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Facial Asymmetry and Genetic Ancestry in Latin American Admixed Populations

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ABSTRACT Fluctuating and directional asymmetry are aspects of morphological variation widely used to infer environmental and genetic factors affecting facial phenotypes. However, the genetic basis and environmental determinants of both asymmetry types is far from being completely known. The analysis of facial asymmetries in admixed individuals can be of help to characterize the impact of a genome's heterozygosity on the developmental basis of both fluctuating and directional asymmetries. Here we characterize the association between genetic ancestry and individual asymmetry on a sample of Latin-American admixed populations. To do so, three-dimensional (3D) facial shape attributes were explored on a sample of 4,104 volunteers aged between 18 and 85 years. Individual ancestry and heterozygosity was estimated using more than 730,000 genome-wide markers. Multivariate techniques applied to geometric morphometric data were used to evaluate the magnitude and significance of directional and fluctuating asymmetry (FA), as well as correlations and multiple regressions aimed to estimate the relationship between facial FA scores and heterozygosity and a set of covariates. Results indicate that directional and FA are both significant, the former being the strongest expression of asymmetry in this sample. In addition, our analyses suggest that there are some specific patterns of facial asymmetries characterizing the different ancestry groups. Finally, we find that more heterozygous individuals exhibit lower levels of asymmetry. Our results highlight the importance of including ancestry-admixture estimators, especially when the analyses are aimed to compare levels of asymmetries on groups differing on socioeconomic levels, as a proxy to estimate developmental noise. Am J Phys Anthropol 000:000–000, 2015. © 2014 Wiley Periodicals, Inc.

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Facial asymmetries have been the focus of extensive research from several perspectives, including the assessment of variation in facial asymmetries on healthy young adult subjects (Ercan et al., 2008), facial dysmorphologies produced by prenatal alcohol exposure (Klingenberg et al., 2010b), sexual dimorphism (Claes et al., 2012), the relationship among asymmetries and growth and development (Wilson and Manning, 1996; Ferrario et al., 2001; Djordjevic et al., 2013), and the evaluation of dental fluctuating asymmetry (FA) as an indicator of developmental stress on Neanderthals and anatomically modern populations (Barrett et al., 2012), among others. As a whole, this vast array of studies suggest that the interest of researchers about asymmetry is not only focused on its genetic basis, but also on the putative environmental effects that affect its expression and, obviously, on the way in which both effects interact during the development.

Asymmetry, defined as the lack or absence of symmetry (Van Valen, 1962), has three observable patterns in nature: fluctuating asymmetry (FA), antiasymmetry (AS), and directional asymmetry (DA). FA constitute small, random departures from perfect symmetry, and its variation is normally distributed around a mean of zero; AS involve a pattern of left-right variation distributed about a mean of zero, but the frequency distribution departs from normality in the direction of platykurtosis or bimodality; finally, DA displays a pattern of left-right variation distributed about a mean that is significantly different from zero. Mardia et al. (2000) and Schaefer et al. (2006) defined the individual asymmetry (IA) component as the additive decomposition of DA, FA, and measurement error. Among the different asymmetries defined by Van Valen (1962), FA can be seen as the most relevant concerning studies of facial variation because it is considered a common indicator of development instability that can be measured directly on bilateral human phenotypic attributes (Palmer and Strobeck, 1986; Møller and Swaddle, 1997; Milne et al., 2003; Little et al., 2008; DeLeon and Richtsmeier, 2009; Özener and Fink, 2010; Özener, 2010; Weisensee, 2013), and it is observable in other animals as well (e.g., Leary and Allendorf, 1989; Clarke, 1993; Møller, 1996; Allenbach et al., 1999; Lens and Van Dongen, 2008; Little et al., 2012). Some previous analyses, however, challenged the existence of such straightforward relationship between FA and developmental instability (McKenzie and Clarke, 1988; Graham et al., 1993; Bjorksten et al., 2000; Lens et al., 2002).

The main goal of the investigations focused on IA has been the characterization of the "*deviation*" of a normal *bauplan*, as a response to a wide array of factors such as sexual selection (Gangestad et al., 2001, 2010), heavy working conditions and socioeconomic status (Özener and Fink, 2010; Özener, 2010a), or attractiveness (Scheib et al., 1999; Gangestad and Thornhill, 2003; Little and Jones, 2003; Rhodes et al., 2009; Zaidel and Hessamian, 2010; Neby and Ivar, 2013). In addition, DA has been reported in humans (McIntyre and Mossey, 2002; Schaefer et al., 2006; DeLeon, 2007; Ercan et al., 2008; Klingenberg et al., 2010b), although it is considered a subtle phenomenon.

Recently, Bigoni et al. (2013) reported a significant relationship between DA/FA and socioeconomic structure. Their analysis identified highest values of DA in the lower socioeconomic levels of the sample. In general, DA is considered to be more determined by genetic factors, since in principle, one can knows the probability of which side of the face will be more or less developed (Møller and Swaddle, 1997). Other studies in non-human animals have consistently found evidence of DA in wing shape of bees (Smith et al., 1997; Klingenberg et al., 2001), mouse mandibles (Leamy et al., 1997), and flies (Klingenberg et al., 1998).

Up to date, the way in which both, directional and fluctuating facial asymmetries, are determined by genetic and/or environmental effects and their putative interaction is far from being completely known. For instance, in their review on the genetics basis of FA, Leamy and Klingenberg (2005; p. 9) stated that "given that the precise relationship between FA and developmental instability remains speculative, our present state of knowledge of the extent of additive genetic variation for developmental instability is even less than that for FA". A possible explanation for this lack of precision is that the data available to explore the genetic basis of facial asymmetries is scarce. In addition, some heritability estimation of FA are contradictory, reporting low and nonsignificant values for mice mandibular characters (Leamy, 1999); while others authors report significant FA heritability estimation on several species of flies (Scheiner et al., 1991; Santos, 2002). To the best of our knowledge, there are still no case reports providing heritability values FA attributes in human faces. Regarding the genetic basis of DA, Leamy et al. (2000) found three significant QTLs for DA affecting the size of mice mandible characters and accounting for 1% of the total phenotypic variation in DA, which is less than the 3–6% obtained on previous estimations made on mandible characters (Leamy, 1984, 1999; Leamy et al., 1997).

