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Indirect Evidence for the Genetic Determination of Short Stature in African Pygmies

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ABSTRACT Central African Pygmy populations are known to be the shortest human populations worldwide. Many evolutionary hypotheses have been proposed to explain this short stature: adaptation to food limitations, climate, forest density, or high mortality rates. However, such hypotheses are difficult to test given the lack of long-term surveys and demographic data. Whether the short stature observed nowadays in African Pygmy populations as compared to their Non-Pygmy neighbors is determined by genetic factors remains widely unknown. Here, we study a uniquely large new anthropometrical dataset comprising more than 1,000 individuals from 10 Central African Pygmy and neighboring Non-Pygmy populations, categorized as such based on cultural criteria rather than height. We show that climate, or forest density may not play a major role in the difference in adult stature between existing Pygmies and Non-Pygmies, without ruling out the hypothesis that such factors played an important evolutionary role in the past. Furthermore, we analyzed the relationship between stature and neutral genetic variation in a subset of 213 individuals and found that the Pygmy individuals' stature was significantly positively correlated with levels of genetic similarity with the Non-Pygmy gene-pool for both men and women. Overall, we show that a Pygmy individual exhibiting a high level of genetic admixture with the neighboring Non-Pygmies is likely to be taller. These results show for the first time that the major morphological difference in stature found between Central African Pygmy and Non-Pygmy populations is likely determined by genetic factors. Am J Phys Anthropol 145:390–401, 2011.

The word "Pygmy" derives from the Greek "pygmaios", a measure of the distance from the elbow to the wrist, and was used by Homer in reference to a mythic population of dwarfs who are at war with birds (Iliad, song III v.1–6). European explorers chose this name, in the second half of the 19th century, to designate more than 25 human populations of small-stature throughout the Central African rain-forest (Du Chaillu and Owen, 1867; Schweinfurth, 1873; Bahuchet, 1993b). Pygmy populations are indeed found in the lower extreme of the worldwide distribution of population average adult height (Froment, 1993). However, anthropologists have shown that there is no discontinuity in average height between Pygmies and Non-Pygmies, and other short-stature populations in Africa are considered locally as Non-Pygmies (Hiernaux, 1974; Froment, 1993). Therefore, rather than using only a stature threshold as a diagnostic criterion, numerous cultural criteria (e.g., way of life, social organization) should be considered to categorize various populations as Pygmies or Non-Pygmies (Cavalli-Sforza, 1986; Bahuchet, 1993b; Froment, 1993; Hewlett, 1996).

The time and location of the emergence of the short stature of African Pygmies remain widely unknown due to the lack of ancient human remains in the acidic soils of the equatorial forest (Cornelissen, 2002; Mercader, 2003; Phillipson, 2005). Only three archaeological sites providing ancient human remains have been excavated throughout Central Africa to date (Lavachery, 2001), and none of these sites have provided evidence of an ancient *Homo sapiens* population of particularly short stature.

Numerous evolutionary hypotheses have been proposed to explain the short stature of Pygmies (Perry and Dominy, 2009). It could be an adaptation to food scarcity (Bailey et al., 1989), to the difficulties of thermoregulation in a hot and humid environment (Cavalli-Sforza, 1986), to dense forest environments where mobility is difficult (Diamond, 1991) or to a trade-off between growth cessation and age at first reproduction caused by a high mortality rate (Migliano et al., 2007; Becker et al., 2010). Using population genetics approaches, Pygmy populations have been found to have diverged

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The first two authors contributed equally to this work.

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from Non-Pygmy populations between 50,000 and 70,000 years ago (Batini et al., 2007; Quintana-Murci et al., 2008; Patin et al., 2009; Verdu et al., 2009; Batini et al., 2010). It is thus reasonable to assume that the Pygmy and Non-Pygmy ancestral populations experienced different evolutionary processes that, in turn, influenced their stature. Unfortunately, due to the lack of archaeological data, it is impossible to know whether Non-Pygmy populations became taller, or whether Pygmy populations evolved towards shorter stature; nor is it possible to know whether such phenotypic changes are recent or in fact more ancient.

Adult height is known to be a highly heritable trait (>80%; Perola et al., 2007) and recent powerful genome-wide association studies have identified numerous (~180) SNPs significantly associated with height in large cohorts of healthy individuals. These cohorts were primarily of European origin (Gudbjartsson et al., 2008; Lettre et al., 2008; Sanna et al., 2008; Weedon et al., 2008; Estrada et al., 2009; Johansson et al., 2009; Soranzo et al., 2009; Lango Allen et al., 2010) but studies have also been conducted in individuals of Japanese (Takeuchi et al., 2009) and African American origin (Shriner et al., 2009). Taken altogether, these genetic polymorphisms account for 10% of the total variation of adult height within populations. Indeed, it is challenging to explain a large proportion of the variability in height within a population, since adult height is influenced not only by complex polygenic systems, but also by environmental factors (Katzmarzyk and Leonard, 1998; Silventoinen et al., 2000; Silventoinen et al., 2003; Hur et al., 2008).

Endocrinologists have described the physiological determination of the African Pygmies' short stature: serum levels of Insulin-Like Growth Factor 1 (IGF1) and of Growth Hormone Binding Protein (GHBP) are abnormally low, whereas the levels of Growth Hormone (GH) and IGF2 do not differ from Non-Pygmy controls (Rimoin et al., 1969; Merimee et al., 1972; Merimee et al., 1981; Merimee et al., 1987; Baumann et al., 1989). In this context, Merimee (1987) proposed that the short stature of African Pygmies could be attributed to the absence of a growth spurt during puberty and that the genetic factor(s) implicated in the Pygmy stature were to be found in the GH-IGF1 axis (Merimee et al., 1981). A recent gene-expression study further showed a slight (1.8-fold) under-expression of GH and a more dramatic (8-fold) under-expression of the GH receptor in adult African Pygmies, which was not found in Non-Pygmy Bantu speakers (Bozzola et al., 2009). However, the only genetic study focusing specifically on Pygmies' stature, failed to find allele frequency differences in the promoter region of the gene encoding IGF1 between two African Pygmy populations and Non-Pygmy controls (Bowcock and Sartorelli, 1990). In this context, whether the Pygmy populations' short stature is solely due to environmental pressures experienced by individuals during growth (i.e., phenotypic plasticity), or to a complex genetic mechanism, remains to be demonstrated.

In this study, we analyzed new anthropometric data in a uniquely large sample of Pygmy and their immediate neighboring Non-Pygmy populations from Central Africa (Cameroon, Central African Republic and Gabon; Fig. 1), categorized as such based on numerous cultural criteria (see below). For a subset of this sample, genetic data from a previous study was also available (Verdu et al., 2009). We were thus able to investigate the role played by genetic factors in the determination of the differences in average adult stature found between Central African Pygmy and Non-Pygmy populations. We found significant positive correlations between the adult height of Pygmies and individual levels of genetic similarity with the Non-Pygmy gene-pool, which showed, for the first time to our knowledge, that genetic factors were very likely to play an important role in the major differences in average adult height observed between Pygmy and Non-Pygmy populations from Africa.

