RAPD Analysis of South Pacific Coconut Palm Populations

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ABSTRACT

The South Pacific region represents a large potential genetic resource for improvement of coconut palms (*Cocos nucifera* L.). A study of the genetic diversity in the species was made in 1992-1993 by means of RAPD analysis on a representative sample from 17 distinct South Pacific coconut populations to characterize the germplasm present in the region. A moderate level of genetic diversity was found, although very few RAPD markers were unique to specific populations. Approximately 60% of the observed diversity occurred within-populations, but this level varied between the various populations. This indicated the generally low but variable influence of gene migration between populations, the establishment of populations by few individuals that comprised a fraction of the genetic variation of their parental populations (founder effects), and subsequent selection by the local human population. Although the coconut populations of the region generally displayed a pattern of continuous variation, this continuum could be divided into discrete groups by cluster analysis. This division comprised a southern group of populations, a northern-eastern group, and single-population groups from the Marquesas and Hawaii groups. The population from Rennell Island diverged from the main genetic continuum, apparently because of isolation and artificial selection. Collection and conservation strategies have been devised for coconut germplasm in the South Pacific region based on existing genetic diversity.

THE COCONUT PALM is an important crop plant in the coastal humid tropics for both subsistence agriculture and as a major trade commodity. The crop evolved in the Asia-Pacific area (Harries, 1990) and represents a large potential source of genetic variability for use in coconut palm improvement programs. Initial surveys of the South Pacific region using fruit characters have revealed some of the expected variation (Whitehead, 1966; Parham, 1966; Foale, 1987; Ashburner, 1994).

Collection and conservation of coconut germplasm are costly and logistically difficult. Since coconuts are exclusively seed-propagated and have recalcitrant, bulky seeds, germplasm must be maintained as perennial field plantings (Ashburner, 1995). Strict quarantine measures are also currently applied to germplasm exchange (Frison et al., 1993). Furthermore, a high degree of genetic variation is expected since coconut palms generally have a mixed mating system with dominant cross pollination. However, dwarf populations exhibit strict self pollination and West African Tall populations exhibit strict cross pollination (Ashburner, 1995).

In situ surveys are required to identify diverse populations so that efficient collection and conservation strategies can be implemented. In situ surveys using morphological descriptors (IBPGR, 1992) are not satisfactory because of the large environmental effect on phenotype (Coomans, 1975) and an absence of heritability estimates for those characters. One exception may be fruit component analysis (Whitehead, 1966) as refined by Harries (1978, 1981). RAPD analysis has been successfully used to survey the genetic resources of economically important plant species, especially perennial tropical species such as *Theobroma cacao* L. (Russell et al., 1993), *Elaeis guineensis* Jacq. (Shah et al., 1994), *Gliricidia* HBK sp. (Chalmers et al., 1992), and *Coffea* L. sp. (Orozco-Castillo et al., 1994). Various methods for estimating genetic distance have been used (Skroch and Nienhuis, 1995). Some authors recommend scoring monomorphic characters (Skroch et al., 1992), whereas others suggest that they should be omitted from the analysis (Link et al., 1995). Furthermore, various methods exist for partitioning the source of genetic variability into within- and between-population components. Among these, the method described by Huff et al. (1993) assumes that population variances are equal and the method of Lynch and Milligan (1994) assumes Hardy-Weinberg equilibrium. A more statistically robust method uses the Shannon-information index (Russell et al., 1993).

The objective of this paper was to survey coconut variability in the South Pacific region by means of RAPD analysis.

MATERIALS AND METHODS

Sample Collection

Leaf material was collected in 1992-1993 from frond number 12 of 25 randomly selected mature coconut palms at each of 14 locations in the South Pacific Region (Table 1). Each palm was at least 200 m from the previously sampled palm. Five additional populations were sampled from germplasm collections in Papua New Guinea and the Solomon Islands (Table 1); however, only five individuals were available in each case. For sporadically occurring genotypes found in association with villages, such as Vanuatu Red Dwarf (VRD) and Niu Leka Dwarf (NLD), and those populations sampled from germplasm collections, sampling occurred at random because the 200 m distance rule could not be effectively applied. The collected leaves were trimmed and sealed in plastic bags. The leaves were then either air-freighted, air-mailed, or carried as hand-baggage to Melbourne, Australia, where they were kept at −20°C. Even after 2 wk in transit at ambient temperature, satisfactory DNA was extracted from the leaves.

