Genetica (2006) 126:251–260
DOI 10.1007/s10709-005-1454-z

Y chromosome diversity in Brazilians: switching perspectives from slow to fast evolving markers

Denise R. Carvalho-Silva[1], Eduardo Tarazona-Santos[1,2], Jorge Rocha[3], Sérgio D.J. Pena[1,*] & Fabrício R. Santos[2]

[1]*Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Av. Afonso Pena 3111/9, 30130-909, Belo Horizonte, Brazil;* [2]*Departamento de Biologia Geral, Universidade Federal de Minas Gerais, 31270-910, Belo Horizonte, Brazil;* [3]*Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal; *Author for correspondence: (*Phone: +5531-3284-8000; Fax: +5531-3227-3792; E-mail: spena@dcc.ufmg.br*

Key words: αh heteroduplex system, Brazilians, differentiation, microsatellites, unique event polymorphisms, Y chromosome

Abbreviations: AMOVA – analysis of molecular variance; ANOVA – analysis of variance; CI – Confidence Interval; DNA – deoxyribonucleic acid; UEPs – unique event polymorphisms; Y-μsat – Y microsatellites

Abstract

We have previously shown that the Y chromosomes of 'white' Brazilians have their immediate geographical origin in Europe, with low frequency of sub-Saharan African chromosomes and virtual absence of Amerindian contribution. The typing of slow evolving polymorphisms on the Y chromosome also revealed no differences between Brazilians and Portuguese, the bulk of European immigrants to Brazil, and even among Brazilians from distinct regions of Brazil, the latter being in sharp contrast with mtDNA data. In order to test if the lack of differentiation is a sex-biased and not a marker-biased phenomenon, we decided to study faster evolving Y chromosome markers in samples from Brazil and Portugal previously studied. The population structure revealed by this work confirmed that there were indeed no significant differences between Brazil and Portugal and no population differentiation within the four geographical regions of Brazil, suggesting that this phenomenon is unrelated to the nature of the markers typed. Nevertheless the fast evolving markers did uncover a higher within population diversity in Brazil than Portugal, which could be explained by the input of diverse European Y chromosomes carried by several migration waves to Brazil. Our present data highlight the significance of typing and combining Y markers that evolve according to distinct mutational paces to usefully assess the levels of diversity in a given population, and can be applied in the study of populations derived from distinct geographical origins such as the Brazilians.

Introduction

Admixture studies have been shown particularly interesting to apply to Latin America populations (Sans, 2000). The historical records about the peopling of Brazil suggest that its population is a mixture of three major geographical groups: Europeans (mainly Portuguese), Africans and Amerindians (Ribeiro, 1995). In previous works, we have tried to assess the relative contribution of these three major founding populations to the gene pool of the current white Brazilian population by studying the genetic variability of maternal (Alves-Silva et al., 2000) and paternal (Carvalho-Silva et al., 2001) lineages (using genetic polymorphisms of mtDNA and Y-chromosome, respectively) in individuals from four major geographical Brazilian regions. We found genetic

evidence corroborating the historical records of directional mating between European males (Carvalho-Silva et al., 2001) and Amerindian and African women (Alves-Silva et al., 2000). While the study of the Y-chromosome diversity showed that the immense majority of white Brazilian Y-chromosomes had European origins, the analysis of mtDNA variability in the same samples revealed that Amerindian and African females together contributed as much as 61% of the lineages.

