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Geographic Survey of Genetic Variation in Kava (*Piper methysticum* Forst. f. and *P. wichmannii* C. DC.)[1]

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ABSTRACT: A survey of the genetic resources of kava (*Piper methysticum* Forst. f. and *P. wichmannii* C. DC.) was conducted throughout the Pacific. Leaf tissues of more than 300 accessions, collected on 35 islands, were analyzed for isozyme variation in eight enzyme systems including ACO, ALD, DIA, IDH, MDH, ME, PGI, and PGM. Isozymes in *P. methysticum* cultivars from Polynesia and Micronesia were monomorphic for all enzyme systems examined; however, cultivars from Melanesia were polymorphic for ACO, DIA, MDH, and PGM. The genetic base of this crop is much narrower than previous morphological and biochemical studies suggest. Most of the morphotypes and chemotypes apparently originated through human selection and preservation of somatic mutations in a small number of original clones. Isozymes of *P. wichmannii* confirmed its status as the wild progenitor of kava. *Piper methysticum* cultivars and *P. wichmannii* and *P. gibbilimbum* C. DC. wild forms were all found to be decaploids with $2n = 10x = 130$ chromosomes, but there was no firm evidence that interspecific hybridization has played a role in the origin of *P. methysticum*.

KAVA, *Piper methysticum* Forst. f., is the only cultivated plant of economic importance with a geographic range restricted entirely to the Pacific Islands. Kava is used in Oceania to make a psychoactive drink that is prepared by grinding the roots of the perennial shrub. Recent work (Lebot and Lévesque 1989) has shown that two botanical species names have been applied to kava; *P. methysticum* refers to reproductively sterile domesticated forms, while *P. wichmannii* C. DC. identifies the seed-producing wild progenitor. Experimental studies have shown that the roots of these species contain up to 20% of active ingredients, called kavalactones, with physiological properties (Hänsel 1968, Lebot and Cabalion 1986, Lebot and Lévesque 1989). These are the only two species in the genus *Piper* from which these flavones and chalcones have been isolated. Kavalactones are presently used by the European pharmaceutical industry, but there is potential for greater exploitation.

The wild form, *Piper wichmannii*, is geographically limited to Melanesia, including New Guinea, the Solomon Islands, and northern Vanuatu. Its closest relative, *P. gibbilimbum* C. DC., has been collected only in Papua New Guinea. Domesticated *P. methysticum* has been cultivated since prehistoric times throughout Polynesia and on the Micronesian islands of Pohnpei and Kosrae. Unlike other Pacific species of *Piper* (e.g., *P. minatum*, *P. abbreviatum*, *P. wichmannii*, and *P. gibbilimbum*), *P. methysticum* has a discontinuous distribution in Melanesia. In eastern Melanesia, it is an important and ancient component of agriculture in Fiji and Vanuatu, and in western Melanesia, it is found in scattered locations in Papua New Guinea, including the Fly River area in the south (Serpenti 1962), Madang on the north coast, and in the Admiralty islands of Lou and Baluan. On the north shore of Papua New Guinea, at least, it was established before European contact, since

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Macklay (1874) observed the preparation of kava in Astrolabe Bay in 1872. In the vast intervening region including New Caledonia, the Solomon Islands, New Britain, and New Ireland, there is no evidence that kava was ever cultivated (Figure 1).

Little was known about the genetic diversity existing within kava and between *P. methysticum* and *P. wichmannii* until a recent survey of genetic resources of kava in the Pacific (Lebot and Lévesque 1989) (Table 1). Substantial variability was found among cultivars, both in morphology and kavalactone composition. Remarkable morphological variability in growth habit, internode color, and lamina shape and pigmentation permitted 118 different kava clones to be distinguished. This diversity was attributed to the effect of natural selection operating under many different climatic and ecological conditions in tropical oceanic archipelagos. However, no clear relationship was observed between phenotypic variation and geographic distribution, a situation that was partly explained as a result of human dispersal of the crop. A High Performance Liquid Chromotography (HPLC) analysis of kavalactone composition in more than 300 root samples revealed nine chemotypes, each with different pharmacological effects and cultural uses. Field trials indicated that chemotypes were genetically controlled and were not affected by environmental factors or plant age. No correlation was observed between morphotypes and chemotypes.

Piper species generally have chromosome numbers that are multiples of a basic genome of 13 chromosomes (Jose and Sharma 1985, Okada 1986, Samuel 1986). Among cultivated members of the genus, including *P. betle* and *P. nigrum*, the existence of genetic races with different ploidy levels has been demonstrated (Jose and Sharma 1985, Samuel 1986). The origin of genetic races in these asexually propagated crops is attributed to endomitosis and subsequent selection of vegetative sports with doubled ploidy level. Chromosome numbers have not been reported previously for *P. methysticum*, *P. wichmannii*, or closely related species, and the extent of ploidy variation in kava is unknown.

The numerous *P. methysticum* cultivars identified by the chemical and morphological survey (Lebot and Lévesque 1989) might have arisen by independent domestications of different genotypes selected from the range of genetic variation in the *P. wichmannii* progenitor, or alternatively, by accumulation of somatic mutations within a narrow genetic base or even a single domesticated clone. One of our objectives was to address the question of the breadth of the genetic base of kava by using isozyme analysis. This technique is often reported in the literature as suitable for assessing how much genetic diversity is present in a crop and has the potential to provide a unique "fingerprint" for each genetically distinct clone, a useful means of identifying different cultivars.

Another objective was to determine the origin of sterility in kava cultivars. Sterility could result from genetic changes occurring in a vegetatively propagated clone, such as somatic mutations or autopolyploidy, or it might result from interspecific hybridization. These alternatives were investigated through isozyme analyses and chromosome counts of kava and related species, including *P. wichmannii* and *P. gibbilimbum*.

MATERIALS AND METHODS

Stem cuttings collected throughout Oceania were planted in the greenhouse of the University of Hawaii at Manoa, Honolulu. Plants were grown, at ambient temperatures in about 80% shade, in pots filled with a mixture of vermiculite and peat moss (2 : 1). Kava accessions originating from Vanuatu were analyzed for isozyme variation using leaves collected in the field and preserved in liquid nitrogen. Entire young leaves were sealed in disposable microcentrifuge tubes, immersed in liquid nitrogen in a shipping container in the field, and transported to the University of Hawaii for analysis.

Cytological examination of *P. methysticum* cultivars originating from southern and northern Papua New Guinea (6 accessions), Vanuatu (1 acc.), Fiji (8 acc.), Samoa (7 acc.), Hawaii (12 acc.), and Pohnpei (2 acc.) was