BDNA Extraction and RAPD Analysis

Extraction of DNA was performed from 300 mg of frozen leaf sample by means of the method of Rogers and Bendich (1985). The DNA concentration was estimated with a DNA fluorometer (Hoeffer, San Francisco, CA) as well as by visualization with ethidium bromide staining after electrophoresis in a 10 g L^{-1} agarose gel at 100 V for 1 h in TAE buffer.

Approximately 25 μg of genomic DNA (1/10 dilution of

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Abbreviations: RAPD, random amplified polymorphic DNA.

Table 1. Locations of South Pacific coconut populations sampled for RAPD analysis.

Code	Population	Origin			
		Area	Country	Latitude	Longitude
CIT†	Kiritimati Tall	Kiritimati	Kiribati	2°N	157°30′W
CKT	Rarotonga Tall	Rarotonga	Cook Islands	21°15′S	159°45′W
FIT	Fiji Tall	Taveuni	Fiji	17°10′S	180°
GET	Gazelle Pen Tall	New Britain	Papua New Guinea	4°20′S	152°10′E
HIT	Hawaii Tall	Hawaii	USA	19°40′N	155°W
KIT	Kiribati Tall	Tarawa	Kiribati	1°30′N	173°E
KRT‡	Kar Kar Island Tall	Madang	Papua New Guinea	4°20′S	146°E
NLD	Niu Leka	Taveuni	Fiji	17°10′S	180°
MAT	Mo'orea Tall	Mo'orea	French Polynesia	17°30′S	149°50′W
MMT‡	Markham Valley Tall	Morobe	Papua New Guinea	6°40′S	146°30′E
MST	Marquesas Tall	Nuku Hiva	French Polynesia	10°30′S	140°W
RAT	Rangiroa Tall	Rangiroa	French Polynesia	16°S	146°50′W
RLT	Rennell Tall	Rennell Id.	Solomon Islands	11°30′S	160°E
ROT†	Rotuma Tall	Rotuma Id.	Fiji	13°20′S	176°40′E
SNT	Russell Tall	Russell Is.	Solomon Islands	9°10′S	159°E
TAT	Tongatapu Tall	Tongatapu	Tonga	21°20′S	175°20′W
VRD	Vanuatu Red Dwarf	Espiritu Santo	Vanuatu	15°30′	165°30′E
VOT†	Vanikoro Tall	Vanikoro	Solomon Islands	11°30′S	167°E
VUT	Vanuatu Tall	Espiritu Santo	Vanuatu	15°30′S	165°30′E

† Sampled from the collection at Lever Solomon Ltd., Yandina, Solomon Islands.
‡ Sampled from the collection at Cocoa and Coconut Research Institute, Kerevat, Papua New Guinea.

DNA extract in sterile distilled water) was amplified in 25-μL reactions containing 67 mM Tris-HCl pH 8.8, 16.6 mM $(NH_4)_2SO_4$, 4.5 mL L^{-1} Triton X-100, 0.2 g L^{-1} gelatin, 4 mM $MgCl_2$, 200 μM each of dATP, dCTP, dGTP, dTTP, 0.25 μM of 10-mer primer (Operon Technologies Inc., Alameda, CA), and 1 U of *Taq* DNA polymerase (Biotech International, Perth, Australia) by means of a thermal cycler (Perkin-Elmer, Norwalk, CT) programmed for 45 cycles of 60 s at 94°C, 60 s at 36°C and 2 min at 72°C. Each reaction vial was overlaid with 1 drop paraffin oil. Amplification products were visualized by electrophoresis in a 15 g L^{-1} TAE agarose (Sigma, St. Louis) gel at 75 V for 4.5 h with a standard of λ phage DNA (Promega, Madison, WI) digested with *Eco*47I (syn. *Ava*II) (Bresatec, Adelaide, Australia). Amplification products were visualized by ethidium bromide staining under UV light (λ = 204 nm) and photographed (Polaroid, MP-4, Cambridge, MA). Each amplification product was considered a RAPD marker and polymorphisms were confirmed by running duplicate samples.

