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Genetic relationships among subspecies of *Vigna unguiculata* (L.) Walp. based on allozyme variation

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Abstract The cowpea [*Vigna unguiculata* (L.) Walp.] is a morphologically and genetically variable species composed of wild perennial, wild annual, and cultivated forms that are mainly used for edible seeds and pods. In this study, genetic variation in 199 germplasm accessions of wild and cultivated cowpea was evaluated using an allozyme analysis. The results from this survey showed that wild cowpea exhibits genetic variation perfectly fitted with the existing morphological classification. The cowpea gene-pool is characterized by its unusually large size. It encompasses taxa (ranked as subspecies) that could be considered as different species considering the high genetic distances observed between accessions belonging to different taxa. These subspecies can be classified into three groups characterized by their breeding systems: perennial outcrossers, perennial out-inbreds, and inbred annuals. Allozyme data confirm this grouping. Perennial outcrossers look primitive and are more remote from each other and from perennial out-inbreds. Within this large gene-pool, mainly made of perennial taxa, cultivated cowpeas (ssp. *unguiculata* var. *unguiculata*) form a genetically coherent group and are closely related to annual cowpeas (ssp. *unguiculata* var. *spontanea*) which may include the most likely progenitor of cultivated cowpeas.

Key words Cowpea · *Vigna unguiculata* · Allozymes · Crop evolution · Genetic diversity

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Introduction

The genus *Vigna* Savi belongs to the family *Leguminosae*, subfamily *Papilionoideae*. Interest in this pantropical genus arises from the fact that it contains several cultivated species: cowpea [*V. unguiculata* (L.) Walp.], Bambara groundnut [*V. subterranea* (L.) Verdc.], and some Asian species: [*V. mungo* (L.) Hepper, *V. radiata* (L.) R. Wilczek, *V. aconitifolia* (Jacq.) Maréchal, *V. angularis* (Willd.) Ohwi & Ohashi, and *V. umbellata* (Thunb.) Ohwi & Ohashi]. On a global basis, cowpea is one of the most important pulse crops. It is estimated that cowpea is now cultivated on at least 12.5 million hectares, with an annual production of over 3 million tons world-wide (Singh et al. 1997). While cowpea is grown on some 80 000 hectares in the U.S. (Fery 1990), Central and West Africa account for more than half of the cultivated area, followed by Central and South America, Asia, East and Southern Africa (Singh et al. 1997). Most of these are characterized by farming systems that make limited use of purchased inputs, which compels breeding programs to concentrate on screening wild and cultivated germplasm for sources of disease and insect resistance, as well as to identify other desirable traits, and on combining multiple resistances. Therefore, the distribution of genetic diversity within and among subspecies and the genetic relationships among them warrant investigation. This information will help to provide an insight into subspecies divergence and to exploit genetic resources for hybridization breeding.

Vigna unguiculata is a diploid species, with $2n = 22$. Within the genus *Vigna*, *V. unguiculata* is a homogeneous species, characterized by striking morphological features like a spurred stipule, an almost symmetric purple flower (sometimes white in cultivated forms), a style with short beak, and a curved pod beak (Maréchal et al. 1978). Within the genus *Vigna*, *V. unguiculata* is well isolated. Between subspecies

reproductive barriers are weak (Rawal et al. 1976; Sakupwanya et al. 1989), while they are strong between *V. unguiculata* and closely related species like *Vigna vexillata* (Barone et al. 1992). Cultivated cowpea originated in Africa, the only continent where wild forms are encountered (Piper 1913; Faris 1965; Maréchal et al. 1978). Wild cowpea is highly variable genetically across Africa. Although morphological characters overlap somewhat, they are sufficiently discrete that formal taxa have been proposed. Morphological studies (Pienaar and Van Wyk 1992; Mithen and Kibblewhite 1993; Padulosi 1993; Pasquet 1993 a) show some divergences, but agree that the cowpea gene pool consists of several perennial taxa and one annual taxon. The last taxonomic treatment (Pasquet 1993 a, c, 1997) divides cowpea into ten perennial subspecies and one annual subspecies, ssp. *unguiculata*. It splits ssp. *unguiculata* into var. *unguiculata* (cultivated cowpea) and var. *spontanea* (Schweinf.) Pasquet (annual wild cowpea).

The 11 subspecies have been classified into three groups according to the breeding system. Outcrossing and inbreeding accessions (as well as herbarium specimens) can be separated according to the disposition of anthers and stigma. In outcrossing accessions, the relative position of the anthers and stigma prevents selfing in the absence of hand-pollination or insect tripping, while in inbreeding accessions, the stigmatic surface and the anthers are in contact (Lush 1979). Subspecies which include only outcrossing accessions (and herbarium specimens) are pooled in the perennial outcrossers. Subspecies which include both outcrossing and inbreeding accessions, (usually in different ecologies) are pooled in the perennial out-inbreds. Subspecies *unguiculata*, which includes almost only inbreeding accessions, is considered to be in the annual inbred group (Pasquet 1994). The perennial outcrossers include ssp. *baoulensis* (A.Chev.) Pasquet, ssp. *burundiensis* Pasquet, ssp. *letouzeyi* Pasquet, ssp. *aduensis* Pasquet, and ssp. *pawekiae* Pasquet. The perennial out-inbreds include ssp. *dekindtiana* (Harms) Verdc. *sensu stricto*, ssp. *stenophylla* (E.Mey.) Verdc., ssp. *tenuis* (E.Mey.) Maréchal, Mascherpa and Stainier, ssp. *alba* (G.Don) Pasquet, and ssp. *pubescens* (R.Wilczek) Pasquet. The annual inbreds include ssp. *unguiculata* var. *spontanea* (Schweinf.) Pasquet and var. *unguiculata*.

Most intraspecific relationships based on molecular studies have been determined using allozymes (Panella and Gepts 1992; Pasquet 1993 b; Vaillancourt et al. 1993), seed storage proteins (Panella et al. 1993; Fotso et al. 1994), and cpDNA (Vaillancourt and Weeden 1992). However, all these studies included a small number of wild accessions, where perennial subspecies were poorly represented.

The data presented here are more comprehensive than all previous molecular studies. We include 91 perennial accessions from eight of the ten perennial subspecies identified to more fully investigate the cowpea gene pool organization and evolution.

Materials and methods

Plant material

The plant material used in this study included eight perennial subspecies, which is all of the material available in living seed collections. Among the eight perennial subspecies, 91 accessions were assayed (Table 1). For the annual subspecies, 95 wild accessions were selected so that most of the geographical and morphological ranges (Pasquet 1994) were sampled. Thirteen cultivated accessions (four from cv gr Unguiculata, four from cv gr Biflora, three from cv gr Melanophthalmus, one from cv gr Sesquipedalis, one from cv gr Textilis) were selected to represent the allozyme polymorphism observed while studying 191 accessions (Pasquet 1994). The wild accessions were obtained from the World *Phaseolinae* collection maintained in the Jardin Botanique National de Belgique (BR), Meise, Belgium (NI accessions); the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (TVNU accessions); the International Plant Genetic Resources Institute (IPGRI), Harare, Zimbabwe (MT accessions); and the ORSTOM collection maintained in Montpellier, France (SP accessions). Cultivated accessions were obtained from the USDA-ARS, Charleston, USA; the Zentralinstitut für Genetik und Kultupflanzenforschung (GAT), Gatersleben, Germany; the Agricultural University (WAG), Wageningen, Netherlands; and the ORSTOM collection maintained in Montpellier, France. Additional information about the accessions studied can be obtained directly from the author.

Each accession is composed of 2–5 autogamous lines, and maintained as such; each of these lines was derived from one seed of the original stock. A minimum of two to a maximum of five plants were assayed per accession.

Isozyme electrophoresis

Preliminary experiments revealed that better resolution and staining quality of bands could be obtained from seed rather than from leaf extracts. Consequently, seed extracts were used throughout the study, with the exception of αEST, GOT, and SOD for which leaf extracts were employed. Seeds were imbibed overnight and cotyledons were then ground in water, while young leaf tissue was ground in 0.1 M Tris-HCl, pH 7.5, 5% sucrose, 0.1% mercaptoethanol (Wendel and Weeden 1989). All isozymes were assayed in the citrate/histidine pH 6.0 buffer system (electrode buffer: 0.41 M citrate pH 6.0; gel buffer: 5 mM L-Histidine mono HCl, pH 6.0), and the gel consisted of 14% starch (Second and Trouslot 1980).

Twenty one enzyme systems revealing 35 putative loci were screened: namely, alcohol dehydrogenase (ADH), aminopeptidase (AMP), NADH diaphorase (DIA), endopeptidase (ENP), esterase (EST), fluorescent esterase (FLE), formate dehydrogenase (FDH), glucose-6-phosphate dehydrogenase (G6PD), β-glucosidase (βGLU), glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), glutathione reductase (GR), isocitrate dehydrogenase (IDH), mannose phosphate isomerase (MPI), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucomutase (PGM), phosphogluconate dehydrogenase (PGD), phosphoglucose isomerase (PGI), shikimate dehydrogenase (SDH), and superoxide dismutase (SOD). Enzyme-specific staining was carried according to Wendel and Weeden (1989) using either leucine-β-naphtylamide or alanine-β-naphtylamide for AMP, and 4-methyl-umbelliferyl

Table 1 *V. unguiculata* (L.) Walp. accessions assayed for putative allozyme variation and their origin[a]