The analysis of asymmetries on admixed populations is an intuitive avenue of research, since potential differences on the pattern and magnitude of DA and FA on subsamples carrying variable levels of admixture could be indicative of the impact of heterozygote genome on the developmental basis of both kinds of asymmetries. Central to this question are the attempts to understand the kinds of genetic effects that might be relevant when widely separated populations admix. As reviewed by Ackermann (2010) a wide range of phenotypic variation is expected when considering expectations for quantitative variation during hybridization. Under a standard polygenic model, where genes with additive effects are responsible for the expression of the continuous traits under study, such traits in hybrids should equal the average of the parental taxa (Falconer and Mackay, 1997). This includes populations of humans that recently diverged and then started to exchange migrants during around five centuries, as is the present case, where it is expected to have fairly small differences in allele frequencies. In such circumstances, F1 hybrids would typically have intermediate phenotypic trait values relative to the parental taxa (Ackermann, 2010). However, admixed individuals can deviate from the average due to many factors that can affect phenotypic variation in a hybrid population, for instance, when in a single gene one allele (e.g., coming from a parental population) masks the effect of other allele in influencing some trait (dominance), or when the action of one gene is modified by one or several other genes (epistasis). Variations produced in such ways often results in substantial variation (Falconer and Mackay, 1997; Ackermann, 2010), including hybrid morphology that is not intermediate (Martínez-Abadías et al., 2006) and/or asymmetries. In

TABLE 1. Sample details concerning age, sex and country for sample of 4,104 volunteers

	Age[a]							
	Young adult		Early adult		Middle adult		Advanced adult	
	Sex							
Country	f	m	f	m	f	m	f	m
Brazil	110	53	336	151	70	49	9	7
Chile	102	184	279	666	61	115	5	1
Colombia	211	129	360	286	1	5	0	0
Mexico	181	87	217	164	45	22	2	2
Peru	65	40	52	34	1	2	0	0
Totals	669	493	1244	1301	178	193	16	10

[a] Young adult (18–20), early adult (20–40), middle adult (40–60), and advanced adult (60 and more).

this context, the exploration of the phenotypic expression of asymmetries in an individual and the results of a population level process like admixture would be useful to characterize individual facial asymmetries and population levels of DA and FA.

Among the vast array of human populations that experienced dramatic gene flow on its recent history, Latin Americans are, perhaps, the most interesting case of a tri-hybrid population shaped on a relatively short time period. Indeed, the history of Latin America has been deeply marked by international migration (Pellegrino, 2000). Denevan (1992) estimated an original population of around 50 millions of Native Americans when the Europeans arrived in 1492 to America. The genetic ancestry of admixed Latino populations varies across regions (Wang et al., 2008; Reich et al., 2012; Ruiz-Linares et al., 2014), and theory predicts that this depends on population density of the immigrant and receptor groups, the migration ratio, and a vast array of socioeconomic factors (Relethford, 2012). From the biological point of view, the admixture process in Latin America can be considered as a population process generating complex, nonlinear genetic and phenotypic patterns (Wang et al., 2008). Therefore, some authors referred to this process as "a natural experiment" (Chakraborty and Weiss, 1988). Among the many genetic topics to be addressed, it is particularly interesting to investigate whether the patterns of gene flow, as the main homogenizing evolutionary factor in the Americas, left an observable pattern of DA or FA indicating development instability.

Regarding the influence of admixture on FA, there is no agreement about the topic. For instance some authors found a negative correlation between heterozygosity and FA (Soulé, 1979; Vrijenhoek and Lerman, 1982; Biémont, 1983; Leary et al., 1984; Livshits and Kobyliansky, 1985; Mitton, 1993), but this vision is challenged by other studies that report absent or weak correlations (Beacham, 1991; Clarke and Oldroyd, 1996; Vøllestad and Hindar, 1997). A meta-analysis conducted by Vøllestad and Hindar (1997), on 118 individual samples of ectothermic and endothermic animals, concluded that heterozygosis and FA relationship is only weakly supported by available data, and that heterozygosis explains a very small amount of the variation in developmental instability among individuals and populations. In humans, Livshits and Smouse (1993) found no relationship between FA and heterozygosis.

Considering all the above, here we aim to characterize the association among genetic ancestry and IA on a sample of Latin-American admixed populations. Particularly, we will test the hypothesis that IA is not related to individual's genetic admixture. In addition, we aim to compare levels of FA at the population level across genome-wide heterozygosity estimates, in order to test the hypothesis that more heterozygous individuals (more admixed or less inbred) with respect to the global populations do not have lower FA values.

MATERIALS AND METHODS

The sample

As part of the CANDELA initiative, we recruited 4,104 volunteers (Table 1) aged between 18 and 85 years (mean = 26.41, s.d. = 9.29), from six Latin-American cities: Mexico City (México), Medellin (Colombia), Lima (Perú), Arica (Chile), Porto Alegre, and Jequié (both in Brazil). The CANDELA consortium aims to evaluate the genetic basis of nonpathological phenotypes differentiated between European, American, and African populations through the analysis of admixed populations (see Ruiz-Linares et al., 2014).

Volunteers with antecedents of craniofacial dysmorphologies, orthodontics treatments or severe facial trauma were not considered in this study. Further sample details are provided in Table 1. Approvals provided by the Ethics Committees of the Universidad Nacional Autónoma de México and Escuela Nacional de Antropología e Historia (México), Universidad de Antioquia (Colombia), Universidad Peruana Cayetano Heredia (Perú), Universidad de Tarapacá (Chile), Universidade Federal do Rio Grande do Sul/Universidade Estadual do Sudoeste da Bahia (Brazil), and University College London (UK) were obtained prior the data collection, and an informed consent were signed by each participant before genetic, socioeconomic, and facial phenotypes data was collected.