Two binary data-sets were created. Data Set 1 was constructed to determine the most discriminatory primers for more in-depth analysis as well to examine geographic pattern in the variation between populations. The data set was constructed by scoring the presence or absence of 123 markers from five plants in each population with 14 10-mer primers [Kit A (Operon Technologies Inc., Alameda CA): 01, 03, 04, 05, 07, 09, 10, 11, 12, 13, 15, 17, 18 and ROG2: CTG TTG CTA C].

Data Set 2 examined the partitioning of genetic variation within- and between-populations (population structure) in the south Pacific region. The data set was constructed by scoring the presence or absence of 39 RAPD markers generated by four primers (A-09, A-11, A-15, and ROG2) in 25 plants of the populations not collected from germplasm collections: CKT, FIT, GET, KIT, MAT, MST, NLD, RAT, RLT, TAT, VRD, and VUT (Table 1).

Statistical Analyses

Data Set 1 was analyzed by fitting generalized linear models to determine which RAPD markers significantly differed in frequency between the populations from the South Pacific region. A similarity matrix was created between all individuals using Jaccard's coefficient (Jaccard, 1908). The similarity matrix was then reduced to population means and subjected to hierarchical cluster analysis by the average-link strategy (UPGMA) (Gordon, 1981). The reduced similarity matrix was also used as input into principal coordinate analysis (Gower, 1966).

Genetic diversity was estimated in Data Set 2 by the Shannon-information index (Chakraborty and Rao, 1991) modified for RAPD analysis, which is defined as

$$H = -\sum_{i=1}^{k} p_i \log_e p_i.$$

For each primer used, H denotes the diversity of RAPD markers in a population, k denotes the number of RAPD markers and p_i denotes the frequency of the ith RAPD marker in a given population. For each population, H was averaged over all the primers to determine the within-population diversity of RAPD markers (H_O). The average diversity of RAPD markers for all populations (H_{POP}) was calculated as the mean of H_O. The diversity of RAPD markers for coconut palms in the region of study (H_{PAC}) was calculated with a p_i based on the whole Pacific region rather than on individual populations. The proportion of diversity that is found within-populations relative to total diversity ($H_{POP/PAC}$) was derived by dividing by $H_{POP} \times H_{PAC}$.

RESULTS

RAPD Profile

A total of 123 different RAPD markers were generated by the 14 primers in Data Set 1. The numbers of markers produced per primer ranged from 3 (A-13) to 15 (ROG2). The number of polymorphic RAPD markers per primer ranged from 1 (A-13) to 14 (A-09). Monomorphic markers totaled 41% in Data Set 1. Only Kiritimati Tall (CIT), Hawaii Tall (HIT), and Vanuatu Red Dwarf (VRD) possessed unique fixed RAPD markers. Significant ($P <$ 0.05) differences in population frequency were found in 35% of the markers. Among all primers measured, A-09, A-11, A-15, and ROG2 gave the largest deviance ratios when comparing the frequencies between populations and were thus the most discriminating primers. In Data

Table 2. Genetic diversity of South Pacific coconut populations calculated by the number of polymorphic RAPD markers per population and the Shannon-information index (H_o).

Population	No. of polymorphic characters per population	Genetic diversity (H_o)†
CKT	14	0.14
FIT	12	0.12
GET	21	0.25
KIT	19	0.19
MAT	20	0.25
MST	18	0.23
NLD	13	0.15
RAT	18	0.23
RLT	22	0.28
TAT	11	0.14
VRD	8	0.05
VUT	16	0.23
Population mean‡	17.1	0.21§

† H_o is the average, over all primers, of the Shannon Information index, $H = -\sum_{i=1}^{k} p_i \log_e p_i$, where k is the number of RAPD markers for a particular primer, and p_i denotes the frequency of the ith RAPD marker in a given population.

‡ NLD and VRD not included in this calculation because they were sporadically occurring genotypes and do not represent the general populations of the area.

§ H_{POP}.