Subspecies	Country	Accessions
baoulensis	CIV	SP 69, 70, 71, 72, 135
	CMR	SP 36, 39, 45, 55, 63, 92, 97, TVNU 390, 573
	GHA	NI 933, SP 136
	LBR	SP 170
	NGA	NI 794, 993, 1026, 1034, 1393, SP 77
letouzeyi	CMR	SP 47, 48, 49, 51, 95
burundiensis	BDI	NI 456
pawekiae	MWI	MT 449, SP 89, TVNU 367
	ZWE	MT 53, 320, TVNU 862
stenophylla	BWA	MT 509, 546, 564, 593, 596, SP 90, TVNU 278, 458
	ZAF	SP 158, 187, 188, 189, 190, 191
alba	AGO	SP 74, 85, 139
	COG	NI 1388, 1389, SP 144, 145, 153, 176
tenuis	MOZ	MT 653
	MWI	MT 340, 357
	ZAF	SP 161, 162, 163, 165, 167, TVNU 714, 716, 885, 1088, 1348, 1349, 1355, 1380, 1381
	ZMB	MT 206, 218
	ZWE	MT 4, 5
pubescens	BDI	NI 1417
	KEN	NI 979, 989, 1186
	TZA	NI 856, 910, 947, 957, 1029, SP 142, TVNU 110, 505
unguiculata var. *spontanea*		
	BDI	NI 1232
	BWA	MT 621, NI 1380, 1381, 1382, 1383, 1384, TVNU 261, 265, 284, 286, 466, 554
	CAF	TVNU 242, 255
	CMR	SP 3, 5, 33, 37, 38, 46, 52, 99, 129
	COG	NI 1390, 1391, SP 148, 149, 151, 152
	GNB	SP 140
	KEN	FLG 4903, 4904, NI 1048, 1228, 1408, SP 87
	MDG	SP 81
	MOZ	SP 141
	MWI	NI 1392, TVNU 363, 371, 435, 437, 439, 440, 442, 742
	NAM	SP 154, 160
	NER	NI 945, 991, SP 66, 80
	NGA	NI 951
	SDN	SP 86
	SEN	NI 963
	TCD	SP 64
	TZA	MT 131, NI 301, 1385, 1386, 1405, SP 75, 83, TVNU 297, 416, 503, 531, 922, 1248
	ZAF	NI 1167, SP 159, TVNU 1089, 1343, 1351
	ZAR	NI 198, 319, 320, 437
	ZMB	MT 651, NI 423, 1171, 1387, TVNU 352, 523, 707, 709, 712
	ZWE	MT 55, 76, 102, NI 817, 874, 1400
unguiculata var. *unguiculata*		
	CMR	CS 15, NO 106, 110, 1878, 2300, 3076
	COL	EX 19
	ETH	ET 3, 5, 14
	IND	EX 11
	ITA	EX 24
	USA	EX 27

[a] Additional information regarding the accessions can be obtained directly from the author

compounds for FLE and βGLU. GR was stained according to Harris and Hopkinson (1976).

For each enzyme system, the presumed loci encoding the most anodally migrating isozyme were designated "1"; with additional loci numbered sequentially in order of decreasing electrophoretic mobility. For each isozyme, the most common allozyme and respective allele has been designated as 100 and the other allozymes have been measured in millimetres in relation to that standard. This procedure was the same as the one utilized by Koenig and Gepts (1989) with *Phaseolus vulgaris* L.

Data analysis

Electrophoretic data at 35 presumed loci were analyzed using BIOSYS-1 version 1.7 (Swofford and Selander 1981) and NTSYS version 1.80 (Rohlf 1993). Data were entered as the allele frequencies of the accessions. In addition, a hierarchical arrangement of the accessions was established at the subspecies/group level so that the data could be analyzed within and among subspecies and summarized by subspecies. The allelic composition of each subspecies/group of accessions was determined at 35 presumed loci.

Genetic variability was assessed using the proportion of polymorphic loci (P), the mean number of alleles among all loci (A) and among polymorphic loci (A_p), and the total diversity (H_t). Total diversity is partitioned into the weighted average diversity within subspecies (H_s), and the between-subspecies gene diversity (D_{st}). These quantities are related by the expression $H_t = H_s + D_{st}$. The proportion of total allelic diversity found among subspecies (G_{st}) is calculated as the ratio D_{st}/Ht (Nei 1973). The genetic distances of Nei (1972) and Rogers (1972) were calculated for all possible pairwise comparisons among the 199 accessions. The unweighted pair group method with arithmetic averaging (UPGMA) was then performed on the distance matrix (Sneath and Sokal 1973). Multivariate relationships among ssp. *pubescens* and ssp. *unguiculata* accessions were revealed through a principal coordinate (PCO) analysis of distances. Finally, the genetic distances were averaged among accessions within subspecies and a Rogers (1972) distance phenogram (calculated from subspecies allele frequencies) was constructed with the Neighbor Joining method of Saitou and Nei (1987).

Results

Genetic variability in *V. unguiculata*

The 21 enzyme systems screened revealed 43 putative loci. However, six polymorphic loci (Amp1, Est2, Fle2, βGlu2, Pgi1 and Sod1) and two monomorphic loci (Mdh4 and Sod3) were poorly resolved or else stained inconsistently under our assay conditions. Because these loci were uninformative with respect to characterizing diversity in *V. unguiculata*, they were excluded from further analysis. Therefore, the 21 systems revealed 35 scorable loci, the presumed products of individual coding loci. These were: *Adh1*, *Adh2*, *Amp2*, *Amp3*, *Amp4*, *Dia1*, *Dia2*, *Enp*, *Est1*, *Est3*, *Fdh*, *Fle1*, *Fle3*, *Gdh*, *βGlu1*, *βGlu3*, *G6pd*, *Got1*, *Got2*, *Gr*, *Idh1*, *Idh2*, *Mdh1*, *Mdh2*, *Mdh3*, *Me*, *Mpi*, *Pgd1*, *Pgd2*, *Pgi2*, *Pgi3*, *Pgm1*, *Pgm2*, *Sdh*, and *Sod2*. All the enzyme bands migrated anodally.

For the 21 enzymes, an estimated 33 polymorphic loci (approximately 1.5 loci per enzyme system) and 143 alleles (approximately 4.0 alleles per locus) were resolved. A summary of the loci and alleles identified in each subspecies or group of accessions is provided in Table 2. Allelic variation among these 33 loci was not partitioned equally, inasmuch as three (*Adh2*, *Mdh2*, *Mdh3*) were minimally variable with only two allozymes per locus. An additional nine loci (*Dia1*, *Dia2*, *βGlu3*, *G6pd*, *Gr*, *Idh2*, *Pgd2*, *Pgm1*, *Pgm2*) were triallelic, and the remaining 21 were multi-allelic, displaying between four and eight alleles per locus. The average allele frequencies for *V. unguiculata* indicated that few polymorphic loci were weakly polymorphic, because only 11 out of 33 loci showed allele frequencies ≥ 0.9 for the most frequent allele. Consequently, as a species, our sample of *V. unguiculata* (wild + cultivated) has a moderately high estimated heterozygosity ($H_t = 0.28$). The mean estimated heterozygosity for individual groups of *V. unguiculata* ranged from a high of 0.27 for ssp. *pawekiae* to a low of 0.08 for cultivated accessions (Table 3).

As expected, wild cowpea is more polymorphic than cultivated cowpea. This trend was evident in the summary statistics (Table 3) and allelic frequencies for wild subspecies (Table 2). As a group, wild cowpea included 143 alleles at 35 loci, yielding an average of 4.08 alleles per locus (4.27 alleles per polymorphic locus). Ninetynine of these alleles were unique to wild cowpea. Subspecies *unguiculata* var. *spontanea* showed the highest number of alleles within the wild group with 100 alleles ($A = 2.86$, $A_p = 3.32$). However, its heterozygosity ($H_t = 0.199$) was not the highest. Higher heterozygosities were calculated with ssp. *tenuis* ($H_t = 0.206$), ssp. *pawekiae* ($H_t = 0.270$), and ssp. *stenophylla* ($H_t = 0.280$). Among groups of wild cowpea, var. *spontanea* and ssp. *stenophylla* showed nine unique alleles.

Cultivated cowpea revealed a total of 44 alleles, with an average of 1.26 alleles per locus and 2.12 per polymorphic locus (Table 3). No allele was unique to cultivated cowpea. However, $Amp2^{102}$ characterized most cultivated cowpea accessions while it was found in only seven wild accessions.

Inter-subspecies relationships in wild *V. unguiculata*

The effect of allele frequency divergence between subspecies on the partitioning of genetic diversity were quantified by the gene diversity statistics of Nei (1973). The total genetic diversity (H_t) was 0.290 over all the wild accessions. Within-accession diversity (H_s) was only 0.022; therefore, between accession diversity (D_{st}) was responsible for most of the genetic diversity, and the coefficient of gene differentiation (G_{st}) was 0.924. Within-subspecies diversity (H_s) was 0.191, between-subspecies diversity (D_{st}) was 0.099 (considering eight subspecies), and the coefficient of gene differentiation (G_{st}) was 0.341. Considering 11 groups, instead of eight subspecies, led to values of 0.161 for H_s, 0.129 for D_{st}, and 0.444 for G_{st}. However, if only perennial subspecies and seven subspecies were considered, H_t rose to 0.337, H_s to 0.183, D_{st} to 0.154, and G_{st} to 0.457. When partitioned between seven subspecies, almost 50% of perennial diversity was found among subspecies. The contribution of different loci to this partitioning of variability indicated that *Gr* and *Idh2* contributed the most, and *Got2*, *Mdh2*, *Mdh3*, and *Pgd1* contributed the least, to the partition (Table 4).

The genetic distances of Nei (1972) and Rogers (1972) were calculated for all possible pairwise comparisons among the 199 accessions. These were clustered by UPGMA. Three interesting features of the cluster analysis are worth mentioning. Firstly, Nei distances (Fig. 1) and Rogers distances (data not shown) yielded the same 11 clusters, i.e. from bottom to top, ssp.