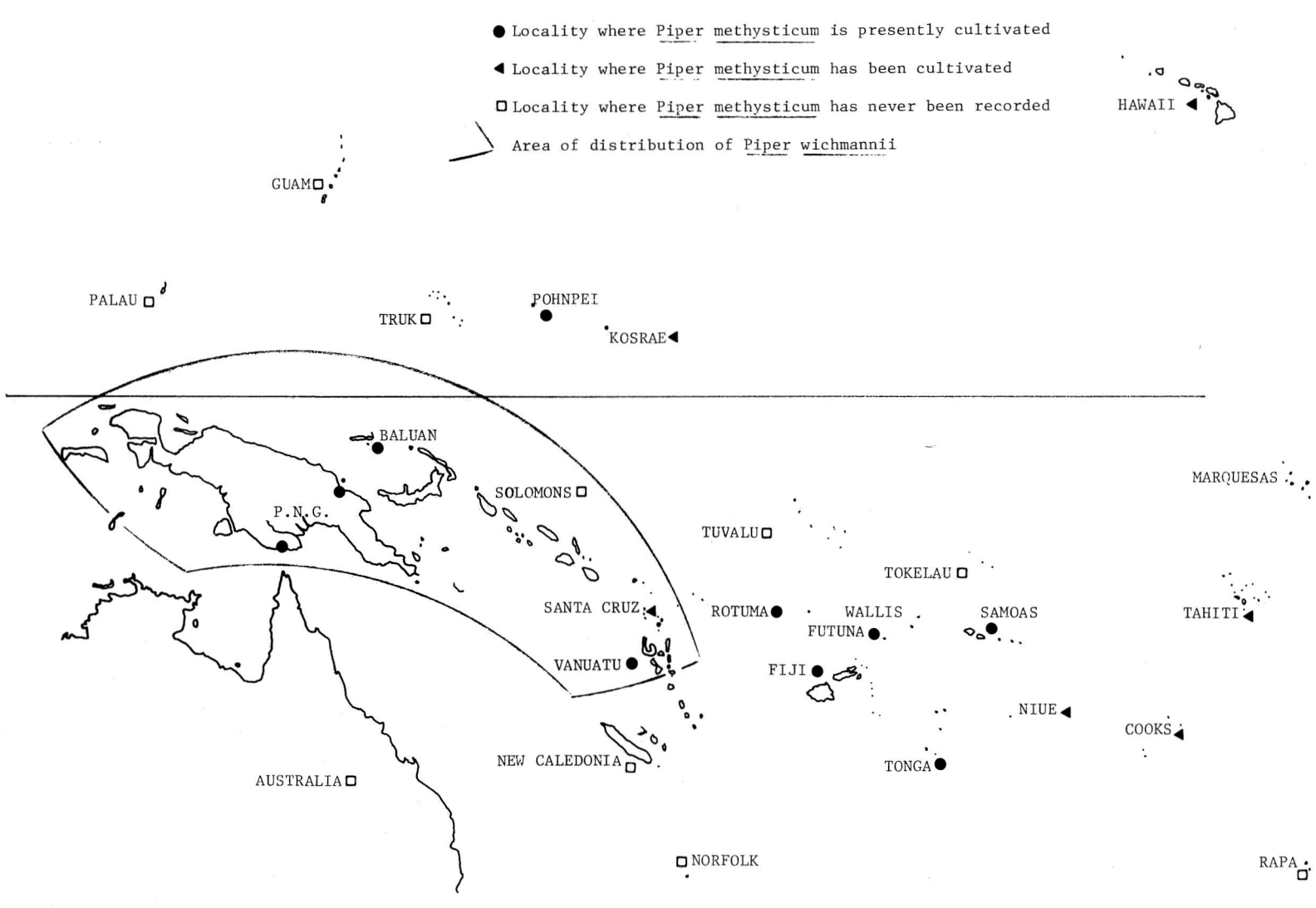


FIGURE 1. Geographic distribution of *Piper methysticum* Forst. f. and *Piper wichmannii* C. DC.

TABLE 1

P. wichmannii AND *P. methysticum* GERMPLASM COLLECTED

COUNTRY	ISLANDS SURVEYED	*P. wichmannii* (WILD FORMS)	*P. methysticum* (CULTIVARS)	CHEMOTYPES*
Papua New Guinea	5	23	4	B, C, D, F
Solomons	3	18	0	B
Vanuatu	23	2	80	A, E, F, G, H
Fiji	3		12	I
Tonga	2		7	E, G
Western Samoa	2		6	G, H, I
American Samoa	1		5	G, H
Wallis and Futuna	2		3	E
Cooks	1		1	I
Tahiti	1		3	I
Marquesas	5		1	E
Hawaii	4		11	E, I
Pohnpei	1		2	E, I
Kosrae	1		1	E
Totals	50	43	118**	

* Kavalactone chemotypes identified through HPLC analysis of root samples (cf. Lebot and Lévesque 1989).
** Some cultivars were collected several times in different island groups.

conducted to study possible variation. Chromosome counts were difficult and time consuming because of the large number and extremely small size of the chromosomes and the very viscous cytoplasm. It was important to induce as much contraction of chromosomes as possible, and a cold treatment of 6 hr was found helpful for this purpose. Chromosome counts were conducted using the following procedure:

Mitosis (on root tips): (1) pretreatment in PDB (para-dichlorobenzene) for 6 hr at 4°C, (2) fixation in acetic acid–ethanol (1 : 3) overnight, (3) hydrolysis for 90 min in HCL 1N, (4) treatment with pectinase (2%) for 30 min, (5) staining for 90 min with Feulgen, (6) preservation in 70% ethanol, (7) squashing in aceto carmine.

Meiosis (on anthers): (1) immature inflorescences were fixed in Carnoy's fluid for 7 days at room temperature, (2) preserved with 70% ethanol at 4°C, and (3) immature anthers were squashed in aceto carmine.

Isozyme electrophoresis: Twenty-five enzyme systems were assayed (Table 2) using a variety of buffer systems, but only histidine citrate, pH 6.5, was found to be useful. The tray buffer consisted of 0.065 M histidine (free base) and 0.007 M citric acid (anhydrous); the gel buffer was 0.016 M histidine and 0.002 M citric acid.

Leaf extracts were obtained using modified Bousquet's buffer (Bousquet et al. 1987) and loaded onto starch gels (12.5%). The extraction buffer composition was as follows: Tris(hydroxymethyl)aminomethane (Tris), 0.1 M; sucrose, 0.2 M; ethylenediamine tetraacetic acid (EDTA disodium), 0.5 mM; dithiothreitol (DTT), 5 mM; cysteine-HCL, 12 mM; ascorbic acid, 25 mM; sodium metabisulfite, 0.02 M; diethyldithiocarbamic acid (DIECA sodium salt), 0.005 M; bovine serum albumine (BSA), 0.1%; polyethylene glycol, MW 20,000 (PEG), 1%; polyoxyethylene sorbitan monooleate (Tween 80), 2%; dimethyl sulfoxide (DMSO), 10%; β-mercaptoethanol, 1%; polyvinylpolypyrrolidone (PVPP), 8 g/100 ml of buffer. The buffer pH was adjusted to 7.5.

Samples were electrophoresed at 4°C. Running conditions were 15V/cm and 40–50 mA for 6 hr. After electrophoresis, the gels were sliced horizontally and stained for aconitase (ACO), aldolase (ALD), diaphorase (DIA), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucose isomerase (PGI), and phosphoglucomutase (PGM), after Soltis et al. (1983). The stained gels were scored for the

TABLE 2

ENZYME SYSTEMS AND BUFFERS INVESTIGATED IN ELECTROPHORETIC SURVEY OF KAVA GERMPLASM

ENZYME SYSTEM	ABBREVIATION	BUFFER SYSTEM*
Acid phosphatase	ACP	HC, TME, SOLTIS #11
Aconitase**	ACO	HC, TC
Alcohol dehydrogenase	ADH	HC, TC
Aldolase**	ALD	HC
Alkaline phosphatase	APH	MC, TEB
Diaphorase**	DIA	HC, TME, SOLTIS #11
Esterase	EST	HC
Endopeptidase	ENDO	HC
Fumerase	FUM	HC
Glutamic oxaloacetic transaminase	GOT	HC
Glutamate pyruvate transaminase	GPT	HC, TME, SOLTIS #11
Glutamate dehydrogenase	GDH	HC, MC, TME, SOLTIS #11, TEB
Glucose-6-phosphate dehydrogenase	G6PD	HC
Isocitrate dehydrogenase**	IDH	HC, TC
Leucine amino peptidase	LAP	HC, MC
Malate dehydrogenase	MDH	HC, TC, TEB
Malic enzyme**	ME	HC, TC, MC, TEB
Menadione reductase	MDR	HC, MC, TME, SOLTIS #11, TEB
Peroxidase	PER	HC, MC
Phosphoglucomutase**	PGM	HC
Phosphoglucose isomerase**	PGI	HC, TC, MC, TME, SOLTIS #11
Shikimic dehydrogenase	SKDH	HC, MC
Uridine diphosphoglucose pyrophosphorylase	UGPP	HC
6-Phosphogluconate dehydrogenase	6-PGD	HC, TC

* Buffer systems: histidine citrate (HC), pH 6.5; Tris citrate (TC), pH 6.1; morpholine citrate (MC), pH 8.1; Tris-maleate (TME), pH 7.4; Na citrate/histidine HCl (SOLTIS #11), pH 7.0; and Tris-EDTA-borate (TEB), pH 8.6.

** Well resolved (on HC buffer) polymorphic enzyme systems yielding data for cluster and principal components analyses.

presence or absence of 53 different electromorphs, including 5 for ACO, 2 for ALD, 6 for DIA, 3 for IDH, 16 for MDH, 5 for ME, 5 for PGI, and 11 for PGM. No interpretation of the genetic significance of the banding patterns was attempted.

Cluster analysis of the binary isozyme data was performed with the assistance of NTSYS-pc software, version 1.21 (Applied Biostatistics Inc., Setauket, NY). Similarity matrix was generated with Jaccard's coefficient. Principal components analysis was also performed on the correlation matrix to compare with the results obtained through the cluster analysis.

