# Dental fluctuating asymmetry in Portugal: developmental instability in Valença and the Coimbra reference sample

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#### Abstract

Fluctuating asymmetry (FA) is commonly used to estimate developmental instability (DI), the organism's response to ontogenic disturbance. This work aims to (1) select nonmetric dental traits for FA testing, according to representativeness and comparability; (2) test DI differences between Valença and Coimbra, two Portuguese samples. Valença (VLN) is a 17th-19th century sample of 31 individuals from a Fortress in the northern border of Portugal. Coimbra (COI) is a 19th/20th century sample of 600 individuals from the Coimbra identified collections, compiled as a Portuguese reference sample. Twenty-nine dental nonmetric traits were scored for Valença and Coimbra. Correlations of FA results between traits were computed. After removal of problematic traits, percentages of FA, Comparable FA (CFA) (removing cases with bilateral trait absence) and Variation FA (VFA) (removing cases with bilateral lack of trait expression) were calculated. FA percentages were compared using Mann-Whitney U rank-sum test and z-ratio (difference of independent proportions test). Samples do not present significant difference in mean individual percentage for FA, CFA, or VFA. Differences in asymmetry proportion were also not statistically distinct for CFA. Yet, proportions differed significantly for FA (VLN: 16.9%, COI: 10.7%; z=-2.34, p=0.0193) and VFA (VLN: 58.6%, COI: 39.3%; z=-2.133, p=0.0346). FA levels suggest Valença suffered from greater levels of DI than Coimbra. Despite of the low socioeconomic status of both samples, Valença individuals possibly had lesser access to adequate nutrition and healthcare due to Valença's rural context and peripheral location.

**Keywords:** bioarchaeology; nutrition and healthcare during development; nonmetric dental traits; modern and contemporary Portugal; peripheral town

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## Introduction

Bilateral asymmetry corresponds to differences in size or shape between the sides of an organism. Asymmetry in populations manifests in one of three ways. Directional asymmetry occurs when one side is more often larger than the other. Antisymmetry means symmetry rarely occurs, despite lack of a preferential side on which the asymmetry is clearly manifested. Finally, the type of bilateral asymmetry concerning this work is fluctuating. In fluctuating asymmetry (FA), asymmetry can occur or not and, when occurring, is manifested randomly in a population, without side preference (1–6).

FA is of interest in biological anthropology because it is considered a consequence of developmental instability (DI). During ontogeny, an organism's resources may be lacking in situations of physiological stress. These situations include exposure to pathogens or parasites, insufficient or inadequate nutrition, inadequate environmental conditions, for example. Maladaptation or exposure to these environmental issues results in DI, which can be measured through FA (2,4,7,8).

FA arises in dental morphology when a given nonmetric trait shows different grade expression between antimeres, or is expressed in one side and not on the other. Few studies were published on dental morphology FA in the last four decades (5,9–14).

Valença is located in the northern border with Spain (in the Minho River). Between the 17th and 19th centuries this city was strategically important, due to its relation to a natural border with Galicia (15), yet peripheral and set within a predominantly rural context. Coimbra is a relatively large city in the center of Portugal, with incipient industrial development and clear commercial activity (16). The Coimbra sample (19th to early 20th century) includes individuals born outside the city, and even abroad. However, this sample has been shown to be representative of the coeval Portuguese biological diversity, and is considered a reference sample for its period (17). The geographic and economic differences between these samples may have had biological impact, eventually causing distinctions in developmental instability.

This work aims to (a) select nonmetric dental traits scored in two Portuguese samples for FA testing, according to representativeness and comparability; (b) test DI differences between the Portuguese samples of Valença and Coimbra.

## Materials and Methods

Valença (VLN) is a peripheral settlement in the northern border of Portugal, in the Minho River. The VLN sample comprises 31 individuals (20 adults and 11 non-adults) from the Fortress of Valença, exhumed in the 2010 excavation of Santa Maria dos Anjos' churchyard, a necropolis from the 17th to 19th centuries. This is a relative chronology, reporting to stratigraphy and the presence of materials (ceramics, coins and military metal uniform buttons) from this period (18). Their churchyard burial suggests VLN individuals were likely of low socioeconomic status (19).