Table 2 Mean allele frequencies for the major groups of *V. unguiculata* examined in this study. Locus/allele nomenclature is described in the Materials and methods. Numbers of accessions are in brackets. Monomorphic loci (*Gdh* and *Sod2*) were omitted

Allele	ssp. *baoulensis* (23)	ssp. *letouzeyi* (5)	ssp. *burundiensis* (1)	ssp. *pawekiae* (6)	ssp. *ste.* ZAF[a] (5)	ssp. *ste.* BWA[b] (9)	var. *spon.* B[c] (14)	ssp. *alba* (9)	ssp. *ten.* ZWE[d] (6)	ssp. *ten.* ZAF[e] (15)	ssp. *pubescens* (12)	var. *spontanea* (81)	Cult. (13)
Adh1 104	0	0	0	0.167	0	0	0	0	0	0.200	0	0	0
102	0	0	0	0	0	0	0	0	0	0.033	0	0	0
100	1	1	1	0.833	0.1	1	1	1	1	0.767	1	1	1
98	0	0	0	0	0.2	0	0	0	0	0	0	0	0
91	0	0	0	0	0.7	0	0	0	0	0	0	0	0
Adh2 100	1	1	1	1	0.3	1	1	1	1	1	1	1	1
91	0	0	0	0	0.7	0	0	0	0	0	0	0	0
Amp2 102	0	0	0	0	0	0	0	0	0	0	0	0.068	0.846
100	0	0	0	0.25	0.9	0.873	1	1	0	0.933	0.208	0.858	0.154
98	0.304	0.6	1	0.75	0.1	0.033	0	0	0.833	0.067	0.708	0.074	0
97	0.696	0	0	0	0	0.089	0	0	0.111	0	0.083	0	0
93	0	0.4	0	0	0	0	0	0	0	0	0	0	0
Amp3 103	0	0	0	0	0.2	0	0	0	0	0.133	0	0.037	0.154
100	0.239	0	1	0.417	0.7	0.222	0.5	1	1	0.733	1	0.833	0.846
97	0.717	0.2	0	0.333	0.1	0.778	0.5	0	0	0.133	0	0.056	0
94	0.043	0	0	0	0	0	0	0	0	0	0	0.062	0
91	0	0	0	0.250	0	0	0	0	0	0	0	0.012	0
0	0	0.8	0	0	0	0	0	0	0	0	0	0	0
Amp4 108	0	0	0	0	0	0	0	0.944	0	0	0	0.025	0
104	0.022	0	1	0	0.1	0	0	0	0.833	0	0	0.043	0
100	0.848	1	0	0.833	0.7	0.167	0.021	0	0.083	0.967	0.042	0.636	1
96	0.130	0	0	0	0.2	0.833	0.921	0	0.083	0.033	0.667	0.241	0
93	0	0	0	0.167	0	0	0.057	0.056	0	0	0.292	0.031	0
89	0	0	0	0	0	0	0	0	0	0	0	0.025	0
Dia1 103	0.043	0	0	0	0	0	0	0	0	0	0	0	0
100	0.957	0.8	1	1	1	1	1	1	0	0.933	1	0.988	1
97	0	0.2	0	0	0	0	0	0	1	0.067	0	0.012	0
Dia2 112	0.457	0	0	0.083	0	0	0	0	0	0	0	0	0
100	0.543	1	1	0.917	1	1	0.964	1	1	1	1	0.988	1
88	0	0	0	0	0	0	0.036	0	0	0	0	0.012	0
Enp 111	0	0	0	0	0	0	0	0	0	0	0	0.012	0
109	0.043	0	0	0	0	0	0	0	0	0	0	0	0
107	0.152	0	0	0	0	0	0	0	0	0	0	0	0
105	0.804	1	0	0.167	0.2	0.222	0.964	0.833	0.667	0.9	0.964	0.173	0.385
103	0	0	0	0	0.2	0.611	0	0	0	0	0	0.006	0
100	0	0	0	0.667	0.6	0.111	0.036	0.167	0.333	0.1	0.036	0.759	0.385
98	0	0	0	0	0	0.056	0	0	0	0	0	0.049	0.231
95	0	0	1	0.167	0	0	0	0	0	0	0	0	0
Est1 110	0	1	1	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0.333	0	0.667	0	0	0.167	0	0	0.099	0
100	1	0	0	0	0.8	0.333	1	0.889	0.833	1	1	0.889	1
95	0	0	0	0	0.2	0	0	0.111	0	0	0	0.012	0
90	0	0	0	0.667	0	0	0	0	0	0	0	0	0

Est3	*109*	0.174	0	1	0	0	0.256	0.929	0	0.083	0	0	0.037	0
	103	0.804	0.5	0	0.167	0	0	0	0.333	0.083	0	0	0.025	0
	100	0.022	0.5	0	0.417	0.3	0.200	0.036	0.500	0.833	1	1	0.932	1
	97	0	0	0	0.083	0.7	0	0	0.111	0	0	0	0	0
	91	0	0	0	0.167	0	0	0	0	0	0	0	0.006	0
	82	0	0	0	0.167	0	0.544	0.036	0.056	0	0	0	0	0
Fdh	*104*	0	0	0	0	0	0	0.071	0	0.333	0	0	0	0
	102	0	0	0	0.333	0	0.222	0.143	0	0.333	0.8	0	0.179	0.385
	100	1	1	1	0.667	1	0.778	0	1	0.333	0.2	1	0.772	0.615
	96	0	0	0	0	0	0	0.786	0	0	0	0	0.049	0
Fle1	*104*	0	0	0	0	0	0	0	0	0.167	0	0	0	0
	100	0.891	1	0	0	1	1	0.321	1	0.833	1	1	0.975	1
	97	0.065	0	0	1	0	0	0.143	0	0	0	0	0.006	0
	94	0.043	0	1	0	0	0	0.536	0	0	0	0	0.019	0
Fle3	*104*	0	0	0	0	0	0	0.464	0	0	0	0	0.049	0
	100	1	1	1	1	0	0.944	0.536	0.889	0.167	0.300	1	0.796	0.385
	96	0	0	0	0	0.8	0	0	0.111	0.833	0.633	0	0.154	0.615
	92	0	0	0	0	0.2	0.056	0	0	0	0.067	0	0	0
βGlu1	*112*	0	0.6	0	0	0	0	0	0	0	0	0	0	0
	106	0	0.2	1	0.333	1	0.667	0.679	0.556	0.917	0.533	0	0.105	0
	100	0.196	0.2	0	0.667	0	0.333	0.321	0.444	0.083	0.467	1	0.895	1
	94	0.804	0	0	0	0	0	0	0	0	0	0	0	0
βGlu3	*100*	0.087	0.8	1	0.167	0	0.056	0	0	0	0	0	0	0
	98	0.717	0	0	0.500	0	0.111	0	0	0	0	0	0	0
	0	0.196	0.2	0	0.333	1	0.833	1	1	1	1	1	1	1
G6pd	*105*	0	0	0	0	0	0	0	0	0	0	0	0.204	0.077
	100	0.13	0.2	0	0.333	0	0.333	1	0.889	0	0.2	0.833	0.704	0.923
	95	0.87	0.8	1	0.667	1	0.667	0	0.111	1	0.8	0.167	0.093	0
Got1	*107*	0	0	0	0	0	0.056	0	0	0	0	0	0	0
	103	0	0	0	0.083	0	0	0	0	0	0	0	0.006	0
	100	1	1	0	0.750	1	0.944	1	1	1	1	1	0.988	1
	93	0	0	1	0.167	0	0	0	0	0	0	0	0.006	0
Got2	*115*	0	0	0	0	0	0	0	0.111	0	0	0	0	0
	110	0	0	0	0	0.2	0.389	0.357	0	0	0	0.083	0.148	0.308
	105	0	0	0	0	0	0.111	0	0	0	0	0	0	0
	100	1	1	1	1	0.8	0.500	0.643	0.778	1	0.967	0.917	0.852	0.692
	90	0	0	0	0	0	0	0	0.111	0	0.033	0	0	0
Gr	*102*	0	0	0	0	0	0	0.071	0	0	0	0	0.012	0
	100	0	0.8	0	0	0.3	0.833	0.929	1	1	0.933	1	0.932	1
	97	1	0.2	1	1	0.7	0.167	0	0	0	0.067	0	0.056	0
Idh1	*110*	0	0.2	0	0	0	0	0	0	0	0	0	0	0
	105	0	0	0	0	0	0.5	0	0	0	0	0	0	0
	100	0.391	0.8	1	1	1	0.5	1	1	0.667	1	1	0.994	1
	95	0	0	0	0	0	0	0	0	0.167	0	0	0	0
	90	0.609	0	0	0	0	0	0	0	0	0	0	0.006	0
	80	0	0	0	0	0	0	0	0	0.167	0	0	0	0