RESULTS

Counts of about 130 mitotic chromosomes were obtained for *P. methysticum* (Figure 2*a*), *P. wichmannii* (Figure 2*c*), and *P. gibbilimbum* (Figure 2*d*). In *P. methysticum*, 12 chromosomes were four to five times the average size of the others (Figure 2*a*), while chromosomes in the other taxa were more uniform. No obvious variation in chromosome numbers was apparent between *P. methysticum* clones representing different morphotypes and chemotypes or between monoecious and dioecious plants. Chromosome counts obtained from pollen mother cells of *P. methysticum* showed about 65 bivalents (Figure 2*b*). Although tetrad formation appeared normal, cotton blue staining revealed poorly formed pollen grains. Meiotic counts were not conducted for *P. wichmannii* or *P. gibbilimbum* because of lack of material.

The zymotypes of wild and cultivated kava forms are given for eight enzymes in Table 3 and illustrated in Figure 3. Resolution and banding intensity were constant regardless of

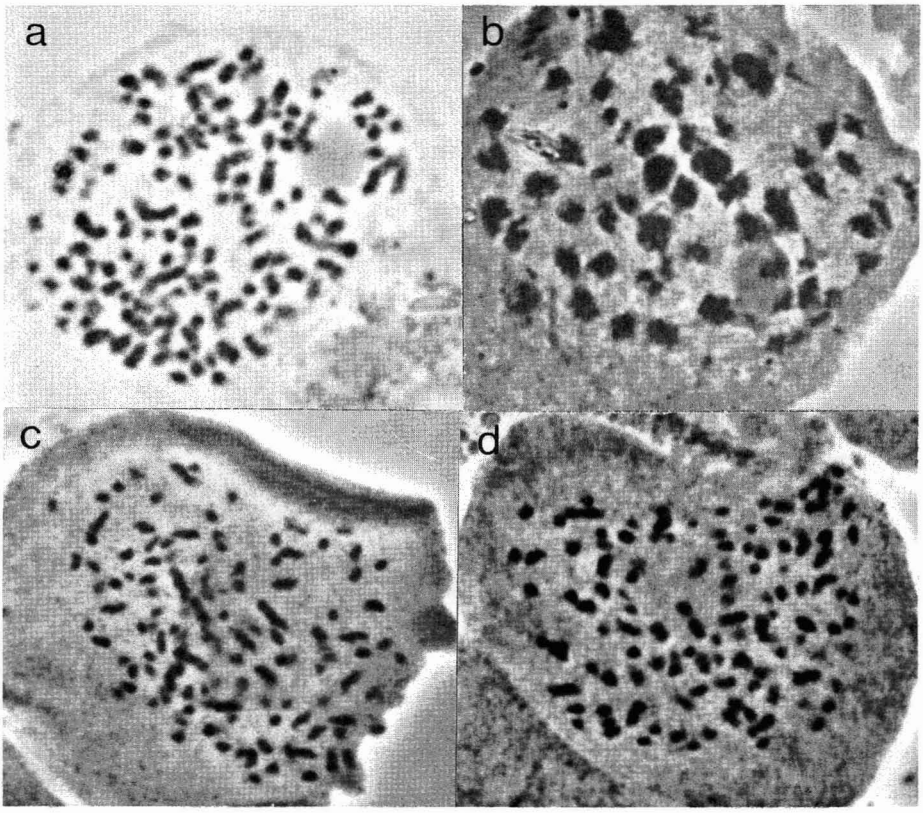


FIGURE 2. *a*, Mitotic chromosomes at metaphase or prophase of *Piper methysticum*, $2n \cong 130$; *b*, *P. methysticum*, meiosis showing about 65 bivalents; *c*, mitotic chromosomes of *Piper wichmannii* C. DC., $2n \cong 130$; *d*, mitotic chromosomes of *Piper gibbilimbum* C. DC., $2n \cong 130$.

TABLE 3

ISOZYME PHENOTYPES (ZYMOTYPES) OF KAVA ACCESSIONS

ACCESSIONS	ENZYME SYSTEMS								ZYMOTYPE
	MDH	ACO	PGM	PGI	IDH	DIA	ME	ALD	
P. wichmannii (193)	A	A	A	A	A	A	A	A	1
P. wichmannii (seedlings)	B	A	B	A	A	A	A	A	2
P. wichmannii (191 to 192)	C	B	C	B	B	B	A	B	3
P. wichmannii (188 to 190)	D	B	D	C	A	C	A	B	4
P. wichmannii (187)	E	C	E	D	C	C	A	B	5
P. wichmannii (174 to 186)	F	D	F	E	B	B	A	B	6
P. wichmannii (171 to 172)	E	D	G	D	D	C	A	B	7
P. methysticum (163 to 170)	E	D	H	D	C	C	B	B	8
*P. methysticum** and *P. wichmannii* (173)	F	D	H	D	C	B	B	B	9
*P. methysticum**	G	E	I	D	C	D	B	B	10

NOTE: Letters represent different isozyme banding patterns within each enzyme system, as shown in Fig. 3.
* Refer to Table 4 for identification and origin of *P. methysticum* accessions.

TABLE 4

ORIGIN OF KAVA ACCESSIONS SUBJECTED TO GEL ELECTROPHORESIS.

NO.	ORIGIN AND IDENTIFICATION	LATITUDE	LONGITUDE	ELEVATION (m)	M*	C**	Z***
Hawaii:							
001	Oahu, Harold Lyon Arboretum	157°48′ W	21°20′ N	280	101	E	10
002	Oahu, H. L. A.	157°48′ W	21°20′ N	280	103	I	10
003	Oahu, H. L. A.	157°48′ W	21°20′ N	280	7	E	10
004	Oahu, H. L. A.	157°48′ W	21°20′ N	280	95	E	10
005	Oahu, H. L. A.	157°48′ W	21°20′ N	280	104	E	10
006	Oahu, H. L. A.	157°48′ W	21°20′ N	280	79	E	10
007	Oahu, H. L. A.	157°48′ W	21°20′ N	280	108	E	10
008	Oahu, H. L. A.	157°48′ W	21°20′ N	280	105		10
009	Oahu, H. L. A.	157°48′ W	21°20′ N	280	66		10
010	Oahu, H. L. A.	157°48′ W	21°20′ N	280	106		10
011	Oahu, H. L. A.	157°48′ W	21°20′ N	280			10
012	Oahu, H. L. A.	157°48′ W	21°20′ N	280			10
013	Oahu, H. L. A.	157°48′ W	21°20′ N	280			10
014	Oahu, H. L. A.	157°48′ W	21°20′ N	280			10
015	Oahu, H. L. A.	157°48′ W	21°20′ N	280			10
016	Oahu, H. L. A.	157°48′ W	21°20′ N	280			10
017	Oahu, H. L. A.	157°48′ W	21°20′ N	280			10
018	Oahu (University of Hawaii, Agron. Dept.)	157°50′ W	21°18′ N	80			10
019	Oahu (UH, Agron. Dept.)	157°50′ W	21°18′ N	80			10
020	Oahu (UH, Agron. Dept.)	157°50′ W	21°18′ N	80			10
023	Oahu (UH, Ethnobotanical garden)	157°50′ W	21°18′ N	60			10
024	Oahu (UH, Ethnobot. g.)	157°50′ W	21°18′ N	60			10
025	Oahu (UH, Ethnobot. g.)	157°50′ W	21°18′ N	60			10
026	Oahu (UH, Ethnobot. g.)	157°50′ W	21°18′ N	60			10
027	Oahu (Waiahole Valley)	157°45′ W	21°25′ N	200			10
028	Oahu (Waiahole Valley)	157°45′ W	21°25′ N	200			10
029	Oahu (Waiahole Valley)	157°45′ W	21°25′ N	200			10
030	Oahu (Waiahole Valley)	157°45′ W	21°25′ N	200			10
031	Kauai (UH Research station)	159°25′ W	22°20′ N	250			10
032	Kauai (UH Research station)	159°25′ W	22°20′ N	250			10
033	Kauai (UH Research station)	159°25′ W	22°20′ N	250			10
034	Kauai (UH Research station)	159°25′ W	22°20′ N	250			10
035	Kauai (UH Research station)	159°25′ W	22°20′ N	250			10
036	Kauai (UH Research station)	159°25′ W	22°20′ N	250			10
037	Kauai (UH Research station)	159°25′ W	22°20′ N	250			10
038	Kauai (Nat. Trop. Bot. Gard.)	159°30′ W	22°18′ N	50			10
039	Kauai (NTBG)	159°30′ W	22°18′ N	50			10
040	Hawaii (Waipio Valley)	155°42′ W	20°10′ N	80			10
041	Hawaii (Waipio V.)	155°42′ W	20°10′ N	80			10
042	Hawaii (Waipio V.)	155°42′ W	20°10′ N	80			10
043	Hawaii (Waipio V.)	155°42′ W	20°10′ N	80			10
044	Hawaii (Waipio V.)	155°42′ W	20°10′ N	80			10
Carolines:							
045	Pohnpei, 'Rahmdel'	158°15′ E	6°50′ N	150	110	I	10
046	Pohnpei, 'Rahmweneger'	158°15′ E	6°50′ N	150	111	E	10
047 to 060	Kosrae	163°00′ E	5°20′ N	200			10
Fiji:							
061	Taveuni, 'Kabra'	180°00′ E	17°30′ S	40	52	I	10
062	Taveuni, 'Qila leka'	180°00′ E	17°30′ S	40	89	I	10
063	Taveuni, 'Qila balavu'	180°00′ E	17°30′ S	40	90	I	10
064	Taveuni, 'Damu'	180°00′ E	17°30′ S	40	92	I	10
065	Vanua Levu, 'Loa kasa leka'	179°20′ E	17°40′ S	40	83	I	10
066	Vanua Levu, 'Loa kasa balavu'	179°20′ E	17°40′ S	40	84	I	10

