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CHLOROGENIC ACIDS AND CAFFEINE AS POSSIBLE TAXONOMIC CRITERIA IN *COFFEA* AND *PSILANTHUS*

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Key Word Index—*Coffea*; *Psilanthus*; Rubiaceae; Cinchonoideae; seeds; chemotaxonomy; chlorogenic acids; caffeine; alkaloids.

Abstract—This paper reports the amount of chlorogenic acids, chlorogenic acid-like components, caffeine and other 276 nm-absorbing components in seeds of *Coffea* and *Psilanthus*. Twenty species have been grouped according to their seed composition and the groupings compared critically with the existing classification scheme based upon morphology. Concordance is generally good, although classification on seed composition suggests that *Coffea* subgen. *Coffea* could be further subdivided and that *Psilanthus* subgen. *Afrocoffea* may merit recognition at generic rather than subgeneric level. It also appears that such analyses may provide a better understanding of the morphologically variable taxa such as *C. congensis*, *C. stenophylla* and the libero-excelsoides group.

INTRODUCTION

Coffees belong to the Rubiaceae subfamily Cinchonoideae tribe Coffeae. This tribe has recently [1] been restricted to two genera, *Coffea* and *Psilanthus*. Currently three subgenera are recognised in *Coffea* and two in *Psilanthus* [2–4] which listed in order of relationship to *Coffea* subgen. *Coffea* are:

(i) *Coffea* subgen. *Coffea*—*ca* 85 species, including all the commercially useful species, found in Africa, Madagascar and the Mascarene Islands.

(ii) *Coffea* subgen. *Psilanthopsis*—containing only *P. kapakata* found in Angola.

(iii) *Coffea* subgen. *Baracoffea*—including two or three Madagascan species and the north east African *C. rhamnifolia*.

(iv) *Psilanthus* subgen. *Afrocoffea*—*ca* 18 species found in Africa, India and Malesia to the Torres Strait Islands.

(v) *Psilanthus* subgen. *Psilanthus*—containing only *P. mannii*, and possibly another poorly known species, found in west and central Africa.

Because the morphological criteria traditionally used by taxonomists to separate the species in *Coffea* are very variable, there is still some difference of opinion concerning the grouping of species within subgen. *Coffea* and in some cases the exact limits of certain species (or infraspecific taxa). Also the question of whether the placement of subgen. *Afrocoffea* in the genus *Psilanthus* is more appropriate than full generic recognition remains open to re-examination [5].

The seeds (beans) of the commercially more important species *Coffea arabica* (commonly known as arabicas) and *C. canephora* (commonly known as robustas) of subgen. *Coffea* have been extensively analysed and are known to contain relatively large, but distinguishable, amounts of chlorogenic acids (CGA) and caffeine [6,7]. In contrast the seeds of at least some wild or non-commercial species of coffee have much smaller amounts of CGA and caffeine and in some cases approaching zero [8–12]. Although the seed composition varies with seed maturity [13, 14] these reports suggested that a systematic analysis of the seeds of wild coffees might provide data to assist classification based on morphological criteria. This paper presents the results from the analysis of 57 seed samples covering 20 species within the tribe Coffeae and including 17 named commercial varieties of arabicas and three of robustas.

RESULTS AND DISCUSSION

Moisture contents fell in the range of 8 to 11% fresh weight. When caffeine and CGA were present at concentrations in excess of the lower limit of integration they have been recorded in Tables 1 to 2 on a % dry mass basis (dmb). Components visually detectable on the chromatograms at concentrations less than the lower limits of integration (0.04% as 5-CQA; 0.02% as caffeine) have been denoted in the Tables by the symbol (+). The chlorogenic acids are referred to using the IUPAC [15] numbering system and the system of abbreviations proposed by Clifford [6, 16].

