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Relations between and inheritance of chlorogenic acid contents in an interspecific cross between *Coffea pseudozanguebariae* and *Coffea liberica* var 'dewevrei'

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Abstract Chlorogenic acids (CGA) are phenolic compounds commonly found in green coffee beans. The main CGA classes are caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), and feruloylquinic acids (FQA). Each contains three isomers differing in the number and identity of the acylating residues. An interspecific cross between *Coffea pseudozanguebariae* (low CGA content) and *C. liberica* var 'dewevrei' (high CGA content) was investigated for CGA contents in F_1 and back-cross hybrids. Relations within and between CGA classes were studied and confirmed the known biosynthesis pathway. A single major gene was noted for the 3-FQA isomer; absence was dominant. Additivity was found for most other isomers either with or without the transformation of variables. Conversely, most ratios were not additive, due to a curvilinear relation between some isomers. The consequences for breeding both in terms of cup taste improvement and disease resistance are discussed.

Key words Chlorogenic acids · Quantitative inheritance · Interspecific cross · *Coffea pseudozanguebariae* · *Coffea liberica* var 'dewevrei'

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Introduction

The esters of quinic acid are commonly named chlorogenic acids (CGA) and constitute the major phenolic secondary metabolites found in the plant kingdom. They control germination and cell growth, participate in defense mechanisms against phytopathogens, or act as lignin precursors. CGA are particularly abundant in the coffee plant (Streuli 1970; Amorin et al. 1974; Maier 1987), especially in green beans and their derivatives – roasted coffee beans, soluble coffee powders, and coffee brews (Clifford 1985a; Balyaya and Clifford 1995). Six classes of CGA are present in coffee trees: caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), feruloylquinic acids (FQA), *p*-coumaoylquinic acids (*p*CoQA), caffeoylferuloylquinic acids (CFQA), and feruloylcaffeoylquinic acids (FCQA). CGA-like components where the cafeic unit is coupled with an amino acid such as tyrosine are also present in trace amounts (Clifford et al. 1989a; Correia et al. 1995). The first three classes represent about 98% of the CGA content (Clifford and Staniforth 1977; Morishita et al. 1989). Each of these classes is commonly divided into three isomers on the basis of the number and position of the acylating residues (Clifford 1985b).

The CGA content of coffee beans modifies cup taste through direct and indirect effects. The direct effect is due to the presence of CGA after roasting [36% in *C. canephora* var 'Robusta' (Lentner and Deatherage 1959; Feldman et al. 1969)]. For example, diCQA is known to increase astringency (Ohiokpehai et al. 1982; Clifford and Ohiokpehai 1982). The indirect effects are due to molecular changes during roasting which have positive or negative influences. Degradation of CGA into phenolic derivatives (Leloup et al. 1995) and the inhibition of pyrazine formation by Maillard's reactions (Guyot et al. 1997) have negative effects. Conversely, the CGA reaction with trigonelline, sucrose, and amino acids which enriches headspace volatile profiles recorded by gas chromatography is a positive effect (De Maria et al. 1994). To summarize, the quality of the beverage increases when the CGA content decreases. This largely explains taste differences between Robusta and Arabica coffees (Clifford 1985b; Guyot et al. 1988, 1996).

Chlorogenic acid content increases resistance to *Ceratocystis fimbriata* at high concentrations but stimulates pathogen growth at low concentrations (Echandi and Fernandez 1962; Zuluaga et al. 1971). Coffee species producing more CGA will be well protected. It seems that the effects of CGA content on taste and disease resistance conflict.

CGA content in green coffee beans varies greatly between species (Clifford and Jarvis 1988; Clifford et al. 1989b; Anthony et al. 1993; Rakotomalala et al. 1993) – from 0.14% dry matter basis (dmb) in *Coffea rhamnifolia* Bridson to 9.90% dmb in *C. sessiliflora* Bridson. The level of CQA ranges from 61% CGA content in *C.* sp *Mouloundou* to 100% in *C. farafanganensis* Leroy. The diCQA level reaches 36% CGA content, whereas that of FQA decreases to 12% CGA content in *C. canephora*. If we consider the African continent, a CGA gradient exists between East African and West African species (Anthony et al. 1993). The large range of CQA, diCQA, and FQA contents in the genus *Coffea* and their implications for organoleptic qualities and disease resistance emphasize the importance of CGA in breeding programs.

C. pseudozanguebariae Bridson (PSE), which has about 1.3% dmb CGA (Anthony et al. 1993) was hybridized with *C. liberica* var 'dewevrei' (DEW), an anciently cultivated species from West Africa with approximately 6.9% dmb CGA (Anthony et al. 1993). F_1 and backcross hybrids were obtained (Louarn 1982, 1992). The objectives of the study presented in this paper were (1) to investigate the quantitative inheritance of CGA and (2) to study the relations between the isomers.

Materials and methods

Plant material

Plant material was maintained at the Agricultural Station ORSTOM-IDEFOR (Man, Côte-d'Ivoire). Parental species from the 1996 harvest were investigated. For each species, beans were separately harvested from seven trees, which were native to the Republic of Central Africa or Kenya for DEW and PSE, respectively. The F_1 generation included 7 hybrids from a controlled cross between genotype 8044 (PSE) and 5851 (DEW) parents. Biochemical analysis was performed on the 1995 harvest. The second generation included 14 hybrids which consisted of 7 $F_1 \times$ DEW (BCDEW) and 7 $F_1 \times$ PSE (BCPSE). Twelve hybrids BCDEW were harvested in both 1995 and 1997 for the environmental effects study.

Sample preparation

All coffee cherries were harvested at complete maturity and depulped using the wet processing method. Fifty green beans per tree were immediately frozen using liquid nitrogen to avoid CGA degradation by heat during the crushing. A 2-min crushing in a ball mill (Dangoumill) yielded a fine powder, which was divided into six samples, three to estimate water content and three for the extraction and analysis of CGA.