In our previous study on the Y-chromosome variability in the white Brazilian population (Carvalho-Silva et al., 2001), we used a battery of UEPs which are slow-evolving markers and we were not able to detect statistically significant differences in the frequency of Y-haplogroups among four major Brazilian regions (North, Northeast, Southeast and South) or between Brazilians (as a whole) and the Portuguese. This absence of differentiation is puzzling in view of the historical records reporting distinct levels of immigration to Brazil from geographical regions other than Portugal, including Italy, Spain, Germany, Japan and the Middle East (Ribeiro, 1995). In principle, this could be due to one or more of the following explanations: (1) the number of non-Portuguese immigrants that arrived in Brazil after the XIX century was not sufficient to change significantly the population structure of Y chromosomes established earlier by the Portuguese; (2) the level of Y-chromosome differentiation among the Portuguese and the other immigrant populations was not sufficient to make a significant imprint in the Y-haplogroup frequency of Brazilians; (3) the UEPs due to their lower mutation rate were not useful to detect extant Y-haplogroup differentiation between Brazilians and the Portuguese and faster evolving markers should be tested instead. The first explanation seems to be unlikely in the face of official numbers from the Brazilian Institute of Geographical Statistics (IBGE, 2000), which show very substantial levels of non-Portuguese immigration. For instance, in the period 1884–1939 Brazil received 4,158,717 immigrants, of which only 1,204,394 (29%) were from Portugal (IBGE, 2000). However, the fact that from 1500 to 1808 practically the only European immigrants to Brazil were Portuguese (apart from a 30-year Dutch invasion in the Northeast during the 17th century), gave them a head-start advantage. The second alternative is not supported by recent studies that show a sizeable degree of differentiation of Y-haplogroup frequency among continents and even within Europe (Rosser et al., 2000; YCC, 2002). The third choice thus appears the most likely and we decided to test it experimentally.

In this article, we present the results of our analysis with fast evolving polymorphic Y-marker namely the αh heteroduplex system (Santos, Pena & Tyler-Smith, 1995; Santos, Bianchi & Pena, 1996; Santos et al., 1999) and microsatellites *DYS19*, *DYS389*II, *DYS389*I, *DYS390*, *DYS391* and *DYS393* (Santos, Pena & Epplen, 1993; Tyler-Smith & Jobling, 1995) in the same individuals previously typed for the slow evolving UEPs (Carvalho-Silva et al., 2001). With such typing we could generate compound haplotypes of UEPs and αh types (UEP–αh) as well as microsatellite haplotypes (Y-μsat) and performed population structure analysis of Y chromosome genetic variability among different regions of Brazil and also between Brazil and Portugal.

Subjects and methods

DNA samples and Y chromosome polymorphisms

The studied population consisted of 200 'white' Brazilians from Northern ($n=49$), Southern ($n=52$), Southeast ($n=50$), and Northeast ($n=49$); and 93 Portuguese males from Northern Portugal. Further details about these subjects are given in a previous publication (Carvalho-Silva et al., 2001). The DNA samples were typed for the αh heteroduplex system according to Santos et al. (1999) and for six Y microsatellites (*DYS19*, *DYS389*II, *DYS389*I, *DYS390*, *DYS391* and *DYS393*) according to Carvalho-Silva et al. (1999). Data on 12 UEPs for the Brazilians and the Portuguese have been published elsewhere (Carvalho-Silva et al., 2001). The distinct Y chromosomes defined by both UEPs and αh types (UEP–αh) were designated by composite names where the first part refers to the UEP haplogroup (the UEP haplogroup nomenclature is according to YCC, 2002; Jobling & Tyler-Smith, 2003), whereas the Roman numeral refers to αh types (Santos et al., 1999). The allele nomenclature used for the microsatellites was as proposed by Kayser et al. (1997) with the exception of the *DYS389* locus.

The nomenclature for the latter was according to Roewer et al. (2001) considering that the sum of p and q stretches corresponds to *DYS389*I. However, *DYS389*II alleles were named by subtracting the *DYS389*I stretch to the total number of repeats $[(n+m+p+q)-(p+q)]$ (Rolf et al., 1998). The six loci were combined to establish Y chromosome microsatellite haplotypes (Y-μsat).

Data analysis

The genetic structure of the Y-chromosome variability in our samples was analyzed at three hierarchical levels: within population, among populations and between groups, where the four Brazilian regions constitute populations, and Brazilians and Portuguese constitute groups. The apportionment of genetic variance was assessed by means of AMOVA (Excoffier, Smouse & Quattro, 1992) using the Arlequin software (version 2.0; Schneider, Roessli & Excoffier, 2000), which was applied to two sets of data: UEP–αh types and Y-μsat. For the UEPs-αh, we used a phylogenetic approach by manually constructing the most parsimonious tree based on ancestral/derived allelic states at all UEPs (reviewed in Santos et al., 1999) and on the molecular evolving mechanisms of the αh system (Santos, Bianchi & Pena, 1996). From this tree, a distance matrix was constructed by using the number of mutation steps between each pair of Y-chromosomes. We calculated the apportionment of diversity for Brazilians and Portuguese with populations defined as Brazil as a whole, Brazil subdivided into four populations (from N, NE, S and SE), and Portugal. Among population comparisons were also made using the exact test of differentiation (Raymond & Rousset, 1995) where putative differences in the frequency of UEP–αh types were assessed. For the microsatellite haplotypes, AMOVA was performed by taking into account the differences in the number of repeats of the microsatellite alleles.