The seven known CGA resolved were 3-CQA, 4-CQA and 5-CQA (reported as total CQA); 3,4-diCQA, 3,5-diCQA and 4,5-diCQA (reported as total diCQA) and 5-FQA reported as such since the quantitatively-minor isomers 3-FQA and 4-FQA are either not resolved, or cannot be attributed to particular peaks on the chromatogram. A number of unidentified components were observed, and those absorbing more strongly at 313 nm than at 276 nm have been described as CGA-like components and their content reported as 5-CQA equivalents. Each CGA-like component is identified by its retention time relative to 5-CQA, and a code number from the sequence 1 to 18, which corresponds to the code used in a previous publication [17]. The identity of these CGA-like

Table 1. Chlorogenic acids and chlorogenic acid-like components

Species and variety	Origin	Cultivation or source	1000 Bean mass	Chlorogenic acids: Total CQA	FQA	Total diCQA
A *Coffea* subgen. *Coffea*						
C. arabica var. *bourbon N*39		Tanzania	118	5.20	0.36	0.46
var. *bourbon vermelho*		Campinas	114	6.63	0.24	0.62
var. *purpurescens*		Tanzania	115	5.03	0.34	0.73
var. *laurina* (12/55)		Tanzania	139	5.13	0.24	0.59
var. *erecta* (181–185)		Tanzania	156	5.50	0.48	0.56
var. *semperflorans*		Tanzania	158	5.04	0.33	0.56
var. *murta*		Tanzania	102	5.12	0.42	0.78
var. *bourbon amarelo*		Tanzania	178	5.89	0.40	0.54
var. *bourbon amarelo*		Campinas	98	6.41	0.32	0.51
var. *mokka*		Tanzania	167	4.43	0.27	0.47
var. *Maragogype*		Palm House, Kew	294	5.44	0.29	1.19
var. *San Ramon* (19/53)		Tanzania	144	4.85	0.29	0.62
var. *caturra* (11/55)		Tanzania	150	4.85	0.33	0.72
var. *polysperma* (1/54)		Tanzania	109	4.73	0.41	0.64
var. *arabica KP*423		Tanzania	162	5.12	0.30	0.57
var. *typica*		Tanzania	167	4.76	0.33	0.54
var. *mundo novo*		Campinas	133	4.83	0.26	0.66
var. *mundo novo*		Campinas	128	4.99	0.25	0.65
var. *catuai vermelho*		Campinas	124	6.13	0.29	0.73
var. *catuai vermelho*		Campinas	119	5.56	0.29	0.70
var. *catuai vermelho*		Campinas	128	4.77	0.27	0.62
C. sessiliflora	Kenya	Ivory Coast	47	6.44	0.20	0.21
C. sessiliflora	Kenya, Kwale district Koubi 417	Ivory Coast/Kew Herbarium +	33	5.20	0.10	0.31
C. mufindiensis	Tanzania, Mufindi Davies C41 1712/31 1981	Kew Herbarium +	60	6.77	0.08	0.29
C. racemosa		Ivory Coast	63	5.09	0.12	0.72
C. racemosa		Campinas 1986	43	4.36	0.13	0.42
C. racemosa		Campinas 1987	44	4.79	0.04	0.50
C. eugenioides		Campinas 1986	44	4.24	0.17	0.15
C. eugenioides	Kenya, Hepper and Maley 7738	Ivory Coast/Kew Herbarium +		4.30	0.10	0.13
C. eugenioides		Ivory Coast	52	3.89	0.32	0.33
C. eugenioides (501–505)		Tanzania	69	5.69	0.27	0.31
C. stenophylla (1073–13)		Campinas 1986	58	6.04	0.16	0.46
C. stenophylla	Ivory Coast, Mt. del'Ira, Hepper and Maley 7728	Ivory Coast/Kew Herbarium +		5.41	0.10	0.53
C. stenophylla (561–565)		Tanzania	55	4.62	0.19	0.24
C. stenophylla		Ivory Coast	140	5.94	0.34	0.56
C. humilis	Ivory Coast	Ivory Coast	128	5.80	0.21	0.67
C. dewevrei	Central African Republic	Ivory Coast	70	6.10	0.61	0.98
C. liberica	Ivory Coast	Ivory Coast	172	6.47	0.32	0.69
C. liberica	Ivory Coast, Indenie	Ivory Coast	198	9.37	0.53	0.73
C. excelsa Sumatra		Ivory Coast	192	6.65	0.38	0.64
C. congensis var. *excelsa* (VC 606–610)		Tanzania	124	5.20	0.67	1.37
C. brevipes (*C. staudtii*)	Cameroun	Ivory Coast	157	4.80	0.32	1.29
C. canephora var. *Ugandae*		Tanzania	111	5.20	0.86	1.64
C. canephora var. *robusta* (VC 1156–1160)		Tanzania	106	4.23	0.98	1.36
C. canephora var. *robusta*		Campinas	94	6.17	0.81	1.31
C. canephora var. *kouillou* (VC 286–290)		Tanzania	105	4.58	1.03	1.14
C. canephora	Central African Republic	Ivory Coast	121	6.91	0.78	1.52
C. congensis	Central African Republic	Ivory Coast	72	6.27	0.50	0.89
C. x arabusta	Ivory Coast	Ivory Coast	120	6.02	0.65	1.25