Coimbra (COI) is a 19th/20th centurv Portuguese reference sample mainly from the Coimbra (central Portuguese) region. COI individuals were mostly of low socioeconomic status, since their crania were gathered after temporary interment in public cemetery plots (20-22). The present COI sample is comprised of 600 individuals, collected and coupled with their identification for anthropological research purposes in the University of Coimbra. Most of the sample (569 individuals) is from the Trocas Internacionais collection, gathered by Eusébio Tamagnini between 1932 and 1942; the remainder (31 individuals) pertains to the Escolas Médicas collection, acquired by Bernardino Machado between 1886 and 1903 (20,21,23-25). This sample's FA analysis (10), dental morphology observer error (26) and phenotypic intrasample variation (17) were previously published.

Dental nonmetric traits were scored using ASUDAS (27) and Marado and Silva's adaptation of Weets' MMPT scoring method (28), after selecting twenty-nine traits from all permanent teeth. For these traits, three FA calculations were computed: (a) simple FA (present when bilateral traits were not of equal grade expression), (b) Comparable FA, or CFA (after removal of bilaterally absent cases) and (c) Variation FA, or VFA (after removal of cases with bilateral grade 0). Each FA calculation was analyzed using mean individual and tooth count percentages.

Kendall's T-b correlations were calculated between FA, CFA and VFA scores for each trait. One trait from trait pairs with strong (T>0.3 or T<-0.3) significant coefficients was removed. FA, CFA and VFA mean individual percentages were compared using Mann-Whitney U ranksum test, while FA, CFA and VFA tooth count

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percentages were compared through the z-ratio (difference of independent proportions test).

#### Results

Selected traits included in all the analyses are listed in Table 1. Table 2 displays traits excluded from one or more analyses because of strong correlation ( $\tau$ >0.3 or  $\tau$ <-0.3) to other trait(s) or impossibility to score in a sample.

Figure 1 shows the percentage of individual asymmetry for each sample and each FA type. Mann-Whitney U test results are shown in Table 3. Neither FA (VLN: 15.3%, COI: 12.3%; U=4226.5, p=0.560), CFA (VLN: 49.2%, COI: 44.6%; U=2503.5, p=0.612) or VFA (VLN: 54.9%%, COI: 40.5%; U=2299.5, p=0.110) show statistically significant difference in mean individual percentage.

Figure 2 reports the tooth count percentages for all samples and FA types. Z-ratios are presented in Table 4. Differences in CFA asymmetry proportion were not statistically detected (VLN: 57.7%, COI: 43.9%; z=-1.408, p=0.1591). However, the z-ratio showed significant (p<0.05) difference between the samples for FA (VLN: 16.9%, COI: 10.7%; z=-2.34, p=0.0193) and VFA (VLN: 58.6%, COI: 39.3%; z=-2.133, p=0.0346) comparisons.

Double shoveling (UI1)	C6 (LM1)	
Interruption grooves (UI2)	C7 (LM1)	
Peg incisors (UI2)	Distal trigonid crest	
	(LM1)	
Accessory cusps (UP3)	Protostylid (LM1)	
Enamel extensions (UM1)	MMPT (LM1)	
Hypocone (UM2)	Groove pattern (LM2)	
Lingual cusp number (LP4)	C6 (LM2)	
Deflecting wrinkle (LM1)	C7 (LM2)	
Anterior fovea (LM1)	Groove pattern (LM3)	
Groove pattern (LM1)	C5 (LM3)	
C5 (LM1)	C7 (LM3)	

Table 1. Selected traits (and observed teeth) included in all analyses.

## Discussion

Dental morphology fluctuating asymmetry (FA) may be used to infer levels of developmental instability (DI) in samples (5,10). DI is a form of phenotypic plasticity, which is contrary to stabilizing selection and therefore reduces population fitness (29). DI can signal the organism about unfavorable environmental conditions, so FA is likely the result of a trade-off between higher early post-natal growth rate and lower symmetry (30), yet can result in nefarious adult health consequences (31).

