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Qualitative relationship between caffeine and chlorogenic acid contents among wild *Coffea* species

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Abstract

Chlorogenic acids, *sensu largo* (CGA), are secondary metabolites of great economic importance in coffee: their accumulation in green beans contributes to coffee drink bitterness. Previous evaluations have already focussed on wild species of coffee trees, but this assessment included six new taxa from Cameroon and Congo and involved a simplified method that generated more accurate results. Five main results were obtained: (1) Cameroon and Congo were found to be a centre of diversity, encompassing the entire range of CGA content from 0.8% to 11.9% dry matter basis (dmb); (2) three groups of coffee tree species – CGA1, CGA2 and CGA3 – were established on the basis of discontinuities; (3) means were 1.4%, 5.6% and 9.9% dmb, respectively; (4) there was a qualitative relationship between caffeine and ACG content distribution; (5) only a small part of the CGA is trapped by caffeine as caffeine chlorogenate.

Keywords: *Coffeae*; Caffeine; Chlorogenic acids

1. Introduction

Two coffee species, *C. canephora* and *C. arabica*, are cultivated worldwide. However, these species only represent a small proportion of the world's coffee genetic resources as, in the wild, the *Coffea* subgenus (*Coffea* genus, Rubiaceae family) includes more than 80 species or taxa endemic to intertropical forest zones of Africa, Madagascar, Mauritius, Comoros and Réunion (Anthony, 1992; Bridson & Verdcourt, 1988; Charrier, 1978; Chevallier, 1947; Lebrun, 1941; Stoffelen, 1998).

Chlorogenic acids (CGA) are secondary metabolites of great economic interest in coffee because of their degradation into phenolic compounds when the beans are roasted, responsible for coffee bitterness (Leloup, Louvrier, & Liardon, 1995). The major CGA subgroups are quinic acid esters with caffeic acid [caffeoylquinic acids (CQA) and dicaffeoylquinic acids (diCQA)] or with ferulic acid [feruloylquinic acids (FQA)] and represent 98% of all CGAs (Clifford, 1985; Morishita, Iwahashi, & Kido, 1989). There are also some minor compounds, such as esters of feruloyl-caffeoylquinic acids (FCQA), caffeoyl-feruloylquinic acids (CFQA) or *p*-coumaric acid (*p*-CoQA).

In previous studies, green bean CGA content (species mean) has been evaluated in some wild species. It varied between 0.14% dry matter basis (dmb) in *C. rhamnifolia* Bridson (Anthony, Noirot, & Clifford, 1993) and 11.3% dmb in *C. canephora* (Ky et al., 2001). Differences were also observed between trees within species, wild or not. In *C. canephora*, for example, CGA content ranges from 7.9% to 14.4% dmb (Ky et al., 2001). Content variations are mostly under genetic additive control, with environmental effects ranging from 22% to 41% according to the CGA subgroup (Ky et al., 1999).

CQA are supposed to form a complex with caffeine (Payen, 1846) in a 1:1 molecular ratio (Sondheimer, Covitz, & Marquisee, 1961). They may bind with other purine alkaloids, such as paraxanthine, theophylline,

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theobromine or theacrine (Kappeler, Baumann, & Greutert, 1987). Complexes are also formed, but at a lower rate, with CQA precursors such as quinic or caffeic acids (Horman & Viani, 1972). All of these compound combinations could explain the close correlation observed between CGA and caffeine contents within the subgenus *Coffea* (Anthony et al., 1993).

In this paper, we present a biochemical evaluation of CGA content in a panel of wild *Coffea* genetic resources, including 15 species and six new taxa that have not yet been botanically described. Similar to variations noted in caffeine content (Campa, Doulbeau, Dussert, Hamon, & Noirot, in press), our study highlighted a discontinuous distribution of CGA content and enabled us to define five qualitative classes in the caffeine-CGA relationship.

2. Material and methods

2.1. Plant material

The 15 species and six new taxa are field-maintained at the IRD coffee breeding station of Man (Côte-d'Ivoire). All species are diploid ($2n = 2x = 22$). Table 1 gives the geographical origin of the species and taxa. Each species or taxon was represented by four accessions.