MATERIALS AND METHODS

Ethics statement

Appropriate oral or video-recorded informed consent was obtained from all volunteer donors for the anthropologic, ethnographic and genetic data here presented. The French, Cameroonian, Gabonese and Central African Republic governments provided research authorizations.

Population sampling

We gathered anthropometric and genetic data from 1,103 adult individuals from 10 populations sampled in Cameroon (Kola, Central Baka, Eastern Baka, Nzimé, Bangando, Tikar), Gabon (Koya, Eastern Bongo, Southern Bongo) and Central African Republic (Aka) (Fig. 1, Table 1). Populations were *a priori* categorized as Pygmy (seven out of 10: Aka, Eastern Bongo, Southern Bongo, Koya, Kola, Eastern Baka, Central Baka) or Non-Pygmy (three out of 10: Tikar, Nzimé, Bangando), based on numerous ethnologic criteria such as self identification, way of life, language, music, or social interactions in a pluri-ethnic context, rather than simply based on stature (Cavalli-Sforza, 1986; Bahuchet, 1992a; Bahuchet, 1993b,c; Froment, 1993; Hewlett, 1996).

Following Central African ethnographers and anthropologists (Turnbull, 1965; Bahuchet and Guillaume, 1982; Bahuchet, 1992b; Bahuchet, 1993a; Fürniss and Bahuchet, 1995; Hewlett, 1996; Joiris, 2003), we conducted with the various populations present at each sampling site, extensive ethnographic interviews during numerous interdisciplinary field-works between 2002 and 2008 in order to categorize *a priori* as "Pygmy" a population that:

1. distinguished itself as a separate community with a different name from other neighboring ethnic communities (although often speaking closely related languages);
2. was designated as "Pygmy" (or its literal translation in local languages) or at least was designated as "other than self" by neighboring outsiders;
3. was recognized by outsiders as specialized for forest activities such as hunting-gathering and medical and magical knowledge of the rain forest;
4. shared complex socio-economic relationships with specific neighboring outsiders, for instance by exchanging forest products (e.g., game, wild honey) for iron tools (e.g., fishing hooks, iron blades, or spear heads);
5. had musical instruments and musical practices often different and recognized as such by outsiders.

No accurate age data was available for these individuals since most of the communities included here did not keep track of birth-dates. However, only adults were included in this study and elderly people were not considered.

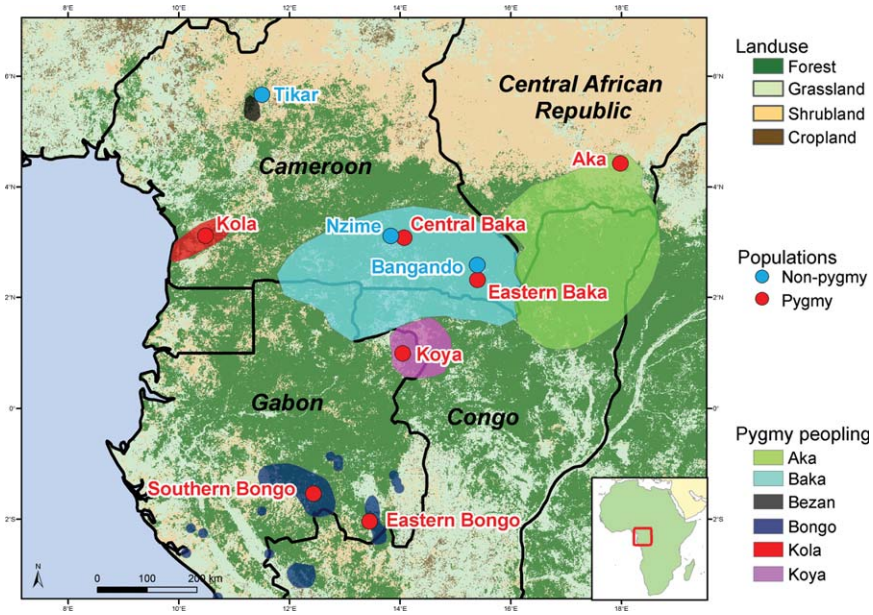

Fig. 1. Geographical locations of Pygmy and Non-Pygmy populations. Populations were categorized as Pygmy or Non-Pygmy based on numerous cultural criteria including way of life, language, music, socio-economic interactions in a pluri-ethnic context, as well as by endogenous and exogenous identification (see Materials and Methods for details). Pygmy peopling areas were inferred from our ethnographic field-work. Map sources: Global Land Cover Facility.

TABLE 1. Population estimated mean values of standing height (H in cm) with standard deviations (SD in cm), for 10 populations from Western Central Africa categorized as Pygmies and Non-Pygmies based on numerous cultural criteria

			Men		Women	
Category	Geographic location	Population*	*N***	$\hat{E}(H)$; SD	*N***	$\hat{E}(H)$; SD
Pygmies	Central African Republic	Aka[1]	125 (0)	155.1; 7.0	104 (0)	145.9; 5.0
	Cameroon East	Eastern Baka[2]	36 (26)	155.6; 5.3	9 (0)	149.5; 4.2
	Cameroon Centre	Central Baka[2]	182 (13)	155.6; 5.7	184 (16)	146.5; 4.9
	Gabon North-East	Koya[3]	20 (18)	156.9; 6.4	11 (11)	145.8; 3.8
	Cameroon South-West	Kola[4]	82 (0)	155.1; 6.1	99 (0)	147.1; 5.3
	Gabon East	Eastern Bongo[5]	25 (18)	159.7; 7.6	19 (11)	151.2; 4.8
	Gabon South	Southern Bongo[5]	12 (7)	159.7; 3.0	9 (5)	151.8; 5.7
		Average Pygmies	482 (82)	155.8; 6.3	435 (43)	146.8; 5.1
Non-Pygmies	Cameroon North	Tikar[2]	22 (18)	167.2; 4.2	16 (11)	156.7; 6.4
	Cameroon Centre	Nzimé[2]	42 (5)	168.5; 6.5	58 (26)	155.8; 6.8
	Cameroon East	Bangando[2]	29 (21)	163.9; 6.6	19 (7)	156.0; 5.9
		Average Non-Pygmies	93 (44)	166.8; 6.3	93 (44)	156.0; 6.5

For averages over Pygmy and Non-Pygmy populations, estimated mean and SD values were calculated with all individuals pooled together.
* Anthropometric data gathered by: [1]Bahuchet and Pagezy, 1977; [2]Froment and Gessain, 2002–2007; [3]Le Bomin, 2006; [4]Pagezy, 2004; [5]Verdu, 2006–2007.
** *N*, Number of individuals for which anthropometric data were gathered. The number of individuals for whom both anthropometric and genetic data were available is indicated between parentheses.

Anthropometrical data and analyses

All anthropometrical statistical analyses were performed using the statistical package R (R Development Core Team, 2007).