Table 2 Continued

Allele	ssp. *baoulensis* (23)	ssp. *letouzeyi* (5)	ssp. *burundiensis* (1)	ssp. *pawekiae* (6)	ssp. *ste.* ZAF[a] (5)	ssp. *ste.* BWA[b] (9)	var. *spon.* B[c] (14)	ssp. *alba* (9)	ssp. *ten.* ZWE[d] (6)	ssp. *ten.* ZAF[e] (15)	ssp. *pubescens* (12)	var. *spontanea* (81)	Cult. (13)
Idh2 105	0	0	0	0	0	0	0	0	0	0	0	0.012	0
100	1	1	1	1	1	1	1	0.056	1	1	1	0.975	1
95	0	0	0	0	0	0	0	0.944	0	0	0	0.012	0
Mdh1 105	0	0	0	0	0	0	0	0.111	0	0	0	0	0
100	0.609	0.6	1	1	1	0.889	0.929	0.500	1	1	1	0.994	1
95	0.391	0.4	0	0	0	0.111	0	0.111	0	0	0	0	0
90	0	0	0	0	0	0	0.071	0.278	0	0	0	0.006	0
Mdh2 100	1	1	1	1	1	0.944	1	1	1	1	1	1	1
92	0	0	0	0	0	0.056	0	0	0	0	0	0	0
Mdh3 100	1	1	1	1	1	0.944	1	1	1	1	1	1	1
92	0	0	0	0	0	0.056	0	0	0	0	0	0	0
Me 103	0	0	0	0.083	0.2	0.389	0	0	0	0	0	0.004	0
100	1	0.6	1	0.417	0.5	0.500	0.036	0.333	0.167	0.500	0.5	0.349	1
97	0	0.4	0	0.333	0.3	0	0.964	0.667	0.833	0.433	0.5	0.553	0
94	0	0	0	0	0	0	0	0	0	0	0	0.044	0
91	0	0	0	0.083	0	0	0	0	0	0.067	0	0.043	0
88	0	0	0	0.083	0	0.111	0	0	0	0	0	0.006	0
Mpi 106	0	0	0	0	0	0	0.071	0.167	0	0	0	0.012	0
103	0	0	0	0	0	0	0	0.333	0	0	0	0.012	0
101	0	0	0	0	0	0	0.500	0.500	0	0.033	0	0.117	0
100	1	1	1	0.917	0	0.111	0.393	0	0.833	0.600	0.75	0.821	1
95	0	0	0	0	0	0	0	0	0.167	0	0	0.006	0
91	0	0	0	0.083	1	0.889	0.036	0	0	0.367	0.25	0.031	0
Pgd1 106	0	0	0	0	0.3	0	0	0	0	0	0	0	0
100	1	1	1	0.417	0.7	1	0.036	0.722	1	0.567	1	0.981	1
94	0	0	0	0.583	0	0	0.964	0.278	0	0.367	0	0.019	0
91	0	0	0	0	0	0	0	0	0	0.067	0	0	0
Pgd2 100	0.783	0.5	1	1	0.9	0.167	1	1	1	0.233	0.917	0.981	1
98	0	0	0	0	0	0	0	0	0	0	0.083	0	0
96	0.217	0.5	0	0	0.1	0.833	0	0	0	0.767	0	0.019	0
Pgi2 115	0	0	0	0	0	0	0	0	0	0.067	0	0.090	0
107	0	0	0	0	0	0	0	0	0	0	0.583	0.037	0.154
100	0.043	1	1	0.500	1	0.889	1	1	1	0.933	0.417	0.844	0.846
92	0.957	0	0	0.333	0	0.111	0	0	0	0	0	0.028	0
84	0	0	0	0.167	0	0	0	0	0	0	0	0	0
Pgi3 116	0	0	0	0.167	0	0	0	0	0	0	0	0	0
112	0	0	0	0.167	0.38	0	0	0	0	0	0	0	0
108	0.935	0	1	0.417	0	0	0.071	1	0.167	0	0.083	0.133	0
100	0.022	1	0	0.250	0.38	1	0.929	0	0.833	0.933	0.917	0.857	1
92	0	0	0	0	0	0	0	0	0	0.067	0	0.010	0
84	0.043	0	0	0	0.24	0	0	0	0	0	0	0	0

Pgm1	*100*	0.783	1	1	0.75	1	1	1	1	1	1	0.975	1	
	96	0.217	0	0	0.25	0	0	0	0	0	0	0.012	0	
	92	0	0	0	0	0	0	0	0	0	0	0.012	0	
Pgm2	*105*	0	0	1	0	0	0	0	0	0	0	0	0	
	100	0.956	1	0	1	1	1	0.893	1	1	1	0.938	1	
	95	0.043	0	0	0	0	0	0.107	0	0	0	0.062	0	
Sdh	*110*	0	0	0	0.500	0	0	0	0	0	0	0.006	0	
	105	0	0	1	0.250	0	0	0	0	0	0	0.062	0	
	100	0.957	1	0	0.167	0.4	0.167	0.036	0	0	0.333	0.833	0.648	1
	95	0.043	0	0	0.083	0.6	0.883	0.964	1	0.667	0.667	0.167	0.284	0
	90	0	0	0	0	0	0	0	0	0.333	0	0	0	0

[a] ssp. *stenophylla* ZAF includes accessions from South Africa
[b] ssp. *stenophylla* BWA includes accessions from Botswana
[c] ssp. *spontanea* BWA includes the 14 accessions from Southern Africa separated by the cluster analysis
[d] ssp. *tenuis* ZWE includes inland accessions from Malawi, Zambia, and Zimbabwe
[e] ssp. *tenuis* ZAF includes coastal accessions from South Africa and Mozambique

burundiensis, ssp. *pawekiae*, ssp. *baoulensis*, ssp. *stenophylla* BWA (from Botswana), ssp. *letouzeyi*, ssp. *stenophylla* ZAF (from South Africa), var. *spontanea* BWA (14 accessions from Botswana and vicinity), ssp. *tenuis* ZWE (from inland areas), ssp. *alba*, ssp. *tenuis* ZAF (from coastal areas), and a last cluster including ssp. *pubescens* and ssp. *unguiculata*. Secondly, these clusters fitted the morphological classification almost perfectly. With the exception of ssp. *unguiculata* and ssp. *pubescens*, which share the same cluster, and five accessions related to ssp. *tenuis* (MT 55, TV 554, SP 141, SP 158, and SP 162), accessions from each cluster belonged to a single subspecies. However, ssp. *stenophylla*, ssp. *tenuis*, and var. *spontanea* were each split into two clusters, according to the geographic origin of the accessions. Thirdly, ssp. *pubescens* and ssp. *unguiculata* were intermixed in the same clusters. Within this cluster, Nei distances (Fig. 1) and Rogers distances (data not shown) yielded different patterns: the Rogers distance ssp. *pubescens* cluster included NI 1417 and TV 505 but excluded SP 142 and TV 110, while cultivated accessions (minus NO 2300 and NO 3076) were better grouped in a cluster which excluded TV 437, SP 37, and SP 80.

Mean allele frequencies for wild and cultivated *V. unguiculata* across 35 loci are presented in Table 2 for each group from the cluster analysis. Inspection of this table reveals that allele frequencies at several loci differ markedly among subspecies. Most loci show differences between subspecies regarding the most common allele (27 out of the 33 polymorphic loci) and a G_{st} higher than 0.2 (26 out of the 33 polymorphic loci). Thirteen loci (*Amp2*, *Dia2*, *Est1*, *Est3*, *βGlu1*, *βGlu3*, *G6pd*, *Gr*, *Idh1*, *Idh2*, *Pgd2*, *Pgi2* and *Pgi3*) showed a G_{st} higher than 0.35 (Table 4).

The genetic-distance coefficients were averaged among accessions within subspecies and cluster analysis groups, and Rogers genetic distances calculated from the group allelic frequencies were reported (Table 5). The smallest genetic distance values were observed between ssp. *pubescens* and both ssp. *unguiculata* varieties, and between ssp. *tenuis* ZAF and var. *spontanea*, while the distance value between var. *spontanea* and var. *spontanea* BWA was much higher. The highest genetic distances values were observed between ssp. *baoulensis*, ssp. *letouzeyi*, ssp. *burundiensis*, and ssp. *pawekiae*.

To evaluate systematic relationships among groups of accessions, we constructed a Neighbor-Joining phenogram (Saitou and Nei 1987) derived from the Rogers genetic distances (Rogers 1972). Cultivated cowpeas consistently represent a discrete lineage which is a sister to var. *spontanea*. Both ssp. *stenophylla* groups clustered together as did both ssp. *tenuis* groups, while var. *spontanea* from Botswana clustered with ssp. *alba*. Subspecies *baoulensis*, ssp. *pawekiae*, ssp. *burundiensis*, and ssp. *letouzeyi* appeared at the bottom of the phenogram (Fig. 2).

Table 3 Genetic diversity statistics for wild and cultivated accessions of *V. unguiculata*

Group	*N*[a]	*A*	*Ap*	*P*	*Hs*	*Ht*	*U*
ssp. *baoulensis*	23	1.74	2.36	0.54	0.011	0.158	4
ssp. *letouzeyi*	5	1.37	2.08	0.34	0.006	0.141	4
ssp. *burundiensis*	1						1
ssp. *pawekiae*	6	2.00	2.75	0.57	0.064	0.270	3
ssp. *stenophylla* ZAF[b]	5	1.63	2.37	0.45	0.072	0.191	4
ssp. *stenophylla* BWA[c]	9	1.88	2.41	0.63	0.042	0.214	5
ssp. *stenophylla* (total)	14	2.20	2.55	0.77	0.053	0.280	9
ssp. *alba*	9	1.54	2.46	0.37	0.017	0.134	2
ssp. *tenuis* ZWE[d]	9	1.51	2.28	0.40	0.008	0.135	4
ssp. *tenuis* ZAF[e]	15	1.71	2.31	0.54	0.016	0.165	2
ssp. *tenuis* (total)	24	2.06	2.61	0.66	0.015	0.206	6
ssp. *pubescens*	13	1.37	2.18	0.31	0.004	0.103	1
var. *spontanea* BWA[f]	14	1.68	2.33	0.51	0.016	0.133	0
var. *spontanea*	77	2.80	3.25	0.80	0.025	0.168	6
var. *spontanea* (total)	91	2.86	3.32	0.80	0.023	0.199	9
Wild accessions	186	4.08	4.27	0.94	0.022	0.290	
Cultivated accessions	13	1.26	2.12	0.23	0	0.084	

[a] Abbreviations for gene diversity statistics include *N* (number of accessions), *A* (average number of alleles per locus), *Ap* (average number of alleles per polymorphic locus), *P* (proportion of loci polymorphic), *Hs* (mean diversity within accessions), *Ht* (total diversity), and *U* (number of unique alleles per group)
[b] ssp. *stenophylla* ZAF includes accessions from South Africa
[c] ssp. *stenophylla* BWA includes accessions from Botswana
[d] ssp. *tenuis* ZWE includes inland accessions from Malawi, Zambia, and Zimbabwe
[e] ssp. *tenuis* ZAF includes coastal accessions from South Africa and Mozambique
[f] ssp. *spontanea* BWA includes the 14 accessions from Southern Africa separated by the cluster analysis

Interrelationships between cultivated and wild *V. unguiculata*

The closest taxa to the cultivated cowpea among all subspecies studied were var. *spontanea* and ssp. *pubescens* (Figs. 1, 2 and 3, Tables 2 and 5). To investigate relationships between ssp. *pubescens*, var. *spontanea*, and cultivated (var. *unguiculata*) accessions, PCO analyses were performed on the distance matrix. Accessions were plotted by their first two principal coordinates which accounted for 53.0% of the total variance in the distance matrix (Fig. 3). This analysis shows that cultivated cowpea is a relatively homogeneous assemblage. The cultivated cowpea overlaps with var. *spontanea* but not with ssp. *pubescens*, while var. *spontanea* overlaps with ssp. *pubescens*. The cultivated cowpea is separated from var. spontanea by *Amp2* alleles (Table 2). Most cultivated accessions and the three wild accessions (SP 37, SP 38 and SP 80) which plotted within the cultivated group possess allozyme $Amp2^{102}$, while the cultivated accessions (NO 2300 and NO 3076) plotted within var. *spontanea* accessions are cv gr Textilis accessions which possess the $Amp2^{100}$ allozyme. Cultivated cowpea is separated from ssp. *pubescens* by two alleles: $Amp2^{102}$ versus $Amp2^{98}$ and $Amp4^{100}$ versus $Amp4^{96}$ (Table 2).