Facial shape data collection

The 3D facial shape was captured using photogrammetric methods applied to three series, each consisting of five digital photographs from left side (0°), left angle (45°), frontal (90°), right angle (135°), and right side (180°) views. All photos were taken manually from ~1.5 meters at eye level using a Nikon D90 and a fixed 50mm AF Nikkor lens at aperture f/11, as implemented in previous studies (Galantucci et al., 2008; de Menezes et al., 2009; Cooper et al., 2012). These settings give a depth of field of 40 cm, about twice the dimensions of

TABLE 2. Facial landmark anatomical definitions including 8 sagittal and 13 bilateral landmarks

No.	Name	Definition
Sagital landmarks		
1	Glabella	The smooth area between the eyebrows just above the nose
18	Nasion (sellion)	The midpoint of the nasofrontal suture
19	Pronasal	The most protruded point of the nasal tip
21	Subnasal	The junction between the lower border of the nasal septum and the cutaneous portion of the upper lip in the midline
23	Labiale superious	The midpoint of the vermilion border of the upper lip
26	Stomion	The midpoint of the labial fissure when the lips are closed naturally
29	Labiale inferious	The midpoint of the vermillion border of the lower lip
30	Gnathion	The lowest point in the midline on the lower border of the chin
Bilateral landmarks		
2, 10	Frontotemporale	The most medial point on the temporal crest of the frontal bone
3, 11	Superaurale	The highest point of the free margin of the ear
4, 12	Tragion	The tip of tragus
5, 13	Subaurale	The lowest point of the ear lobe
6, 16	Exocanthion	The outer corner of the eye fissure where the eyelids meet
7, 15	Palpebrale superiorus	The superior point of the eyelid
8, 14	Endocanthion	The inner corner of the eye fissure where the eyelids meet
9, 17	Palpebrale inferiorus	The inferior point of the eyelid
20, 22	Alare	The most lateral point on the nasal ala
24, 28	Crista philtre (upper lip point)	Highest point of the upper vermillion
25, 27	Cheilion	The outer corner of the mouth where the outer edges of the upper and lower vermilions meet
31, 33	Otobasion superiorious	The superior point on the union of the lobule and the head
32, 34	Otobasion inferiorous	The basal point on the union of the lobule and the head

See Figure 1 for a visual reference.

an average human head, ensuring that the whole face was in focus in all the photographs. We used the flash integrated to the camera. From the three series formed by five photographs each, the one presenting the most neutral expression was selected for the 3D reconstruction.

A set of 34 standard facial landmarks (Table 2; Fig 1) where placed using the software Photomodeler (http://www.photomodeler.com/; Eos Systems, Vancouver, Canada). We have followed the standard recommendations for quality and accuracy of the software (i.e., residual values inferior to 5.0, optimal camera calibration, camera resolution, photo redundancy, etc.). Several types of lens distortion are fixed during the camera calibration procedure automatically implemented in Photomodeler.

Several previous articles have entailed precision and accuracy experiments of 3D human faces reconstructed after photogrammetry (Galantucci et al., 2008, 2010; de Menezes et al., 2009; Alias et al., 2010), and some of them, using similar conditions to the ones implemented by us, report that the advantage of the presented (photogrammetric) method over laser scanning or

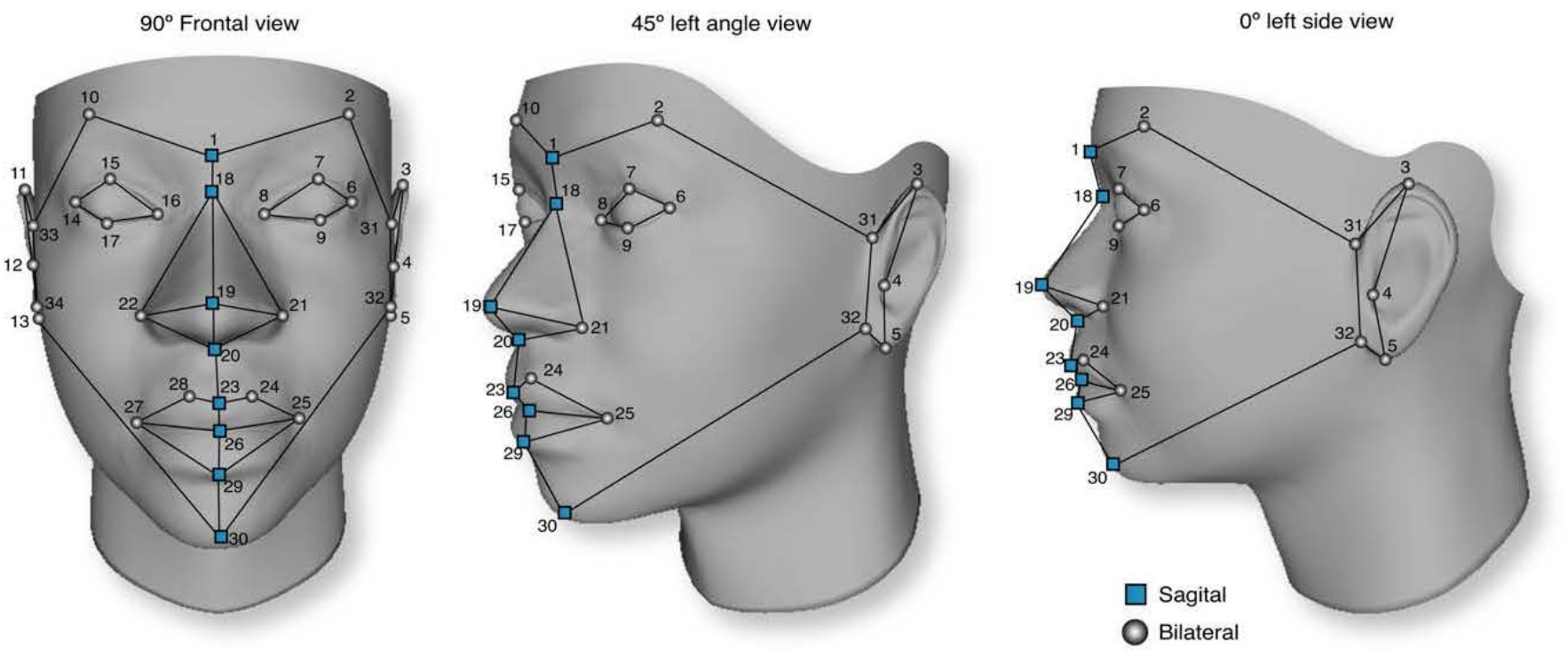

Fig. 1. Anatomical location of the landmarks used in this study. Frontal, frontal-lateral, and left side views. Gray squares and black points indicate sagittal and bilateral landmarks, respectively (see Table 2 for anatomical definitions) [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

electromagnetic digitizer is the acquisition of the source data in short time (D'Apuzzo,1998, 2002; Galantucci et al., 2008; de Menezes et al., 2009), and show that systematic errors between direct measurements versus photogrammetric reconstructions using Photomodeler were found insignificant (below 2 mm). In terms of measurement precision, the 3D photos were clearly better than direct anthropometry, and they had the highest overall precision, without systematic biases found between repeated measurements on the same photographs or on different photographs (Weinberg et al., 2006; Abdulkareem and Al-Mothaffar, 2012).