Set 2, a higher proportion of the markers were polymorphic as compared with Data Set 1 (90% as opposed to 59%, respectively), and of these markers, a higher proportion showed significantly different frequencies between populations (67% as opposed to 35%, respectively).

Within- and Between-Population Genetic Diversity

The average diversity of RAPD markers for tall coconut populations in the South Pacific region (H_{PAC}) was 0.35. The average RAPD diversity (H_O) for individual populations ranged from 0.05 (Vanuatu Red Dwarf) to 0.28 (Rennell Tall) (Table 2). As the most discriminating primers were used in this calculation, the diversities were therefore higher than would be expected if a random selection of markers was used. For example, H_{PAC} calculated with Data Set 1 (a random choice of markers) was 0.10. The mean within-population diversity of tall populations in the south Pacific (H_{POP}) was 0.21. The proportion of the total diversity found within-populations ($H_{POP/PAC}$) was calculated to be 0.60, making the proportion of the diversity found between-populations 0.40.

Geographic Analysis of Genetic Variation

Pairwise population similarity indices varied moderately (Table 3), ranging from 0.763 [Rennell Tall (RLT) and Hawaii Tall (HIT)] to 0.928 [Kar Kar Island Tall (KRT) and Markham Valley Tall (MMT)].

Five distinct population clusters were defined corresponding to the northern and eastern parts of the south Pacific, the southern part of the south Pacific, Hawaii Tall, Rennell Tall, and Marquesas Tall (Fig. 1 and 2). The northern-eastern group included populations from Papua New Guinea. Within these broad groups, further geographic groupings were evident, such as the close associations of the Papua New Guinea populations (GET, MMT, KRT) and close associations of the Solomon Islands (SNT), Vanikoro (VOT), and Rotuma (ROT) populations.

A low amount of variation (35%) was contained within the first three principal coordinates of the ordination (Fig. 3) and did not represent the whole complexity of the relationships between the respective populations. However, it presented the main relationships present and demonstrated some geographic patterns. The first two principal coordinates separated one large group of populations from Rennell Tall (RLT). Within the large group, further division could be made between the southern and northern-eastern clusters, although no clear distinction was found; the variation was continuous in nature. The Hawaii Tall (HIT) population was more closely aligned with populations from the northern-eastern group, whereas the Marquesas Tall (MST) population had closer affinities with those from the southern group.

DISCUSSION

The number of RAPD markers generated per primer varied because of primer sequence and individual genotype and was consistent with studies in other crops (Williams et al., 1993). Using Data Set 1 as an indicator of general RAPD diversity, the percentage of monomorphic markers in South Pacific coconut germplasm was intermediate at 41%, and was comparable to that found in

Table 3. Similarity matrix of south Pacific coconut populations derived from the RAPD markers of data set 1.

CIT	1.000																
CKT	0.827	1.000															
FIT	0.850	0.851	1.000														
GET	0.871	0.837	0.836	1.000													
HIT	0.807	0.802	0.824	0.821	1.000												
KIT	0.858	0.814	0.821	0.869	0.822	1.000											
KRT	0.859	0.841	0.822	0.901	0.811	0.861	1.000										
MAT	0.870	0.839	0.834	0.858	0.812	0.874	0.857	1.000									
MMT	0.837	0.849	0.827	0.880	0.801	0.835	0.928	0.843	1.000								
MST	0.826	0.813	0.838	0.832	0.807	0.824	0.824	0.820	0.810	1.000							
RAT	0.807	0.821	0.825	0.852	0.830	0.831	0.845	0.811	0.844	0.889	1.000						
RLT	0.777	0.815	0.821	0.782	0.763	0.774	0.775	0.771	0.803	0.797	0.784	1.000					
ROT	0.808	0.851	0.833	0.822	0.828	0.784	0.896	0.822	0.818	0.787	0.824	0.773	1.000				
SNT	0.839	0.879	0.841	0.855	0.842	0.827	0.828	0.832	0.844	0.805	0.856	0.797	0.898	1.000			
TAT	0.841	0.841	0.838	0.831	0.787	0.803	0.824	0.816	0.808	0.816	0.809	0.813	0.829	0.847	1.000		
VOT	0.826	0.860	0.842	0.858	0.833	0.829	0.835	0.819	0.845	0.809	0.861	0.794	0.880	0.934	0.829	1.000	
VUT	0.859	0.852	0.874	0.852	0.816	0.822	0.833	0.834	0.824	0.814	0.828	0.779	0.838	0.871	0.850	0.861	1.000
	CIT	CKT	FIT	GET	HIT	KIT	KRT	MAT	MMT	MST	RAT	RLT	ROT	SNT	TAT	VOT	VUT