Moreover, we assessed the within population variability by using three measures of diversity: (1) the number of UEP–αh types in the sample, (2) the haplotypic diversity, in the case of the UEP–αh types; and the average gene diversity among the six microsatellite loci, in the case of the μsat haplotypes (Nei, 1987), and (3) the mean variance in the number of repeats among the Y-μsat haplotypes. In the first approach, to test the null hypothesis that Brazilian populations have similar diversity as the Portuguese one, we used coalescent simulations under the Wright–Fisher model, to construct 95% CIs for the expected number of alleles, given sample sizes of 50 and 200, and assuming as θ value, its Ewen's estimator for the Portuguese population, θ_k, which is based on the number of alleles and the Ewen's sampling formula (Nei, 1987). We performed the simulations as implemented in the software DNAsp 4.0 (Rozas et al., 2003). In the second case, significant differences among populations were tested using 95% CIs constructed by the bootstrap technique (sampling the haplotypes 5000 times), using the software GENETIX (Belkhir et al., 2004). In the third case, we tested the significance of differences among populations or groups of populations by performing a nonparametric ANOVA (Kruskal–Wallis test) for independent samples on the distribution of individual average deviances among the six loci, calculated as suggested in Tarazona-Santos et al. (2001).

Results

Typing with the αh polymorphism

The αh polymorphism is a unique system that uses the generation of heteroduplexes to detect variation of the sequence diversity and number of loci in the Y centromere alphoid DNA. It is hypervariable, with 53 different αh types described so far (Santos, Pena & Tyler-Smith, 1995; Santos, Bianchi & Pena, 1996; Santos et al., 1999). Most of the αh types represent unique event polymorphisms, but the simplest ones like types I and II can be recurrently derived by deletion events from the most complex types (Santos, Bianchi & Pena, 1996). The typing of the αh polymorphism allowed us to subdivide our Y haplogroups defined by the UEPs into more discrete types, revealing previously cryptic diversity. The 25 observed Y UEP-αh haplogroups (Table 1) could be connected in a parsimonious network for both the Brazilians (Figure 1a) and Portuguese (Figure 1b).

The Brazilian sample ($n=200$) contained 22 different UEP–αh haplogroups while the Portuguese ($n=93$) displayed 14. Assuming an infinite allele model, the Ewen's θ estimator for the Portuguese population is 4.35. Under the hypothesis that Portuguese Y-chromosome population is the