(%dmb) in the seeds of Coffeae

Chlorogenic acid-like components														
1 (0.57)	3 (1.20)	5 (1.25)	6 (1.42)	7 (1.49)	8 (1.55)	9 (1.60)	10 (1.89)	13 (1.95)	14 (2.00)	15 (2.10)	16 (2.25)	17 (2.30)	18 (2.45)	Total CGA
		+												6.02
+	+	+												7.49
		+												6.10
		+												5.96
	+	+												6.54
	+	+												5.93
	+	+												6.32
	+	+												6.83
+	+	+												7.24
	+	+												5.17
+	+	+												6.92
	+	+												5.76
	+	+												5.90
	+	+												5.78
	+	+												5.99
	+	+												5.63
	+	+												5.75
	+	+												5.89
+	+	+												7.15
+	+	+												6.55
	+	+												5.66
+		0.14												6.99
	+	0.07												5.68
														7.14
+		0.03	0.07											6.03
+		+	0.06											4.97
0.05		+	0.03											5.41
+		+												4.56
+		+												4.53
		+												4.54
		+												6.27
+	+	+												6.66
		+												6.04
		+												5.05
+		0.10					+	0.04	0.04			0.18	0.30	7.50
+		+						+		+		+	+	6.68
		+					+	0.04		0.06	+			7.79
		+					+	+		+				7.48
		+					+	+		+				10.63
0.03		0.08					+	+		+				7.78
		+	+	0.05	+		0.12	0.10	0.25					7.76
+		+					+	0.06	0.12	+		0.06	0.09	6.74
+		+	+	0.35	+	+	0.12	0.13	0.30		+	0.06	0.08	8.81
+		+		0.07	+		0.10	0.09	0.30	0.08	+	0.11		7.39
+		+		+	+		0.14	+	0.20	+	+	0.04		8.67
+		+		+			0.10	0.09	0.28		+	+		7.25
		0.03					+	0.08	0.20	0.04		0.07		9.63
		+				+	0.13	0.04	0.11	0.04				7.98
+		+					+	0.04		0.08				8.04

Table 1. (*Contd.*)

Species and variety	Origin	Cultivation or source	1000 Bean mass	Chlorogenic acids: Total CQA	FQA	Total diCQA
C. pseudozanguebariae	Kenya, Shimba Hills, Zabi Koubi No. 382	Ivory Coast/Kew Herbarium +	40	0.80	0.07	
C. pseudozanguebariae	Kenya	Ivory Coast +	29	1.57	0.06	0.12
C. salvatryx		Campinas 1986 +	36	1.95	0.16	0.14
C. salvatryx (651–655)	Madagascar?	Tanzania 1986	50	1.39	0.11	0.18
B *Coffea* subgen. *Psilanthopsis*						
Psilanthopsis kapakata (221–225)		Tanzania	46	4.11	0.34	0.61
C *Coffea* subgen. *Baracoffea*						
C. rhamnifolia	Somalia, Garrard SRS 327/1	Kew Herbarium +	42	0.14		
D *Psilanthus* subgen. *Afrocoffea*						
Psilanthus ebracteolatus	Ivory Coast	Ivory Coast	51	0.18	0.06	0.03
Psilanthus ebracteolatus	Sierra Leone	Kew Herbarium +		+		
E *Psilanthus* subgen. *Psilanthus*						
Psilanthus mannii	Ivory Coast	Ivory Coast	202	0.35	0.03	

+ Voucher specimen available at Kew

*Figures in parentheses are R_f relative to 5-CQA

Key CQA = caffeoylquinic acids; FQA = feruloylquinic acids; diCQA = dicaffeoylquinic acids