2.2. Sample preparation

Coffee cherries were harvested at full maturity and depulped using the wet processing method. After desiccation on silica gel, 50 green beans per tree were frozen in liquid nitrogen before crushing in a ball mill (Dangoumill) for 2 min. The fine powder was split into six samples, three to estimate the water content and three for extraction and analysis.

Table 1
Geographical origin of the species and taxa

Species and taxa	Geographical origin
C. brevipes	Cameroon
C. canephora	Côte d'Ivoire
C. congensis	Congo Democratic Republic
C. eugenioides	Kenya
C. heterocalyx	Cameroon
C. humblotiana	Comores
C. humilis	Côte d'Ivoire
C. kapakata	Angola
C. liberica dewevrei	Central African Republic
C. liberica liberica	Côte d'Ivoire
C. pocsii	Tanzania
C. pseudozanguebariae	Kenya
C. racemosa	Tanzania
C. salvatrix	Tanzania
C. stenophylla	Côte d'Ivoire
C. sp. Bakossi	Cameroon
C. sp. Congo	Congo Democratic Republic
C. sp. Koto	Cameroon
C. sp. Ngongo 2	Congo Democratic Republic
C. sp. Moloundou	Congo Democratic Republic
C. sp. N'koumbala	Cameroon

2.3. Extraction and HPLC analysis

CGAs were extracted using a slightly modified version of the method of Ky et al. (1999). To 50 ml conical tubes (Falcon) containing the powdered sample (50 mg) and sodium bisulfite (50 mg), 50 ml of methanol/water (70/30 v/v) were added. Tubes were then shaken overnight at 4 °C in darkness on a stirring table at 125 rpm. After 18 h, the organic extracts were treated with Carrez reagents (50 µl each). After centrifugation (1500 rpm, 2 min), extracts were filtered (0.2 µm pore-size filter) and directly analysed by HPLC.

Analyses were carried out on an HPLC system (Waters), consisting of a LiChrospher 100 RP-18 (5 µm) column (250 mm × 4 mm, Merck), a C_{18} guard column and a photodiode-array detector (Waters 996). The elution system (0.8 ml min^{-1}) involved two filtered (0.2 µm pore-size filter), degassed and sonicated (Ney, 300 ultrasonik) solvents, namely solvent A (phosphoric acid, 2 mM, pH 2.7) and solvent B (methanol). The gradient was: 0 min, 25% solvent B; 0–30 min, 100% solvent B, linear; 30–33 min, 100% solvent B, isocratic; 33–38 min, 25% solvent B, linear; 38–50 min, 25% solvent B, isocratic. The calibration curve was plotted using three replicate points for a standard solution (5-CQA, Fluka) at 10, 20, 30 and 40 mgl^{-1}. Identification and quantification of 3-, 4-, and 5-feruloylquinic acids (FQA), 3- and 4-/5-caffeoylquinic acid (CQA) and 3,4-, 3,5- and 4,5-dicaffeoylquinic acids were done at room temperature at 325 nm by comparison with standard, according to the technique of Ky, Noirot, and Hamon (1997). CGA content was calculated by adding the nine isomer contents. Compound content was expressed as a percentage of dry matter (% dmb).

2.4. Statistical analysis

All results were analysed using the Statistica software package (5.1 Version, 1997, for Microsoft Windows).

Each tree was characterized by its mean CGA content and the previously evaluated mean caffeine content (Campa et al., in press). Statistical analysis only concerned the between-species variation, which was tested using a one-way ANOVA.

3. Results and discussion

3.1. Diversity in CGA content

CGA content, evaluated for the 21 species or taxa, ranged from 0.8% dmb in *C.* sp. Bakossi to 11.9% dmb in *C.* sp. N'koumbala (Table 2).