Extraction and purification

Extraction and purification of CGA were accomplished using a fast and accurate method. Coefficients of variation are 3–5% for CQA, 1–2% for FQA and 5–7% for diCQA (Ky et al. 1997). Each sample was extracted in a 250-ml Erlenmeyer flask. Each flask contained about 250 mg of powder, 100 ml of a methanol : water (70/30 v/v) mixture and 0.5% sodium bisulfite. The Erlenmeyer flasks were shaken overnight at 4°C in darkness on a stirring table at 125 rpm. The organic extracts were directly treated with Carrez reagents (0.5 ml each). Solutions were then filtered through a 0.2-µm filter and directly analyzed by high pressure liquid chromatography (HPLC).

Analytical HPLC

Chromatography was carried out on a system consisting of two Waters Associates Model 510 pumping units, an automated sample injector (Waters 717 plus autosampler), a variable-wavelength UV detector (Waters 996 Photodiode Array Detector), a C_{18} pre-column and a 250 × 4-mm Merck Superspher 100 RP 18 column, 5-µm particle size. The elution program used two solvents, A and B. Solvent A was 2 m*M* phosphoric acid, pH 2.7, containing 5% methanol. Solvent B was methanol containing 5% 2 m*M* phosphoric acid, pH 3.9. These two mobile phases were filtered (0.2 µm), degassed and sonicated (Ney, 300 ultrasonik) before use. Sample or standard (10 µl) was analyzed at room temperature using the following elution program: A–B mixture (75/25 v/v) to pure solvent B in 45 min of linear gradient. Flow rate was 0.8 ml/min. UV detection was at 325.2 nm, which corresponds to maximum CGA absorption.

The processing order of extracts was fully randomized for HPLC analysis. Every ten extracts, a 5-CQA standard (ref. D11.080-9, Aldrich-Chimie; concentration 100 mg/l) was used to verify the stability of the HPLC measurements. Every 40 extracts, the C_{18} pre-column and the 100 RP 18 column were washed overnight with a solution of pure methanol at a flow rate of 0.2 ml/min.

CGA identification and quantification

Chlorogenic acid isomers were identified by means of their chromatograms (at 280 nm and 325 nm), retention times, and UV spectrum (Ky et al. 1997), which were compared to Rakatomalala's results (1992). Identification of CQA isomers was confirmed by isomerization of 5-CQA (ref. D11.080-9, Aldrich-Chimie) following the method proposed by Trugo and Macrae (1984).

Quantification was achieved by peak-area measurement (PA) and by comparison with the standard 5-CQA. A 5-CQA calibration curve ($C = 2.768\ 10^{-5} \times PA$) was generated using three replicate points at 25, 50, 75, 100, 125, and 150 mg/l. CGA isomer contents were expressed in percentage of dry matter basis (% dmb).

Statistical analysis

All results were analyzed using Statistica software.

The relative importance of between-group (PSE, BCPSE, F_1, BCDEW, and DEW) and within-group diversities was analyzed using the nested model of ANOVA with two factors: group (fixed effects) and plants nested in group (random effects).

Differences between harvest years in the 12 BCDEW hybrids were tested using a crossed model of ANOVA with two factors: hybrids (random effects) and harvest year (fixed effects).

Additivity was tested using linear fitting between expected values (PSE = 0, BCPSE = 0.25, F_1 = 0.5, BCDEW = 0.75, DEW = 1) and observed data.

Relations between isomers were supposed to be allometric ($y = ax^b$). Fitting was done after double transformation of the variables [$Y = \log(y)$ and $X = \log(x)$] to obtain a linear model. When estimation of the b parameter was close to 1, a classic linear regression without transformation was applied to data. Figures represented relations for initial data, but R^2 estimations are those obtained from transformed data. Within-group relations were studied using covariance analysis (within-group regression and parallelism tests).

Results

Eight peaks were observed in DEW instead of the nine expected. Isomerization of pure 5-CQA led to two peaks for 3 isomers showing that 5- and 4-CQA peaks were in fact confounded (Ky et al. 1997).

Table 1 Chlorogenic acid contents. Contents are expressed in percentage dry matter basis (%dmb). Table includes main group characteristics (2nd to 5th columns) with averages (top number) and minimum-maximum values (lower number), and *F* tests (6th and 7th columns) with test results (top number) and factorial contributions (lower number in brackets). Results of the Newmann & Keuls test are indexed with letters

