved since the time they were separated. The source population may even have disappeared. But it must have been be related to the present time populations from the same region. We thus grouped the Pacific coconut populations into 11 regional groups and adopted the following similarity index:

$$C_{Li} = \sum_{a} f_{PLa} \times f_{G_iLa}$$

where indices L and a stand, respectively, for the loci and the alleles. Index P corresponds to the Panama and G_i to one of the 11 above-mentioned groups. This similarity index represents the probability of obtaining the same allele when sampling at random one allele from the Panama Tall and one from the populations of the ith region at the considered locus. We calculated this index for all locus-group combination and averaged it for each region.

Results

The average values of the similarity index for the 11 regions are represented in Fig. 1. Evolution since the Panama Tall was separated from its parent population may have altered allele frequencies, especially at rare loci but our index is especially sensitive to the variations of frequent alleles, which are more stable. Surprisingly, the Polynesian populations were the least similar to the Panama Tall, while the Filipino populations were the most similar. This means that, in spite of a considerable loss of diversity, the Panama Tall still presents a number of distinctive molecular features found in the present days Filipino populations.

Table 2 compare the scores of the Philippines and Polynesia at all loci. Since a founder effect (and the subsequent genetic drift) may have altered considerably allele frequencies, similarity at a particular locus doesn't necessarily reflect the actual degree of relatedness. However, the score of the Philippines exceeded that of Polynesia at 27 out of the 30 tested loci ($\chi^2 = 19.6$***). The ratio between the similarity indexes of these regions exceeded 1000:1 at one locus and 10:1 at four further loci. It was slightly below 1:1 at only two loci. This makes a fortuitous inversion of the scores highly unlikely. Indeed, at one-third of the loci, the most frequent allele of the Panama Tall was at a fairly high frequency in the Philippines, but absent or rare in Polynesia.

Regarding the other regions, the score of the Mexican group was close to that of the Philippines; the other South-East Asian regions (continental and Indonesian) had higher scores than the South Pacific represented by Melanesia, Micronesia and Polynesia.

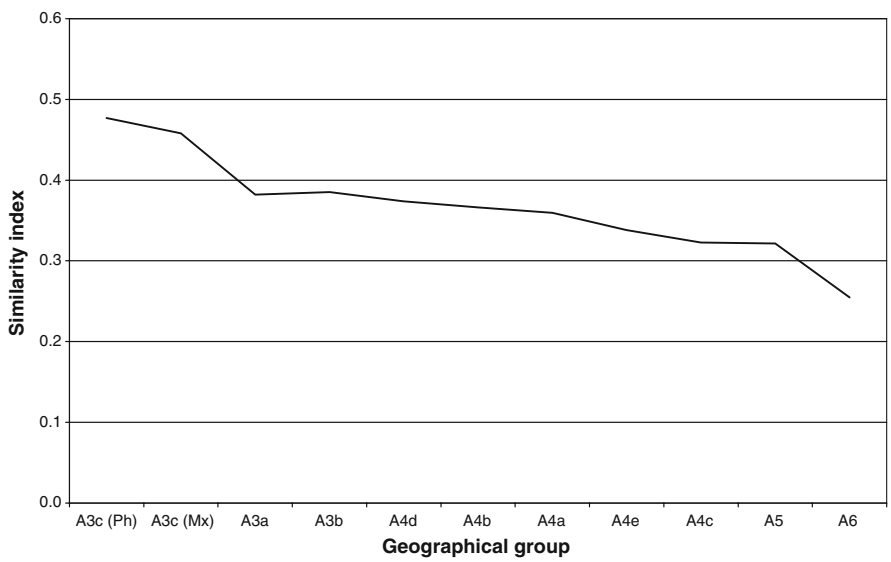

Fig. 1 Value of the mean similarities for 11 possible regions of origin based on 30 microsatellite loci (see Table 1 for the meaning of the geographical group codes)

Table 2 Comparison between the similarity indexes of the Filipino and the Polynesian regions at 30 loci

Locus	Philippines	Polynesia	Ratio	Locus	Philippines	Polynesia	Ratio
CNZ40	0.425	4×10^{-4}	1073	CnCirE2	0.035	0.017	2.06
CnCir2	0.628	0.022	28.4	CnCir 215	0.742	0.466	1.59
CnCir119	0.320	0.021	15.0	CnCirH4′	0.329	0.211	1.56
CnCirF2	0.302	0.024	12.7	CnCirE12	0.492	0.322	1.53
CnCir147	0.241	0.021	11.6	CnCirE1	0.117	0.079	1.47
CnCirA3	0.491	0.058	8.41	CnCirA9	0.460	0.335	1.37
CnCirH7	0.539	0.080	6.72	CNZ03	0.943	0.729	1.29
mEgCIR2739	0.581	0.106	5.49	CnCirG11	0.348	0.272	1.28
CnCirC12	0.717	0.153	4.68	CnCirB6	0.525	0.457	1.15
CNZ42	0.666	0.150	4.43	CnCirC7	0.640	0.558	1.15
mEgCIR3400	0.395	0.090	4.38	CnCirI4	0.934	0.826	1.13
CnCirH11	0.268	0.062	4.30	mEgCIR3750	0.487	0.442	1.10
CnCirB12	0.210	0.062	3.42	CnCir126	1.000	1.000	1.00
CnCir206	0.267	0.112	2.38	CnCirF3′	0.156	0.159	0.98
CnCirE10	0.908	0.431	2.11	CnCirC5	0.092	0.117	0.79

This ranking is consistent with the known genetic gradients throughout the Indian Ocean.