Table 2
Mean chlorogenic acid (CGA) and caffeine (CAF) contents for different coffee species (expressed in percent of dry matter) and their class membership according to Newmann and Keuls test

Species or taxa	CGA		CAF[a]		Classes	
	Mean	Range	Mean	Range	CGA	CAF[a]
C. sp. Bakossi	0.79	0.61–1.05	0.00	0.00–0.03	CGA1	CAF1
C. humblotiana	1.00	0.90–1.09	0.00	0.00–0.01	CGA1	CAF1
C. pseudozanguebariae	1.47	1.30–1.61	0.00	0.00–0.00	CGA1	CAF1
C. salvatrix	2.18	1.74–3.00	0.03	0.01–0.06	CGA1	CAF1
C. eugenioides	5.17	4.55–6.08	0.51	0.44–0.60	CGA2	CAF2
C. sp. Moloundou	5.55	5.40–5.73	0.58	0.52–0.61	CGA2	CAF2
C. racemosa	5.33	4.78–5.57	1.05	0.86–1.25	CGA2	CAF3
C. heterocalyx	6.25	6.11–6.52	0.92	0.86–0.99	CGA2	CAF3
C. liberica Dewevrei	7.62	6.22–8.78	0.94	0.81–1.10	CGA3	CAF3
C. liberica Koto	8.85	8.81–8.89	1.31	0.91–1.70	CGA3	CAF3
C. liberica liberica	9.80	8.75–10.7	1.24	1.12–1.39	CGA3	CAF3
C. congensis	8.44	8.15–8.77	1.47	1.08–1.83	CGA3	CAF3
C. kapakata	9.71	8.84–10.7	1.20	1.04–1.39	CGA3	CAF3
C. pocsii	10.65	9.77–11.5	1.27	1.04–1.71	CGA3	CAF3
C. stenophylla	8.23	6.76–9.39	2.27	2.05–2.43	CGA3	CAF4
C. humilis	8.65	7.87–10.3	1.93	1.67–2.27	CGA3	CAF4
C. sp. Congo	9.26	7.65–10.3	2.27	2.11–2.37	CGA3	CAF4
C. sp. N'gongo2	10.77	10.1–11.3	2.12	1.90–2.32	CGA3	CAF4
C. brevipes	11.12	10.4–12.3	2.54	2.36–2.96	CGA3	CAF4
C. canephora	11.34	9.76–14.4	2.64	1.51–3.33	CGA3	CAF4
C. sp. N'koumbala	11.90	11.1–12.7	2.36	1.89–2.89	CGA3	CAF4

[a] Data from Campa et al. (in press).

Table 3
Comparison of current data with the Anthony's (1992) results for 14 species or taxa

Species or taxa	Current data	Anthony et al.
C. canephora	11.3	7.81
C. brevipes	11.1	6.41
C. sp. Nkoumbala	11.9	8.73
C. pocsii	10.7	7.92
C. liberica liberica	9.80	7 48
C. humilis	8.60	6.16
C. congensis	8.40	7.66
C. stenophylla	8.20	7.12
C. liberica Dewevrei	7.60	7.68
C. sp. Moloundou	5.60	4.65
C. racemosa	5.50	5.40
C. eugenioides	5.20	5.12
C. salvatrix	2.20	1.96
C. pseudozanguebariae	1.50	1.25

Data are expressed in percentage of dry matter (dmb).

CGA has already been biochemically evaluated by Anthony et al. (1993). Fourteen species were common to those assessed in our study, so the CGA content results were compared (Table 3). A discrepancy was noted for species with high CGA content. For example, our evaluations in *C. canephora*, *C. brevipes* and *C.* sp. Nkoumbala were 44.7%, 73.2% and 36.3% higher, respectively. Nevertheless, there was a linear regression between the old (X) and new (Y) data [($r = 0.915$; $p < 0.001$): $Y = 1.347X - 0.52$]. Consequently, the discrepancy cannot be attributed to a sampling difference. According to the relationship, there was a mean gain of about 35% in the new data. This increase in extraction yield may be attributed to our use of the procedure of Ky et al. (1997).

The CGA bean content range differed between eastern, central and western Africa. The lowest range was recorded in western Africa, where CGA varied from 8.6% in *C. liberica* subsp. *liberica* to 11.3% in *C. canephora*. In eastern Africa, CGA content varied from 1.5% in *C. pseudozanguebariae* to 10.7% dmb in *C. pocsii*. The range was similar in central Africa (Central African Republic, Congo, Cameroon), where CGA content ranged from 0.8% in *C.* sp. Bakossi to 11.9% in *C.* sp. Nkoumbala. Two features characterised central Africa: (1) with the highest range, it appeared to be a centre of diversity for CGA content as already noted with respect to caffeine content (Campa et al., in press); (2) two yet undescribed taxa had the most extreme CGA contents, thus highlighting the interest of collecting them for breeding purposes.