A scale factor was assessed using the nasion-gnathion distance that was measured directly on the individuals using a standard caliper. Chelion-chelion distance was also measured directly on the individuals, enabling the comparison of two scale factors obtained from the 3D reconstruction using these two facial distances as reference. A very high concordance (>93% correlation) indicates accuracy of the Photomodeler method, consistent with other studies (Lynnerup et al., 2003).

Raw 3D coordinates obtained from Photomodeler were saved in a text file and were used on subsequent analyses.

Estimation of genetic ancestry

On each individual, blood samples were collected and DNA extraction was performed following standard laboratory procedures. Genomic data involving 730,525 marker SNPs was obtained from these samples (see further details in Ruiz-Linares et al., 2014). The SNPs were pruned to remove Linkage Disequilibrium, and after removing correlated SNPs, 90,000 SNPs were left for analysis. Ancestry estimation was performed with this SNP data. Genome-wide average heterozygosity was estimated from this data using PLiNK (Purcell et al., 2007; Yang et al., 2010), which provides a measure of excess heterozygosity compared to the overall sample. It is calculated as 1—excess homozygosity, while excess homozygosity is estimated using the inbreeding coefficient as the average excess of homozygous alleles across all SNPs for an individual as compared to the overall sample.

Intra and interobserver error analysis

Since landmark data was obtained by two different observers (MQS and LC), and considering that measurement error was identified as an important noising factor in asymmetry studies (Palmer and Strobeck, 1986; Graham et al., 1993; Merila and Bjorklund, 1995; Björklund and Merilä, 1997; Dongen, 1998; Palmer, 2000) we performed specific tests to measure between and within-individual measurement errors. To do so, we collected fifteen observations per observer of one individual (an adult male) selected randomly from the whole sample. The raw landmark coordinates were superimposed using the Generalized Procrustes fit implemented in MorphoJ 1.05 (Klingenberg, 2011). The error assessment was performed following Singleton (2002). First, the Euclidean distance of each landmark to its respective centroid was computed. Second, landmark deviations were calculated relative to the individual landmark mean and mean deviations and percentage errors were calculated for individual landmarks and subsequently averaged to give a mean deviation and percentage error for each observer across all landmarks. Finally, a one-way analysis of variance (ANOVA) was conducted for each landmark by observer, and the root mean squares [root mean square error (RMSE)] were examined. The root of the within-groups mean squares (RMSE) corresponds to the intra-observer error (Sokal and Rohlf, 1995), while the root of between-groups mean squares corresponds to interindividual (among replicates) error (for details Singleton, 2002). Additionally, another indicator of error in the experiment was the implementation of a Procrustes ANOVA; where measurement error is computed from the variation among replicate measurements, see below (Klingenberg et al., 2002).

Multivariate characterization of facial asymmetry

As a first exploratory analysis, the asymmetric component of shape was submitted to a PCA analysis in order to identify the main trends of asymmetrical morphological variation (Klingenberg, 2011). Then, a Procrustes ANOVA and multivariate analysis of variance (MANOVA) were used to characterize the asymmetric component of shape variation in the facial phenotype (Klingenberg and McIntyre, 1998). Following Klingenberg et al. (2002, 2010a), organisms displaying object symmetry need a special procedure to characterize the relative amounts of symmetric and asymmetric shape variation components. The analyses for landmark configurations with object symmetry separates the original landmark configuration into components of symmetric variation and asymmetry by Procrustes superimposition of the original configurations and their mirror images (Klingenberg et al., 2002), in order to get individual facial fluctuating asymmetry (FFA) scores. The Procrustes ANOVA model estimates the significance of an individual effect, a side effect informative of DA, an individual-by-side interaction effect that indicates the amount of FA, and the measurement error effect (Klingenberg and McIntyre, 1998; Mardia et al., 2000). *P* values were calculated using a permutation test based on 100,000 iterations of the original data. To estimate the error term we performed a repeated landmarking of the faces, where we landmarked again a subset of 200 faces chosen across all the five countries. Considering that most biological forms present a nonisotropic distribution of variance across landmarks (Klingenberg et al., 2002), we also took into consideration the results of a MANOVA test that further considers the nonisotropic nature of landmark configurations to estimate DA and FA effects. Sex is included as a covariate contributing another main effect in the ANOVA.

Correlation and regression of FFA score on covariates

FFA scores were estimated from the Procrustes landmarks using procedures described in Klingenberg and McIntyre (1998) under a nonisotropic model, thus avoiding the homogeneity assumption, using the Mahalanobis distance metric. The FFA score was considered to be the trait of interest, and its regression against all the covariates was then evaluated. The list of covariates includes age, sex, BMI, weight, height, education, wealth-index, melanin, heterozygosity, and continental ancestries. In the case of BMI, it is noteworthy to mention that some previous studies have found a relationship between FA and BMI (Milne et al., 2003, Windhager et al., 2013).

As the three ancestry variables add up to 100%, to avoid colinearity problems we considered Native

TABLE 3. Procrustes ANOVA and MANOVA results with sex as covariate

Procrustes ANOVA

Effect	SS	MS	*df*	*F*	*P* (param.)
Sex	0.4987866	0.0097801	51	163.98	<.0001
Individual	12.6538792	0.0000596	212160	6.64	<.0001
DA	0.0746448	0.0016965	44	188.74	<.0001
FA	1.6456741	0.0000090	183084	1.24	<.0001
Error	0.1132016	0.0000072	15675		

MANOVA

Symmetric component			Asymmetry component		
Effect	Pillai tr.	*P* (param.)	Effect	Pillai tr.	*P* (param.)
Sex	0.66	<.0001	Sex	0.1	<.0001
Individual	51.52		DA	0.75	<.0001
			FA	43.02	<.0001

ancestry as the baseline and included African and European ancestry in the regression (Ruiz-Linares et al., 2014). All shape changes were visualized using warped surfaces (Wiley et al., 2005). Additionally, the shape changes depicted by the regression of shape on genetic ancestry (after removing the effects of covariates) were combined with a triplot graph obtained from the distribution of ancestry estimates for the three parental groups.