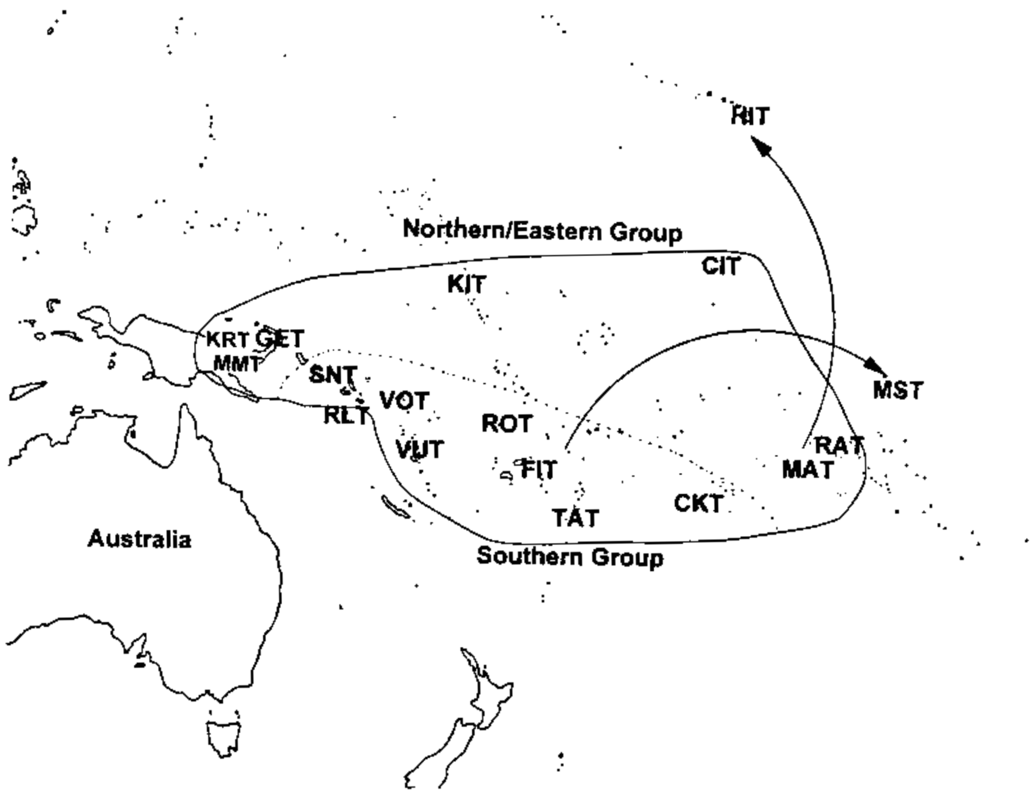

Fig. 1. Locations of the coconut palm populations studied in the south Pacific. Groupings shown are derived from cluster analysis of RAPD markers (Fig. 2).

other plant species (Hu and Quiros, 1991; Huff et al., 1993; Russell et al., 1993; Huen et al., 1994, Link et al., 1995).

The relatively high between-population diversity in the coconut populations of the south Pacific region suggested that differentiation has probably arisen because of the establishment of populations by few individuals that comprised a fraction of the genetic variation of their parental population (founder effects) from natural or artificial migration, followed by reproductive isolation between populations, and mass propagation of desirable types during commercial plantation establishment. Founder effects cause genetic shifts in populations but do not generally reduce diversity in outcrossing species (Nei et al., 1975). The level of within-population diversity is within the range of values found in isozyme studies of other outcrossing woody perennial species (Hamrick, 1989). The lack of fixed unique characters for specific