Trait (and tooth)	Excluded from:	Correlated to:	
Shoveling	FA	LM1 MMPT	
(UI1)		(τ=0.570, p<0.001,	
		n=76) in COI	
Distal	All	Not scorable in VLN	
accessory			
ridge (UC)			
Carabelli's	CFA	LP4 lingual cusp	
trait (UM1)		number (τ=-0.425,	
		p=0.011, n=37) in	
		COI	
Metaconule	CFA	LM2 C5 (τ=-0.810,	
(UM1)		p=0.010, n=11) in	
		COI	
Parastyle	FA	LM1 C7 (τ=0.331,	
(UM3)		p=0.003, n=83) in	
		COI	
C5 (LM2)	VFA	UM1 C5 (τ=-0.810,	
		p=0.010, n=11) in	
		COI	
C6 (LM3)	CFA	LM3 C5 (τ=0.397,	
		p=0.014, n=39) and	
		LM3 groove pattern	
		(τ=0.592, p=0.040,	
		n=13) in COI	

Table 2. Selected traits (and observed teeth) excluded from one or more analyses due to correlation to other traits. FA – fluctuating asymmetry; CFA – comparable fluctuating asymmetry; VFA – Variation fluctuating asymmetry; COI – Coimbra; VLN – Valença.

Previous studies on FA, as calculated from different structures, found significant differences indicating groups with lower access to resources because of socioeconomic (32–37) and chronological (6,38) differences suffered from greater DI.

In the period leading to, and during industrialization, Portugal remained a conservative country, clinging to the old regime.

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Figure 1. Mean individual percentages and standard deviations (SD) of Valença (VLN) and Coimbra (COI) samples, comparing fluctuating asymmetry (FA), Comparable FA (CFA) and Variation FA (VFA) scores.

Mann-Whitney U rank-sum	U	р	n
FA	4226.5	0.560	593
CFA	2503.5	0.612	510
VFA	2299.5	0.110	534

Table 3. Mann-Whitney U rank-sum test results from the comparison between VLN and COI of FA, CFA and VFA scores. FA – fluctuating asymmetry; CFA – comparable fluctuating asymmetry; VFA – Variation fluctuating asymmetry.

z-ratio (difference of independent proportions)	Z	р
FA	-2.340	0.0193
CFA	-1.408	0.1591
VFA	-2.133	0.0346

Table 4. Z-ratio of the difference of independente proportions of VLN and COI, comparing FA, CFA and VFA scores. FA – fluctuating asymmetry; CFA – comparable fluctuating asymmetry; VFA – Variation fluctuating asymmetry.

Agriculture was technically traditional and industry was incipient, which was reflected in the prevalence of poverty, economic underdevelopment, late and slow urban growth, high childhood mortality and high emigration rates (16,39–44).

The samples from Valença and Coimbra are of low socioeconomic status, according to their origin: Valença individuals were interred in a churchyard, a resting place of low prestige (19), while Coimbra individuals were interred in temporary public plots for lack of funds or family claim of the deceased (21,22).

This preliminary study indicates the Valença sample may have been subjected to higher levels of DI than COI, since two FA analyses suggest Valença has significantly higher FA frequency (Table 4). The FA discrepancies found may be justified by either geographical, chronological or economic differences between the samples, or by a combination of these factors. Valença occupies a strategic, yet peripheral, position in the Portuguese border with Spain along the Minho River (15). Between the 17th and 19th centuries (VLN's chronology), Portugal had a predominantly agricultural economy which growth was dependent on demographic growth (45,44). The city of Coimbra is central and close to the shore. In the 19th/20th century, Coimbra had a relatively strong commercial class and a concentration of artisans and skilled workers, notwithstanding its incipient industrialization (16).

Despite similar social standing, Valença's peripheral location and predominantly rural setting was possibly related to greater poverty, inequality and economic underdevelopment when compared to Coimbra, even within the general context of Portugal. If so, Valença's geo-economic status likely lead to a lower access to adequate nutrition, proper health care and appropriate living conditions for low social classes.



Figure 2 – Tooth percentages of Valença (VLN) and Coimbra (COI) samples, comparing fluctuating asymmetry (FA), Comparable FA (CFA) and Variation FA (VFA) scores.

#### Conclusions

Dental morphology fluctuating asymmetry was analyzed as a proxy for developmental instability in Valença and a Portuguese reference sample (Coimbra). Preliminary results indicate

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Valença's low status individuals could have greater physiological obstacles to the growth process than Coimbra's. Economic indicators suggest COI, a post-industrial sample from a more developed city, likely provided better access to adequate nutrition, health care and living conditions than a pre- and early postindustrial sample from a peripheral town (VLN).

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