	Averages and ranges					Between-group differences	Within-group differences
	DEW	BCDEW	F_1	BCPSE	PSE	$F_{4,30}$	$F_{30,70}$
CGA	7.97[a]	5.81[b]	4.36[c]	2.50[d]	1.51[e]	114***	41.2 ***
	6.2–8.8	5.0–6.6	3.6–5.2	1.9–3.1	1.3–1.8	(93.9%)	(5.7%)
CQA	6.33[a]	4.41[b]	3.34[c]	1.81[d]	1.40[d]	103***	53.9***
	5.1–7.5	3.6–4.9	2.7–3.8	1.3–2.3	1.2–1.7	(93.3%)	(6.3%)
diCQA	1.18[a]	0.97[b]	0.67[c]	0.45[d]	0.04[e]	54.2***	32.5***
	0.9–1.4	0.8–1.2	0.5–0.8	0.2–0.9	0.02–0.06	(87.8%)	(11.2%)
FQA	0.465[a]	0.431[a]	0.344[a]	0.236[a,b]	0.075[b]	6.11***	178***
	0.24–0.82	0.13–0.90	0.20–0.61	0.09–0.43	0.04–0.13	(41.9%)	(57.1%)
CQA/CGA	0.794[b]	0.760[a,b]	0.767[a,b]	0.725[a]	0.925[c]	25.8***	126***
	0.76–0.86	0.71–0.81	0.72–0.83	0.63–0.81	0.87–0.95	(77.7%)	(21.7%)
diCQA/CGA	0.149[a]	0.167[a]	0.155[a]	0.176[a]	0.025[b]	17.2***	127***
	0.11–0.18	0.14–0.19	0.11–0.19	0.10–0.32	0.01–0.04	(69.4%)	(29.9%)
FQA/CGA	0.058[a]	0.072[a]	0.078[a]	0.099[a]	0.049[a]	1.95 NS	345***
	0.03–0.08	0.02–0.14	0.06–0.12	0.05–0.15	0.03–0.09	–	(99.22%)
4-&5-CQA	5.80[a]	4.15[b]	3.21[c]	1.74[d]	1.37[d]	80.3***	63.7***
	4.4–6.9	3.3–4.6	2.6–3.7	1.3–2.2	1.2–1.6	(91.7%)	(8.0%)
3-CQA	0.532[a]	0.257[b]	0.134[c]	0.073[d]	0.026[e]	158***	38.8***
	0.43–0.66	0.24–0.31	0.12–0.15	0.04–0.14	0.020–0.032	(95.5%)	(4.1%)
4-&5-CQA/CQA	0.914[a]	0.941[b]	0.959[c]	0.960[c]	0.981[d]	22.9***	73.2***
	0.87–0.93	0.91–0.95	0.95–0.97	0.94–0.98	0.97–0.99	(75.3%)	(23.7%)
4,5-diCQA	0.596[a]	0.512[a]	0.325[b]	0.214[c]	0.025[d]	45.5***	22.0***
	0.36–0.71	0.41–0.69	0.28–0.42	0.09–0.40	0.012–0.045	(85.4%)	(12.8%)
3,5-diCQA	0.321[a]	0.254[b]	0.227[b]	0.166[b]	0.007[c]	27.3***	42.1***
	0.24–0.39	0.24–0.29	0.14–0.30	0.08–0.40	0.002–0.015	(78.3%)	(20.3%)
3,4-diCQA	0.262[a]	0.204[b]	0.122[c]	0.071[d]	0.005[e]	60.6***	20.2***
	0.22–0.30	0.15–0.28	0.08–0.17	0.02–0.14	0.004–0.007	(88.6%)	(9.8%)
4,5-diCQA/diCQA	0.501[b]	0.527[b]	0.488[b]	0.481[b]	0.657[a]	12.6***	4.15***
	0.40–0.55	0.43–0.59	0.58–0.74	0.41–0.54	0.58–0.74	(52.8%)	(24.2%)
3,5-diCQA/diCQA	0.273[b]	0.264[b]	0.332[a]	0.371[a]	0.184[c]	14.5***	7.18***
	0.25–0.31	0.25–0.28	0.27–0.38	0.27–0.48	0.08–0.29	(60.2%)	(26.8%)
3,4-diCQA/diCQA	0.225[a]	0.209[a,b]	0.180[a,b]	0.148[b]	0.159[b]	4.09**	7.02***
	0.19–0.28	0.18–0.29	0.15–0.21	0.12–0.20	0.06–0.29	(28.3%)	(60.2%)
5-FQA	0.382[a]	0.296[a]	0.253[a]	0.200[a,b]	0.064[b]	5.14**	271***
	0.18–0.66	0.05–0.63	0.17–0.48	0.05–0.35	0.036–0.12	(37.0%)	(62.3%)
4-FQA	0.049[a]	0.115[b]	0.091[b]	0.036[a]	0.011[a]	11.5***	19.6***
	0.02–0.10	0.05–0.23	0.04–0.13	0.01–0.05	0.007–0.016	(67.3%)	(32.7%)
3-FQA	0.036[a]	0.020[b]	0.000[c]	0.000[c]	0.000[c]	18.7***	89.6***
	0.019–0.064	0.0–0.041	0.0–0.0	0.0–0.0	0.0–0.0	(71.3%)	(27.7%)
5-FQA/FQA	0.818[a]	0.662[b]	0.734[a,b]	0.810[a]	0.849[a]	5.27**	70.0***
	0.76–0.86	0.41–0.81	0.62–0.82	0.77–0.91	0.76–0.90	(37.2%)	(60.2%)
4-FQA/FQA	0.106[a]	0.298[b]	0.266[b]	0.190[a,b]	0.151[a]	5.49**	72.5***
	0.07–0.13	0.18–0.59	0.18–0.38	0.09–0.23	0.10–0.24	(38.4%)	(59.1%)
3-FQA/FQA	0.076[a]	0.040[b]	0.000[c]	0.000[c]	0.000[c]	42.5***	20.6***
	0.06–0.13	0.00–0.07	0.0–0.0	0.0–0.0	0.0–0.0	(84.4%)	(13.5%)

*** Very highly significant ($P < 0.001$)

[a] For explanation of abbreviations see Materials and methods (plant material)

Within-group variation was detected for all traits studied (Table 1). Its relative importance, as regards the general variation, varied among traits from 4% (3-CQA) to 99% (FQA/CGA). Some trends can be highlighted: within-group variation was lower for CQA isomer contents than for FQA and diCQA isomer contents, and was lower for isomer contents than for ratios. Otherwise, within-group variation was related to species average (CGA, CQA, 4-&5-CQA) in some cases and was similar in all groups (diCQA, 4,5-diCQA) or group-dependent (3,5-diCQA) in other cases.