Discussion

Molecular evidence presented here suggests that the Panama Tall is more related to the present coconut populations of the Philippines than to those from any other regions. Based on genetic similarity and proximity, Mexico would be a reasonable alternative but historical considerations show that the genetic relationships between Mexico and Panama result from their common ancestry in the Philippines rather than from a direct germplasm movement from Mexico to Panama: coconut was already abundant in Panama before the Spaniards imported it from the Philippines to Mexico. Such movement would thus have increased genetic diversity rather than reduced it. In addition, recorded coconut seed shipment between these countries suggest a modest contribution of the Panamanian coconuts to the Mexican germplasm (Zizumbo Villarreal 1996) and not the reverse. In this context, the low genetic diversity observed in the Panama Tall confirms that it remained untouched by introgression from Mexico. But it must have come from elsewhere.

Ancient trans-Pacific voyages have long been debated but recent findings made it possible to date the introduction of a Polynesian breed of chicken to Chile at least 1200–1300 AD (Borell 2007; Storey et al. 2007), thus providing hard evidence for trans-Pacific navigation in the pre-Columbian period. Such voyages occurred in both directions since the American sweet potato was introduced to the Cook Islands, apparently at the same period (Hather and Kirch 1991). In the case of coconut, human dissemination was considered seriously after Ward and Brookfield (1992) concluded that it was more likely than unaided drifting caused by winds and sea currents: a floating coconut would probably have started germinating (and soon died) before arriving to the American coasts. This conclusion is reinforced by the morphology and the physiology of the Panama Tall fruit: it belongs to the *niu Vai* type—the domesticated type according to Harries (1978), which is poorly adapted to long distance dissemination by sea currents.

However, our main result implies that the Panama Tall and more generally the pre-Columbian coconuts are more likely to originate in South-East Asia than in Polynesia. The first consequence is to rule out completely the possibility of natural dispersion. The second one is that the introductions of chicken and of coconut to Americas have to be totally independent events: the Southeast Asian chicken breeds don't have the haplotypes that are common to Chile and Polynesia. On the other hand, ecological conditions would have prevented any introduction of coconut

below the latitude of the north of Peru. But was it possible to reach America from South-East Asia in the pre-Columbian times?

Five thousands year ago, Austronesian speaking people left South china to reach Taiwan and then the South-East Asian archipelago. About 1,500 years later, their descendants, known as proto-Polynesians expanded eastward, eventually colonizing the Pacific Ocean. Another branch ventured to the West and reached Madagascar, introducing their own coconut variety (Lebrun et al. 1998). In fact, the Austronesians were not only long range navigators, but farmers and didn't fail to carry animals and useful plants in their large canoes. They would be used as food supply but could also be introduced in the event a new island was discovered. Coconut was part of the travel along with other plants: Langdon (1993), argues that the plantain banana (from Southeast Asia) already existed in Latin America in pre-Columbian times and reports similarities between the Quechuan words for bamboo and their equivalent in languages from the Philippines (Langdon 2001).

By themselves, our data do not provide indications to date the introduction of coconut to America, but it is interesting to note that they seem to corroborate the interpretation of archaeological findings in Ecuador as related to Southeast Asian cultures by Estrada and Meggers (1961). These findings could, in turn, suggests a plausible date and a place of arrival for coconut. A series of pottery objects found in the Bahía de Caraquez is dated about 250 BC and includes "house models with saddle roofs (such as the one represented in Fig. 2), neck rests, rectanguloid net weights, "golf-tee" ear plugs, and panpipes graduated from each side toward the center". These authors note that "they were restricted to the Bahía Culture in the New World, but were widespread at an earlier time in Southeast Asia and Indonesia, implying a transpacific introduction". After their introduction to Ecuador, maritime trade along the Pacific coast suggested by Langdon (2001) could have promoted the distribution of coconut northward to Columbia and Panama and possibly southward to northern Peru.

Although suggestive, the cited evidence in favour of an introduction of the coconut about 1,000 before the introduction of chicken in Chile is only indirect. It could only be confirmed by the recovery of archaeobotanical remains of coconut tissues such as the woody endocarp (Di Piazza 1998; Kirch 1989; Morcote-Ríos and Bernal 2001) or the fibrous mesocarp. Coconut pollen has been used successfully in the South Pacific but, in America, the presence of closely related species would demand scanning electron microscopy (Maloney 1993). The hypothesis of two distinct introduction events could also be tested directly on chicken if bones were available for mtDNA in Peru: Pizarro found that chicken were integrated in the Incan culture (Storey et al. 2007) making an introduction by the Spaniards unlikely. More DNA studies would certainly be useful to test hypotheses involving the introduction of various plant from America to Easter Island and farther Polynesian islands (Langdon 1993). Finally, the results presented here should encourage more field testing of varieties originating from South-East Asia, as an effort to find more diversified sources of resistances factors to the Lethal Yellowing disease.

Fig. 2 House-shaped vessel from Bahía de Caraquez, Manabí (Ecuador), presenting a deep saddle roof, similar to those found in South-East Asia (With permission of B. Meggers)

Acknowledgements We thank the Cogent network, which facilitated to us obtaining the plant material and the Genotyping Platform of the Languedoc Roussillon Genopole, hosted by CIRAD (C. Billot and R. Rivallan) where all the SSR data have been obtained.

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