Table 2. Caffeine and other 276 nm-absorbing components

Species and variety	276 nm-Absorbing components*: A (0.48)	B (0.73)	C (0.77)	D (0.84)	E (0.89)	F (0.94)	G (1.11)	H (1.28)
A *Coffea* subgen. *Coffea*								
C. arabica var. *bourbon N39*								1.33
var. *bourbon vermelho*								1.26
var. *purpurescens*								0.76
var. *laurina* (12/55)								0.79
var. *erecta* (181–185)								1.49
var. *semperflorans*								1.48
var. *murta*								1.75
var. *bourbon amarelo*								1.49
var. *bourbon amarelo*								1.54
var. *mokka*								0.87
var. *Maragogype*								1.39
var. *San Ramon* (19/53)								1.44
var. *caturra* (11/55)								1.59
var. *polysperma* (1/54)								1.65
var. *arabica KP423*								1.57
var. *typica*								1.67
var. *mundo novo*								1.57
var. *mundo novo*								1.59
var. *catuai vermelho*								1.82
var. *catuai vermelho*								1.53
var. *catuai vermelho*								1.28
C. sessiliflora			0.05					0.55
C. sessiliflora			0.05					0.46
C. mufindiensis			0.08					1.29
C. racemosa								0.76
C. racemosa								0.92
C. racemosa								1.16

Table 1. (*Contd.*)

Chlorogenic acid-like components*														
1 (0.57)	3 (1.20)	5 (1.25)	6 (1.42)	7 (1.49)	8 (1.55)	9 (1.60)	10 (1.89)	13 (1.95)	14 (2.00)	15 (2.10)	16 (2.25)	17 (2.30)	18 (2.45)	Total CGA
			0.06											0.93
			0.04											1.79
		0.11	0.05											2.41
		0.04	0.04											1.76
		+					+	0.06		+				5.12
														0.14
														0.27
														+
														0.38

(% dmb as caffeine) in the seeds of Coffeae

I (1.48)	J (1.56)	K (1.66)	L (1.76)	M (1.80)	N (1.84)	O (1.88)	P (2.00)	Q (2.03)	R (2.06)	S (2.12)	T (2.15)	U (2.38)	Total 276 nm-absorbing
													1.33
	+												1.26
													0.76
													0.79
	+												1.49
	+												1.48
	+												1.75
	+												1.49
	+												1.54
													0.87
	+												1.39
	+												1.44
	+												1.59
	+												1.65
	+												1.57
	+												1.67
	+												1.57
	+												1.59
	+												1.82
	+												1.53
	+												1.28
													0.60
													0.51
													1.37
	+												0.76
	+												0.92
	+												1.16

Table 2. (*Contd.*)

Species and variety	276 nm-Absorbing components*							
	A (0.48)	B (0.73)	C (0.77)	D (0.84)	E (0.89)	F (0.94)	G (1.11)	H (1.28)
C. eugenioides								0.55
C. eugenioides								0.47
C. eugenioides								0.65
C. eugenioides (501–505)								0.63
C. stenophylla (1073–13)								1.58
C. stenophylla								1.50
C. stenophylla (561–565)								0.62
C. stenophylla								2.64
C. humilis			0.08					2.04
C. dewevrei				0.18				1.97
C. liberica		+		0.04			+	1.81
C. liberica				+				1.28
C. excelsa Sumatra								1.84
C. congensis var. *excelsa* (VC606–610)								3.16
C. brevipes (*C. staudtii*)								2.16
C. canephora var. *Ugandae*								3.02
C. canephora var. *robusta* (VC 1156–1160)								2.29
C. canephora var. *robusta*								2.48
C. canephora var. *kouillou* (VC 286–290)								2.22
C. canephora								3.19
C. congensis								2.13
C. x arabusta								2.29
C. pseudozanguebariae					0.06	0.48	0.13	+
C. pseudozanguebariae					0.04	0.16	0.13	+
C. salvatryx	0.36			0.07	0.23			0.28
C. salvatryx (651–655)	0.40			0.07	0.17			0.08
B *Coffea* subgen. *Psilanthopsis*								
Psilanthopsis kapakata (221–225)	+	0.20						1.04
C *Coffea* subgen. *Baracoffea*								
C. rhamnifolia								0.06
D *Psilanthus* subgen. *Afrocoffea*								
Psilanthus ebracteolatus								0.03
Psilanthus ebracteolatus								0.02
E *Psilanthus* subgen. *Psilanthus*								
Psilanthus mannii								

nd = not detected

*Figures are R_t relative to 5-CQA

components has not been established unequivocally, but there are good reasons to expect that 5-CoQA; 4-FQA; 4-C, 5-FQA; 4-F, 5-CQA and caffeoyltryptophan [16, 18–20] will be present.