For all 1,103 adult individuals (575 men and 528 women) considered here, we measured adult height using a height gauge, according to standard biometrical procedures (Weiner and Lourie, 1981). We compared the variance of individuals' height between pairs of populations by performing pairwise F-tests for men and women separately, and corrected the *P*-values hereby obtained for multiple testing between pairs of populations using a Bonferroni correction. We compared the estimated mean values of height between Pygmies and Non-Pygmies as well as between pairs of populations by performing Student's tests assuming equality or inequality of variance where appropriate.

Genetic data

For 213 individuals, coming from eight populations among the 10 considered (Table 1), DNA was also available and had previously been genotyped at 28 independent nuclear tetranucleotide microsatellite loci (Verdu et al., 2009). These authors investigated the structure in the genetic variation among a wider sample set including 604 Pygmy and Non-Pygmy individuals from West-

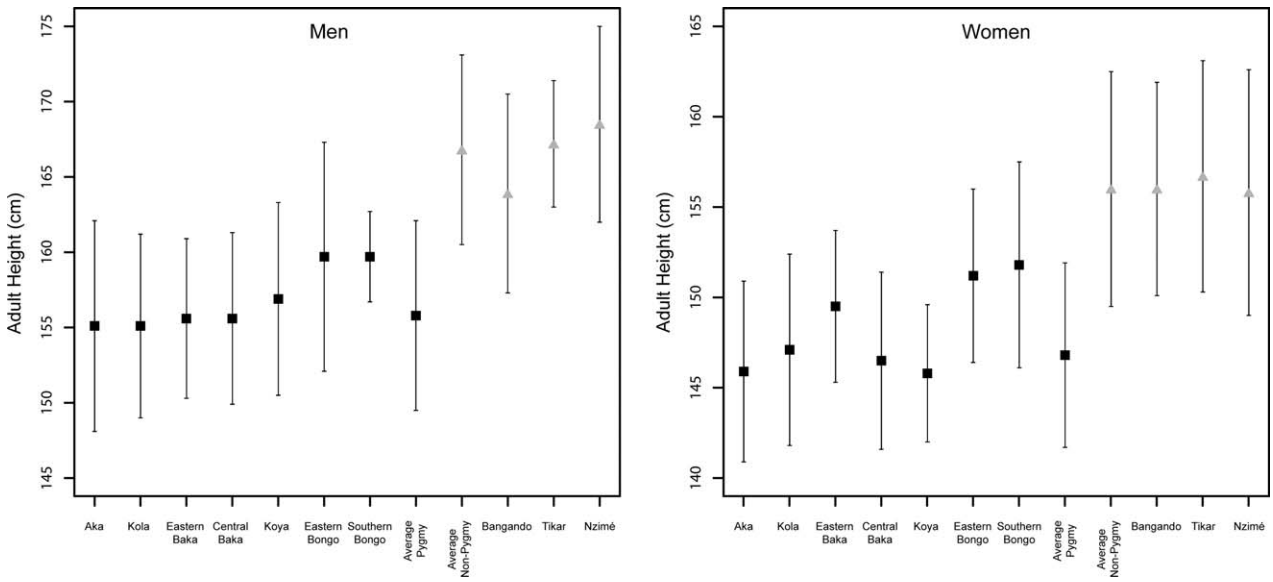

Fig. 2. Estimated mean adult height (in cm) for Pygmy and Non-Pygmy populations. Estimated population means are indicated by black squares for Pygmy populations and gray triangles for Non-Pygmy populations. Bars show estimated standard deviations among individuals within populations. "Average Pygmy" and "Average Non-Pygmy" are estimated means calculated by considering all Pygmy individuals pooled and all Non-Pygmy individuals pooled, respectively. For these latter averaged estimates, estimated standard deviations are calculated across all Pygmy and Non-Pygmy individuals, respectively.

ern Central Africa by using the Bayesian clustering algorithm implemented in the software STRUCTURE v.2.1 (Falush et al., 2003; Pritchard et al., 2000). This software uses a Monte-Carlo Markov-Chain (MCMC) algorithm to first build a set of K clusters (where the user specifies K) based on the allele frequencies at each locus, without considering the individuals' populations of origin. STRUCTURE then assigns each locus genotyped in each individual to one of the K clusters. For each individual, the proportion of genotypes assigned to a specific cluster is called the membership proportion. The individual membership proportion can thus be viewed as the individual's level of genetic similarity to a given cluster between the K inferred genetic clusters, which does not necessarily correspond to the population of origin.

Using this clustering method with $K = 2$, it was shown that Western Central African Pygmy individuals exhibited different levels of membership proportions in a cluster (blue), in which the Non-Pygmy individuals exhibited very high membership proportions (Verdu et al., 2009). For the 213 individuals for which stature data was available, we extracted these membership proportions in the blue cluster for $K = 2$ using 50 independent runs of STRUCTURE and analyzed for consistency across runs (see Supporting Information for details) using the software CLUMPP (Jakobsson and Rosenberg, 2007). For each individual, we first considered the membership proportions for $K = 2$, averaged across the 50 independent runs giving all similar clustering results. Second, we considered the individuals' membership proportions independently for each one of the 50 runs.

We then performed new runs of STRUCTURE using, in addition to the 604 individuals studied in Verdu et al. (2009), 119 individuals from six Continental African populations from the HGDP-CEPH panel for which genotypes were previously published (Rosenberg et al., 2002). See Supporting Information for details on how the two data sets were merged. We used the same parameterization (see Supporting Information and Verdu et al., 2009) of STRUCTURE and CLUMPP to analyze this combined data set comprising 27 populations from Continental Africa genotyped for 26 microsatellite loci. Similarly as previously, membership proportions for each individual were first averaged across the 50 independent runs giving similar clustering results and were also considered independently for each one of the 50 runs. Results were plotted using the program DISTRUCT (Rosenberg 2004).

Statistical analyses comparing genetic and anthropometrical data

Analyses using membership proportions based on previous data by Verdu et al. (2009). We compared (see Fig. 2) individuals' membership proportions in the blue cluster from Verdu et al. (2009) and individuals' adult height by using Pearson correlation tests (Table 2). All tests were performed at the individual level on men and women separately. To account for potential structure in the genetic data, we first (1) considered Pygmy and Non-Pygmy individuals pooled together (Table 2). Second (2) we considered the individuals from the five Pygmy populations pooled together (Table 2). Third (3) we considered the potential role of each one of the five different Pygmy populations (Table 3). Finally (4), we performed the Pearson correlation tests considering individuals within each Pygmy population separately. We conducted analyses (1) and (2) using both the individual membership proportions averaged over 50 independent STRUCTURE runs, and separately for each one of the 50 runs.