Table 2 Quantitative effects of hybrid, harvest year, and interaction on chlorogenic acid contents and ratios. Factorial contributions are in brackets

	Hybrid $df = 11, 48$	Year $df = 1, 11$	Interaction $df = 11, 48$
CGA	48.4***	0.25 NS	7.80***
	(77.7%)	–	(22.3%)
CQA	71.4***	1.49 NS	11.0***
	(77.8%)	–	(22.2%)
diCQA	23.5***	24.7***	2.35*
	(65.3%)	(26.9%)	(7.8%)
FQA	121***	20.8***	9.48***
	(71.3%)	(18.6%)	(22.0%)
CQA/CGA	82.2***	66.4***	8.63***
	(42.7%)	(49.3%)	(8.0%)
diCQA/CGA	56.3***	54.0***	2.06*
	(73.2%)	(24.0%)	(2.8%)
FQA/CGA	166***	15.3***	18.0***
	(68.2%)	(17.8%)	(14.1%)
5-& 4-CQA	77.8***	1.14 NS	12.0***
	(77.7%)	–	(22.3%)
3-CQA	21.5***	8.46*	4.60***
	(61.3%)	(17.1%)	(21.6%)
5-& 4-CQA/CQA	55.6***	4.63 NS	6.07***
	(84.3%)	–	(15.7%)
4,5-diCQA	21.6***	13.9**	2.18*
	(74.4%)	(17.0%)	(8.6%)
3,5-diCQA	22.0***	21.0***	2.78**
	(62.0%)	(27.4%)	(10.5%)
3,4-diCQA	13.9***	29.0***	2.69**
	(44.8%)	(43.5%)	(11.7%)
4,5-diCQA/diCQA	4.26***	4.90*	1.50 NS
	(76.9%)	(23.1%)	–
3,5-diCQA/diCQA	5.54***	0.026 NS	2.55*
	(59.5%)	–	(40.5%)
3,4-diCQA/diCQA	8.50***	6.27*	3.09**
	(52.1%)	(18.9%)	(29.1%)
5-FQA	121***	20.6***	7.45***
	(76.3%)	(15.5%)	(8.2%)
4-FQA	65.1***	4.49*	15.8***
	(62.4%)	(8.9%)	(28.7%)
3-FQA	261***	12.2**	24.9***
	(73.4%)	(13.1%)	(13.5%)
5-FQA/FQA	173***	0.074 NS	42.7***
	(67.3%)	–	(32.7%)
4-FQA/FQA	232***	0.001 NS	51.2***
	(69.7%)	–	(30.3%)
3-FQA/FQA	205***	4.58 NS	10.4***
	(91.6%)	–	(8.4%)

* Significnt ($0.01 < P \leqslant 0.05$); ** highly significant ($0.001 < P \leqslant 0.01$); *** very highly significant ($P < 0.001$). NS, Not significant

Some environmental effects (between years 1995 and 1997) were also studied. Between-tree differences were recorded again for all isomer contents (Table 2), but in particular, most traits showed both year and interaction effects. Some traits, mainly ratios, showed only interaction effects, whereas the 4,5-diCQA/diCQA ratio presented a year effect without interaction. The importance of environmental effects (years + interactions) varied between traits from 8.4% (3-FQA/FQA) to 57% (CQA/CGA). In general, minor isomers showed greater environmental effects.

Quantitative inheritance of total chlorogenic acid content (CGA)

Chlorogenic acid contents differed between parental species (Table 1): CGA content of green beans of DEW was 8.0% dmb, i.e. about 5 times that of PSE. In the F_1 hybrids, CGA content was similar to that of the mid-parental species and differed significantly from those of parental species. CGA content in BCPSE was intermediate between F_1 and PSE averages, whereas CGA content in BCDEW was between F_1 and DEW values. This suggests an additivity hypothesis for this trait, as can be verified in Fig. 1.

Quantitative inheritance of caffeoylquinic acid content (CQA)

CQA levels represented 79% and 93% of the CGA content in DEW and PSE, respectively, and these values are significantly different (Table 1). Nevertheless, the quantitative inheritance of the ratio was not simple. Indeed, F_1 and BCDEW showed a ratio close to that of DEW, but the BCPSE ratio (72%) was in particular significantly lower than the DEW ratio. Conversely, quantitative inheritance of CQA content was simpler than for the ratio. CQA content, which was 4.5-fold higher in DEW than in PSE, showed an intermediate value between parental species in F_1 hybrids,

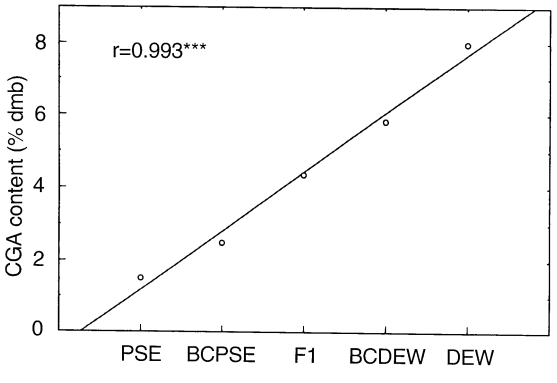

Fig. 1 Regression analysis showing additivity of CGA content

Table 3 Biochemical traits showing additivity either without or with data transformation. Non-additive traits are not presented. Correlation coefficient (*r*) and its significance from regression analysis as in Fig. 1

Biochemical trait	Transformation	*r*	Significance
CQA	No transformation (y = x)	0.981	**
4-&5-CQA/CQA	No transformation (y = x)	0.965	**
4-&5-CQA	No transformation (y = x)	0.984	**
3-CQA	$y = \sqrt{x}$	0.984	**
DiCQA	No transformation (y = x)	0.993	***
4,5-diCQA	No transformation (y = x)	0.993	***
3,5-diCQA	$y = x^2$	0.998	***
3,4-diCQA	No transformation (y = x)	0.998	***
FQA	$y = x^2$	0.994	***
5-FQA	$y = x^2$	0.984	**

** Highly significant ($0.001 < P \leqslant 0.01$); *** very highly significant ($P = 0.001$)

suggesting additivity. This was confirmed by values observed in the two backcrosses (Table 3).

The contents of 4- and 5-CQA isomers varied from 1.37% dmb in PSE to 5.80% dmb in DEW, and the additivity hypothesis fitted the data well (Table 3). 4- and 5-CQA levels represented 91% of the CQA content in DEW and 98% in PSE, the two values being significantly different (Table 1). Quantitative inheritance of the ratio seems additive (Table 3), but discrepancies between observed and expected values were larger than for CGA and CQA. This is probably due to the relative importance of the within-group variation associated with the small number of trees used to estimate each average.