RESULTS

Intra- and interobserver error analysis

The mean landmark deviation for the inter-replicate (within-observer) error was 0.0012 in units of Procrustes distances (min. = 0.0004, max. = 0.0059), and 0.0010 (min. = 0.0004, max. = 0.0026), for observer 1 (MQS) and 2 (LC), respectively. Mean landmark deviation for the interobserver error was 0.0435 (min. = 0.0004, max. = 0.1047). The interobserver differences are two orders above the inter-replicate error in all landmarks and both observers. The ANOVA results showed that the mean interobserver (MQS/LC) RMSE is 0.00088 (0.91%), and 0.00067 (1.08%) for the intraobserver comparison. The lowest interobserver repeatability errors were detected on the landmarks superior lateral tragion (left), subaurale (left), and subaurale (right), whereas the greatest errors were detected on endocanthion (left), endocanthion (right), and subnasale. The Procrustes ANOVA showed that mean squares for the error component present far lower values than the mean squares for FA (Table 3) Considering the relatively large size of the faces studied here, and that the interobserver and inter-replicate errors are lower than the interindividual differences, these margins of error were considered acceptable.

Both sex, DA and FA appear highly significant in the Procrustes ANOVA (Table 3) and in the nonisotropic model (MANOVA), which is not limited by the assumption of isotropy in the data (Klingenberg et al, 2002; Table 3)

The asymmetric shape was submitted to a PCA analysis in order to identify the main trends in asymmetrical morphological variation. The shape changes associated to PC1 (22.015% of explained variance) are focused in the ear lobes, nose and mouth, showing a left DA tendency in the positive axis. Ear lobes fluctuate along the first PC regarding its anterior-posterior position, reaching more anterior positions on the right side of the face. The mouth, nose and to a greater extent the eyes follow the general directional shifts observed in the face. The second PC (11.04% of explained variance) describes superior-inferior DA changes such as a left displacement of the chin and mouth, and a more basal position of the left ear lobes and. Conversely the nose, eyes and right ear lobe change to right superior positions (changes observable toward the positive values of PC2). Finally, PC3 (9.61% of explained variance) express changes related to the DA of the ears, attachment and protrusion (Fig. 2).

The triplot (Fig. 3) representing genetic ancestry and associated asymmetric shape changes indicates that Amerindians exhibit right asymmetrical shifts related to ear attachment, the chin, nose, and lower part of the frontal (nasion). Conversely, asymmetric changes associated to the European vertex describe changes at the left side of the face (Fig. 3). Finally, African vertex describes changes associated to ear attachment and protrusion in the left side, accompanied by changes in the nose and the left side of the frontal area (Fig. 3).

Correlation and regression of FFA score on covariates

As depicted in Table 4 almost all of the correlations were significant at $P < 0.00001$. Sex, height, melanin, education, wealth, African ancestry, and heterozygosity have negative correlations with FFA. In contrast, age, weight, BMI and American ancestry showed positive correlations. Multiple regression results (Table 5) show that FFA scores strongly depend on age, sex, ancestry, and heterozygosity (whole model R^2: 9184%, $P < 0.00001$). Asymmetry increases with age (Fig. 4a), while FFA is lower in males. FFA shows a negative correlation with heterozygosity (Fig. 4b), and European and African ancestries are negatively associated with FFA while Native ancestry is positively correlated. Height and BMI do not seem to be associated to FFA. Education appears to be positively associated with FA, but the correlation with education can be seen as a by-product of the correlation with age (age and education are obviously correlated). Wealth index is only slightly correlated with FA.