TABLE 4 (continued)

NO.	ORIGIN AND IDENTIFICATION	LATITUDE	LONGITUDE	ELEVATION (m)	M*	C**	Z***
067	Vanua Levu, 'Vula kasa leka'	179°20′ E	17°40′ S	40	85	I	10
068	Vanua Levu, 'Vula kasa balavu'	179°20′ E	17°40′ S	40	85	I	10
069	Vanua Levu, 'Dokobana vula'	179°20′ E	17°40′ S	40	87	I	10
070	Vanua Levu, 'Dokobana loa'	179°20′ E	17°40′ S	40	88	I	10
071	Viti Levu, 'Matakaro'	178°35′ E	18°18′ S	40	91	I	10
072	Viti Levu, 'Matakaro leka'	178°35′ E	18°18′ S	40	93	I	10
Samoas:							
073	Upolu, 'Ava lea'	172°50′ W	13°55′ S	40	85	H	10
074	Upolu, 'Ava la'au'	172°50′ W	13°55′ S	40	86	I	10
075	Savai'i, 'Ava mumu'	172°40′ W	13°35′ S	40	84	I	10
076	Savai'i, 'Ava talo'	172°40′ W	13°35′ S	40	95	G	10
077	Savai'i, 'Ava sa'	172°40′ W	13°35′ S	40	96	I	10
078	Tutuila, 'Ava samoa'	170°48′ W	14°20′ S	40	94	H	10
079	Tutuila, 'Ava ulu'	170°48′ W	14°20′ S	40	95	G	10
Tonga:							
080	Vava'u, 'Leka hina'	174°00′ W	18°38′ S	30	85	G	10
081	Vava'u, 'Akau'	174°00′ W	18°38′ S	30	86	G	10
082	Tongatapu, 'Leka huli'	175°10′ W	21°15′ S	30	83	I	10
083	Tongatapu, 'Akau huli'	175°10′ W	21°15′ S	30	84	E	10
084	Tongatapu, 'Valu'	175°10′ W	21°15′ S	30	97	G	10
085	Tongatapu, 'Fulufulu'	175°10′ W	21°15′ S	30	98	G	10
Cooks:							
086 to 093	Mangaia, 'Vaine rea'	157°55′ W	21°12′ S	20		I	10
Vanuatu:							
114	Vanua Lava, 'Giemonlagakris'	167°30′ E	13°59′ S	40	13		9
115	Vanua Lava, 'Tarivar'	167°30′ E	13°59′ S	40	8	E	9
116	Vanua Lava, 'Ranranre'	167°30′ E	13°59′ S	40	11		9
117	Vanua Lava, 'Gelava'	167°30′ E	13°59′ S	40	12		9
118	Vanua Lava, 'Visabana'	167°30′ E	13°59′ S	40	1	E	9
119	Vanua Lava, 'Gemime'	167°30′ E	13°59′ S	40	7	H	9
173†	Vanua Lava, 'Vambu'	167°30′ E	13°59′ S	40	14	A	9
094	Santo, 'Kar'	167°10′ E	15°20′ S	340	7	H	10
095	Santo, 'Palavoke'	167°10′ E	15°20′ S	340	32	E	10
096	Santo, 'Malogro'	167°10′ E	15°20′ S	340	44	F	10
120	Santo, 'Fock'	167°10′ E	15°20′ S	340	18	E	9
121	Santo, 'Marino'	167°10′ E	15°20′ S	340	42	E	9
122	Santo, Thyei'	167°10′ E	15°20′ S	340	43	F	9
123	Santo, 'Yevoet'	167°10′ E	15°20′ S	340	41	F	9
124	Santo, 'Tudei'	167°10′ E	15°20′ S	340	45	E	9
125	Santo, 'Visul'	167°10′ E	15°20′ S	340	40	H	9
126	Santo, 'Parisi'	167°10′ E	15°20′ S	340	26	E	9
127	Santo, 'Merei'	167°10′ E	15°20′ S	340	10	F	9
130	Malo, 'Malo'	167°10′ E	15°40′ S	40	9	E	9
135	Ambae, 'Melomelo'	167°50′ E	15°20′ S	80	15	G	9
129	Maewo, 'Tarivarus'	168°10′ E	15°10′ S	90	8	E	9
097	Pentecost, 'Melmel'	168°10′ E	15°40′ S	220	18	E	10
098	Pentecost, 'Borogu'	168°10′ E	15°40′ S	220	15	G	10
099	Pentecost, 'Memea'	168°10′ E	15°40′ S	220	7	H	10
101	Pentecost, 'Maita'	168°10′ E	15°40′ S	220	24	G	10
131	Pentecost, 'Ronrongwul'	168°10′ E	15°40′ S	220	22	H	9
132	Pentecost, 'Abogae'	168°10′ E	15°40′ S	220	8	E	9
133	Pentecost, 'Laklak'	168°10′ E	15°40′ S	220	25	E	9
134	Pentecost, 'Tamaevo'	168°10′ E	15°40′ S	220	34	E	9
213	Pentecost, 'Tabal'	168°10′ E	15°40′ S	220	47	E	10
214	Pentecost, 'Gorogoro'	168°10′ E	15°40′ S	220	24		10

TABLE 4 (continued)