The 3-CQA content varied from 0.026% dmb in PSE to 0.532% dmb in DEW. Its quantitative inheritance was not additive. In fact, only the square root of its content was additive (Table 3).

An inter-group and curvilinear relations was emphasized between 3-CQA and 4- and 5-CQA contents (Fig. 2a). The allometric model ($y = ax^b$) statistically accounted for 89% of the variance and the b parameter was close to 2 (b = 1.803), meaning that the relation is quadratic. The curvilinearity explains why the 4- and 5-CQA/CQA ratio varied between groups. Conversely, no relation was observed within groups ($F_{1,29} = 1.93$; $P = 0.17$).

Quantitative inheritance of dicaffeoylquinic acid content (diCQA)

DiCQA levels represented 15% and 2.5% of the CGA content in green beans of DEW and PSE, respectively; these values being significantly different (Table 1). Quantitative inheritance of the ratio was not additive: the averages of BCDEW, F_1, and BCPSE hybrids did not differ from the DEW average. Note the presence of a BCPSE hybrid in which the diCQA level accounted for up to 32% of CGA content. No explanation can be given for this exception as for all other chlorogenic acids, it had values expected for a BCPSE hybrid. Quantitative inheritance of diCQA content was simpler than that of the ratio. diCQA content was 29-fold higher in DEW, and F_1 hybrids showed an intermediate value between parental species. Additivity was confirmed by values observed in the two backcrosses (Table 3).

The 4,5-diCQA content varied from 0.025 in PSE to 0.596 in DEW (Table 1). F_1 hybrids showed an average intermediate between those of the parental species, and additivity was confirmed in the backcrosses (Table 3). These contents represented 50% and 66% of diCQA content in DEW and PSE, respectively, and these ratios were significantly different (Table 1). Hybrid groups (BCDEW, BCPSE, and F_1) did not differ from DEW for the 4,5diCQA proportion.

The 3,5-diCQA content was 46-fold higher in DEW than in PSE (Table 1). Additivity can be assumed, but discrepancies between expected and observed values were not negligible. In contrast, additivity became evident when data were transformed using $y = x^2$ (Table 3). This isomer represented 27% and 18% of diCQA content in DEW and PSE, respectively, and these ratios were significantly different (Table 1). Genetic behavior of the 3,5-diCQA ratio was complex: BCDEW did not differ from DEW, whereas F_1 and BCPSE showed the highest values (35%).

The 3,4-diCQA content was 52-fold higher in DEW than in PSE (Table 1). Table 3 shows clearly that additivity can be fully accepted for this isomer content. Proportions of 3,4-diCQA in diCQA were similar to, but slightly lower than, proportions of 3,5-diCQA. As for other ratios, quantitative inheritance was not simple: the additivity hypothesis can be accepted for F_1 and BCDEW hybrids and rejected for BCPSE hybrids.

An inter-group and linear relation was noted between 3,4-diCQA and 3,5-diCQA contents (Fig. 2b). The allometric model [$y = a(x + c)^b$] statistically accounted for 97% of the variance and the b parameter was close to 2 (b = 2.17), indicating that the relation is quadratic. Parameter c was necessary to linearize the relation using logarithmic transformation, and this means that 3,4-diCQA could theoretically be present