The 31 wild accessions closest to the cultivated group [the average Nei (1972) distance between these wild accessions and the 13 cultivated accessions was lower than 0.15] included 29 var. *spontanea* accessions, and also NI 1186, and SP 162. The latter two accessions are perennial accessions introgressed by ssp. *unguiculata*. The 29 var. *spontanea* accessions come from 11 countries scattered throughout Africa, i.e. Cameroon, Central African Republic, Guinea-Bissau, Kenya, Madagascar, Malawi, Niger, Senegal, South Africa, Tanzania, and Zaire. In the same way, the seven wild accessions displaying the $Amp2^{102}$ allele, common in most cultivated accessions, come from six different countries scattered throughout Africa, i.e. Cameroon, Congo, Kenya, Malawi, Niger, and South Africa.

Discussion

Variability of wild *V. unguiculata* across its geographic range

In contrast to previous studies (Panella and Gepts 1992; Vaillancourt et al. 1993), we found evidence that the genetic variation in wild cowpea from different subspecies is structured. First, there are alleles which characterize subspecies (Table 2). For example, in the case of ssp. *baoulensis*, $Dia1^{103}$, Enp^{109}, Enp^{107} and $\beta Glu1^{94}$ are not encountered in other subspecies, while $Amp2^{97}$, $\beta Glu1^{94}$, $Idh1^{90}$ and $Pgi2^{92}$, are not the "most common alleles" in other subspecies. Second, there is

Table 4 Partitioning of total diversity (H_t) for each polymorphic locus and averaged for the 35 loci: diversity within subspecies (H_s), between subspecies gene diversity (D_{st}), and proportion of total allelic diversity found among subspecies (G_{st})

Loci	H_t	H_s	D_{st}	G_{st}
Adh1	0.094	0.077	0.017	0.181
Adh2	0.037	0.028	0.009	0.236
Amp2	0.513	0.288	0.225	0.439
Amp3	0.484	0.348	0.136	0.280
Amp4	0.611	0.461	0.150	0.245
Dia1	0.102	0.080	0.023	0.221
Dia2	0.126	0.082	0.044	0.350
Enp	0.592	0.422	0.171	0.288
Est1	0.286	0.165	0.122	0.424
Est3	0.534	0.342	0.192	0.360
Fdh	0.444	0.350	0.094	0.212
Fle1	0.216	0.146	0.070	0.324
Fle3	0.392	0.303	0.089	0.227
βGlu1	0.544	0.326	0.218	0.400
βGlu3	0.273	0.097	0.176	0.643
G6pd	0.562	0.338	0.223	0.398
Got1	0.042	0.029	0.013	0.319
Got2	0.245	0.222	0.023	0.093
Gr	0.363	0.120	0.244	0.671
Idh1	0.216	0.126	0.090	0.418
Idh2	0.107	0.026	0.081	0.755
Mdh1	0.179	0.129	0.050	0.278
Mdh2	0.005	0.005	0.000	0.033
Mdh3	0.005	0.005	0.000	0.033
Me	0.580	0.467	0.113	0.194
Mpi	0.475	0.324	0.151	0.317
Pgd1	0.267	0.236	0.031	0.115
Pgd2	0.269	0.174	0.094	0.352
Pgi2	0.412	0.204	0.208	0.504
Pgi3	0.443	0.214	0.228	0.516
Pgm1	0.088	0.075	0.012	0.141
Pgm2	0.088	0.075	0.012	0.140
Sdh	0.566	0.408	0.148	0.266
All 35 loci	0.290	0.191	0.099	0.341

subspecies partitioning of genetic variation as measured from G_{st} (Table 4). Third, there are different genetic distances between accessions of the same and different subspecies (Table 5). Distances between accessions of different subspecies (or cluster analysis groups) are markedly higher than distances between accessions belonging to the same subspecies. Most of the inter-subspecific distances are higher than 0.4, which is the grand mean Nei (1972) distance for populations of congeneric species (Crawford 1989). Fourth, there is a clustering of the UPGMA phenogram (Fig. 1), which is fairly stable, whatever the genetic distance employed.

The different conclusions drawn from these studies are partly due to different electrophoretic or staining techniques, especially regarding the choice of the enzymes studied. First, in the present work 35 loci were studied whereas Panella and Gepts (1992) examined 24 loci and Vaillancourt et al. (1993) 26. Second, most of the loci studied by Panella and Gepts (1992) or Vaillancourt et al. (1993), and not studied here, were monomorphic (*Ald*, *Rbco*, *Xdh* and *Sod3*) or poorly variable (*Aco1*, *Fk*, *Sod1*, *Tpi1* and *Tpi2*). The latter loci yielded rare variants not helpful for subspecies distinction and G_{st} was lower than 0.15 for these five loci. Only *Aco2* ($G_{st} = 0.43$ in Vaillancourt's study and 0.46 in Panella's study) and *Prx* ($G_{st} = 0.83$) were of interest regarding subspecies distinction and mainly characterized ssp. *baoulensis*. On the other hand, the loci studied here and not by other authors, i.e. *Amp3*, *Enp*, *Est1*, *Est3*, *βGlu1*, *βGlu3*, and *Gr*, include some of the most polymorphic loci and the most suitable for separating subspecies (Table 4).

However, the different conclusions drawn by these studies are mainly the result of the greater diversity of the material examined. Thirty out of the forty three wild accessions studied by Vaillancourt et al. (1993) and 39 out of the 56 wild accessions studied by Panella and Gepts (1992) were included in the present study while 107 out of our 186 wild accessions were never previously examined. Regarding the perennial material, the difference is striking: Vaillancourt et al. (1993) analyzed only 17 accessions from six different perennial subspecies, and Panella and Gepts (1992) examined 29 accessions from only six different perennial subspecies. By contrast the present work included 91 accessions from eight subspecies. Therefore, wild cowpea diversity, and especially the number of alleles per loci, is much higher ($H_t = 0.290$, $A = 4.08$) than in both the Panella and Gepts (1992) ($H_t = 0.110$, $A = 2.00$) and the Vaillancourt et al. (1993) ($H_t = 0.168$, $A = 2.42$) studies.

Both the Panella and Gepts (1992) and Vaillancourt et al. (1993) conclusions were hampered by the use of the Mithen and Kibblewhite (1993) morphological classification. Vaillancourt et al. (1993) reported that accessions of var. *protracta sensu* Mithen were found dispersed among four different clusters. However, once the accessions had been correctly identified, the three ssp. *pubescens* accessions clustered together within a larger var. *spontanea* cluster and NI 1186 was within the cultivated cluster, just as in the present analysis. They also reported that three accessions of var. *tenuis* clustered together with a number of accessions from var. *dekindtiana* and *huillensis*, while two other accessions of var. *tenuis* did not cluster with any other accessions. Using proper identification shows that six of the seven ssp. *tenuis* accessions are in the same cluster with five var. *spontanea* accessions from Southern Africa. Vaillancourt et al. (1993) were obliged to conclude that a general lack of agreement between clustering and botanical nomenclature was evident. In the same way, using the Mithen and Kibblewhite (1993) morphological classification, Panella and Gepts (1992) concluded that it was possible that populations not clearly fitting into any other classification have been lumped together as var. *dekindtiana* by default, since their cluster analysis first separated the four ssp. *baoulensis* accessions identified as var. *dekindtiana sensu lato* in Mithen's classification.