The Pearson correlation test assumes that data are approximately Gaussian distributed, an assumption that may be violated for the membership proportions in some of our analyses. Therefore, we further tested the correlation between individuals' membership proportions and adult height by performing random permutation procedures. We first (Analysis 1) produced 10,000 permuted data sets by randomly permuting both individuals' height and individuals' membership proportions among

TABLE 2. Pearson correlation tests between individuals' adult height (H) and individuals' genotype membership proportions from STRUCTURE at K = 2

Individuals (sample size)		Western Central Africa		
		Pearson correlation r_0	*P*-value	10,000 permutations $r > r_0$*
All	Men (126)	0.46	$6.04*10^{-8}$	2
	Women (87)	0.55	$3.63*10^{-8}$	6
Pygmy	Men (82)	0.30	0.006	28
	Women (43)	0.49	0.0007	11

Pearson correlation tests were first conducted for 213 Pygmy and Non-Pygmy individuals pooled together. Second, tests were conducted for the 125 individuals categorized as Pygmies based on numerous cultural criteria without considering height information a priori.
10,000 permuted data sets were obtained by permuting randomly both height and membership proportions first among 213 Pygmy and Non-Pygmy individuals pooled together, second, only among the 125 individuals categorized as Pygmies. All analyses were conducted for men and women separately.
Western Central Africa: We consider here individuals' membership proportions previously obtained with STRUCTURE (Pritchard et al., 2000; Falush et al., 2003) using 28 microsatellites genotyped in 604 individuals from Cameroon and Gabon (Verdu et al., 2009).
* Number of permuted data sets with a Pearson correlation (r) greater than the value estimated for the corresponding real data set r_0.

all individuals in the real data set. Second (2), we produced 10,000 permuted data sets by randomly permuting both individuals' height and individuals' membership proportions among individuals from all Pygmy populations. Third (3), we produced five sets of 10,000 permuted data sets each by randomly permuting both individuals' height and individuals' membership proportions among individuals from four Pygmy populations leaving in turn each one of the five Pygmy populations out. Then, for men and women separately, we performed Pearson correlations at the individual level on each permuted data set, and compared the values of the Pearson correlation (r) found for each permuted data set with the original estimates obtained with the corresponding real data sets (Tables 2 and 3). For the fourth analysis (4), we conducted a Fisher Combined Probability test by combining the Pearson correlation tests' *P*-values obtained for men and women in each Pygmy population separately.

The analyses conducted at the individual level within each Pygmy population separately (Analysis 4) were likely to lack power due to small sample sizes when considering men and women separately. We increased the sample sizes within each Pygmy population by transforming the height data measured in women according to male stature from the same population. We then conducted Pearson correlation tests within each population separately by pooling all individuals together without distinguishing genders. In order to do so, we first calculated the *Zscore* of height (H) for each woman as $Zscore = (H - \hat{E}(H)_{\female})/\hat{\sigma}_{\female}$, where $\hat{E}(H)_{\female}$ is the estimated population mean of women height and $\hat{\sigma}_{\female}$ is the estimated standard deviation of height across females from the same population. We then corrected the height values for each woman ($Height_{modif}$) by considering them as men having the same *Zscore* value: $Height_{modif} = Zscore * \hat{\sigma}_{\male} + \hat{E}(H)_{\male}$, where $\hat{E}(H)_{\male}$ is the estimated population mean of male height and $\hat{\sigma}_{\male}$ is the estimated standard deviation of height across males from the same population. We then performed Pearson correlation tests between individuals' membership proportions and height similarly as previously. Finally, as before, we conducted a Fisher Combined Probability test by combining the Pearson correlation tests' *P*-values obtained for each one of these Pygmy populations separately.

Analyses using membership proportions based on 27 populations from Continental Africa. Since results from STRUCTURE can vary when a different number of markers or a different set of populations is used, we repeated these correlation analyses using the 213 individuals for which we had both genetic and height data, with the individuals' membership proportions obtained using STRUCTURE including Verdu et al.'s (2009) 604 Western Central African individuals and the 119 individuals from the six Continental African populations from the CEPH-HGDP. Similarly as previously, we performed Pearson correlation tests to evaluate the correlations between individuals' membership proportions and height both using individuals' membership proportions averaged over 50 runs, and for each one of the 50 runs separately. Finally, as in analyses (1) and (2), we performed permutation analyses with 10,000 permuted data sets by randomly permuting both individu-

TABLE 3. Pearson tests of correlation between individuals' height (H) and genotype membership proportions, comparison between 10,000 permuted data sets and the original real data set

Population left out	Real data		10,000 permuted data sets
	Pearson correlation r_0	*P*-value	$r > r_0$*
a) Men			
Central Baka	0.25	0.038	186
Eastern Baka	0.469	0.0003	2
Koya	0.261	0.039	191
Eastern Bongo	0.221	0.082	430
Southern Bongo	0.282	0.015	82
b) Women			
Central Baka	0.486	0.01	32
Eastern Baka	0.495	0.0007	1
Koya	0.506	0.003	12
Eastern Bongo	0.33	0.065	365
Southern Bongo	0.517	0.0009	5

Five separate Pearson tests of correlation were conducted for individuals from four Pygmy populations, leaving out in turn each one of the Pygmy population.
10,000 permuted data sets were obtained by permuting both height and membership proportions among individuals from four Pygmy populations pooled together, leaving out in turn individuals from each one of the Pygmy population.
For the "real data", we consider for each individual the genotypes membership proportions averaged across 50 independent runs of STRUCTURE at $K = 2$ previously obtained by Verdu et al. (2009).
All analyses were conducted for men (a) and women (b) separately.
*Number of permuted data sets with a Pearson correlation (r) greater than the value estimated for the corresponding real data set r_0.