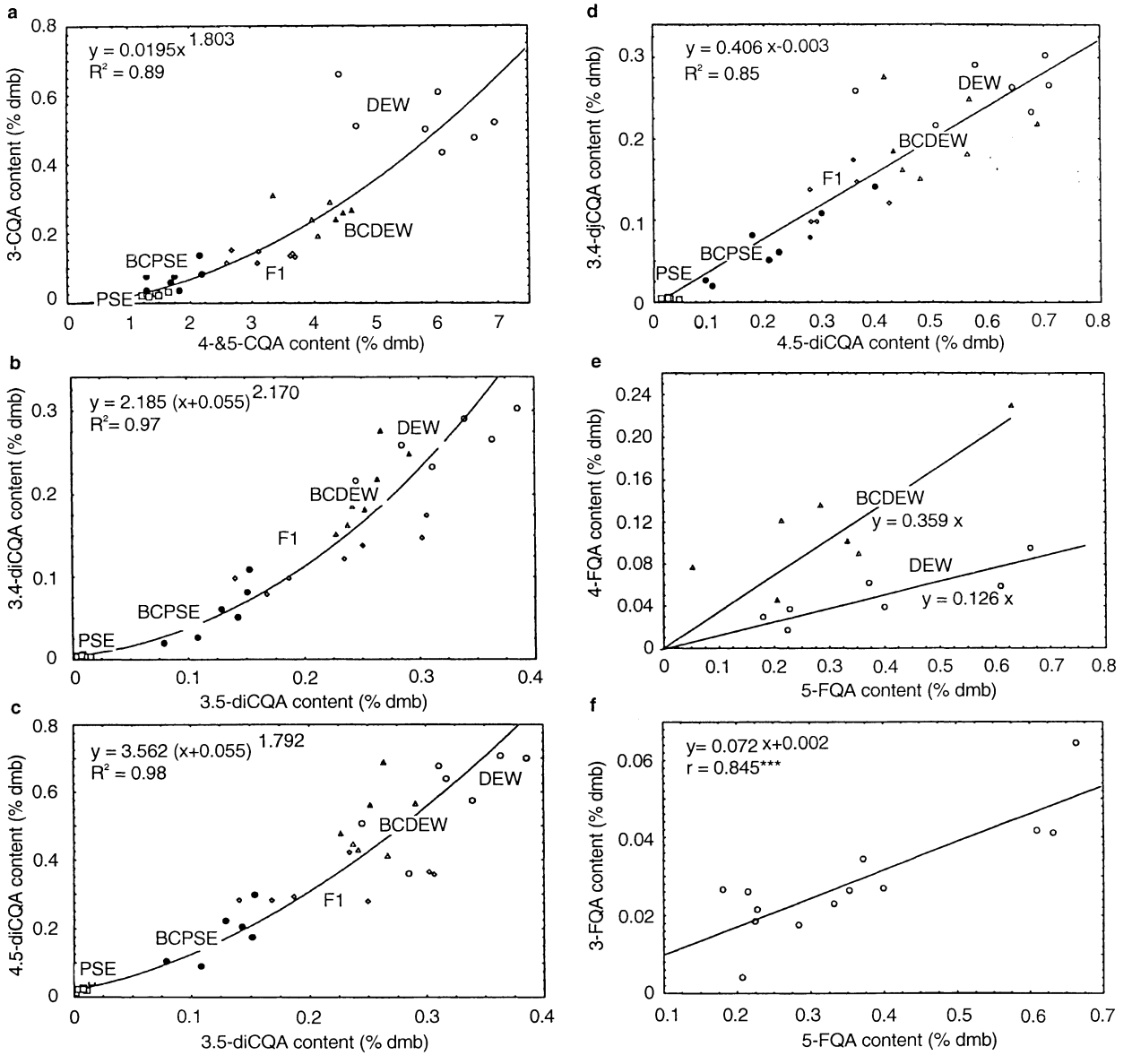

Fig. 2a–f Relations between isomer contents within CGA classes

even if 3,5-diCQA is absent. A within-group relation was also detected ($F_{1,28} = 26.3$; $P = 0.00002$) with different slopes (parallelism test: $F_{4,24} = 9.33$; $P = 0.0001$). In fact, slopes of within-group relations seemed close to the tangent to the curve, i.e., increased linearly with the 3,5-diCQA content.

An inter-group and curvilinear relation was also recorded between 4,5-diCQA and 3,5-diCQA contents (Fig. 2c). The allometric model [$y = a\ (x + c)^b$] statistically explained 98% of the variance, and the b parameter was again close to 2 (b = 1.79). Note that the c parameter had the same value as in the 3,4-diCQA-3,5-diCQA relation. A within-group relation was again noted ($F_{1,28} = 14.1$; $P = 0.0008$), with different slopes ($F_{4,24} = 3.48$; $P = 0.022$).

Another inter-group, but linear, relation was observed between 3,4-diCQA and 4,5-diCQA contents (Fig. 2d). Linearity can be easily explained when we consider the two other relations as quadratic (b = 2). Again, a within-group relation was noted ($F_{1,28} = 12.0$; $P = 0.0017$), but with a common slope ($F_{4,24} = 1.13$; $P = 0.37$).

Quadratic relations explain why the three diCQA ratios differed between species and are consistent with our results on 3,5-diCQA additivity after transformation.

Quantitative inheritance of feruloylquinic acid content (FQA)

The FQA level represented 7% of the CGA content on average, and no significant difference was observed

between groups for the ratio (Table 1). In contrast, FQA content differed greatly between groups, especially between DEW and PSE. As for 3,5-diCQA, additivity can be recorded when we take into account the transformed values using "$y = x^2$" (Table 3).

The 5-FQA isomer accounted for the same proportion of FQA (83%) in the two parental species (Table 1). As expected, F_1 hybrids and BCPSE hybrids showed the same ratio as the parental species, but BCDEW hybrids gave a significantly lower ratio. In contrast with the ratio, 5-FQA content varied greatly between species, with PSE presenting a sixfold lower ratio (Table 1). As for CQA, additivity was accepted for 5-FQA (Table 3).

The proportion of 4-FQA in FQA did not differ significantly between parental species (Table 1). Nevertheless, the ratio increased linearly from PSE (15%) to BCDEW (30%). More surprising, the 4-FQA content showed a genetic behavior close to the ratio: content increased linearly from PSE to BCDEW, but DEW had a twofold lower content than BCDEW.

The 3-FQA isomer was a minor isomer. It was absent in PSE, BCPSE and F_1, and represented only 7.6% of FQA, i.e., 0.4% of the CGA content in DEW. Nevertheless, this isomer is genetically interesting: indeed, results could be interpreted as a dominance of the absence of 3-FQA. In addition, when we assume 50% heterozygotes in BCDEW, we expect a BCDEW average intermediate between those of F_1 and DEW, as observed (Table 1).

A relation between 5-FQA and 4-FQA was detected in four groups. It was linear, but weak (only 37% of the 4-FQA content variation was statistically explained by 5-FQA content variation). In fact, two situations can be defined: (1) absence of a relation within PSE, BCPSE, and F_1; and (2) presence of a relation for BCDEW and DEW, i.e., in groups with the highest 5-FQA content. Nevertheless, the relation was different within DEW and BCDEW: the slope was threefold higher in BCDEW than in DEW (Fig. 2e).

A linear relation was also recorded between 3-FQA and 5-FQA for trees with 3-FQA, i.e., in DEW and in BCDEW. The two within-group relations did not differ in slope ($F_{1,9} = 0.11$; $P = 0.75$) or intersect ($F_{1,10} = 3.00$; $P = 0.11$). A common relation was then estimated (Fig. 3a), for which the intersect can be considered as equal to 0 ($t = 0.484$; $df = 11$; $P = 0.637$).

Relations between CGA classes

An almost linear between-group relation existed between diCQA and CQA content (Fig. 3a), but PSE was unrelated. The allometric model [$y = a(x)^b$] statistically explained 81% of the variance and the b parameter was again close to 1 ($b = 0.95$) meaning that the linear model could be appropriate. A within-group relation was noted ($F_{1,22} = 4.63$; $P = 0.043$), with a common slope ($F_{3,19} = 0.29$; $P = 0.830$).