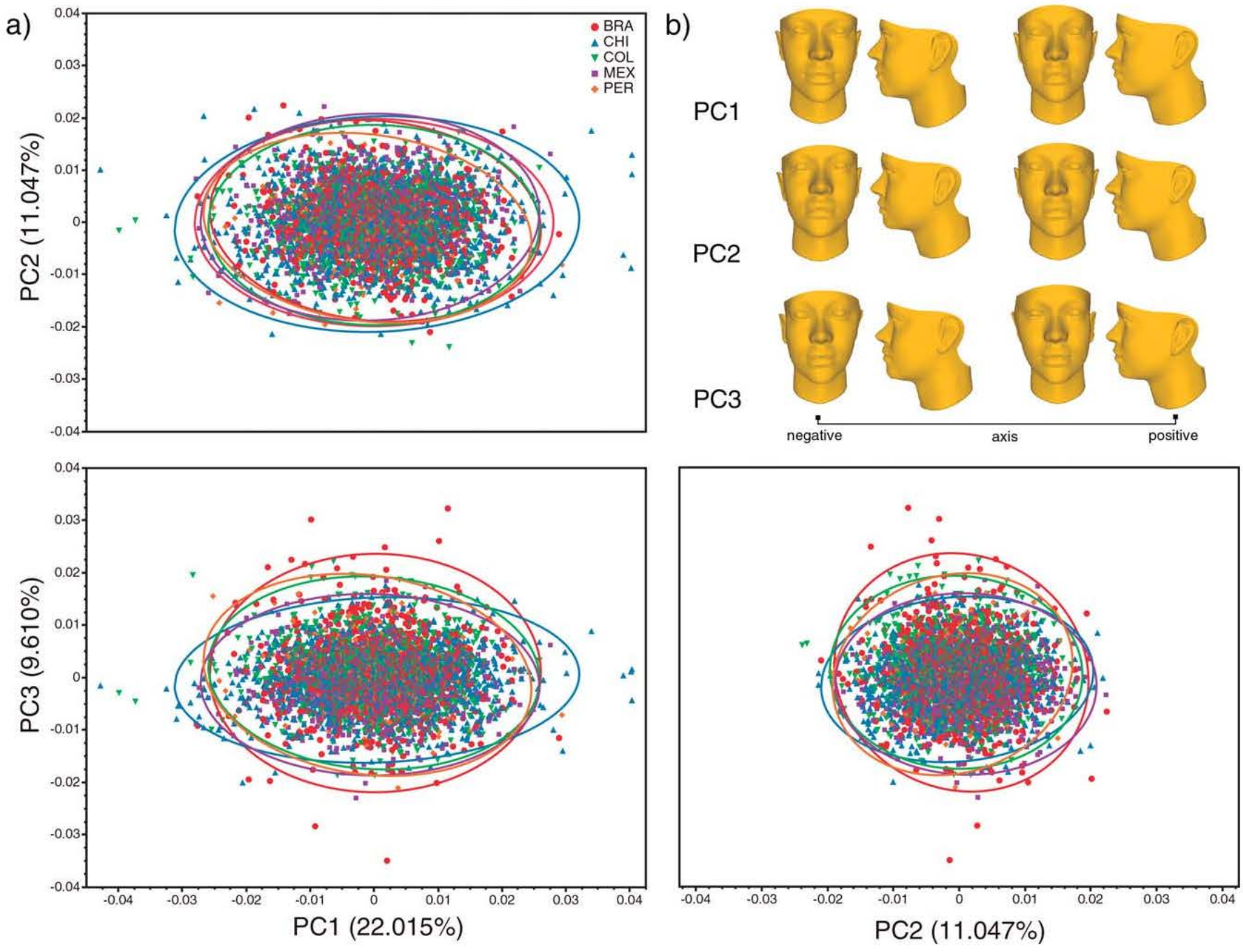

Fig. 2. Principal Components Analysis. Scatterplots and morphings of the asymmetric component of shape variation (**a**). Ellipses represent the 99% of the variance. Morphings represent the extreme shapes observed on the negative and positive scores of each PC (**b**). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

A central goal of many hybridization studies is to measure the degree to which hybridization results in introgression of genetic material between populations (Strasburg and Rieseberg, 2013), with Mayr's idea in mind (Mayr, 1963) that phenotypic divergence is often correlated with the degree of isolation. Gene flow has the effect of homogenizing the genetic composition of a population and thus can be seen as a constraining evolutionary force (Slarkin, 1985), with an opposite effect to that of diversifying natural selection. Additionally, migration has two main effects: it reduces the between-group differences, and increases variances within demes (Konigsberg, 2000; Hamilton, 2009). The analysis of human admixed samples contributes by identifying the morphological effects of hybridization in populations that have diverged during the human diaspora in the Pleistocene and Early Holocene. In this context, our study sheds light on some aspects regarding dominance/epistasis by exploring if admixed individuals are a balanced mixture of parental traits (asymmetries, in this case), intermediate between parental populations. As discussed previously (Ackermann, 2010; Ackermann et al., 2006), admixed individuals can show a range of morphologies, resembling one parental group or the other, or displaying novel phenotypes, depending on dominance and epistatic interactions between alleles fixed or predominant in either parental group. In this context, asymmetries are expected in highly admixed individuals, due to the putative developmental disturbances introduced by hybridization at the genetic and environmental levels. By large, previous research aimed to empirically test these predictions were focused on detecting craniofacial variations in size and shape in the context of

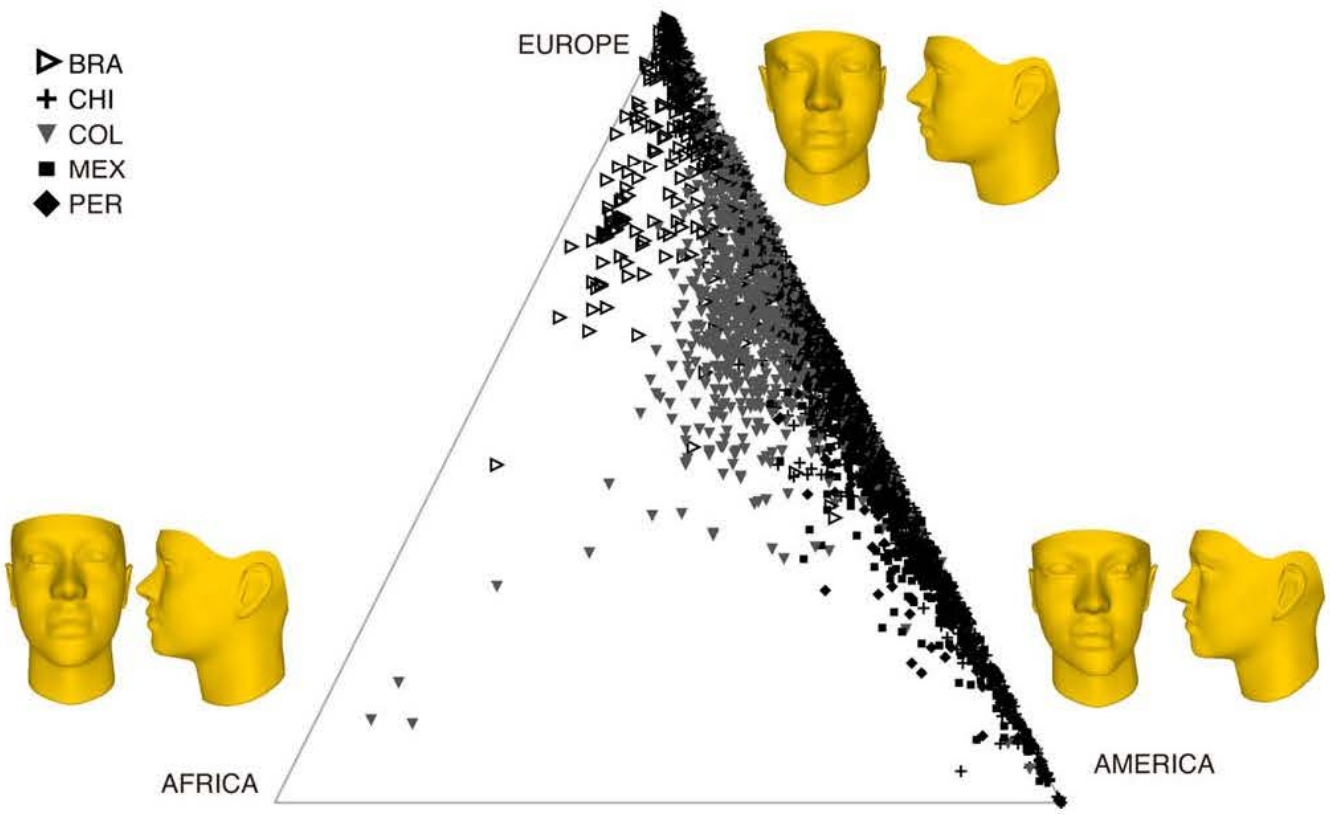

Fig. 3. Triplot of the genome-wide genetic ancestry estimation and their respective asymmetric shape changes, obtained after the regression of the asymmetric component of shape and genetic ancestry. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

variable genetic admixture. For instance, Wijsman and Cavalli-Sforza (1984) reviewed the implications of gene flow in human populations showing that theoretically, in presence of large migratory events, migrants produce a dialectic hybrid populations by intermating with native populations (Wijsman and Cavalti-Sforza, 1984). On their comparison among Mexican colonial skull samples, Martínez-Abadías et al. (2006) showed that the craniofacial phenotypes resulting from Spanish-Amerindian admixture departs from an intermediate position in the morphospace between parental groups, thus suggesting that heterosis phenomena underlie the behavior of craniofacial phenotypes in admixed populations. On the same line, in their study on baboon hybrids produced in captivity and on natural populations, Ackermann et al. (2006) further verified the significant signals of craniofacial heterosis, suggesting caution against simple assumptions that hybrids will display the average of parental phenotypes (Ackermann, 2010).