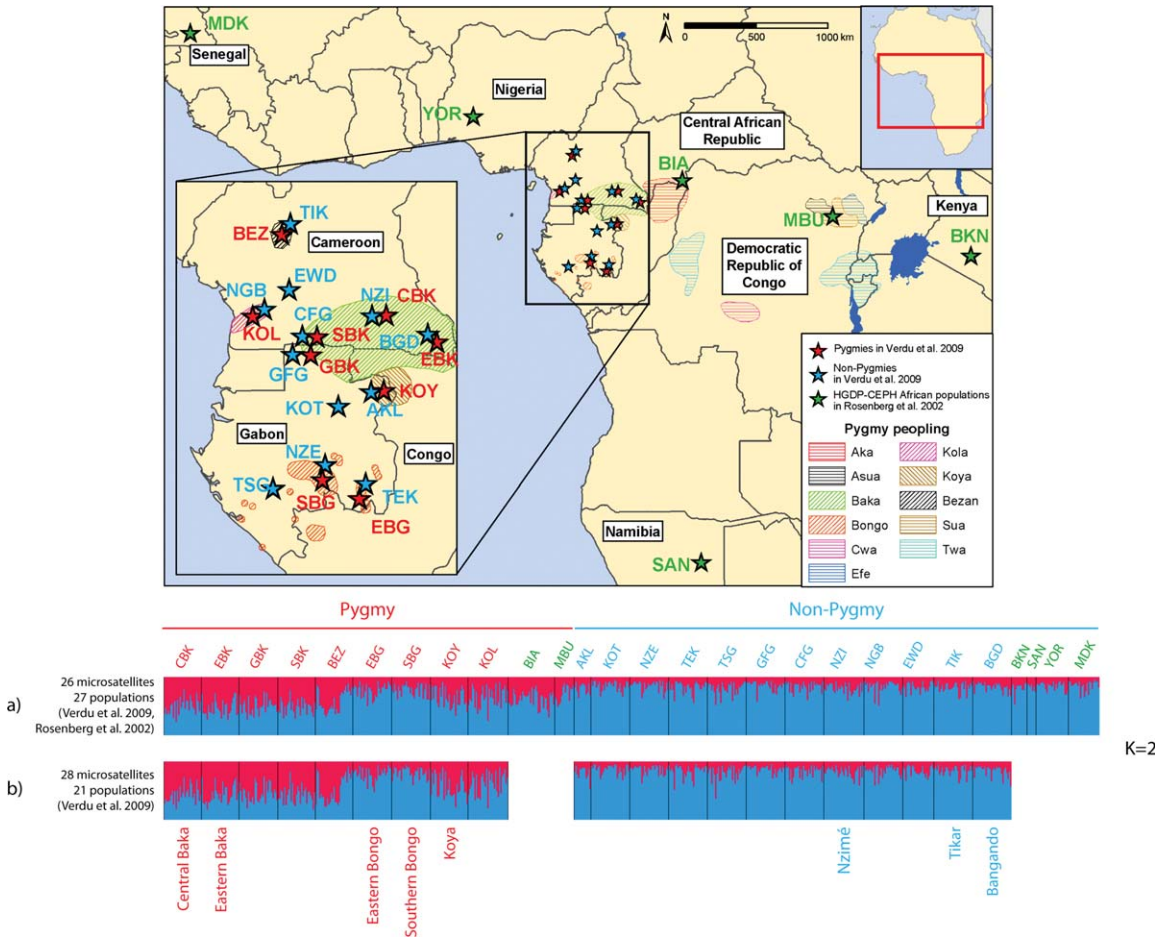

Fig. 3. Genetic structure of African populations. Map showing the geographical locations of the populations from Verdu et al. (2009) and Rosenberg et al. (2002) considered in the STRUCTURE (Pritchard et al., 2000; Falush et al., 2003) analyses. Population codes for Verdu et al.'s (2009) data set are noted in blue (Non-Pygmy populations) and in red (Pygmy populations): CBK, Central Baka; EBK, Eastern Baka; GBK, Gabonese Baka; SBK, Southern Baka; BEZ, Bezan; EBG, Eastern Bongo; SBG, Southern Bongo; KOY, Koya; KOL, Kola; AKL, Akele; KOT, Kota; NZE, Nzébi; TEK, Teke; TSG, Tsogho; GFG, Gabonese Fang; CFG, Cameroonese Fang; NZI, Nzimé; NGB, Ngumba; EWD, Ewondo; TIK, Tikar; BGD, Bangando. Population codes for Rosenberg et al. (2002) Continental African data are noted in green: BIA, Biaka Pygmies from Central African Republic; MBU, Mbuti Pygmies from Democratic Republic of Congo; BKN, Bantu speakers from Kenya; SAN, Bushmen !Kung San from Namibia; YOR, Yoruba from Nigeria; MDK, Mandenka from Senegal. Pygmy peopling areas were inferred from our ethnographic field-work. (a) STRUCTURE results averaged for each individual over 50 independent runs obtained for $K = 2$ using 26 microsatellites genotyped in 604 Western Central African (Verdu et al., 2009) and 119 Continental African individuals (Rosenberg et al., 2002). (b) STRUCTURE results averaged for each individual over 50 independent runs obtained for $K = 2$ using 28 microsatellites genotyped in 604 Western Central African individuals, previously published in Verdu et al. (2009). Individuals' membership proportions in each cluster ("blue" or "red") are represented by a single vertical line divided in two colors for each individual. Black lines separate individuals from different predefined populations. Populations considered in the present paper for comparison between individuals' membership proportions and individuals' adult height data are indicated below the bar plots.

als' height and individuals' membership proportions among the corresponding (1 and 2) sets of individuals. We further performed Pearson correlations on these sets of 10,000 permuted data sets and compared the results with the Pearson correlation tests obtained on the corresponding real data sets.

RESULTS AND DISCUSSION

African Pygmies categorized as such based on cultural criteria are on average shorter than Non-Pygmies

We found that adult height was considerably lower in Pygmies, categorized as such based on numerous cultural criteria rather than height (see Materials and Methods), than in the neighboring Non-Pygmies, for both men and women (Table 1, Fig. 3). Pygmy males from the seven populations pooled together had, on average, an estimated mean height ($\hat{E}(H)$) of 155.8 cm (SD = 6.3 cm across individuals), as compared with 166.8 cm (SD = 6.3 cm) on average for Non-Pygmy males from the three populations pooled together. Similarly, Pygmy women pooled together had, on average, an estimated mean height of $\hat{E}(H) = 146.8$cm (SD = 5.1 cm across individuals) as compared to 156.0 cm (SD = 6.5 cm) on average for Non-Pygmy females pooled together. Hence (see also populations' details in Table 1 and Fig. 3), the Pygmy individuals from the seven different populations categorized as such based on numerous cultural criteria

without considering height data a priori are found, on average, in the lower extreme of the worldwide distribution of population average adult height, which has a mean of 164.8 cm for men and 154.3 cm for women averaging across 1,172 human populations worldwide (Hiernaux, 1974; Froment, 1993).

We wondered if this short average height in Pygmy populations was coincident with a reduced variability of height across individuals within the various Pygmy populations as compared to Non-Pygmies. We performed *F*-tests to compare the variance in height between all possible pairs of Pygmy and Non-Pygmy populations and found no major reduction in height variability across individuals within Pygmy populations as compared to Non-Pygmy populations: 97.6% of the tests comparing variance in height across individuals (100% for men and 95.2% for women) were not significant between Pygmy and Non-Pygmy pairs of populations, which indicates that the variability of height is not lower in Pygmies consistently with previous observations (Hiernaux, 1974; Froment, 1993).

It has been proposed that having a short adult stature could be advantageous for individuals living in hot, humid and dense environments, such as the equatorial forest, in terms of improving body-temperature regulation and energetic expenditure (Katzmarzyk and Leonard, 1998; Tilkens et al., 2007; Taylor-Weale and Vinicius, 2008). This hypothesis has also been proposed to explain the short stature of Central African Pygmies as compared with Non-Pygmies (Cavalli-Sforza, 1986; Diamond, 1991). In this context, we expect that Pygmy and Non-Pygmy individuals who have grown up in the same environment will achieve the same adult stature. To test this assumption, we compared respectively the Central Baka Pygmies with their Nzimé Non-Pygmy neighbors and the Eastern Baka Pygmies with their Bangando Non-Pygmy neighbors. These pairs of Pygmy and Non-Pygmy populations have close socio-economic relationships with one another and live most of the time in the very same villages. Therefore, for these pairs of populations, Pygmy and Non-Pygmy individuals were more likely to have grown in similar climatic and ecological environments and, under the hypothesis of determination of height by climatic and forest density conditions through phenotypic plasticity, should have achieved similar adult stature.