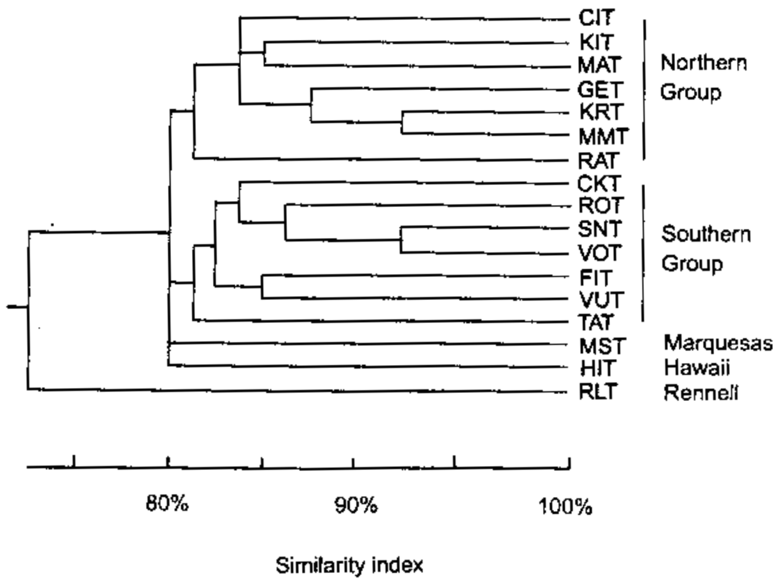

Fig. 2. Average-link dendrogram of south Pacific coconut populations based on a pair-wise similarity matrix formed using Jaccard's coefficient of RAPD markers from Data Set 1.

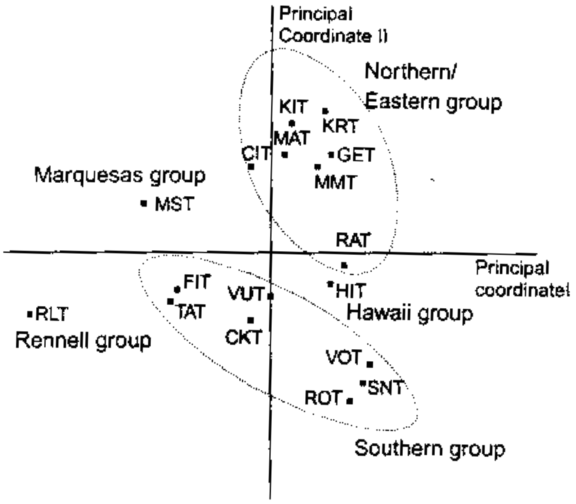

Fig. 3. Ordination of south Pacific coconut populations based on principal coordinate analysis of the RAPD markers of Data Set 1. The first and second principal coordinates describe 14 and 12% of the total variation, respectively.

populations suggested that strict genetic isolation has not occurred. Although coconut palms naturally disperse by floating, this trait has been compromised to some extent by selection for more domesticated characters, such as a lower husk-to-nut ratio (Harries, 1981).

Within-population diversity (H_O) varied distinctly between south Pacific populations, indicating that genetic constrictions or bottle-necks probably have occurred to varying degrees. For example, the lower diversity found in the populations of the south central Pacific (Cook Islands, Fiji, and Tonga) may be a reflection of the selection of seed-nuts from limited sources in those countries during commercial plantation establishment within the last 100 yr. Alternatively, genetic diversity may have been restricted as a result of culling by local inhabitants, or as a result of natural disasters such as cyclones. The low within-population diversity measured for Vanuatu Red Dwarf is consistent with its high self-pollination rate (Whitehead, 1966).