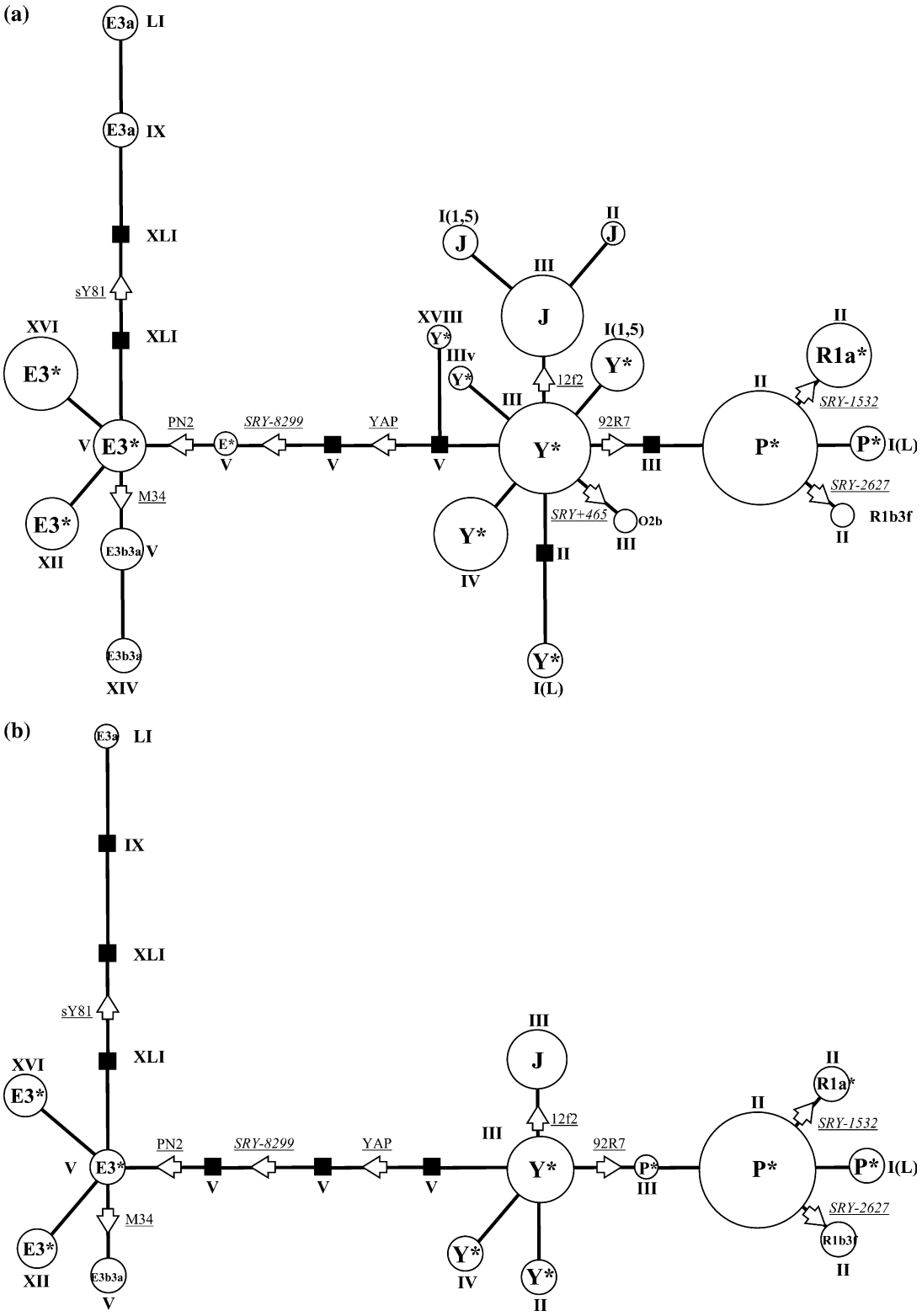
Table 1. Number of Y chromosomes defined by both UEPs and the αh types observed in 'white' Brazilians and in Portuguese

Haplogroups YCC				Brazil				
a1	a2	αh types	*N*	NE	S	SE	Total	Portugal *N*
1	P*	II	25	31	22	28	106	56
		I(L)	1	1	0	0	2	2
		III	0	0	0	0	0	1
3	R1a*	II	1	1	5	0	7	2
		I(1,5)	1	1	0	0	2	0
9	J	III	7	0	1	5	13	6
		II	0	0	1	0	1	0
22	R1b3f	II	0	0	0	1	1	2
		IX	0	1	0	1	2	0
8	E3a	LI	0	1	0	1	2	0
		XIV	0	0	0	0	0	1
AF	E*	V	1	0	0	0	1	0
		XVI	4	1	2	3	10	4
PN2	E3*	XII	0	0	2	3	5	3
		V	1	3	1	0	5	2
M34	E3b3a	V	1	0	2	1	4	2
		XIV	0	0	1	1	2	0
		III	4	5	6	2	17	8
		IIIv	0	1	0	0	1	0
		IV	1	3	3	3	10	2
2	Y*	I(1,5)	0	0	4	1	5	0
		XVIII	0	0	1	0	1	0
		II	0	0	0	0	0	2
		I(L)	1	0	1	0	2	0
20	O2b	III	1	0	0	0	1	0
Total			49	49	52	50	200	93
Nei's	UEP[b](95% CI)			0.67 (0.53–0.79)	0.54 (0.37–0.68)	0.72 (0.62–0.79)	0.65 (0.50–0.77)	0.66 (0.59–0.72)	0.57 (0.45–0.68)
diversity	αh (95% CI)			0.65 (0.51–0.75)	0.56 (0.38–0.72)	0.68 (0.52–0.80)	0.64 (0.46–0.77)	0.64 (0.56–0.71)	0.53 (0.41–0.64)
	UEP + αh (95% CI)			0.71 (0.55–0.83)	0.58 (0.40–0.73)	0.79 (0.66–0.87)	0.67 (0.50–0.80)	0.70 (0.63–0.78)	0.62 (0.49–0.74)