However, we found that both these Pygmy populations were highly significantly smaller in stature than their respective Non-Pygmy immediate neighbors (Central Baka vs Nzimé: $t = -12.83$, df = 222, P-value = 2.20×10^{-16} for men and $t = -9.69$, df = 76.3, P-value = 3.22×10^{-15} for women; Eastern Baka vs. Bangando: $t = -5.68$, df = 63, P-value = 3.63×10^{-7} for men and $t = -2.96$, df = 26, P-value = 0.0065 for women). Therefore, it was reasonable to assume that the differential stature found nowadays between Pygmies and neighboring Non-Pygmies may not have been immediately due to major environmental differences in the temperatures, humidity levels or forest densities that individuals experienced during their growth period. This difference in stature may thus not be explained by phenotypic plasticity.

This result does not rule out the possibility that these environmental factors played an important evolutionary role in the past. Indeed, if ancestral Pygmy and Non-Pygmy populations did not occupy the same climatic and ecological environments, they could have gone through different morphological adaptations to their respective environments. Such different evolutionary processes may have resulted in the stature dissimilarities found between Pygmies and Non-Pygmies, despite the fact that some of these populations now share the same climatic and ecological environment. It is still challenging to thoroughly explore this evolutionary hypothesis, since, in the lack of extensive archaeological and paleo-climatic data, it is still widely unknown where and in which climatic and ecological environments the ancestors of existing Central African Pygmy and Non-Pygmy populations evolved.

Genetic factors underlying African Pygmies' short stature. Although sharing a recent common ancestor about 2,800 years ago (Verdu et al., 2009), Western Central African Pygmy populations exhibit significant pairwise genetic differences as determined using 28 independent nuclear microsatellites (AMOVA $F_{ST} = 0.014$ in Verdu et al., 2009). Concerning Pygmy populations, the individual membership proportions in the blue cluster (see Fig. 4) estimated by STRUCTURE for $K = 2$ can be interpreted as individual levels of genetic similarity with the Non-Pygmy gene-pool. If the short stature of Pygmies was determined by genetic factors, we would expect that the Pygmy individuals with high levels of genetic similarity with the Non-Pygmies will be taller than Pygmy individuals with low levels of genetic similarity with the Non-Pygmies. We would therefore expect a positive correlation between Pygmy individuals' height and their respective membership proportions in the Non-Pygmy cluster obtained with STRUCTURE at $K = 2$.

We investigated this hypothesis for the 213 Pygmy and Non-Pygmy individuals for whom both height and genetic data were available (Table 1; Fig. 2). To account for potential population structure and for the binary categorization of individuals as Pygmies and Non-Pygmies, we investigated the correlation between individual height and membership proportions in the Non-Pygmy genetic cluster, using four different levels of groupings of individuals. We also conducted the above correlation analyses using membership proportions estimated by STRUCTURE based on 27 Continental African populations genotyped at 26 microsatellite loci.

Positive correlation between Pygmies' adult height and levels of genetic similarity with the Non-Pygmies

Analyzing Pygmy and Non-Pygmy individuals pooled together. Considering all Pygmy and Non-Pygmy individuals pooled together, we found (Table 2) a significant positive correlation between individuals' membership proportions in the "blue" cluster and individuals' adult height, separately considering men pooled together (Pearson correlation test: $r = 0.46$, P-value = 6.04×10^{-8}) and women pooled together (Pearson correlation test: $r = 0.55$, P-value = 3.63×10^{-8}) regardless of their categorization in Pygmy or Non-Pygmy (Fig. 2, Table 2). Since the statistical assumptions underlying the Pearson correlation test may be violated, we further performed Pearson correlations on 10,000 permuted data sets, for which both height and membership proportions were randomly permuted among all individuals. Among the 10,000 permuted data sets, only 0.02% and 0.06% permuted data sets, respectively for males and females, gave a value of the correlation (r) greater than the one obtained with our real data (Table 2). This strongly suggests that the positive correlations obtained here between individuals' adult height and their respective