3.2. Species classes according to CGA content

According to the Newman and Keuls test, two discontinuities split the CGA content range into three classes (Fig. 1). The first class (CGA1) consisted of four species with low CGA content (mean 1.4% dmb). Its range (0.8–2.2% dmb) showed significant interspecific differences. The second class (CGA2) included four species. Its CGA content ranged from 5.2% to 6.3% dmb, with a mean of 5.6% dmb. The third class (CGA3) con-

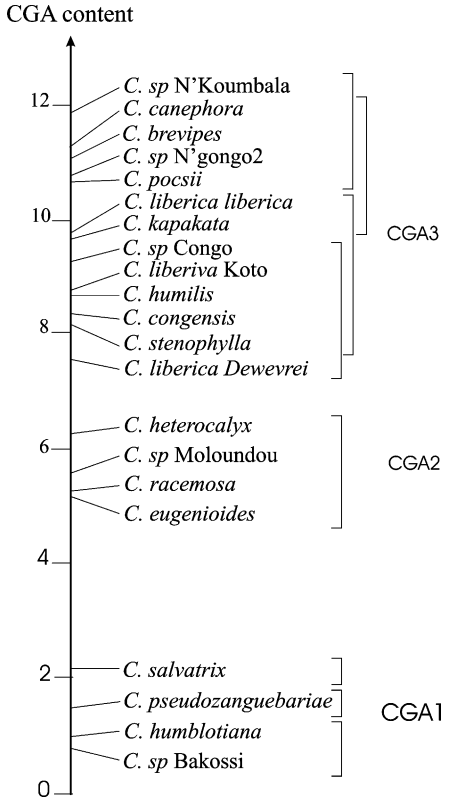

Fig. 1. Within-subgenera *Coffea* clusters for CGA contents (expressed in % dmb). Brackets indicate Newmann and Keuls test results.

sisted of 13 species with high CGA content (mean 9.8% dmb). As in CGA1, its range (7.6–11.9% dmb) showed significant between-species variations, but with a large overlap.

On the basis of the chromatographic profiles, Clifford, Williams, and Bridson (1989) defined three groups – group 1, group 2 and group 3 – which correspond to CGA2, CGA3 and CGA1, respectively. Only two small pseudo-differences were noticed between both studies, but they have a great impact at the taxonomic level. The first concerns *C. sessiliflora.* For a long time, *C. pocsii* Bridson was considered as belonging to *C. sessiliflora*. In our biochemical analysis, *C. pocsii* Bridson belongs to CGA3, whereas *C. sessiliflora* would belong to CGA2 according to Clifford et al. (1989). This confirms *C. pocsii* Bridson as a new species. The second pseudo-difference concerns *C. stenophylla*. For this species, biochemical heterogeneity was emphasized, corresponding to two distinct populations of Côte-d'Ivoire (Western and Eastern) and associated with morphological variation (Clifford et al., 1989). According to these authors, the Western population would belong to CGA3, whereas the Eastern one had to be included in CGA2. Note that in our case, results concerned the Western population. Consequently, there is a strong similarity between the *C. sessiliflora C. pocsii* taxonomic situation and the Western–Eastern one observed in *C. stenophylla*. Such similarity underlines the interest of using CGA chromatographic profiles as one criterion for taxonomic studies.

Clustering has also been documented for caffeine content, using the same species and four classes (CAF1 to CAF4) defined (Campa et al., in press). As with caffeine clustering, four main differences appeared for CGA: (1) no groups showed only trace quantities of CGA; (2) there were between-species differences within groups; (3) the discriminating power (between-group variance/within-group variance) was lower, due to the lower genetic contribution to the phenotypic variance, i.e., 78% in CGA vs. 94% in caffeine (Barre et al., 1998; Ky et al., 1999), and (4) the group means did not evolve according to multiplicative effects. In fact, the mean determined for CGA2 (5.3% dmb) was between CGA1 (1.4% dmb) and CGA3 (9.8% dmb).