Cochromatography with authentic standards indicated that 1,3-diCQA [21] caffeic acid, ferulic acid, aesculin, scopolin and scopoletin were not detectable, but that component 1 may be 3,4-dihydroxybenzoic acid (protocatechuic acid). This has been reported in the fruit of *C. arabica* var. *caturra* but absent from those of *C. arabica* var. *bourbon* [22].

Monitoring the eluates at 276 nm reduced the intensity of the peaks corresponding to the CGA and CGA-like components but permitted the detection of caffeine and, in some samples, one or more unidentified components. For convenience these components have been quantified using a caffeine standard, but it should not be assumed that they are necessarily structurally related to caffeine. These 276 nm-absorbing components have been described by their retention time relative to 5-CQA and a code letter from the sequence A to U.

On co-chromatography with authentic standards 1-methylxanthine, 3-methylxanthine, 7-methylxanthine, 1,3-dimethylxanthine (theophylline), 1,7-dimethylxanthine (paraxanthine), 3,7-dimethylxanthine (theobromine), uric acid, 1-methyluric acid, 1,3-dimethyluric acid, 1,7-dimethyluric acid, quinine, quinidine, cinchonine and cinchonidine could not be detected.

On the basis of the chromatographic profiles the coffees have been assembled into groups having similar seed composition, as shown in Tables 1 and 2.

Group 1

The species assigned to Group 1 produce seeds having moderate contents of total CGA and caffeine, but without

Table 2. (*Contd.*)

I (1.48)	J (1.56)	K (1.66)	L (1.76)	M (1.80)	N (1.84)	O (1.88)	P (2.00)	Q (2.03)	R (2.06)	S (2.12)	T (2.15)	U (2.38)	Total 276nm-absorbing
													0.59
													0.47
													0.69
	0.04												0.67
	0.04												1.62
													1.50
	+												0.62
													2.64
													2.12
													2.15
													1.85
													1.28
													1.84
													3.16
													2.16
													3.02
													2.29
													2.48
													2.22
													3.19
													2.13
													2.29
+	2.35												3.02
+	2.71												3.04
	2.56			+									3.53
	2.28			+									3.00
	0.07												1.31
													0.06
	0.29	1.91	0.43	0.05	0.05	0.21	0.47	0.18	0.21	0.04	0.04	0.43	4.34
0.05	0.21	0.52	0.58	0.17	0.30	0.26	0.30	0.02	+	0.87	0.05	0.17	3.42
													nd

any CGA-like components [10–18] eluting after the diCQA.

Subgroup 1a

Subgroup 1a is restricted to the tetraploid *C. arabica*, including the commercial varieties, that are characterized by relatively large seeds. Caffeine is the major alkaloid in this species, but traces of another 276 nm-absorbing substance (J) were present in the seeds from many of the named varieties. The low caffeine content observed for *C. arabica* var. *laurina* is consistent with the data obtained by Carelli *et al.* [10]. The chlorogenic acid contents are, as might be expected, very similar to those reported [6, 16] for commercial arabicas. The within-cultivar variation in total CQA content observed in this study may reflect slight differences in average fruit maturity at harvest [13, 14], especially in samples of Brazilian origin where it is traditional to pick the fruit by stripping the plant, even if some are not fully ripe. That this may have occurred, is also suggested by the relatively low 1000 bean mass for the Brazilian sample of var. *bourbon amarelo.*

Subgroup 1b

Subgroup 1b includes the diploid species which produce smaller seeds than most varieties of *C. arabica.* The current data for the total CGA content (4.43 to 6.27%) and caffeine content (0.47 to 0.65%) of *C. eugenioides* seeds are consistent with the corresponding values of 5.67% and 0.23 to 0.51% reported by Carelli *et al.* [10] and Charrier and Berthaud [11]. Current values of 4.97 to 6.03% total CGA and 0.76 to 1.16% caffeine for the *C. racemosa* seeds, are similar to literature values of 6.68% and 0.58 to 1.20%. For *C. racemosa* it has been reported that the caffeine content depend upon the subspecies (*C. swynnertonii* or *C. ibo*) and origin [10, 23]. However, there are no formally recognised subspecies of *C. racemosa. C. swynnertonii* is a synonym of *C. racemosa*, while *C. ibo* is a synonym of the closely related *C. zanguebariae.*

The current data (0.46 to 0.56%) for the caffeine content of the *C. sessiliflora* seeds are consistent with values (0.52 to 0.71%) reported by Hamon *et al.* [24] for what was then known as *C.* sp *A*.