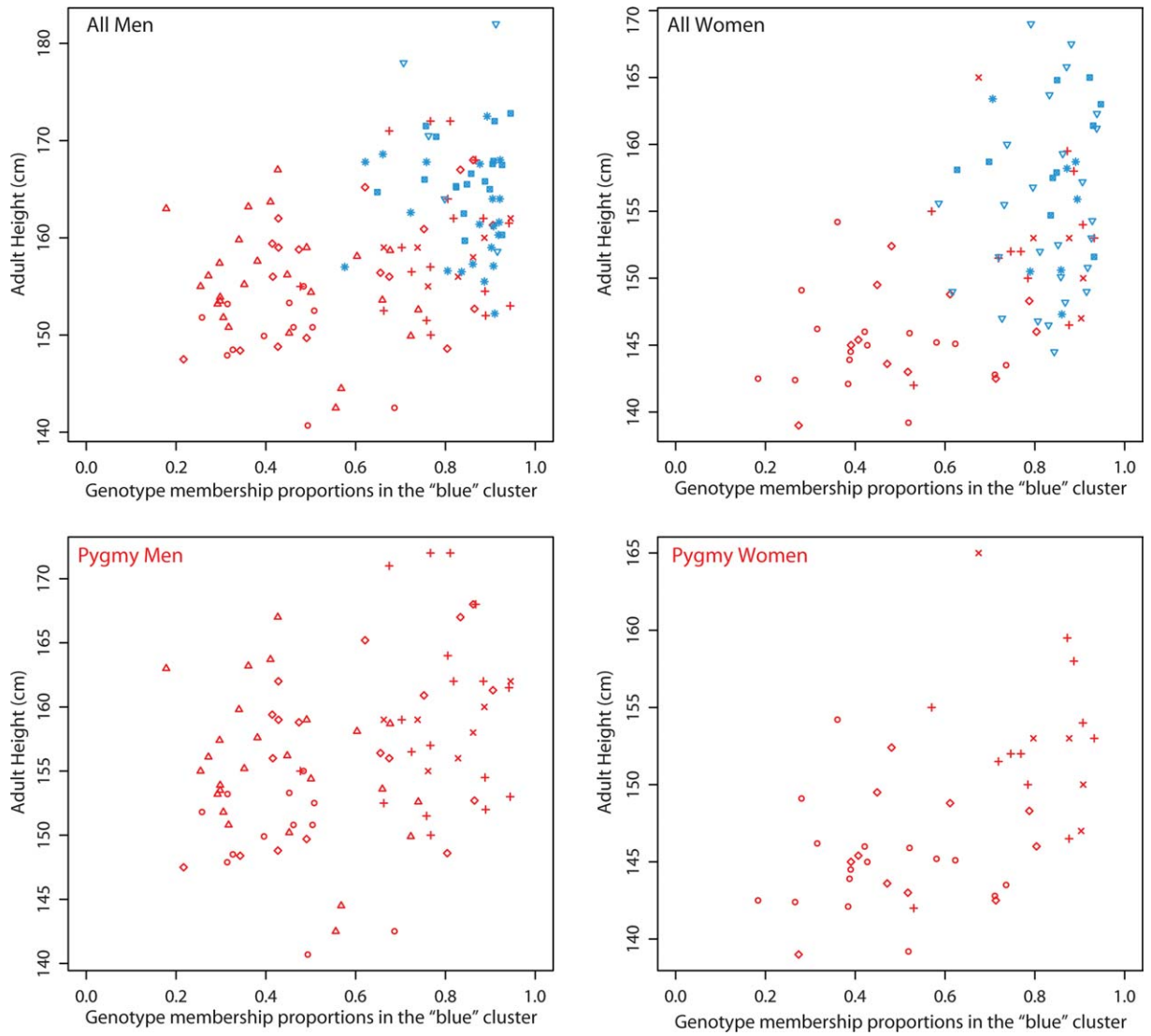

Fig. 4. Individuals' adult height (in cm) as a function of individuals' genotype membership proportions, for men and women separately. Individuals' genotype membership proportions in the "blue" cluster (see Fig. 3b) obtained previously (Verdu et al., 2009) are given on the *x*-axis. Corresponding individuals' adult heights are given on the *y*-axis. Pygmy (in red) and Non-Pygmy (in blue) individuals are categorized as such based on numerous cultural criteria rather than height. The two upper graphs present data considering all Pygmy and Non-Pygmy individuals for men (on the left) and women (on the right) separately. The two lower graphs present data considering only Pygmy individuals for men (on the left) and women (on the right) separately. Population symbols: Central Baka (○); Eastern Baka (△); Eastern Bongo (+); Southern Bongo (×); Koya (◇); Nzimeé (▽); Tikar (⊠); and Bangando (*).

membership proportions were very unlikely to have been obtained only by chance.

Analyzing Pygmy individuals pooled together. These positive correlations could potentially be simply due to the few individuals at both the tails of the distribution of height and membership proportions in our data set when pooling Pygmies and Non-Pygmies together: at one extreme, very short Pygmy individuals with low membership proportions in the blue cluster and, at the other extreme, very tall Non-Pygmy individuals with high membership proportions in the same blue cluster (see Fig. 2). If the significant positive correlations between individuals' height and membership proportions were just due to these two sets of extreme individuals, we should not expect to find such significant positive correlations when considering only Pygmy individuals pooled together.

Nevertheless, significant positive correlations (Fig. 2; Table 2) were found when pooling together only the individuals categorized as Pygmies (Pearson correlation test: $r = 0.30$, P-value $= 0.006$ for Pygmy men and $r = 0.49$, P-value $= 7 \times 10^{-4}$ for Pygmy women). Similarly as previously, we further tested the significance of correlations between Pygmy individuals' levels of genetic similarity with the Non-Pygmy gene-pool and adult height using a random permutation approach. Among 10,000 permuted data sets, for which both height and membership proportions were randomly permuted among Pygmy individuals only, only 0.28% and 0.11% (respectively for males and females) permuted data sets gave a value of the Pearson correlation (r) greater than the one obtained with our real data (Table 2). Therefore, it is very

unlikely that the positive correlations between Pygmy individuals' height and their respective levels of genetic similarity with the Non-Pygmies were obtained simply by chance when considering only the Pygmy individuals pooled together.

Finally, we estimated the correlation between individuals' height and membership proportions for each one of the 50 independent runs of STRUCTURE separately. We performed this analysis for all individuals pooled together (1) and for Pygmy individuals only (2). As expected given the high levels of similarity among STRUCTURE runs (see Supporting Information and Verdu et al., 2009), we found systematically similar results to those obtained when considering the individuals' membership proportions averaged over the 50 independent runs (results not shown). Altogether, our results showed that the stature of Pygmy individuals was significantly positively correlated with the level of genetic similarity with the Non-Pygmy gene-pool: Pygmy individuals who were more similar genetically to Non-Pygmies were likely to be taller.

Analyzing sets of Pygmy individuals leaving one Pygmy population out in turn. We wanted to evaluate the contribution of each Pygmy population to the positive correlations previously found between individuals' height and membership proportions when pooling all Pygmy individuals together. To do so, we removed the individuals from each one of the five Pygmy populations in turn and performed Pearson tests of correlation between individuals' height and membership proportions using only the Pygmy individuals from the four remaining populations pooled together.

We found a significant positive correlation (Table 3) between individuals' height and individuals' levels of genetic similarity with the Non-Pygmy gene-pool for all "leave one population out" analyses, except when leaving out the Eastern Bongo individuals, although this latter correlation was still close to significant (r = 0.22, P-value = 0.08 leaving out Eastern Bongo men and r = 0.33, P-value = 0.07 leaving out Eastern Bongo women). The nonsignificant result for Eastern Bongo Pygmies could have been due to the violation of the assumption of Gaussian distributions underlying the Pearson correlation tests in this particular test. Indeed, when considering 10,000 permuted data sets for which both individual height and membership proportions were randomly permuted among all individuals from the four Pygmy populations excluding the Eastern Bongo individuals, we found only 4.3% and 3.65% permuted datasets, respectively for males and females, giving a value of the Pearson correlation (r) greater than the one obtained with our real data (Table 3). Therefore, although the Pearson correlation test was only marginally significant here, it is unlikely that the positive correlation between individuals' height and membership proportions found for all Pygmy individuals except the Eastern Bongo individuals was obtained only by chance. Altogether, our results indicated that all Pygmy populations contributed to the positive correlation between the height of Pygmy individuals and their genetic similarity with the Non-Pygmy gene-pool.

Analyzing Pygmy individuals within each population separately. We investigated the correlation between individuals' membership proportions and individuals' adult height within each one of the Pygmy populations separately, and found no significant positive correlation at the within-population level. Moreover, a Fisher combined probability test, combining the Pearson correlation test's P-values calculated independently for each one of the Pygmy populations, was not significant (χ^2 = 15.1, df = 10, P-value = 0.13) for the five populations of Pygmy males, and was marginally significant (χ^2 = 15.4, df = 8, P-value = 0.05) for the four populations of Pygmy females. The absence of a significant positive correlation at the within-Pygmy-population level could be due to a lack of power due to the small sample sizes used when considering men and women separately within each Pygmy population (Table 1).

In order to overcome this small sample size limitation, we increased the sample sizes within each Pygmy population by transforming the height data measured in women according to male stature from the same population and then considering all individuals pooled together without distinguishing between genders (see Materials and Methods). For each one of the Pygmy populations, in which males and females were confounded and the sample sizes hence increased, the Pearson correlations (r) between height and membership proportions increased at the within-Pygmy-population level (results not shown) and even achieved significance (Pearson r = 0.39, P-value = 0.035) in the Koya Pygmy population from Gabon. A Fisher combined probability test, combining the Pearson correlation test's p-values calculated independently for each one of these five Pygmy populations, was close to significance (χ^2 = 17.4, df = 10, P-value = 0.06).