[a1] Haplogroup nomenclature according to Carvalho-Silva et al. (2001); [a2] Haplogroup nomenclature according to YCC (2002) and Jobling & Tyler-Smith (2003). [b] Values calculated from data published elsewhere (Carvalho-Silva et al., 2001).

ancestral of the Brazilian one, the expectation for the number of alleles (i.e. UEP–αh haplogroups) is 22, (11–24; 95% CI) for $n = 200$ and 11.4 (7–17; 95%CI) for $n = 50$. As shown in Table 1, all the observed values of number of alleles in Brazilian samples (considered each region separately or all regions grouped) fall within the 95% confidence intervals. Therefore, despite the recurrent migration of males from different continents and diverse European countries to Brazil during the last centuries, we were no able to detect a statistically significant increment in the number of UEP–αh alleles in the Brazilian samples from those populations.

We next partitioned the Brazilian Y chromosome diversity into within and among population components using AMOVA (Excoffier, Smouse & Quattro, 1992) and by comparing with the


(a)
E3a LI
E3a IX
XLI
sY81
XLI
XVI
E3*
V
E3*
PN2
E*
V
SRY-8299
V
YAP
V
XVIII
Y*
IIIv
Y*
III
12f2
I(1,5)
J
II
J
III
J
I(1,5)
Y*
92R7
III
Y*
II
R1a*
SRY-1532
II
P*
P*
I(L)
SRY-2627
R1b3f
II
SRY+465
O2b
III
M34
E3*
XII
E3b3a
V
Y*
IV
E3b3g
XIV
Y*
I(L)
(b)
E3a LI
IX
XLI
sY81
XLI
XVI
E3*
V
E3*
PN2
V
SRY-8299
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III
J
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Y*
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II
R1a*
SRY-1532
II
P*
P*
I(L)
SRY-2627
R1b3f
II
M34
E3*
XII
E3b3a
V
Y*
IV
Y*
II

Figure 1. Y chromosome networks in Brazilians (a) and Portuguese (b). Sampled Y chromosomes are indicated by circles (with area proportional to the absolute frequency), whereas those not observed in our sample, but essential to draw the network are indicated by black squares. Arabic numerals (in combination with letters, e.g. E3*) refer to the haplogroups constructed with biallelic markers (Carvalho-Silva et al. 2001), whereas Roman numerals refer to αh types. Each link corresponds to one mutational event and the mutations referring to biallelic markers (names above arrows) are indicated by arrows pointing to the derived state. Please note that haplogroup P* means P* (xR1a*, R1b3f, Q3).

Portuguese data we observed that virtually all variation was found to be concentrated at the within population level (between population component of variance, $F_{ST} = 0$; $p > 0.05$, and between-groups component, $F_{CT} = 0$, $p > 0.05$). By performing the exact differentiation test, no significant statistical difference was observed between Brazil and Portugal ($p = 0.70$). When we took into account the subdivision of Brazil in four subpopulations and compared them to Portugal we observed significant statistical differences between the South and the following populations: Southeast Brazilians ($p = 0.02$), Northern Brazilians ($p = 0.03$) and Portuguese ($p = 0.04$). However, after applying the Bonferroni correction for multiple comparisons these differences did not remain statistically significant.

Typing with the Y microsatellite loci

The scoring of six microsatellites (*DYS19*, *DYS389*II, *DYS389*I, *DYS390*, *DYS391* and *DYS393*) defined 155 Y-μsat haplotypes (overall haplotypic diversity of 97.3%) in 293 individuals from Brazil and Portugal (see supplementary data in http://publicacoes.gene.com.br/scient/Y-Genetica.xls). Out of 155 Y-μsat, only 47 (30.3%) were observed more than once. Haplotype 14–16–10–24–11–13 was the most frequently seen in both Brazil (10.5%) and Portugal (16%).