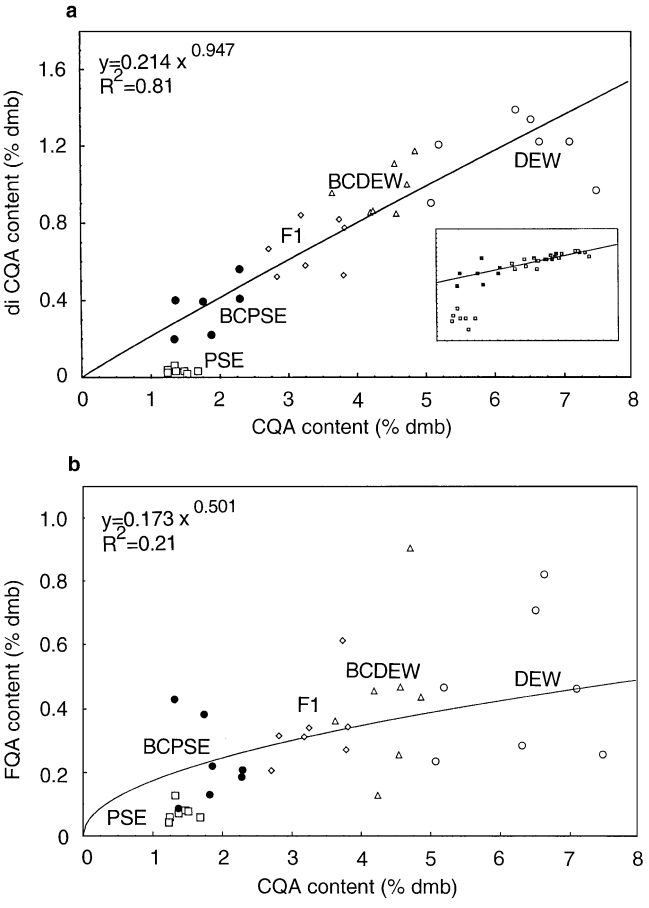

Fig. 3a, b Relations between CGA class contents

Another allometric, but weaker, relation was seen between groups for FQA and CQA contents (Fig. 3b). As before, CQA content seems to be over-represented in PSE. Two fittings were applied: (1) with BCPSE, F_1, BCDEW, and DEW groups using $y = a\ (x)^b$, and (2) with all groups using $y = a\ (x + c)^b$. The two fittings gave similar values of b (0.437 and 0.501, respectively), both close to 0.5. The relation seems to be of the square root type. This is consistent with the fact that FQA must be transformed using X^2 to become additive, while CQA is additive without transformation. Note that CQA additivity would be better if we consider that CQA is over-represented in PSE (Fig. 1).

Discussion

An overview of chlorogenic acid biosynthetic pathways is necessary to interpret our results. In coffee trees, all biosynthetic pathways of CGA formation occur in the leaves (Colonna 1986). CGA is derived from phenylalanine (Villegaas and Kojima 1986). The caffeic

acid unit, which exists as caffeoyl-D-glucose, reacts with the quinate unit to give the 5-CQA isomer. The enzyme catalyzing the reaction is the quinate hydroxycinnamoyl transferase (CQT). The isomeric monosubstituted quinic acids, e.g. 3-CQA and 4-CQA, are produced by enzymatic trans-esterification of 5-CQA.

The 3,5-diCQA was formed by caffeate substitution of 5-CQA in the 3-position by a chlorogenic acid caffeoyl transferase. The other two disubstituted quinates, e.g., 3,4diCQA and 4,5-diCQA, were also formed by enzymatic *trans*-esterification of 3,5-diCQA (Friedman 1997). Therefore, all of the diCQA isomers derived from 5-CQA.

In contrast, FQA isomer formation was independent of 5-CQA synthesis. A quinate transferase other than CQT catalyzes the reaction of feruloyl-CoA with quinate. The 5-FQA is formed first, whereas the 3- and 4-FQA are derived from *trans*-esterification (Ulbrich and Zenk 1979). In CQA and FQA, the migration of the cinnamoyl group is an intramolecular process which occurs in the sequence $5 \Leftrightarrow 4$ and $5 \Leftrightarrow 3$ (Hanson 1965). Useful descriptions can be found in Morigushi et al. (1988) and Tanaka and Kojima (1991).

CGA are then exported to beans where their accumulation is maximum when the fruits are green. During fruit maturation, the CGA level decreases (Petnga 1986). Thus, from their biosynthesis in leaves to their accumulation in beans at complete maturity, a lot of processing occurs which leads to quantitative variation in their contents.

Relations between isomers within CGA classes

Relations between isomers within CGA classes seem to result from the isomerization processes. In the CQA and diCQA classes, our results agreed well with known pathways. The relations between 4-FQA and 5-FQA in the BCDEW and DEW groups is also due to the isomerization process. Its absence in the PSE, BCPSE and F_1 groups could be explained by a lack of accuracy due to the low content of 4-FQA in such hybrids. This is also true for the relation between 3-FQA and 5-FQA (3-FQA was not detected in the PSE, BCPSE, and F_1 groups).

Changes in relations are more surprising: some relations were linear, whereas others were curvilinear. Differences appeared even for isomers belonging to the same CGA class. As an example, in the case of the diCQA class, 4,5-diCQA and 3,4-diCQA were curvilinearly related to 3,5-diCQA, while 3,4-diCQA was linearly related to 4,5-diCQA. A linear relation is expected from enzymatic reactions at equilibrium: the product/reactant ratio was indeed constant. Deviation from linearity could be due to the involvement of 3,5-diCQA in other specific pathways. Indeed, 3,5-diCQA should react with caffeine to form a complex (Horman and Viani 1971; Kappeler et al. 1987; Baumann and Röhrig 1989). The curvilinear relation between 3-CQA and 4- and 5-CQA could be similarly explained: 5-CQA, which contributes mainly to the 4- and 5-CQA peak (Clifford 1985b), is known to complex with caffeine (Baumann et al. 1993). In contrast, the linear relation between 4,5-diCQA and 3,4-diCQA suggests that 4,5-diCQA and 3,4-diCQA would either not complex with caffeine or would complex at a much lower rate.

The slope difference observed for the relation between 4-FQA and 5-FQA in BCDEW and DEW is strange. Because the intersect of straight lines was nil, the slope can be interpreted as a 4-FQA/5-FQA ratio, which would be relatively constant within groups (as expected) but would differ greatly between BCDEW and DEW. Nevertheless, parental species similarity in 4-FQA/5-FQA ratios complicates the interpretation problems of such results, for which further studies appear necessary.