Besides these and other similar studies focused on absolute shape differences, the relationship between admixture and craniofacial asymmetries in humans or other primates received less attention. In this context, departures from ideal conditions during ontogeny (i.e., environmental or genetic stress) may reduce the "efficiency" of normal developmental processes and increase the level of FA (Palmer and Strobeck, 1986; Parsons, 1990; Clarke, 1993; Møller and Swaddle, 1997) and/or DA (McIntyre and Mossey, 2002; Hennessy et al., 2004; Schaefer et al., 2006; DeLeon, 2007; Ercan et al., 2008; Klingenberg et al., 2010b; Barrett et al., 2012; Claes et al., 2012). Associations between heterozygosity and FA have been reported in mammals and other species (Soulé, 1979; Biémont, 1983; Leary et al., 1984; Palmer and Strobeck, 1986; Mitton, 1993; Vøllestad, 1999; Leamy et al., 2001), with some exceptions (Wooten and Smith, 1986; Patterson and Paton, 1990; Beacham, 1991; Britten, 1996; Gilligan et al., 2000; Leamy et al., 2001). Moreover, FA seems to increase with increasing homozygosity, hybridization, inbreeding, mutation (Palmer and Strobeck, 1986, 1992; Clarke, 1992; Møller and Swaddle, 1997). In his meta-analysis of FA in relation to heterozygosity, Vøllestad et al. (1999) found an overall negative correlation between heterozygosity and FA, suggesting that heterozygosity seems to explain only a very small amount of the variation in developmental instability among individuals and populations. In the same line, Windhager et al. (2014) shows lack of correlation between heterozygosity and Procrustes FFA scores, but they recognize a limitation in their results due to the relatively small number of SNPs (102 SNP's) used, which may limit the representative value in terms of an individual's total heterozygosity.

Our results indicate that both DA and FA are present in the analyzed sample, with varying expressions in the three parental groups, and suggested that facial asymmetries tend to decrease in admixed Latin American individuals (Fig. 4b). Overall, these results suggest that, if FA is assumed to be a proxy to developmental instability, then admixed individuals present a slightly more stable development than homozygous ones. This

TABLE 4. Correlations of FFA scores on covariates

Variable	Correlation	*P* value
Sex	−0.073	3.00E−06
Age	0.231	6.30E−51
Weight	0.038	1.50E−02
Height	−0.07	6.40E−06
BMI	0.096	6.90E−10
Melanin	−0.045	4.10E−03
African ancestry	−0.094	1.40E−07
European ancestry	−0.11	6.10E−10
American ancestry	0.129	3.30E−13
Heterozygosity	−0.146	4.00E−16

TABLE 5. Multivariate regression output of FFA scores on covariates

Variable	Estimate	Std. error	*t* statistic	*P* value
Age	0.027281	0.00199	13.712	2.00E−16
Sex	−0.23666	0.052547	−4.504	6.88E−06
Weight	0.001907123	0.001220825	1.56	0.1183
Height	0.001826	0.002905	0.629	0.53
BMI	0.005072	0.004592	1.105	0.269
Melanin	−0.008200114	0.002878823	−2.85	0.0044
African ancestry	−1.66279	0.347125	−4.79	1.75E−06
European ancestry	−0.704101	0.098411	−7.155	1.04E−12
Heterozygosity	−2.76366	0.308706	−8.952	2.00E−16

Whole model adjust R^2: 9.184%, $P < 0.00001$, 100,000 rounds

reinforces the idea that the novel genetic and environmental landscape of the admixed individuals in Latin America does not represent a case of developmental instability enough to trigger the expression of asymmetric phenotypes. Furthermore, and given the negative relationship between heterozygosity and FFA, these results lend support to the idea that the genetic basis of such asymmetries can be seen as a case of underdominance, rather than an additive, dominance, or epistatic scenario (see Ackermann, 2010; Ackermann et al., 2006).

In terms of type of asymmetry, our data indicates that DA is more important than FA as a source of facial asymmetries, bringing support to previous works suggesting a greater component of DA on the asymmetric normal variation in the human cranium, face and dentition (Schaefer et al., 2006; DeLeon, 2007; Ercan et al., 2008; Barrett et al., 2012; Claes et al., 2012), as well as on dismorphological samples (McIntyre and Mossey, 2002; Hennessy et al., 2004; Klingenberg et al., 2010b). Traditional views states that DA is more genetically determined, and thus could be likely used as a proxy to developmental stability (Palmer, 1994). However, other authors suggest that DA could also be indicative of certain mechanisms involving developmental instability (Graham et al., 1993; Møller and Swaddle, 1997; Smith et al., 1997). More specifically, Graham et al. (1993) using a modified Rashevsky-Turing reaction-diffusion model of morphogenesis, showed that both AS and DA can be induced by simply changing the levels of feedback and inhibition in the model. Unfortunately, our data are not appropriate to test the hypothesis of DA as a proxy to developmental instability, but it is interesting to note how DA and FA levels differ among ancestral groups (e.g., among individuals carrying high percentage of European, Amerindian, or African ancestry). This could be indicative that the basal condition for any given population is not perfect symmetry, but some varying level of both, DA and FA (Farrera et al., 2014).

On the other hand, FA patterns were widely assumed as indicators of development instability (Palmer and Strobeck, 1986; Livshits and Kobyliansky, 1991; Møller and Swaddle, 1997; Milne et al., 2003 b; DeLeon, 2007; Little et al., 2008; DeLeon and Richtsmeier, 2009; Özener, 2010a, b, 2011; Özener and Fink, 2010; Weisensee, 2013). Our results indicate that, even though FA explains a smaller fraction of variation than DA, its contribution to the total amount of asymmetrical variation is significant. Interestingly, matrix comparison tests yielded significant degrees of proportionality among individual and asymmetry covariance structures, thus suggesting that the same developmental processes underlie the expression of shape variation at both levels (Klingenberg and McIntyre, 1998). Another important aspect when dealing with comparisons among DA and FA is that, usually, it is assumed that the "normal" expectance, or optimal phenotype for a population is FA = 0 or perfect symmetry, which is not necessarily a strong null hypothesis as Debat and David (2001) argues and Farrera et al. (2014) suggest for a sample of Mexicans.