Although *C. stenophylla* is placed in subgroup 1b only three of the four samples examined have seed compositions clearly showing affinities with Group 1. The fourth sample, which was taken from a natural population in the western Ivory Coast is morphologically distinguishable from natural populations found in the eastern Ivory Coast (Anthony, F. personal communication). It produces seeds with a larger caffeine content (2.64%), which is not only greater than that obtained for the other three samples (0.62 to 1.53%), but also outside the range (0.9 to 1.9%) reported for this species by Charrier and Berthaud [25]. This particular specimen is also peculiar in that the seeds contain five CGA-like components [10, 13, 14, 16, 17] which elute after diCQA. Although Maier and Grimsehl [12] have reported the seed CGA content of a *C. stenophylla*, they did not comment whether or not these late-eluting CGA-like components were present. The total diCQA content was, however, larger (1.47%) than any of those observed in the present study. Porteres (cited by Charrier and Berthaud, [25] has referred to the morphological variation in this species, and the current data suggest that it may be heterogeneous, or that some misidentification may have occurred, perhaps because narrow-leaved forms of other species have been confused with the true *C. stenophylla*.

Group 2

The species in group 2 differ quite extensively in the size of seeds that are produced, and all are characterized by relatively large total CGA and caffeine contents, and by the presence of several CGA-like components which elute after diCQA.

Subgroup 2a

With the exception of *C. humilis*, the taxa in this subgroup belong to the species complex *C. liberica sensu lato*. Bridson [26, 27] accepts Lebrun's simplification [28] of recognising two varieties—*C. liberica* var. *liberica* and var. *dewevrei* (including *C. excelsa*); samples were received as *C. dewevrei*, *C. excelsa*, and *C. liberica*. They produce seeds which contain relatively few of the late-eluting CGA-like components, and these at relatively small concentrations, that together, probably do not exceed 0.20% in total.

Current values for total CGA content are in reasonable agreement with published values (*C. liberica*, 7.48% and 10.63% compared to 7.52% and 9.82%; *C. excelsa*, 7.67% compared to 6.44%; *C. dewevrei*, 7.79% compared to 7.03%) reported by Carelli *et al.* [10] and Maier and Grimsehl [12]. However, neither of these investigators commented upon the presence of any components which might correspond to the late-eluting CGA-like components. The current caffeine contents of 1.28% and 1.81% (*C. liberica*), 1.84% (*C. excelsa*) and 1.97% (*C. dewevrei*) are similar to published values (0.97 to 1.81% for liberio-excelsoides) and 0.52 to 1.80% for *C. dewevrei* [11].

It is interesting to note that *O*(2),1,9-trimethyluric acid, 1,3,7,9-tetramethyluric acid and *O*(2),1,7,9-tetramethyluric acid have been reported in the leaves of *C. liberica sensu lato*, e.g. *C. dewevrei* var. *excelsa*, *C. dewevrei* var. *aruwimiensis*, *C. abeokutae* (*Gros Indenie* Porteres) and in the seeds of *C. arnoldiana* [29–31]. In this study traces of three additional 276 nm-absorbing components (B, D, G) were observed in the seeds of one or more members of this subgroup, but it is not known whether these correspond to the previously reported uric acids.

Subgroup 2b

The species in subgroup 2b produce seeds containing at least five late-eluting CGA-like components which collectively account for some 0.30 to 0.80% of the seed.