3.3. Relationship between CGA and caffeine contents

When taking the interspecific relationship between CGA and caffeine (Anthony et al., 1993) and the existence of caffeine chlorogenate (Payen, 1846) into account, a qualitative relationship would be expected, due to the discontinuous distribution in CGA and caffeine contents. Hence, the relationship between the four caffeine classes (CAF1 to CAF4) and the three CGA classes could be tested (Table 4). Among the twelve theoretical possibilities, only five groups were highlighted. The G1 group (CGA1–CAF1) included the four species belonging to CGA1 and CAF1 (*C. salvatrix*, *C. pseudozanguebariae*, *C. humblotiana* and *C.* sp. Bakossi) and was characterised by trace amounts of caffeine and a mean CGA content of 1.4% dmb. The existence of this group indicates that CGA can be accumulated in beans without being bound to caffeine. The G2 group (CAF2–CGA2) was formed by only two species (*C. eugenioides* and *C.* sp. Moloundou) and showed, on average, about 0.55% dmb of caffeine and 5.4% dmb of CGA. If all caffeine is trapped by CGA, 1% [1] dmb of CGA would be present as caffeine chlorogenate and 4.4% dmb (81.5% of total CGA) as caffeine-free CGA (we called it free-CGA [2]). The free-CGA content was threefold higher than for G1. The G3 group (CAF3–CGA2) was characterised, on average, by 1.0% dmb of caffeine and 5.9% dmb of CGA. It also included two species (*C. heterocalyx* and *C. racemosa*) and differed mainly from G2 by a twofold higher caffeine content. In this group, 1.8% dmb of CGA would bind to caffeine. Then, 4.1% dmb of CGA would be caffeine-free. This value represented 69.5% of the total CGA. The G4 group (CAF3–CGA3) characterised, on average, by 1.2% dmb of caf-

[1] A 1:1 molecular ratio is equivalent to a CAF/CGA content ratio of about 0.55.

[2] Free in regards to caffeine. It could be bound to other compounds.

Table 4
Contingency table for caffeine and chlorogenic acid groups of diploid species

		Caffeine group (% dmb)			
		CAF1 <0.05	CAF2 0.5–0.6	CAF3 0.9–1.5	CAF4 >1.9
CGA group (% dmb)	CGA1 <2.5	G1 4 species	–	–	–
	CGA2 5.0–6.5	–	G2 2 species	G3 2 species	–
	CGA3 7.5–12.0	–	–	G4 6 species	G5 7 species

feine and 9.4% dmb of CGA, included six taxa (*C. congensis*, *C. kapakata*, *C. liberica* subsp. *liberica*, *C. liberica* subsp. *Dewevrei*, *C.* sp. Koto and *C. pocsii*). G4 had the same caffeine content as G3, but a higher CGA content. It differed from G3 by a marked increase in free-CGA content (7.5% dmb vs. 4.1% dmb), which represented 79.8% of the total CGA content. The G5 group (CAF4-CGA3) showed, on average, 2.3% dmb of caffeine and 10.2% of CGA and was formed by seven taxa (*C. canephora*, *C. brevipes*, *C. humilis*, *C. stenophylla*, *C.* sp. Congo, *C.* sp. N'gongo2 and *C.* sp. Nkoumbala). G5 only differed from G4 by a twofold higher caffeine content. It showed a mean 6% dmb free-CGA content, equivalent to 58.8% of the total CGA content.

When comparing the caffeine and CGA contents in green beans of 21 *Coffea* species or taxa, variations in these parameters showed similar trends, with an increase in CGA content always being accompanied by an increase in caffeine content. In fact, there was no group with a low caffeine content and a high CGA content on conversely. Nevertheless, CGAs were always more abundant than caffeine, thus indicating that all the CGA was not trapped as caffeine chlorogenate in the seed. The concentration of free-CGA compared to total CGA was not a constant. It decreased from nearly 100% in G1 group to 58.8% in G5, so the CGA content was not solely dependent on the caffeine content. The proportion of the different isomers forming the CGA pool was not taken into account in this analysis. Their distribution and location within cells constitutes a new objective for adjusting caffeine and CGA content in genetic ways.

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