Geographic analysis of the genetic variation of Pacific populations indicated continuous genetic variation (Fig. 3) that could be arbitrarily divided into two geographically cohesive groups and two single-population groups by cluster analysis (Fig. 2). The southern group roughly corresponded to the Melanesian and Southern Polynesian groups defined by fruit morphology (Ashburner, 1994) and therefore represented populations that exhibited more the domestic-type character of low husk-to-nut ratios. The populations from the northern-eastern group generally exhibited the more wild-type character of a high husk to nut ratio in the fruit, with the exception of the Papua New Guinea populations (Ashburner 1994). On a cautionary note, the genetic relationships between these populations were not as simple as presented, since only a low proportion of the total variation was described by the first two axes of the ordination (Fig. 3). This is apparently common in RAPD analysis of plant species (Russell et al., 1993; Huen et al., 1994, Link et al., 1995) and the complexity involved in trying to assign geographic patterns to genetic variation in landrace populations has been previously noted (Brown, 1978). In the case of coconut palms, this complexity has probably arisen because of artificial selection, migration, introgression, and the rapid expansion of cultivation areas over the past century. Simulation studies of other plant species have shown that between 10 and 20 generations are needed to re-establish equilibrium after such disturbances (Turner et al., 1982; Sokal et al., 1989). In a perennial species, such as coconut that has a generation time of between 20 and 30 yr, this period may be 200 to 600 yr without further disturbances.

The origin of Hawaii Tall from the northern-eastern group of populations is consistent with Polynesian migration patterns. The origin of Marquesas Tall from the southern group can be similarly explained, since this area is thought to be the first area colonized by Polynesians when they migrated from the Central to the Eastern Pacific (Kirch, 1986). Polynesian migration could explain the deviance of the Rennell Island population from the main genetic continuum as it is a Polynesian outlier in Melanesia. These three populations have presumably become more genetically distinct from the other populations either because of isolation and genetic drift, genetic shift, or a combination of both. Hawaii is geographically isolated from other coconut populations and the presence of a pre-human wild population is unlikely (Harries, 1978). Similarly, the Marquesas Islands and Rennell Island have very little land suitable for the natural establishment of coconuts and therefore the populations that were established there would most likely have been isolated from any introgression or further migration, either natural or artificial, and diverged rapidly.

This study helps in the development of ex situ collection and conservation strategies for coconut palms in the south Pacific region. Each population is unique even though very few unique fixed markers were found in any population. Because of the relatively high amount of variation between populations, preference should be given to collecting populations rather than individuals in a population. Cluster analysis can be used, along with morphological and environmental data, to rationalize the collection of germplasm by such methods as core subsets (Brown, 1989). The choice of populations in these groups can be made at random or on the basis of either maximizing the within-population diversity by selecting highly variable populations, or by selecting groups that will maximize genetic distance. With this information and with reference to morphological and environmental variation (Ashburner 1994), coconut collection and conservation should proceed more efficiently.

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REFERENCES

Ashburner, G.R. 1994. Characterisation, collection and conservation of *Cocos nucifera* L. in the south Pacific. Ph.D. thesis, The University of Melbourne, Victoria, Australia

Ashburner, G.R. 1995. Reproductive biology of coconut palms. p. 173–186. *In* C. Oropeza et al. (ed.) Lethal yellowing: Research and practical aspects. Kluwer, Dordrecht, the Netherlands.

Brown, A.H.D. 1978. Isozymes, plant population structure and genetic conservation. Theor. Appl. Genet. 52:145–157.

Brown, A.H.D. 1989. Core collections: a practical approach to genetic resources management. Genome 31:818–824.

Chakraborty, R., and C.R. Rao. 1991. Measurements of genetic variation for evolutionary studies. p. 271–316. *In* C.R. Rao and R. Chakraborty R (ed.). Handbook of statistics, Vol 8. Elsevier, Amsterdam.

Chalmers, K.J., J.I. Sprent, A.J. Simons, R. Waugh, and W. Powell. 1992. Patterns of genetic diversity in a tropical tree legume (*Gliricidia*) revealed by RAPD markers. Heredity 69:465–472.

Coomans, P. 1975. Influence des facteurs climatiques sur les fluctuations saisonnières et annuelles de la production du cocotier. Oléagineux 30:153–159.

Foale, M.A. 1987. Coconut germplasm in the south Pacific islands. ACIAR Technical Reports No 4. Australian Centre for International Agricultural Research, Canberra.

Frison, E.A., C.A.J. Putter, and M. Diekmann (ed.). 1993. FAO/IBPGR technical guidelines for the safe movement of coconut germplasm. Food and Agriculture Organisation and International Board for Plant Genetic Resources, Rome.