NO.	ORIGIN AND IDENTIFICATION	LATITUDE	LONGITUDE	ELEVATION (m)	M*	C**	Z***
215	Pentecost, 'Borogoru tememe'	168°10′ E	15°40′ S	220	7		10
172†	Pentecost, 'Sini Bo'	168°10′ E	15°40′ S	220	14	A	7
225	Pentecost, 'Rara'	168°10′ E	15°40′ S	220	23		10
226	Pentecost, 'Sese jarakara'	168°10′ E	15°40′ S	220	11		9
227	Pentecost, 'Rong rong vula'	168°10′ E	15°40′ S	220	22		9
229	Pentecost, 'Bogongo'	168°10′ E	15°40′ S	220	27		10
205	Pentecost, 'Bukelita'	168°10′ E	15°40′ S	220	26		10
220	Malekula, 'Pade'	167°15′ E	16°05′ S	360	49		10
221	Malekula, 'Tafandai'	167°15′ E	16°05′ S	360	51		10
222	Malekula, 'Daou'	167°15′ E	16°05′ S	360	48		10
217	Paama, 'Toh'	168°15′ E	16°30′ S	40	52		10
100	Epi, 'Lo'	168°10′ E	16°40′ S	20	55	G	10
102	Epi, 'Kelai'	168°10′ E	16°40′ S	20	17	H	10
136	Epi, 'Bagavia'	168°10′ E	16°40′ S	20	36	E	9
137	Epi, 'Pakai'	168°10′ E	16°40′ S	20	56	E	9
138	Epi, 'Purumbue'	168°10′ E	16°40′ S	20	15		9
216	Epi, 'Meawmeia'	168°10′ E	16°40′ S	20	54		10
209	Emae, 'Miela'	168°20′ E	17°05′ S	40	63	H	10
103	Emae, 'Miae'	168°20′ E	17°05′ S	40	63	H	10
104	Nguna, 'Malakesa'	168°20′ E	17°25′ S	120	54		10
105	Nguna, 'Milake'	168°20′ E	17°25′ S	120	64		10
139	Tongoa, 'Piri'	168°30′ E	16°50′ S	80	62		9
140	Tongoa, 'Puariki'	168°30′ E	16°50′ S	80	37	G	9
208	Tongoa, 'Ewo'	168°30′ E	16°50′ S	80	61	F	10
171†	Tongoa, 'Kau'	168°30′ E	16°50′ S	80	14	A	7
228	Tongoa, 'Pualiu'	168°30′ E	16°50′ S	80	67	G	10
207	Erromanga, 'Pore'	169°00′ E	18°45′ S	160	65		9
106	Tanna, 'Alakar'	169°20′ E	19°30′ S	400	65		10
107	Tanna, 'Lulu'	169°20′ E	19°30′ S	400	66		10
108	Tanna, 'Loa'	169°20′ E	19°30′ S	400	67		10
109	Tanna, 'Aigen'	169°20′ E	19°30′ S	400	68	G	10
110	Tanna, 'Paama'	169°20′ E	19°30′ S	400	15		10
141	Tanna, 'Apin'	169°20′ E	19°30′ S	400	69	E	9
197	Tanna, 'Leay'	169°20′ E	19°30′ S	400	71	H	10
198	Tanna, 'Ahouia'	169°20′ E	19°30′ S	400	67	G	10
199	Tanna, 'Tikiskis'	169°20′ E	19°30′ S	400	74	H	10
200	Tanna, 'Fare'	169°20′ E	19°30′ S	400	70		10
201	Tanna, 'Wapil'	169°20′ E	19°30′ S	400	75		10
202	Tanna, 'Tudey'	169°20′ E	19°30′ S	400	45		9
203	Tanna, 'Malamala'	169°20′ E	19°30′ S	400	73	H	10
204	Tanna, 'Ring'	169°20′ E	19°30′ S	400	35		10
206	Tanna, 'Pentecost'	169°20′ E	19°30′ S	400	26		10
210	Tanna, 'Kowarwar'	169°20′ E	19°30′ S	400	79		10
218	Tanna, 'Awke'	169°20′ E	19°30′ S	400	78		10
219	Tanna, 'Awor'	169°20′ E	19°30′ S	400	37		10
223	Tanna, 'Kowariki'	169°20′ E	19°30′ S	400	37		10
224	Tanna, 'Gnare'	169°20′ E	19°30′ S	400	76		10
211	Anatom, 'Ketche'	169°50′ E	20°10′ S	60	80		10
212	Anatom, 'Yag'	169°50′ E	20°10′ S	60	81	H	10
Solomons:							
174†	Malaita	161°30′ E	9°20′ S	400			6
175†	Malaita	161°30′ E	9°20′ S	400			6
176†	Malaita	161°30′ E	9°20′ S	400			6
177†	Guadalcanal	160°10′ E	9°40′ S	550	112		6
178†	Guadalcanal	160°10′ E	9°40′ S	550	112		6
179†	Guadalcanal	160°10′ E	9°40′ S	400	112		6
180†	Guadalcanal	160°10′ E	9°40′ S	400	112		6

TABLE 4 (continued)

NO.	ORIGIN AND IDENTIFICATION	LATITUDE	LONGITUDE	ELEVATION (m)	M*	C**	Z***
181†	Guadalcanal	160°10′ E	9°40′ S	350	112		6
182†	Guadalcanal	160°10′ E	9°40′ S	250	112		6
183†	Guadalcanal	160°10′ E	9°40′ S	200	112		6
184†	Santa Cruz, Ndende	166°25′ E	10°15′ S	110	112		6
185†	Santa Cruz, Ndende	166°25′ E	10°15′ S	110	112		6
186†	Santa Cruz, Ndende	166°25′ E	10°15′ S	110	112		6
Papua New Guinea:							
142	Western Province, Nomad, 'Gowi'	142°10′ E	6°20′ S	120	111		9
143	West. Pr., Nomad, 'Gowi'	142°10′ E	6°20′ S	120	111		9
144	West. Pr., Nomad, 'Gowi'	142°10′ E	6°20′ S	120	111		9
145	West. Pr., Nomad, 'Gowi'	142°10′ E	6°20′ S	120	111		9
146	West. Pr., Dadalibi, 'Gowi'	142°15′ E	6°20′ S	160	111		9
147	West. Pr., Dadalibi, 'Gowi'	142°15′ E	6°20′ S	160	111		9
148	West. Pr., Dadalibi, 'Gowi'	142°15′ E	6°20′ S	160	111		9
149	West. Pr., Dadalibi, 'Gowi'	142°15′ E	6°20′ S	160	111		9
150	West. Pr., Ptifi	142°20′ E	6°20′ S	240	111		9
151	West. Pr., Ptifi	142°20′ E	6°20′ S	240	111		9
152	West. Pr., Ptifi	142°20′ E	6°20′ S	240	111		9
153	West. Pr., Ptifi	142°20′ E	6°20′ S	240	111		9
154	West. Pr., Isago, 'Sika'	142°50′ E	8°05′ S	60	111	F	9
155	West. Pr., Isago, 'Sika'	142°50′ E	8°05′ S	60	111	F	9
156	West. Pr., Isago, 'Sika'	142°50′ E	8°05′ S	60	111	F	9
157	West. Pr., Ume, 'Gamata'	143°05′ E	9°20′ S	20	111	F	9
158	West. Pr., Ume, 'Gamata'	143°05′ E	9°20′ S	20	111	F	9
159	West. Pr., Ume, 'Gamata'	143°05′ E	9°20′ S	20	111	F	9
160	West. Pr., Wando	141°10′ E	9°10′ S	40	111	F	9
161	West. Pr., Wando	141°10′ E	9°10′ S	40	111	F	9
162	West. Pr., Wando	141°10′ E	9°10′ S	40	111	F	9
163	Madang Province, Riwo, 'Koniak'	145°50′ E	4°50′ S	120	117	F	8
164	Madang Pr., Riwo, 'Koniak'	145°50′ E	4°50′ S	140	117	F	8
165	Madang Pr., Karkar, 'Ayou'	146°00′ E	4°35′ S	220	117	F	8
166	Madang Pr., Karkar, 'Ayou'	146°00′ E	4°35′ S	200	117	F	8
167	Madang Pr., Usino, 'Isa'	145°40′ E	5°20′ S	200	117	F	8
168	Madang Pr., Usino, 'Isa'	145°40′ E	5°20′ S	200	117	F	8
169	Madang Pr., Macklay Coast, 'Kial'	145°50′ E	5°20′ S	10	117	F	8
170	Madang Pr., Macklay Coast, 'Kial'	145°50′ E	5°20′ S	10	117	F	8
111	Manus Province, Baluan, 'Kau pel'	147°20′ E	2°40′ S	20	114	F	10
112	Manus Pr., Baluan, 'Kau pwusi'	147°20′ E	2°40′ S	20	115	F	10
113	Manus Pr., Baluan, 'Kau pel'	147°20′ E	2°40′ S	20	115	F	10
187†	Morobe Province, Bundun	146°40′ E	6°50′ S	800	112	B	5
188†	Morobe Pr., Bulolo	146°50′ E	6°50′ S	1600	112		4
189†	Morobe Pr., Bulolo	146°50′ E	6°50′ S	1600	112		4
190†	Morobe Pr., Bulolo	146°50′ E	6°50′ S	1600	112		4
191†	Morobe Pr., Karamengi	146°40′ E	7°20′ S	2100			3
192†	Morobe Pr., Karamengi	146°40′ E	7°20′ S	2100			3
193†	Western Province, Tabubil	141°10′ E	5°50′ S	600			1
194†	Western Pr., Tabubil, seedlings	141°10′ E	5°50′ S	600			2
195††	West Sepik, Strickland, Duvan	142°40′ E	5°20′ S	1200			
196††	West Sepik, Strickland Gorge	142°40′ E	5°20′ S	2010			

*M = morphotype.
**C = Kavalactone chemotype as described in Lebot and Levesque (1989).
***Z = zymotype.
† *Piper wichmannii.*
†† *Piper gibbilimbum.*