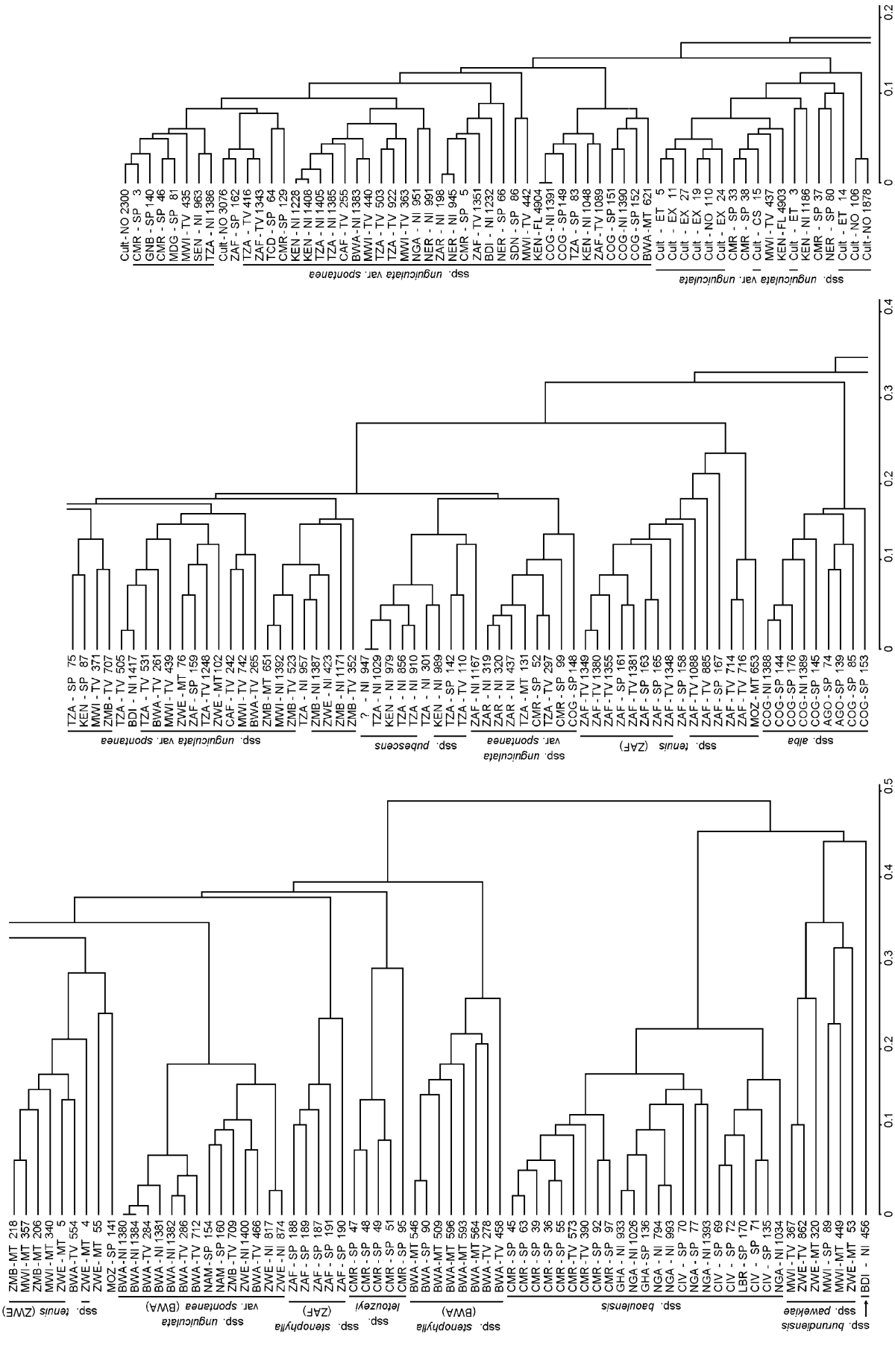

Fig. 1 UPGMA phenogram of 199 accessions of *V. unguiculata* using Nei (1972) genetic distances values. Taxa lines are broken where the accessions do not belong to the taxon

Table 5 The distribution of Nei (1972) genetic distances between accessions within or between groups. The upper line is the minimum distance, the middle line is mean distance (in bold), and the lower line is maximum distance (lower triangle). The distribution of Rogers (1972) distances calculated from group allelic frequencies (upper triangle). For each column, the number of accessions studied is given in brackets

Accession	ssp. *baoulensis* (23)	ssp. *letouzeyi* (5)	ssp. *burundiensis* (1)	ssp. *pawekiae* (6)	ssp. *ste.* ZAF[a] (5)	ssp. *ste.* BWA[b] (9)	var. *spon.* B[c] (14)	ssp. *alba* (9)	ssp. *ten.* ZWE[d] (9)	ssp. *ten.* ZAF[e] (15)	ssp. *pubesces* (13)	var. *spontanea* (77)	Cult. (13)
ssp. *baoulensis* (23)	0.022 **0.171** 0.384	0.417	0.525	0.426	0.488	0.450	0.540	0.506	0.483	0.463	0.429	0.428	0.465
ssp. *letouzeyi* (5)	0.164 **0.399** 0.560	0.075 **0.190** 0.377	0.516	0.441	0.461	0.427	0.522	0.490	0.422	0.387	0.385	0.390	0.410
ssp. *burundiensis* (1)	0.355 **0.522** 0.722	0.377 **0.468** 0.560	–	0.486	0.560	0.568	0.605	0.584	0.536	0.579	0.556	0.542	0.588
ssp. *pawekiae* (6)	0.253 **0.448** 0.623	0.315 **0.465** 0.624	0.315 **0.446** 0.611	0.106 **0.308** 0.437	0.462	0.443	0.499	0.488	0.462	0.417	0.428	0.383	0.453
ssp. *steno.* ZAF[a] (5)	0.332 **0.554** 0.803	0.272 **0.497** 0.616	0.566 **0.579** 0.589	0.366 **0.518** 0.671	0.108 **0.176** 0.285	0.362	0.483	0.443	0.430	0.357	0.396	0.366	0.430
ssp. *steno.* BWA[b] (9)	0.353 **0.543** 0.783	0.283 **0.432** 0.612	0.457 **0.627** 0.708	0.343 **0.557** 0.800	0.265 **0.400** 0.556	0.044 **0.236** 0.427	0.427	0.450	0.433	0.334	0.368	0.339	0.410
var. *spon.* BWA[c] (14)	0.420 **0.657** 0.867	0.405 **0.538** 0.686	0.568 **0.644** 0.737	0.351 **0.548** 0.778	0.338 **0.477** 0.589	0.238 **0.410** 0.578	0 **0.139** 0.234	0.420	0.462	0.394	0.406	0.363	0.453
ssp. *alba* (9)	0.363 **0.547** 0.737	0.348 **0.499** 0.650	0.545 **0.607** 0.768	0.273 **0.557** 0.798	0.321 **0.426** 0.575	0.271 **0.447** 0.679	0.219 **0.342** 0.504	0.037 **0.144** 0.247	0.437	0.391	0.380	0.357	0.427
ssp. *ten.* ZWE[d] (9)	0.398 **0.569** 0.895	0.322 **0.414** 0.545	0.464 **0.505** 0.528	0.351 **0.524** 0.745	0.300 **0.388** 0.481	0.239 **0.466** 0.664	0.245 **0.414** 0.595	0.245 **0.406** 0.545	0.067 **0.163** 0.210	0.333	0.355	0.337	0.391
ssp. *ten.* ZAF[e] (15)	0.315 **0.517** 0.737	0.209 **0.368** 0.570	0.545 **0.647** 0.722	0.260 **0.489** 0.678	0.153 **0.328** 0.481	0.091 **0.356** 0.585	0.132 **0.348** 0.528	0.209 **0.367** 0.596	0.188 **0.302** 0.457	0.022 **0.179** 0.363	0.319	0.258	0.307
ssp. *pubescens* (13)	0.260 **0.420** 0.611	0.216 **0.322** 0.420	0.420 **0.537** 0.665	0.228 **0.431** 0.624	0.217 **0.404** 0.556	0.140 **0.369** 0.611	0.184 **0.340** 0.511	0.164 **0.309** 0.496	0.216 **0.313** 0.473	0.154 **0.289** 0.435	0 **0.116** 0.223	0.225	0.272
var. *spontanea* (77)	0.260 **0.464** 0.730	0.209 **0.354** 0.511	0.384 **0.561** 0.686	0.198 **0.418** 0.700	0.219 **0.361** 0.536	0.116 **0.370** 0.623	0.100 **0.334** 0.560	0.103 **0.305** 0.545	0.098 **0.327** 0.528	0.029 **0.265** 0.473	0.052 **0.185** 0.391	0 **0.163** 0.489	0.220
Cult. (13)	0.260 **0.438** 0.560	0.223 **0.330** 0.420	0.511 **0.592** 0.665	0.223 **0.448** 0.624	0.215 **0.384** 0.556	0.164 **0.418** 0.623	0.223 **0.397** 0.528	0.223 **0.369** 0.596	0.234 **0.338** 0.457	0.029 **0.254** 0.405	0.082 **0.184** 0.336	0.022 **0.179** 0.473	0.029 **0.096** 0.188

[a] ssp. *stenophylla* ZAF includes accessions from South Africa
[b] ssp. *stenophylla* BWA includes accessions from Botswana
[c] ssp. *spontanea* BWA includes the 14 accessions from Southern Africa separated by the cluster analysis
[d] ssp. *tenuis* ZWE includes inland accessions from Malawi, Zambia, and Zimbabwe
[e] ssp. *tenuis* ZAF includes coastal accessions from South Africa and Mozambique

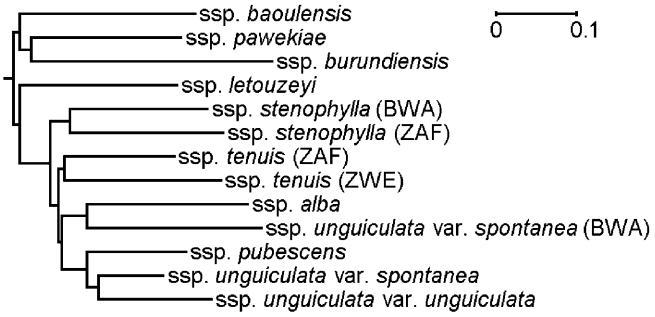

Fig. 2 Neighbor-joining cluster analysis of systematic relationships among subspecies based on the Rogers (1972) genetic distance derived from allele frequencies at 35 allozyme loci. The tree is rooted by midpoint rooting. The scale bar represents a Rogers distance unit

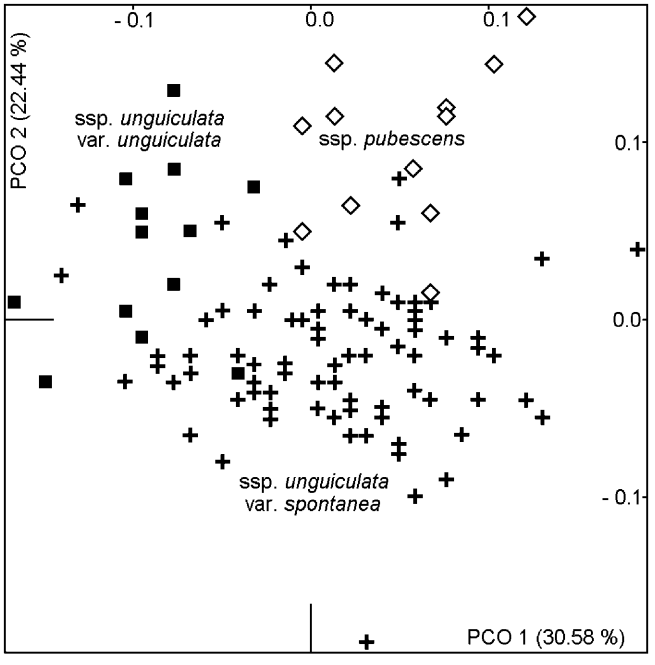

Fig. 3 Principal co-ordinate analysis of 103 accessions from ssp. *pubescens* and ssp. *unguiculata* based on the Nei (1972) distance matrix. The first two axes account for 30.6% and 22.4% of the total variance, respectively