The five samples of *C. canephora* have seeds that are similar, but not identical in size and composition. It is interesting to note that the differences in the pattern of CGA-like components appear to be greater between the seeds of named varieties than between samples of var. *robusta* seeds from plants cultivated in different continents. Commercial robustas, which are the prepared seeds of commercially grown *C. canephora*, but not necessarily var. *robusta*, have compositions [16, 32] which resemble the *C. canephora* seeds analysed in this study. However, among the commercial robustas there are variations, particularly in the pattern of CGA-like components [17]. The composition of the sample of commercial *C. x arabusta* seeds is consistent with previous data [11.16] being intermediate between the parents *C. arabica* and *C. canephora*, but closer to the latter.

The seeds received as '*C. congensis* var. *excelsa*', cultivated in Tanzania contain five late-eluting CGA-like components which total 0.62%. This pattern of CGA-like components, while clearly placing this sample also in subgroup 2b, is very different from that of the Ivory Coast-cultivated *C. congensis* sample which originated in the Central African Republic, and which has a caffeine content close to the range (0.8 to 2.0%) reported for *C. congensis* by Anthony and Le Pierres [33]. Since the seeds of the '*C. congensis* var. *excelsa*' were significantly larger (1000 bean masses 124 versus 72) with a much larger caffeine content (3.11 vs 2.13%) it is probable that these two specimens are not closely related. It has been suggested that the sample supplied as '*C. congensis* var. *excelsa*' might be a *C. x congusta* hybrid derived from *C. congensis* and *C. canephora* which has been backcrossed to *C. canephora*, a pedigree that could explain the large contents of both the late-eluting CGA-like components and of caffeine (Anthony, F., private communication).

Subgroup 2c

The total CGA content for the seeds of *Psilanthopsis kapataka* reported here (5.12%) is smaller than that (6.72%) reported by Carelli *et al.* [10], and this sample appears to have a seed composition which is quite distinct from that of all others examined in the present study. The presence of three late-eluting CGA-like components (10, 13 and 15) exclude this species from Groups 1 and 3 and strongly suggest Group 2. However the small seed size and relatively small contents of caffeine and total CGA separate *P. kapakata* from the other species assigned to Group 2, and thus it has been assigned to subgroup 2c.

Group 3

The species placed in group 3 have seeds which are characterized by small or very small contents of total

CGA and caffeine. With one exception (*Psilanthus mannii*) the seeds are small and contain variable but sometimes significant quantities of at least one late-eluting 276 nm-absorbing component. Baumann and Frischknecht [34] have previously reported that *P. mannii* is a species free from purine alkaloids. Although three uric acid derivatives have been identified [29–31] in the seeds of *C. liberica* and *C. dewevrei*, this appears to be the first reported of such a large number of 276 nm-absorbing components in the seeds of *Psilanthus*, *Psilanthopsis* and other *Coffea* species. Previous reports of the virtual absence of caffeine from the seeds of *C. pseudozanguebariae* [24] and of a low, but unspecified, caffeine content and low (2.70%) total CGA content in *C. salvatryx* seeds [10] are consistent with the current observations.

Concordance with existing classification based upon morphology

The results presented here for a relatively small number of samples, in some cases less than perfectly defined, nevertheless indicate that species within the tribe Coffeeae may differ considerably in terms of their seed composition. It appears that there may be a continuous spectrum ranging from typical commercial coffees, which produce seeds having large contents of CGA and caffeine, through species with seeds containing little or no CGA and caffeine but significant amounts of other 276 nm-absorbing components, to *P. manni* with seeds lacking even these substances. In several respects this spectrum is similar to the relationships proposed by Leory [2–4] and accepted (with the exception of *Nostolachma*) by Robbrecht and Puff [1] and which was summarized in the introduction.

Psilanthus mannii (subgen. *Psilanthus*) is currently separated from *P. ebracteolatus* (subegn. *Afrocoffea*) at the subgeneric level. The analytical data reported here clearly indicate substantial differences between these seeds. Thus *P. ebracteolatus* seeds contain at least thirteen 276 nm-absorbing components equivalent to some 4%, expressed as caffeine while no such compounds could be detected in the seeds of *P. mannii*. Although these two species are similar morphologically when flowering, their fruits are strikingly dissimilar, those of *P. mannii* being much larger and crowned by an accrescent calyx-limb. These differences have led to the suggestion that these species should be separated at full generic level [5], and it is suggested that the analytical data reported here favour such a distinction.