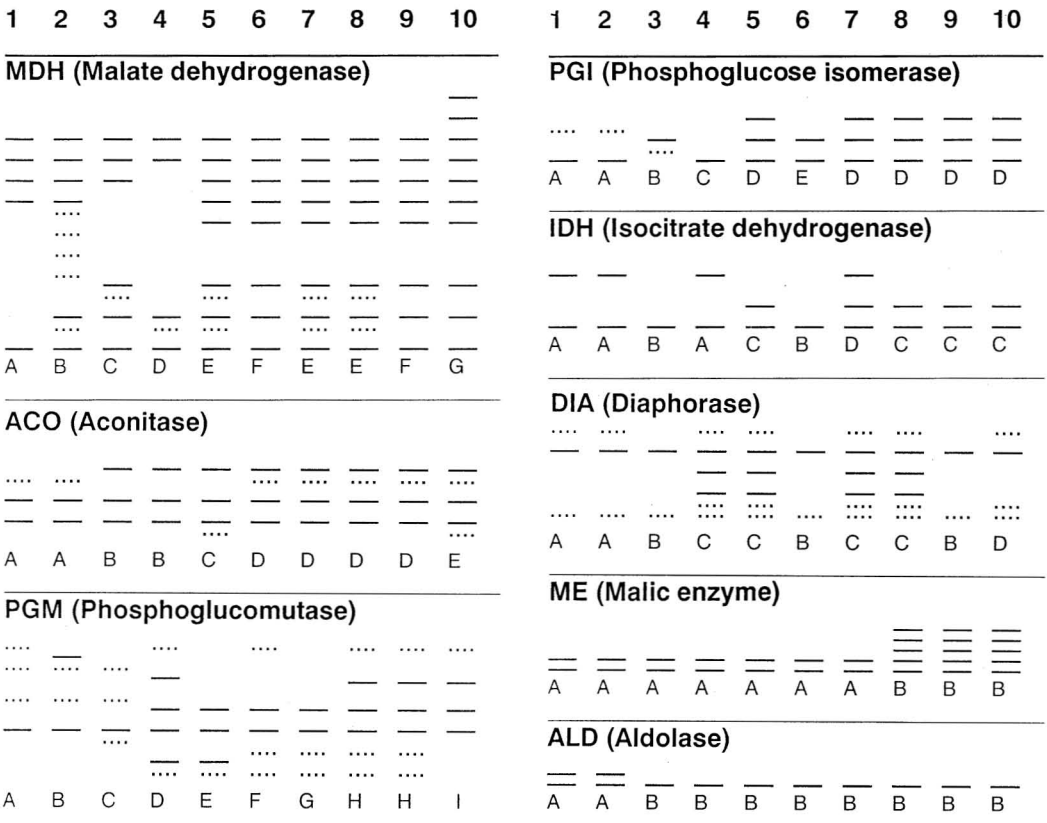
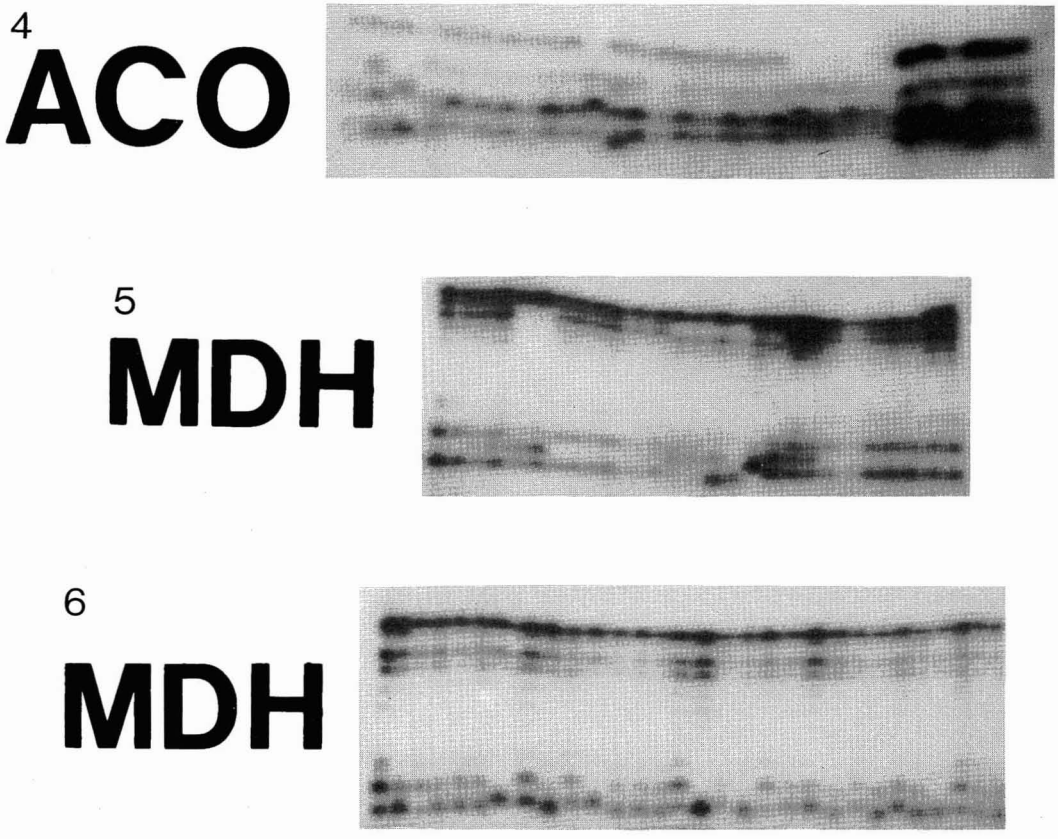


FIGURE 3. Schematic representation of ten zymotypes (1 to 10) observed in kava accessions listed in Table 4. Letters (A, B, C, etc.) indicate polymorphic variants within the eight enzyme systems investigated.

the position of the leaf used or origin of the sample. The most variable isozyme system was PGM, for which nine banding patterns were observed.

All of the enzyme systems were polymorphic in *P. wichmannii* accessions, and a total of eight different zymotypes was observed among the wild materials (Figures 3–5). *Piper wichmannii* zymotypes were most variable in the western part of the natural range (zymotypes 1 through 5 in Papua New Guinea). Less variation is present in eastern Melanesia (zymotypes 6, 7, and 9 only in all of the Solomons and Vanatu). With the exception of the two progenies consisting of about 120 seedlings collected from Western Province of Papua New Guinea (zymotypes 1 and 2), which were segregating for MDH (Figure 6) and PGM, plant populations at any particular collection site were monomorphic with regard to isozyme banding patterns.

Among the cultivated *P. methysticum* accessions there was less variation in isozyme banding patterns. Only four of the eight enzyme systems, including ACO, DIA, MDH, and PGM, were polymorphic, and only three different zymotypes were observed. In all of Polynesia and Micronesia, only one zymotype was identified. From this large geographic area, 93 samples representing 59 cultivars of *P. methysticum*, including male, female, and monoecious plants, were analyzed for isozyme variation and were found to be monomorphic (zymotype 10). Previous work has shown that the Polynesian and Micronesian accessions electrophoresed were differentiated into 28 morphotypes and four chemotypes (Lebot and Lévesque 1989). Slightly more variation



FIGURES 4–6. 4, Polymorphism for ACO; 5, polymorphism for MDH; 6, segregation at MDH loci for *P. wichmannii* (194) progenies.

exists in Melanesia, where analysis of 61 cultivars collected from Papua New Guinea and Vanuatu revealed all three *P. methysticum* zymotypes. Collections from Vanuatu exhibited zymotypes 9 and 10, while in Papua New Guinea, all cultivars collected in the south were uniformly of zymotype 9 and differed from those of the north (zymotype 8) with respect to MDH and DIA banding patterns. Significantly, zymotype 9, which was common among *P. methysticum* cultivars in Vanuatu and in the Fly River region in southern Papua New Guinea, also occurred in one *P. wichmannii* accession (no. 173 from Vanua Lava, Banks Archipelago, Vanuatu).

DISCUSSION

The present work gives the first reports of chromosome numbers for the species studied. It is also the first time that decaploids have been recorded in the genus *Piper*. Based on previous reports by Jose and Sharma (1985), Okada (1986), and Samuel (1986), who concluded that the genus *Piper* is a homogeneous group with a basic number of $x = 13$, we conclude that the three species examined in the present study are decaploids with $2n = 10x = 130$ chromosomes. Despite vegetative propagation, there is uniformity in the chromosome numbers of *P. methysticum* cultivars, and the ploidy level was identical in sterile cultivars of *P. methysticum* and wild forms of *P. wichmannii* and *P. gibbilimbum*.