Relations between CGA classes

The fact that we summed up the individual level of each of the three isomers for each respective CGA class in order to obtain CQA content, diCQA content, or FQA content has a biological significance when biosynthesis pathways are taken into account. For example, CQA content actually reflects the quantity of 5-CQA synthesized before isomerization.

A relation between CQA and diCQA contents is again expected from our knowledge of biosynthesis: diCQA derives directly from CQA. Conversely, the relation between FQA content and CQA content is indirect: FQA does not derive from CQA, but both 5-CQA and 5-FQA synthesis requires a common substrate, the quinate unit. Indirect effects also explain why the FQA-CQA relation was weaker than its diCQA-CQA counterpart. Similarly, the absence of a relation between FQA and diCQA contents is in agreement with the larger distance between their biosynthesis pathways.

The two between-class relations were not linear. Concavity would mean that diCQA and FQA are implicated in biosynthesis pathways other than that for CQA. FQA is known to be involved in lignin biosynthesis pathways (Hahlbrock and Grisebach 1979) and diCQA plays a role in cafeic acid detoxification (Colonna 1979).

Genetic control of the 3-FQA isomer content

The absence of 3-FQA in PSE, BCPSE and F_1 could be an artefact due to a lack of accuracy in measuring individuals with very low contents. Nevertheless, 3-FQA was also absent from BCDEW hybrids for which 5-FQA content was high enough to suggest a detectable 3-FQA content.

3-FQA isomer content seems to be controlled by one major gene with a dominant allele, leading to the absence of 3-FQA. Parental species would be FF and ff for PSE and DEW, respectively, whereas F_1 hybrids would be Ff . All BCPSE would have the F allele, leading to absence of 3-FQA. In contrast, BCDEW hybrids would be Ff or ff in a 1 : 1 proportion, explaining why the average 3-FQA content of BCDEW (0.020%dmb) was intermediate between F_1 (0.00% dmb) and DEW (0.36%dmb).

Physiologically, a compound may fail to accumulate because of the: (1) absence of the enzyme responsible for its biosynthesis, (2) absence of transport from leaves (biosynthesis tissues) to beans (storage tissues), and (3) presence of an enzyme responsible for its transformation before transport, i.e., in leaves or later in fruits. Nevertheless, the dominance of absence favors the third hypothesis. Further studies on leaves and fruits during maturation would provide some explanations.

Additivity

Genetic inheritance of CGA isomer contents showed three types of genetic behavior. The first case concerned traits showing additivity without data transformation: CGA, CQA, diCQA, 5-CQA, 4,5-diCQA, and 3,4-diCQA. The second case concerned traits for which the additive model was present after data transformation. Our results are consistent with previous interpretations on the relations between isomers or between classes. For example, with 5-CQA being additive and 3-CQA being non-linearly related to 5-CQA, the inverse transformation to obtain additivity for 3-CQA is expected to be efficient. We can verify that additivity after data transformation concerns isomers (3,4-diCQA, 4,5-diCQA, 3-CQA) and classes (FQA) which are non-linearly related to true additive traits. An additive model after data transformation has already been encountered in the same interspecific cross for caffeine (Barre et al. 1998). In this example, additivity of the square root of caffeine content has been observed. The third case concerns the contents for which additivity was not or partly observed. For 3-FQA, which was discussed above, additivity was verified for F_1, BCDEW and DEW. The situation was more complex for 4-FQA ($PSE = BCPSE = DEW < F_1 = BCDEW$) and cannot be interpreted easily.

Lastly, note the absence of additivity for most ratios except 4- and 5-CQA/CQA. Here too, curvilinear relations explain well the absence of additivity for most ratios.

Environmental effects and interactions

Genetic effects represented 78% of the variation in the best cases, thereby showing the relative importance of environmental effects and interactions. Environmental effects and interactions would have probably been more important in crosses of less genetically differentiated species. These effects include inter-annual climatic variation during bean maturation, but also maturity variation at harvest and bean-drying conditions after harvest. In particular, the greater importance of interactions for diCQA could be explained by their degradation during bean drying, which would change between years. Observed interactions constitute an example of "genotype × environment" interactions. This implies that CGA are sensitive to all types of environmental changes and, consequently, interactions should exist when genotypes are growing in different regions.

Conclusions and prospects

The consequences for breeding are many and varied: (1) additivity implies the possibility of choosing parents to obtain an expected average in progenies; (2) genetic diversity is the main source of variation allowing clonal selection; (3) the "hybrid-year" interaction is not important (22% of effects) indicating that observations should be recorded over several years to obtain a good estimate of the phenotypic average; (4) the presence of "genotype × environment" interactions should lead to a multilocal selection for coffee tree varieties for taste quality improvement. However, choosing hybrids with low CGA content (i.e., having good cup taste) would conflict with the resistance role played by high CGA content.

Biosynthetically speaking, data on isomers and between-class relations confirmed the presently accepted pathways both in (1) interdependence of each CGA class and (2) formation of each isomer within each class. From a genetics point of view the results showed that the 3-FQA isomer content was a trait governed by a single major gene dominant for 3-FQA absence. Other isomers showed different additive patterns. This study was the first using interspecific progenies to explain both genetic and physiological aspects of the CGA trait for a particular aim: comprehension and improvement of coffee cup taste or disease resistance.

As CGA is a complex trait implicated in aroma, physiology and genetics, further studies are needed. Study of the relations between CGA and other interesting traits such as morphological traits (leaf area, bean weight, etc.), phenological traits (fructification cycle period, etc.) and biochemical traits (sucrose, trigonelline, free amino acid contents, etc.) would lead to a better planning of diploid coffee tree breeding programs. Molecular tools would be helpful in screening for markers near interesting quantitative trait loci (QTL) to develop marker-assisted selection of plantlets.

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