On morphological criteria *C. rhamnifolia* is now placed in *Coffea* subgen. *Baracoffea* where it is considered to provide a link between *Coffea* and *Psilanthus* subgen. *Afrocoffea* [35]. The seeds contained very small quantities of CGA, and caffeine but no other 276 nm-absorbing components, thus distinguishing it from *P. ebracteolatus* and suggesting that such a taxonomic position is not inappropriate.

Psilanthopsis kapakata is now generally recognised as being distinct from *Coffea* only at infrageneric level, the main morphological differences being the larger, dentate calyx-limb and the ribbed fruit. Some affinity to certain eastern African taxa with well developed calyx limbs has been suggested [36, 37] but these taxa are poorly known and no seeds were available for analysis. On compositional criteria there is a tantalising affinity with each of the other groups and subgroups, proposed in this paper, but as discussed previously, these features taken collectively make *P. kapakata* distinguishable from all other species examined. Such a seed composition is therefore consistent with its assignment to *Coffea* subgen. *Psilanthopsis*.

The seeds of 16 species assigned to *Coffea* subgen. *Coffea* have been found to contain in excess of 1.0% CGA, a feature which supports such a taxonomic grouping. However, among these 16 species, variations in seed composition (presence or near absence of caffeine, presence or absence of other 276 nm-absorbing components, presence or absence of late-eluting CGA-like components) are observed. These variations are of a magnitude similar to those used to distinguish the other subgenera and therefore suggest that subgen. *Coffea* could be further subdivided, for example into:

(A) *C. arabica* and diploid species which generally resemble. *C. arabica* by producing seeds containing significant (>4.5%) CGA but lacking the late eluting CGA-like components.

(B) *C. canephora* and species which resemble *C. canephora* by producing seeds containing significant (>4.5%) CGA including the late eluting CGA-like components.

(C) *C. salvatryx* and *C. pseudozanguebariae* which produce seeds containing intermediate amounts of CGA (1–3%), and a moderate amount of caffeine (0.5%) accompanied by significant quantities (2.5% in total) of at least one additional 276 nm absorbing component.

It is interesting to note that *C. x arabusta* falls into Group B along with one of its parents (*C. cancephora*) but that the other parent (*C. arabica*) falls into Group A. Similarly *C. pseudozanguebariae* and *C. sessiliflora*, which are sympatric and very similar in facies are quite distinct in their seed composition. However these proposed compositional subgroups within *Coffea* subgen. *Coffea* neither coincide with any significant morphological characters, nor with geographical distribution. Nevertheless the present study indicates that seed analysis has the potential to assist in understanding the relationships within the Coffeeae. However to achieve fully this potential the seeds of more species, and more samples of each species must be analysed, and such developments are in hand, along with the isolation and characterization of some of the unknown components.

EXPERIMENTAL

Materials. Mature fruit, or the dried seeds therefrom, were kindly supplied from the living collections of the Coffee Research Station at Lyamungu in Tanzania, the ORSTOM Coffee Research Station at Man in the Ivory Coast, and of the Istituto Agronomico at Campinas in Brazil. In some cases voucher specimens were supplied with the seeds and identity confirmed at the Royal Botanic Gardens, Kew, England, the source also of a few fruits from herbarium specimens. The availability of voucher specimens is indicated in the tabulated results.

Methods. Normally the seeds were separated from all outer tissues and visually examined. Damaged seeds were discarded and the remainder counted and weighted (±0.01 g). A sample was ground to pass 0.5 mm and extracted by refluxing with 70% MeOH (usually 0.5 g to 100 ml) as previously described and optimised for the analysis of CGA in commercil green coffee beans [21, 38]. These extracts were analysed by reversed phase HPLC using detection at 313 nm for the CGA, and at 276 nm in order to detect caffeine. In both cases a 15 cm × 4 mm analytical column packed (Hichrom) with Spherisorb ODSI 3 μ solid phase

was used with a 5 cm × 4 mm guard column containing the corresponding 5 μ solid phase. A linear gradient of 0.5% formic acid in 6% aq. MeCN to 0.5% HCO_2H in 30% aq. MeCN, in 22.5 min at a flow rate of 1 ml/min was used to separate the CGA and 276 nm-absorbing components. When sufficient sample was available the mass loss on drying to constant weight (105°) was determined.

In the case of herbarium samples lack of material precluded the determination of moisture content and the data for these samples have been presented assuming a value of 10%.

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