If *P. wichmannii* is dioecious in the wild, then progenies should be segregating at least for male and female types. This implies that this species is fertile and sexual, or at least partly so. Experimental evidence and observations conducted on other *Piper* species (Sem-

ple 1974) indicated poor fruit set in the absence of staminate flowers, suggesting that for good fruit set pollination is required. Our field observations showed that for *P. gibbilimbum* and *P. wichmannii*, wind pollination was unlikely because of the very sticky and glutinous nature of the pollen. We also observed that this pollen was not easily washed away by heavy rainfalls. Fruits of these two species are very small but are not easily dispersed by wind and remain on the mature inflorescence until it falls to the ground. However, bats were observed eating the long (up to 40 cm) inflorescence of *P. wichmannii* and could be responsible for its dispersal in the forest. *Piper wichmannii* is very common in Papua New Guinea and the Solomons, particularly at about an elevation of 800 m. All the inflorescences observed for these two species showed very good fruit set with crowded spikes. *Piper gibbilimbum* is a very successful colonizer of disturbed forests in New Guinea and was found to be an efficient pioneer in the grasslands of the Strickland Gorge, spread over an altitudinal range from 1500 to 2500 m.

If *P. wichmannii* and *P. gibbilimbum* are reproducing sexually rather than apomictically, then *P. methysticum* could be a sterile F_1 interspecific hybrid between two of these. Several different F_1s from genetically variable parent species would explain different zymotypes in *P. methysticum*, although this hypothesis seems unlikely. Field observations suggest that it is unlikely that cross-pollinations occur between *P. wichmannii* and its relative, *P. gibbilimbum*. These species were often found growing close to each other without any evidence of hybridization. Polyploidy alone cannot be considered as the only explanation for the sterility observed in *P. methysticum*. *Piper peepuloides* and *P. kadzura* have been reported (Okada 1986) as being dodecaploids with $2n = 12x = 156$ chromosomes and are both fertile in the wild, as are *P. wichmannii* and *P. gibbilimbum* (decaploids). The limited isozyme segregation observed in more than 120 *P. wichmannii* seedlings from western Papua New Guinea suggests that cross-pollination occurs, but further evidence is needed to confirm this hypothesis. Chew (1972) stated that *P. wichmannii* and *P. methysticum* are dioecious but our field observations have revealed that monoecious plants also exist for the latter species, suggesting that the same phenomenon could occur for *P. wichmannii*.

The most striking result of the present study is the consistently low isozyme variability found in *P. methysticum*. There are several possible explanations for the absence of variability at the isozyme level. The most realistic hypothesis is that *P. methysticum* consists of a group of sterile clones resulting from human selection of somatic mutants. This hypothesis fits well with the results obtained from previous studies on this species' variability. It is possible that only a few genes are responsible for the morphological and chemical variation observed and that none of these are linked with loci controlling isozyme markers.

There is some incongruence in the data obtained. While chemotypes and zymotypes are significantly correlated ($r = 0.66$) at the 1% level of confidence, no correlation exists between morphotypes and zymotypes. All the Hawaiian accessions, for example, present the same zymotype, although there are clear morphological and chemical differences.

Cluster analysis (Figure 7) conducted on data obtained from the banding patterns indicates that *P. wichmannii* accessions originating from the Western Province of Papua New Guinea (zymotypes 1 and 2) are genetically very different from *P. methysticum*. This observation suggests that these *P. wichmannii* populations are unlikely to be the wild progenitors of the cultivated *P. methysticum*. The closest *P. wichmannii* zymotype is found in Vanuatu, where the cultivated form ('Vambu,' from Vanua Lava, Banks Archipelago) presents the same zymotype as cultivars of *P. methysticum* from Vanuatu and southwestern Papua New Guinea (zymotype 9). Both the cluster analysis (Figure 7) and the principal components analysis (Figure 8) suggest that *P. methysticum* (zymotypes 9 and 10) could have been domesticated in Vanuatu from *P. wichmannii* (zymotype 9).

Cultivars from Vanuatu encompass all of the isozyme variability (zymotypes 9 and 10) and most of the chemical variability found in *P. methysticum* throughout Oceania. The isozyme evidence suggests that Polynesian migrants have collected cultivars in Vanuatu

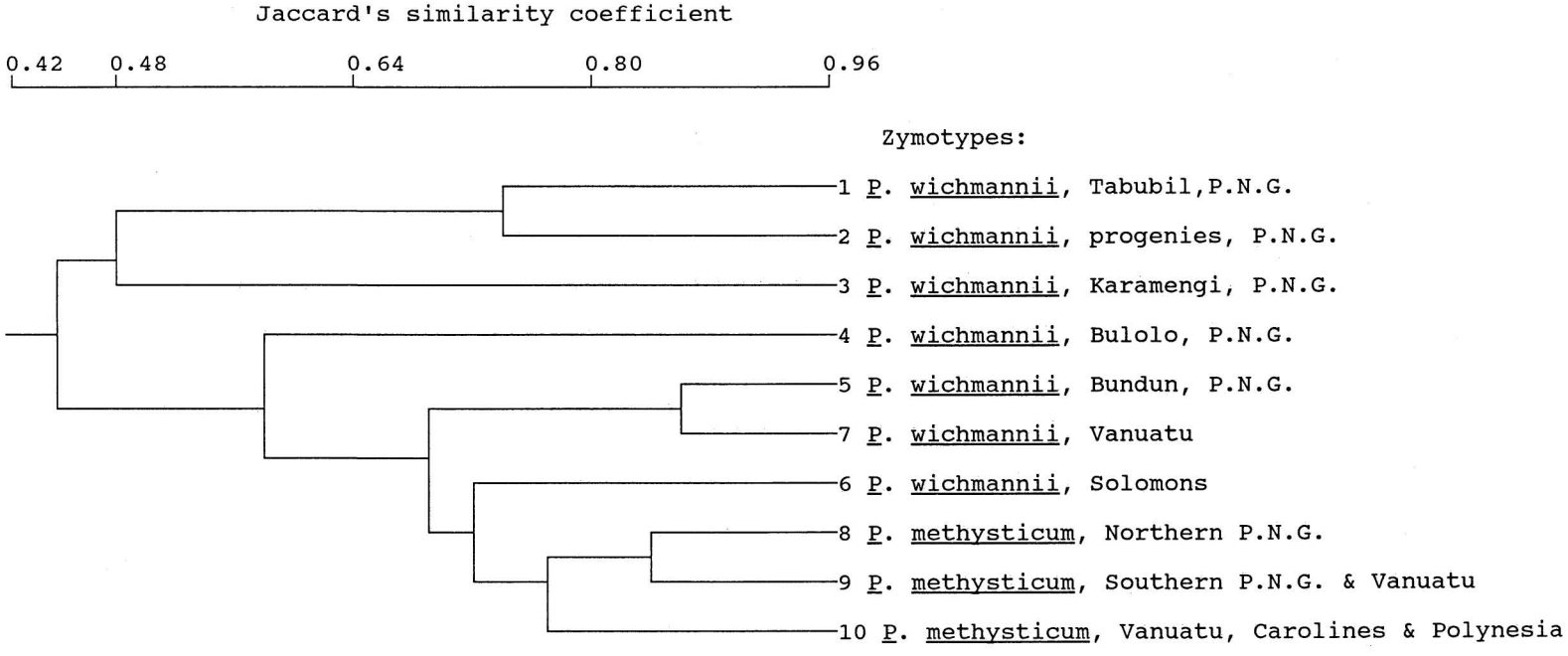


FIGURE 7. UPGMA cluster analysis based on Jaccard's coefficient of similarity among kava zymotypes. Similarity matrix is based on the presence or absence of isozyme bands.