Gordon, A.D. 1981. Classification. Chapman and Hall, London.

Gower, J.C. 1966. Some distance properties of latent root and vector methods using multivariate analysis. Biometrika 53:325–338.

Hamrick, J.L. 1989. Isozymes and the analysis of genetic structure in plant populations. p. 87–105. *In* D.E. Soltis and P.M. Soltis (ed.). Isozymes in plant biology. Chapman and Hall, London.

Harries, H.C. 1978. The evolution, dissemination and classification of *Cocos nucifera* L. Bot. Rev. 44:265–319.

Harries, H.C. 1981. Practical identification of coconut varieties. Oléagineux 36:63–72.

Harries, H.C. 1990. Malesian origin for a domestic *Cocos nucifera*. p. 351–357. *In* P. Baas et al. (ed.). The plant diversity of Malesia. Kluwer, Dordrecht, the Netherlands.

Hu, J., and C.F. Quiros. 1991. Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep. 10:505–511.

Huen, M., J.P. Murphy, and T.D. Phillips. 1994. A comparison of RAPD and isozyme analyses for determining the genetic relationships among *Avena sterilis* L. accessions. Theor. Appl. Genet. 87:689–696.

Huff, D.R., R. Peakall, and P.E. Smouse. 1993. RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.]. Theor. Appl. Genet. 86: 927–934.

IBPGR 1992. Descriptors for coconut. International Board for Plant Genetic Resources, Rome.

Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44:223–270.

Kirch, P.V. 1986. Rethinking Polynesian prehistory. J. Polynesian Soc. 95:9–40.

Link, W., C. Diskens, M. Singh, M. Schwall, and A.E. Melchinger. 1995. Genetic diversity in European and Mediterranean faba bean germplasm revealed by RAPD markers. Theor. Appl. Genet. 90: 27–32.

Lynch, M., and B.G. Milligan. 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3:91–99.

Nei, M., T. Maryuma, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1–10.

Orozco-Castillo, C., K.J. Chalmers, R. Waugh, and W. Powell. 1994. Detection of genetic diversity and selective gene introgression in coffee using RAPD markers. Theor. Appl. Genet. 87:934–940.

Parham, R.W. 1966. Coconut and breadfruit surveys of the south Pacific region. Technical Information Bull. 1, South Pacific Commission, Noumea, New Caledonia.

Rogers, S.O., and A.J. Bendich 1985. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissue. Plant Mol. Biol. 5:69–76.

Russell, J.R., F. Hosein, E. Johnson, R. Waugh, and W. Powell. 1993. Genetic differentiation of cocoa (*Theobroma cacao* L.) populations revealed by RAPD analysis. Mol. Ecol. 2:89–97.

Shah, F.H., O. Rashid, A.J. Simons, and A. Dunsden. 1994. The utility of RAPD markers for the determination of genetic variation in oil palm (*Elaeis guineensis*). Theor. Appl. Genet. 89:713–718.

Skroch, P., and J. Nienhuis. 1995. Impact of scoring error and reproducibility of RAPD data on RAPD based estimates of genetic distance. Theor. Appl. Genet. 91:1086–1091.

Skroch, P., J. Tivany, and J. Nienhuis. 1992. Analysis of genetic relationships using RAPD marker data. p. 26–30. *In* Application of RAPD technology to plant breeding. Joint Plant Breeding Symposia Series, November 1992. CSSA, Madison, WI.

Sokal, R.R., G.M. Jacquez, and M.C. Wooten. 1989. Spatial autocorrelation analysis of migration and selection. Genetics 121:845–855.

Turner, M.E., C. Stephens, and W.W. Anderson. 1982. Homozygosity and patch structure in plant populations as a result of nearest-neighbour pollination. Proc. Natl. Acad. Sci. (USA) 79:203–207.

Whitehead, R.A. 1966. Sample survey and collection of coconut germplasm in the Pacific islands (30 May–5 September 1964). Her Majesty's Stationary Office, London.

Williams, J.G.K., M.K. Hanafey, J.A. Rafalski, and S.V. Tingey. 1993. Genetic analysis using random amplified polymorphic DNA markers. Meth. Enzymol. 218:704–740.