Previous GWA studies focusing on the within population variation of adult height used several thousands of individuals within a population in order to identify mutations that were significantly associated with stature phenotypes (Gudbjartsson et al., 2008; Lettre et al., 2008; Sanna et al., 2008; Weedon et al., 2008; Estrada et al., 2009; Johansson et al., 2009; Shriner et al., 2009; Soranzo et al., 2009; Takeuchi et al., 2009; Lango Allen et al., 2010). In this context, our results suggest that if larger sample sizes were available, we would potentially be able to find a significant correlation between height and levels of genetic similarity with the Non-Pygmy gene-pool at the within-Pygmy-population level. Future analyses considering much greater population sample sizes will be needed to confirm this hypothesis.

Correlation analyses using STRUCTURE results based on 27 Continental African populations. Using a different set of markers as well as including different populations in the STRUCTURE analysis could have led to an insignificant correlation between Pygmy individuals' adult height and their genetic similarity with the Non-Pygmy gene-pool.

STRUCTURE results at K = 2 obtained using 27 Continental African populations and 26 microsatellite markers were very consistent with previous results obtained by Verdu et al. (see Fig. 4 and Supporting Information). With the membership proportions in the blue cluster obtained using this Continental African dataset (see Fig. 4), we also systematically found significant positive correlations between individuals' levels of genetic similarity with the Non-Pygmy gene-pool and individuals' adult height for men and women separately, both when considering (1) all Pygmy and Non-Pygmy individuals pooled together (Supporting Information S1) as well as when considering (2) only individuals from Pygmy populations pooled together (Supporting Information Table S1).

Despite the small sample sizes, the results of our five sets of genetic analyses overall strongly suggest that Pygmy individuals, categorized as such based on numerous cultural criteria, are likely to be taller if they have a higher level of genetic similarity with the neighboring Non-Pygmy populations. Using the software STRUCTURE, Pygmy individuals' membership proportions in the "blue" cluster could also be interpreted as levels of genetic introgression from the Non-Pygmies into the Pygmy populations (Patin et al., 2009; Tishkoff et al., 2009; Verdu et al., 2009), which is consistent with the complex socio-cultural rules regarding marriages between these groups of populations (Destro-Bisol et al., 2004; Verdu et al., 2009). Under the latter interpretation, our results suggest that Central African Pygmy individuals that are more admixed with Non-Pygmy neighbors are also likely to be taller.

Our results therefore showed, for the first time to our knowledge, that complex genetic factors were very likely to play a direct role in the determination of the differential stature of African Pygmies and Non-Pygmies. These yet unknown genetic factors may be found in the genetic pathway of the GH-IGF1 axis, as previously proposed by Merimee (1987). Such hypothesis was further suggested by the resistance of various Efe Pygmy cell-lines to the proliferative action of IGF1 (Geffner et al., 1993; Cortez et al., 1996) and by the decreased transcription of IGF1 receptor gene suggested by another study (Hattori et al., 1996). More recently, Bozzola et al. (2009) found a severe underexpression of the GH receptor in African Pygmies as compared to Non-Pygmies (Bozzola et al., 2009). Interestingly, endocrinology studies in other short stature populations from Papua New Guinea and the Philippines found low levels of serum GH Binding Protein similar to that of African Pygmy populations, which suggests a common physiological basis for short-stature phenotypes across human populations (Baumann et al., 1991; Clavano-Harding et al., 1999; Davila et al., 2002; Bozzola et al., 2009).

African Pygmies have been shown to share a recent common ancestry (Patin et al., 2009; Verdu et al., 2009). However, it is unlikely that these African populations share a recent common ancestry with other short-stature populations from different continents, such as the Aeta from the Philippines, the Maya from Central America or the Inuit from the Arctic Circle. All these worldwide populations are thus likely to have experienced different evolutionary histories and genetic adaptations with respect to stature.

Recent Genome-Wide Association studies have identified more than 180 loci associated with the determination of individual height in human populations, which could also be relevant candidates to explain the differences in stature between various worldwide Pygmy and Non-Pygmy populations (Gudbjartsson et al., 2008; Lettre et al., 2008; Sanna et al., 2008; Weedon et al., 2008; Estrada et al., 2009; Johansson et al., 2009; Shriner et al., 2009; Soranzo et al., 2009; Takeuchi et al., 2009; Lango Allen et al., 2010). Therefore, it remains difficult to predict whether the genetic polymorphisms playing a role in the differential stature of African Pygmies and Non-Pygmies would also be determining the stature of other short-stature populations worldwide. In this context, the identification and the comparison of such polymorphisms would allow studying the convergence of phenotypic traits among human populations with different origins and evolutionary histories. In turn, this would bring new fundamental knowledge to our comprehension of the evolutionary and adaptation mechanisms shaping the human genetic diversity determining stature.

CONCLUSIONS

We have analyzed a uniquely large population sample comprising 1,103 Central African adults and showed that Pygmy populations, categorized as such through extensive interdisciplinary ethnographic inquiries rather than on a height threshold, were, on average, much shorter than their Non-Pygmy neighbors, which is consistent with the historical usage of the word "Pygmy" in Africa. Furthermore, results obtained by comparing Pygmy and nearby neighboring Non-Pygmy populations, suggest that climatic or forest density pressures did not play a major role in the major difference in stature found nowadays between populations through phenotypic plasticity. However, this result does not exclude that such environmental pressures have played an important evolutionary role in the past.

Considering a subset of 213 individuals for which DNA was available, we were able to formally compare the individual variation in height with the neutral genetic variation among individuals from the different Pygmy and Non-Pygmy populations.

Controlling for the binary categorization of individuals as Pygmies or Non-Pygmies, as well as for population substructure, we found strongly significant positive correlations between Pygmy individuals' stature and their levels of admixture with the Non-Pygmy gene-pool estimated using the clustering software STRUCTURE. This result suggests that the major difference in average stature observed between Central African Pygmy and Non-Pygmy populations is likely determined by complex genetic factors.

In this context, Genome Wide Association studies and Admixture Mapping methods will likely reveal the genetic loci involved in the determination of the differences of average height found in existing African Pygmy and Non-Pygmy populations. This will further help us to better understand the determination and evolution of height variation among human populations.

This result, however, does not rule out the environmental hypotheses previously proposed for explaining such morphological differences, such as ecologic parasitological or nutritional differential evolutionary pressures (Perry and Dominy 2009). Indeed, the major genetic differences found nowadays between Central African populations could result from differential natural selection pressures caused by the potential different ecological conditions experienced by the ancestors of these groups of populations. Unfortunately, the lacks of archaeological and paleo-environmental data for the Congo Basin make further thorough investigation of these evolutionary hypotheses challenging. To overcome these limitations, our study suggests that a promising strategy will be to investigate the influence of Natural Selection on determining the patterns of genetic variation between Central African Pygmies and Non-Pygmies. Such an approach will likely reveal the extent to which past differences in environmental pressures affected the genetic determination of differential morphological features among Central African populations.

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