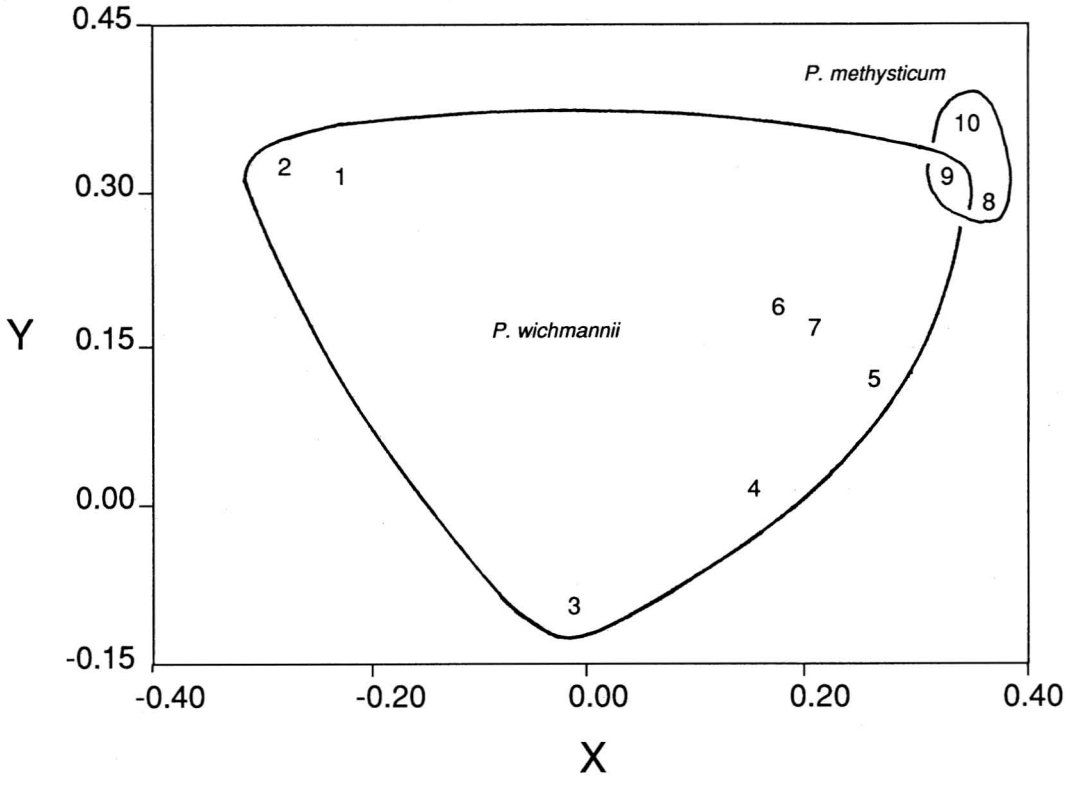


FIGURE 8. Principal components analysis conducted on the correlation matrix of isozyme banding patterns. Axes X (principal component 1) and Y (principal component 2) account for 34.76% and 18.77% of the total diversity, respectively. Numbers refer to zymotypes: 1, *P. wichmannii*, Tabubil, Western Province, P.N.G.; 2, *P. wichmannii* seedlings, Tabubil, P.N.G.; 3, *P. wichmannii*, Karamengi, Morobe Province, P.N.G.; 4, *P. wichmannii*, Bulolo, Morobe, P.N.G.; 5, *P. wichmannii*, Bundun, Morobe, P.N.G.; 6, *P. wichmannii*, Solomons; 7, *P. wichmannii*, Vanua Lava, Vanuatu; 8, *P. methysticum*, Madang Province, P.N.G.; 9, *P. methysticum*, Western Province, P.N.G., and Vanuatu and *P. wichmannii*, Vanua Lava, Vanuatu; 10, *P. methysticum*, Baluan, Vanuatu, Carolines, Fiji and Polynesia.

and distributed them throughout Polynesia as far as Hawaii. Kava in Micronesia, Pohnpei, and Kosrae is most probably an introduction from Vanuatu, directly or via the Admiralty Islands, rather than from Polynesia.

In Papua New Guinea, *P. methysticum* has all the attributes of an introduced species. Plants are always cultivated on coastal plains, around Madang and the Macklay coast, or in the lowlands of Western Province, the highest elevation being 240 m in Nomad. In those areas the two wild relatives, *P. wichmannii* and *P. gibbilimbum*, are absent.

Zymotype 8 is found only in northern Papua New Guinea. However, zymotypes 8 and 9 are so similar that the differences observed in MDH and DIA could probably be explained as somatic mutations, like the other variants. In Papua New Guinea, zymotypes 8 and 9 also have the same chemotype (F, cf. Lebot and Lévesque 1989), and their morphotypes are not very different (111 and 117). Zymotype 9 has probably been introduced in Western Province of Papua New Guinea (Fly River and Strickland River areas). In that region of lowland swamps, mangroves, and savannas, farmers claim that the plant is very difficult to cultivate and the species exhibits no variation. Zymotype 8, from the north coast, is so similar that it is tempting to speculate that kava in Western Province was introduced from the Astrolabe Bay area. Considering the natural geographic barrier made by the highlands, this hypothesis seems un-

reasonable. It is more likely that in both cases the plant was introduced from an overseas source.

Previous work (Lebot and Lévesque 1989) has shown that chemotypes were probably selected from *P. wichmannii* in northern Vanuatu (zymotypes 7 and 9). Our isozyme study supports this hypothesis and that locality seems to be the area of domestication of *P. methysticum*. Clones of *P. methysticum* cultivated in Papua New Guinea in Western Province (zymotype 9), in the Madang area (zymotype 8), or on Baluan Island (zymotype 10) presumably originated in Vanuatu. However, it is difficult to say if the plant was introduced in the Admiralty Islands from the Carolines and Pohnpei or vice versa. *Piper methysticum* was not cultivated in the Solomon Islands, probably because the plant was never introduced there. In Kosrae, Tahiti, the Marquesas, and the Hawaiian Islands, it is still possible to collect plants escaped from cultivation and surviving in the wild through natural vegetative reproduction. Suitable environmental conditions are necessary for such survival, but these conditions exist in the Solomons, and so far *P. methysticum* has never been collected in that archipelago (Whitmore 1966). Few plants were sighted at the beginning of the century in the Polynesian outliers of Tikopia and Vanikoro (Kirch and Yen 1982), but it is likely that they have been introduced by Polynesian migrants.

This isozyme study also supports previous remarks on the lack of taxonomical and nomenclatural validity for the species *P. methysticum* and *P. wichmannii* (Lebot and Lévesque 1989). *Piper wichmannii* has eight discrete zymotypes and *P. methysticum* has three. Zymotype 9 appears in accessions of both taxa, which appear to represent a single species. As *P. methysticum* was described first (Forster 1786), it has priority, and De Candolle's *P. wichmannii* (1910) is superfluous.

CONCLUSIONS

In this paper we have attempted to make a number of points: (1) The taxonomic distinction between *P. methysticum* and *P. wichmannii* is not supported by isozymes or chromosome counts. The "species" overlap (zymotype 9). (2) Wild plants appear to reproduce sexually in western Papua New Guinea, but this is less obvious in eastern Papua New Guinea, the Solomons, and Vanuatu, where apomixis may be predominant. This conclusion is based on the occurrence of greater isozyme variation in the western part of the range, suggesting outcrossing. (3) Kava was domesticated through vegetative propagation from a narrow genetic base in wild fertile progenitors, as indicated by the similarity of zymotypes in cultivated clones. It may have become sterile through accumulation of mutations affecting fertility. However, there is some cytological evidence that, alternatively, kavas could have arisen through interspecific hybridization, since the one genome of larger chromosomes found in cultivars is apparently not found in wild forms. (4) Morphological and kavalactone variability observed in kavas is the result of human selection and preservation of somatic mutations in a few genetically similar, vegetatively propagated clones. (5) Vanuatu is the center of origin of kava cultivars. Kava may be a relatively recent domesticate, considering the arrival date of Austronesians in Vanuatu only 2500 to 3000 years ago. (6) Papua New Guinea kavas are probably introductions from Vanuatu, Micronesia or Polynesian outliers. This conclusion is based on the restricted range and genetic uniformity of the cultivated clones in Papua New Guinea and the distant genetic relationship to local wild kavas. (7) Kava is a relatively late introduction into Polynesia, since there is no variation in isozymes in that region.

One of our objectives was to identify isozyme markers that could be used to attribute genetic fingerprints to each accession for the purpose of clonal identification. However, in the case of kava, isozymes cannot be used for this purpose, since clonal variation in morphology and kavalactone content is not tightly linked with the limited isozyme variation. On the other hand, the study of isozymes has made a useful contribution to elucidate the origin of kava, a much-discussed enigma of Oceanian botany.

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