In the present study, both ssp. *stenophylla* and ssp. *tenuis* were split between two clusters. In both cases, one can see: (1) a geographical correlation (inland versus coastal), (2) a correlation with the breeding system (most inland ssp. *tenuis* and coastal ssp. *stenophylla* show an outbreeding floral morphology while most coastal ssp. *tenuis* and inland ssp. *stenophylla* accessions show a floral morphology consistent with an inbreeding breeding system), and (3) a correlation with some morphological characters. Stems and pods of the inland ssp. *stenophylla* accessions are scabrous while these organs are pubescent in the coastal ssp. *steno-phylla* accessions. Inland ssp. *tenuis* accessions have large seeds and a low ovule number, whereas the coastal ssp. *tenuis* accessions have smaller seeds and a higher ovule number. Moreover, the var. *spontanea* accessions associated with the inland ssp. *tenuis* cluster, i.e. MT 55 and SP 141 [and at least MT 62 and MT 65 studied by Panella and Gepts (1992) and Vaillancourt et al. (1993)] can be considered either as var. *spontanea* accessions with tuberous roots or as ssp. *tenuis* accessions with hastate leaves, multi-noded inflorescences and a high ovule number. Therefore, if these accessions are related with ssp. *tenuis* through allozyme profiles, they are also morphologically related with ssp. *tenuis*. The correlation between allozyme and morphological data goes beyond the actual morphological classification.

Morphological studies of these accessions show that the different subspecies can be classified according to breeding system, precisely according to the disposition of anthers and stigma within the flower. Regarding the subspecies studied here, the perennial outcrossers include ssp. *baoulensis*, ssp. *burundiensis*, ssp. *letouzeyi* and ssp. *pawekiae*, while the perennial out-inbreds include ssp. *stenophylla*, ssp. *tenuis*, ssp. *alba* and ssp. *pubescens* (Pasquet 1994). Table 5 and Fig. 1 show that the perennial outcrossers are the group most remote from ssp. *unguiculata* and that these groups are separated by the highest distances. Most mean intergroup Nei (1972) distances higher than 0.4 involve perennial outcrossers, while most mean intergroup distances within perennial out-inbreds are lower than 0.4. On the other hand, ssp. *pubescens* and ssp. *unguiculata* appear very close despite clear morphological distinctions. Mean intergroup distances within ssp. *pubescens* and ssp. *unguiculata* are lower than 0.2. Rogers (1972) distances yield similar features: distances higher than 0.45 concern perennial outcrossers while most mean intergroup distances within perennial out-inbreds are lower than 0.45.

We can thus hypothesize that the first step in wild cowpea evolution was the diversification of perennial outcrossers, which appear primitive, since the genetic distances between perennial outcrosser accessions are as high as those usually reported between different species (Crawford 1989). The second step would have involved the diversification of perennial out-inbreds, which appear to have evolved more recently, since the genetic distances between perennial out-inbreds are lower than those usually reported between different species (Crawford 1989). This is well illustrated by the Neighbor-Joining phenogram (Saitou and Nei 1987) derived from the Rogers genetic distances (Fig. 2). Perennial outcrossers are at the bottom of

the tree while perennial out-inbreds are at a higher position.

Once all the convergences between morphological and allozyme data are highlighted, there remain few discrepancies, and these can be explained by introgression phenomena. The first example is SP 158 (from South Africa), a ssp. *stenophylla* accession which is as close to ssp. *tenuis* (0.09–0.23 with coastal ssp. tenuis, 0.31–0.47 with inland ssp. *tenuis*) as to other ssp. *stenophylla* accessions (0.22–0.42 with inland ssp. *stenophylla*, 0.35–0.43 with coastal ssp. *stenophylla*). However, SP 158 shows pubescent stems and unbeaked keels twisted towards the left, as do all ssp. *stenophylla* accessions, but a uninodal inflorescence, which is a ssp. *tenuis* character. Therefore, SP 158 is a ssp. *stenophylla* accession introgressed by ssp. *tenuis*, which explains its clustering with ssp. *tenuis* (Fig. 1). The second example concerns NI 301 and SP 142. NI 301 is a lineage originating from a seed taken from the var. *spontanea* herbarium specimen Richards 17805 (K). NI 301 is a var. *spontanea* accession which shows few ssp. *pubescens* allozymes ($Amp2^{98}$, Enp^{105}) and clusters with other ssp. *pubescens* accessions (Fig. 1). SP 142 is a lineage obtained from another seed from the same herbarium specimen, but this accession shows long inflorescence internodes and pubescent stems and pods and is identified as a ssp. *pubescens* accession. SP 142 clusters with other ssp. *pubescens* accessions (Fig. 1). Therefore, Richards 17805 (K) is obviously a var. *spontanea* specimen introgressed by ssp. *pubescens*. These introgression phenomena explain the higher var. *spontanea* diversity (Table 3).

The only discrepancy which is not yet fully explained is var. *spontanea* BWA. The cluster var. *spontanea* BWA (Fig. 1) is made up of 14 accessions from Southern Africa: eight from Botswana, three from Zimbabwe, two from Namibia and one from Zambia. More precisely, these accessions come from the area where the geographical distributions of ssp. *stenophylla* and var. *spontanea* overlap (Pasquet, unpublished data). Morphologically, these accessions cannot be separated from other var. *spontanea* accessions. They cluster together because they show marked differences in allele frequencies at ten loci (Table 2). In six of these loci (*Amp3*, *Amp4*, *Enp*, *Est3*, *βGlu1* and *Sdh*) var. *spontanea* BWA allele frequencies are much closer to those of ssp. *stenophylla*, which suggests introgression. However, five of the allozymes encountered at high frequency in var. *spontanea* BWA and at low frequency in var. *spontanea*, i.e. Fdh^{96}, $Fle1^{94}$, $Fle1^{97}$, $Fle3^{104}$ and $Pgd1^{94}$, are not encountered in ssp. *stenophylla*. This could be explained by an insufficient sampling (eight accessions) of ssp. *stenophylla* in this area. However, this could also be explained by introgression from another allozymatically unknown perennial taxon, such as var. *dekindtiana sensu stricto*, encountered in Angola, Zambia, and Zimbabwe.

Genetic variation in wild and cultivated cowpea and insights into the domestication of cowpea

During the initial phases of domestication, it is assumed that only a fraction of the total genetic variation present in an ancestral taxon will be incorporated into a newly evolved domesticate (Doebley 1989). The total genetic diversity in cultivated cowpea ($H_t = 0.084$) was higher than that reported by Panella and Gepts (1992) ($H_t = 0.018$) and Vaillancourt et al. (1993) ($H_t = 0.029$). However, this higher value was due to the present sampling including only cultivated accessions displaying different allozyme profiles. We found a lower value ($H_t = 0.053$), closer to those previously reported, while screening 191 accessions (Pasquet 1994). Nevertheless, cultivated cowpea diversity values are lower than those reported in many other crops (Doebley 1989), and especially legume crops, because authors reported $H_t = 0.140$ in *Glycine max* (Kiang et al. 1987), $H_t = 0.092$ in *Phaseolus acutifolius* (Schinkel and Gepts 1989), $H_t = 0.209$ in *P. vulgaris* (Singh et al. 1991), and $H_t = 0.055$ in *Sphenostylis stenocarpa* (Potter and Doyle 1992). The low diversity of cultivated cowpea suggests that cowpea was domesticated once and only once, unlike *P. vulgaris* (Singh et al. 1991) or rice (Second 1985).

Estimates of genetic variability from this study indicate that wild cowpea is much more diverse genetically than is cultivated cowpea. When wild cowpeas are compared with cultivated cowpeas, the reduction in genetic diversity is manifested in three ways: as a reduction in allelic diversity (A and A_p), as a reduction in the proportion of polymorphic loci (P), and as a reduction in estimated heterozygosity (H_t) (Table 3). This reduction appears important because the diversity in wild cowpea ($H_t = 0.290$) is much higher than that of cultivated cowpea ($H_t = 0.084$), and the bottleneck between wild and cultivated cowpea has been called strong or severe by previous authors (Panella and Gepts 1992; Vaillancourt et al. 1993). Indeed, Weeden et al. (1996) used the words "extreme bottleneck".

However, the closest group to cultivated cowpea among all the subspecies studied is var. *spontanea* (Figs. 1, 2 and 3, Tables 2 and 5). Reducing the cultivated cowpea progenitor to var. *spontanea* is logical because most perennial subspecies could be considered as different species if only genetic distances were considered (Crawford 1989). Other legume crop progenitors are annual inbreeding taxa, just like var. *spontanea*. They show diversity, i.e. $H_t = 0.150$ in *Glycine soja* (Kiang et al. 1987), $H_t = 0.248$ in *P. acutifolius* (Schinkel and Gepts 1989), $H_t = 0.132$ in *P. vulgaris* (Singh et al. 1991) and $H_t = 0.200$ in *S. stenocarpa* (Potter and Doyle 1992), closer to that of var. *spontanea* ($H_t = 0.199$) than to wild cowpea ($H_t = 0.290$). Therefore, if one considers that the sister group and the most probable progenitor of cultivated cowpea is var. *spontanea*, then the bottleneck appears more

reasonable and closer to that encountered in most crop legumes.