Finally, it is important to contextualize that a great amount of research on facial asymmetries is aimed test a potential dependence on socioeconomic, educational, or nutritional status indexes of a wide array of populations and cultural contexts (DeLeon, 2007; Gawlikowska et al., 2007; Gray and Marlowe, 2009; Özener, 2010a, 2011; Bigoni et al., 2013; Hope et al., 2013). In general, these analyses depart from the premise that exposure of nutritional or psychosocial stress during gestation and pre-natal development could derive on an alteration of "normal" (perfect symmetry) developmental pathways leading to directional, but mainly FA. In this context, the regression of asymmetric facial shape on genetic ancestry revealed that its magnitude tend to decrease with admixture. In other words, individuals with larger proportions of a parental genetic background are more asymmetric, and its asymmetrical traits differ depending on the parental population. This result seems to be concordant with previous analyses suggesting that there is no single asymmetry pattern in our species (Farkas and Cheung, 1981; Ras et al., 1994; Ferrario et al., 1995; Shaner et al., 2000; Smith, 2000; Ercan et al., 2008; Klingenberg et al., 2010b; Özener, 2010b).

CONCLUSIONS

Differential patterns and magnitudes of DA and FA are observed among the subsamples of admixed individuals exhibiting greater amounts of Amerindian, African, or European genetic ancestry. As a whole, more admixed individuals exhibit lower levels of asymmetry, which lend support to the notion that the expression of facial asymmetry is not directed by dominant or epistatic effect, and that the genetic and environmental conditions of admixed individuals cannot be seen as a case of developmental instability. DA appears to be the greatest manifestation of asymmetry, in comparison to FA. Our results highlight the importance of considering ancestry-admixture when comparing levels of asymmetries on

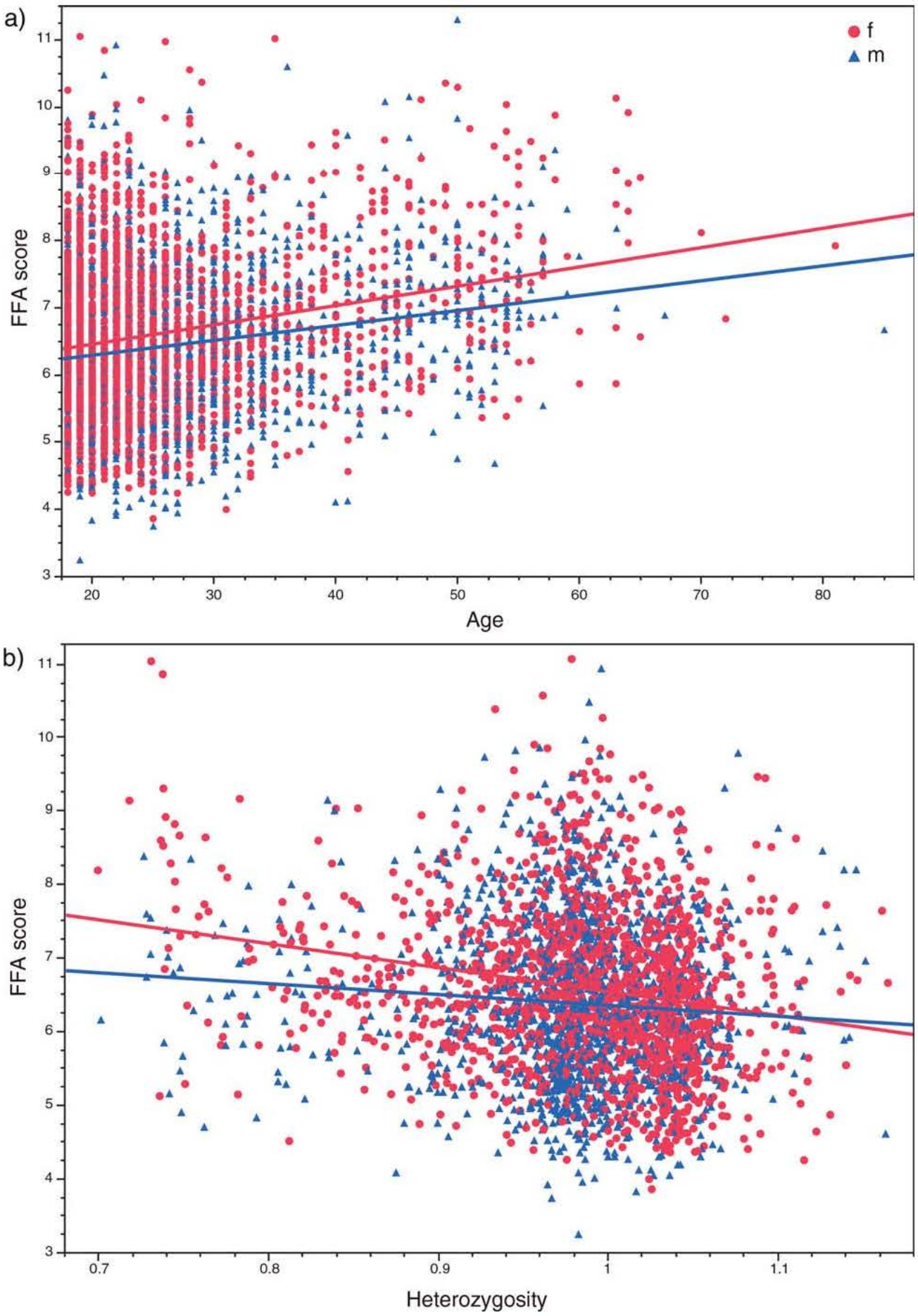

Fig. 4. Plots of the FFA scores on covariates [plotting age (**a**) and heterozygosity (**b**)]. Lines represent the axis of regression by sex (triangles and black line = males, circles and gray line = females). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

groups differing on socioeconomic levels. Future research on the CANDELA database will be focused on testing if putative differences on asymmetries among socioeconomic status vanish when controlling the effects of ancestry.

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