Beyond var. *spontanea*, our data suggest no narrowly defined center of origin for cultivated cowpea. Vaillancourt et al. (1993) reached the same conclusion. Accessions closest to cultivated cowpea are dispersed throughout Africa, from Senegal to South Africa. Considering $Amp2^{102}$, which characterized most cultivated accessions, does not help since var. *spontanea* accessions displaying $Amp2^{102}$ are also dispersed from Niger to South Africa. The point of origin of the cultivated cowpea is obscured by the near absence of available var. *spontanea* accessions from North-East Africa, i.e. Sudan, Eritrea, and Ethiopia. It is also blurred by the existence of numerous weedy forms. These weedy forms can be identified morphologically by their larger seed size (Panella and Gepts 1992; Padulosi 1993) or ecologically if they grow in fields or disturbed areas. In Niger, for example, wild cowpea was always collected in, or in the vicinity of, cultivated areas and was never encountered in undisturbed areas like the National Park (Pasquet, unpublished data), and all accessions and herbarium specimens from Niger should be considered as weedy. Unfortunately, ecological data are missing for most of the accessions studied.

Conclusion

The results of this study yield a renovated image of the cowpea gene pool. This gene-pool is characterized by its unusually large size. It encompasses taxa which could be considered as different species in light of the high genetic distances observed between accessions belonging to different taxa. However, these taxa show a marked morphological homogeneity, weak genetic barriers between them, and introgression phenomena which justify their subspecies rank. These taxa can be classified into three groups characterized by their breeding systems: the perennial outcrossers, the perennial out-inbreds, and the inbred annuals. Allozyme data confirm this grouping. Perennial outcrossers look primitive and are more remote from each other and from out-inbred taxa. With the exception of the 14 var. *spontanea* accessions from Bostwana and its vicinity, there is an excellent fit between the morphological and allozyme data, which has not been reported by other authors.

Within this large gene-pool, mainly consisting of perennial taxa, the annual subspecies (ssp. *unguiculata*) includes both cultivated cowpea (var. *unguiculata*) and wild annual cowpea (var. *spontanea*). Despite the closely related perennial ssp. *pubescens*, ssp. *unguiculata* var. *spontanea* is clearly the progenitor of the wild cowpea. However, var. *spontanea* is widely distributed in Africa and a narrowly defined center of origin for cultivated cowpea cannot be identified as yet.

Regarding the future of cowpea breeding programs, this study shows that the most important material is not var. *spontanea*, which is genetically too close to the cultivated forms to be very helpful in yielding useful genes, but the perennial subspecies and especially the perennial outcrossers. However, this perennial material is poorly represented in living collections (no ssp. *aduensis* accession, one ssp. *burundiensis* accession, five ssp. *letouzeyi* accessions from one country, six ssp. *paweikae* accessions) which are mostly made of annual accessions. Two-thirds of the wild cowpea accessions from the IPGRI collection in Meise are var. *spontanea* accessions (Vanderborght, personnal communication) as are 3/4ths of the wild cowpea accessions from the IITA collection in Ibadan, Nigeria (Padulosi 1993).

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References

Barone A, Del Giudice A, Ng NQ (1992) Barriers to interspecific hybridization between *Vigna unguiculata* and *Vigna vexillata*. Sex Plant Reprod 5:195–200

Crawford DJ (1989) Visualization and interpretation of plant isozymes. In: Soltis DE, Soltis PS (eds) Isozymes in plant biology. Chapman and Hall, London, pp 146–164

Doebley J (1989) Isozymic evidence and the evolution of crop plants. In: Soltis DE, Soltis PS (eds) Isozymes in plant biology. Chapman and Hall, London, pp 165–191

Faris DG (1965) The origin and evolution of the cultivated forms of *Vigna sinensis*. Can J Genet Cytol 7:433–452

Fery RL (1990) The cowpea: production, utilization, and research in the United States. Hort Rev 12:197–222

Fotso M, Azanza JL, Pasquet R, Raymond J (1994) Molecular heterogeneity of cowpea (*Vigna unguiculata*, *Fabaceae*) seed storage proteins. Plant Syst Evol 191:39–56

Harris H, Hopkinson DA (1976) Handbook of enzyme electrophoresis in human genetics. North Holland, Amsterdam

Kiang YT, Chiang YC, Doong JYH, Gorman MB (1987) Genetic variation of soybean germplasm. In: Crop exploration and genetic resources. Taichung District Agricultural Improvement Station, Taiwan, pp 93–99

Koenig R, Gepts P (1989) Segregation and linkage of genes for seed proteins, isozymes, and morphological traits in common bean (*Phaseolus vulgaris*). J Hered 80:455–459

Lush WM (1979) Floral morphology of wild and cultivated cowpeas. Econ Bot 33:442–447

Maréchal R, Mascherpa JM, Stainier F (1978) Etude taxonomique d'un groupe complexe d'espèces des genres *Phaseolus* et *Vigna* (*Papilionaceae*) sur la base de données morphologiques et polliniques, traitées par l'analyse informatique. Boissiera 28:1–273

Mithen R, Kibblewhite H (1993) Taxonomy and ecology of *Vigna unguiculata* (*Leguminosae-Papilionoideae*) in South-Central Africa. Kirkia 14 : 100–113

Nei M (1972) Genetic distance between populations. Am Nat 106 : 283–292

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70 : 3321–3323

Padulosi S (1993) Genetic diversity, taxonomy and ecogeographic survey of the wild relatives of cowpea [*Vigna unguiculata* (L.) Walpers]. Thèse, Université catholique, Louvain la Neuve, Belgium

Panella L, Gepts P (1992) Genetic relationships within *Vigna unguiculata* (L.) Walp. based on isozyme analyses. Genet Res Crop Evol 39 : 71–88

Panella L, Kami J, Gepts P (1993) Vignin diversity in wild and cultivated taxa of *Vigna unguiculata* (L.) Walp (*Fabaceae*). Econ Bot 47 : 371–386

Pasquet RS (1993 a) Classification infraspécifique des formes spontanées de *Vigna unguiculata* (L.) Walp. à partir de données morphologiques. Bull Jard Bot Nat Belg 62 : 127–173

Pasquet RS (1993 b) Variation at isozyme loci in wild *Vigna unguiculata* (L.) Walp. (*Fabaceae*, *Phaseoleae*). Plant Syst Evol 186 : 157–173

Pasquet RS (1993 c) Two new subspecies of *Vigna unguiculata* (L.) Walp. (*Leguminosae* : *Papilionoideae*). Kew Bull 48 : 805–806

Pasquet RS (1994) Organisation évolutive des formes spontanées et cultivées du niébé, *Vigna unguiculata* (L.) Walp. Biosystématique et processus de domestication. Thèse, INAPG, Paris, France

Pasquet RS (1997) A new subspecies of *Vigna unguiculata* (*Leguminosae-Papilionoideae*). Kew Bull 52 : 840

Pienaar BJ, Van Wyk AE (1992) The *Vigna unguiculata* complex (*Fabaceae*) in southern Africa. S Afr J Bot 58 : 414–429

Piper CV (1913) The wild prototype of the cowpea. USDA, Bureau of Plant Industry, Circular No. 124. Miscellaneous Papers. Applied Biostatistics. Government Printing Office, Washington, Setauket, New York, pp 29–32

Potter D, Doyle JJ (1992) Origins of the African yam bean (*Sphenostylis stenocarpa*, Leguminosae): evidence from morphology, isozymes, chloroplast DNA, and linguistics. Econ Bot 46 : 276–292

Rawal KM, Rachie KO, Franckowiak JD (1976) Reduction in seed size in crosses between wild and cultivated cowpeas. J Hered 67 : 253–254

Rogers JS (1972) Measures of genetic similarity and genetic distance. Studies in Genetics VII. University of Texas Publ 7213, Austin, Texas, pp 145–153

Rohlf FJ (1993) NTSYS-PC: numerical taxonomy and multivariate analysis system, version 1.80

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4 : 406–425

Sakupwanya S, Mithen R, Mutangandura-Mhlanga T (1989) Studies on the African *Vigna* gene pool. II. Hybridization studies with *Vigna unguiculata* var. *tenuis* and var. *stenophylla*. Plant Genet Res Newslett 78–79 : 5–9

Schinkel C, Gepts P (1989) Allozyme variability in the Tepary Bean, *Phaseolus acutifolius* A. Gray. Plant Breed 102 : 182–195

Second G (1985) Evolutionary relationships in the Sativa group of *Oryza* based on isozyme data. Génét Sél Evol 17 : 89–114

Second G, Trouslot P (1980) Electrophorèse d'enzymes de riz (*Oryza* sp.). ORSTOM, Paris

Singh BB, Chambliss OL, Sharma B (1997) Recent advances in cowpea breeding. In: Singh BB, Mohan Raj DR, Dashiell KE, Jackai LEN (eds) Advances in cowpea research. IITA-JIRCAS, Ibadan, pp 30–49

Singh SP, Nodari R, Gepts P (1991) Genetic diversity in cultivated common bean. 1. Allozymes. Crop Sci 31 : 19–23

Sneath PHA, Sokal RR (1973) Numerical taxonomy. W.H. Freeman, San Fransisco

Swofford DL, Selander RK (1981) BIOSYS-1. A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J Hered 72 : 281–283

Vaillancourt RE, Weeden NF (1992) Chloroplast DNA polymorphism suggests a nigerian center of domestication for the cowpea, *Vigna unguiculata* (*Leguminosae*). Am J Bot 79 : 1194–1199

Vaillancourt RE, Weeden NF, Barnard J (1993) Isozyme diversity in the cowpea species complex. Crop Sci 33 : 606–613

Weeden NF, Wolko B, Vaillancourt R (1996) Contrasting patterns of partitioning genetic diversity in cultivated legumes. In: Pickersgill B, Lock JM (eds) Advances in legume systematics 8: legumes of economic importance. Royal Botanic Gardens, Kew, pp 1–9

Wendel JF, Weeden NF (1989) Visualization and interpretation of plant isozymes. In: Soltis DE, Soltis PS (eds) Isozymes in plant biology. Chapman and Hall, London, pp 5–45