The Y-μsat haplotypic variance between Brazil and Portugal and among the four Brazilian regions and Portugal were assessed both at the within and among population levels. Table 2 summarizes the statistics used to compare the within population variability indices for our populations and shows that Brazil as a whole has significantly more within population diversity than Portugal, measured by Nei's diversity index and by the variance in the number of repeats averaged among the six microsatellite loci (Kruskal–Wallis ANOVA, one-tail test, $H = 10.45$, $p = 0.0334$). When we divided Brazil in four distinct populations, defined according to the geographical locations, we could specifically detect the presence of higher diversity in South Brazil. By applying the AMOVA and the exact population differentiation tests to assess a geographic structure of the genetic variability, we observed once more that no variation was concentrated at the among population level (F_{ST} and $F_{CT} = 0$, $p > 0.05$). Therefore, the analysis of Y chromosome microsatellite haplotypes allowed us to detect in the Southern region of the country a significantly higher level of variability in comparison with the Portuguese population.

Discussion

Three major geographical groups have contributed to the gene pool of the Brazilian population since the arrival of Portuguese to Brazil in 1500. The Amerindians (~2.4 million in Brazil by the XVI century), the Europeans (~6 million among Portuguese, Italians, Spanish and Germans until the XX century) and approximately 4 million African slaves arrived in Brazil between 1550 and 1850 (IBGE, 2000). We have determined that the genetic contribution of Amerindians, Europeans or Africans to the current gene pool of Brazilians has been mediated differentially by males and females (Alves-Silva et al., 2000; Carvalho-Silva et al., 2001). Our previous analysis on Y UEPs showed that the geographical origin of the vast majority of the Y chromosomes in 'white' Brazilians are from Europe, very likely from Portugal, since no significant differences on the Y haplogroup frequencies between Brazilians and Portuguese were detected (Carvalho-Silva et al., 2001). Moreover, when we partitioned Brazil into four geographical subpopulations, the Y chromosome UEP data did not to reveal any difference among them, in contrast with the mtDNA data that showed that the amount of Amerindian, European and African

Table 2. Summary statistics for Y chromosomes haplotypes

Population	Sample size	Locus diversity[a] (CI)[b]	Variance ratio[c]	Number of haplotypes	Haplotypic Diversity	Mean number of pairwise differences
Brazil	200	0.57 (0.54–0.60)	1.13 [d]	119	0.98	3.43
N	49	0.56 (0.48–0.61)	0.97	40	0.99	3.38
NE	49	0.54 (0.46–0.61)	0.98	38	0.98	3.29
S	52	0.60 (0.54–0.65)	1.20	41	0.98	3.66
SE	50	0.56 (0.48–0.62)	1.03	38	0.98	3.41
Portugal	93	0.49 (0.44–0.53)	0.82 [e]	57	0.97	3.08

[a] According to Nei (1987) and averaged across all six Y-microsatellite loci; [b] The 95% confidence interval was obtained using GENETIX version 4.05 (Belkhir et al., 2004); [c] According to Jorde et al. (1997); [d] All Brazilian regions merged as a whole sample; [e] By considering Brazil as a whole, the variance ratio to Portugal is 0.87 and not 0.82.

ancestries varies, displaying distinct patterns in the diverse regions (Alves-Silva et al., 2000). This similarity at the Y-UEP variation level between Brazil and Portugal might indicate that non-Portuguese immigrants that arrived in Brazil after the 19th century did not change significantly the genetic structure of the previous Y-chromosome population. Alternatively, these non-Portuguese populations could be similar to the Portuguese at the Y chromosome level. In this regard it is worthwhile to mention that the Portuguese themselves have a fair amount of genetic admixture from other regions of Europe and from North Africa, since along the centuries Portugal was sequentially invaded by Celts, Phoenicians, Romans, Northern-European tribes – Vandals, Suevos (from the modern area of Berlin in Germany) and later Visigoths – and North Africans (especially Berbers), and also received considerable immigration of Jews and Gypsies. Finally, we did not find significant differences at the Y-chromosome level between Brazil and Portugal simply because we have studied slow evolving markers instead of faster evolving ones which in turn show higher mutation rates and therefore could disentangle a different picture when analyzed and compared in the same samples previously typed at UEPs.

There are two characteristics of Y-chromosome variability that render it a particularly suitable tool to study sex-specific admixture in human populations: (1) Because Y-chromosome markers have shown the highest level of structure among human populations (Seielstad, Minch & Cavalli-Sforza, 1998; Oota et al., 2001; YCC, 2002), it is relatively easy to assign Y-chromosomes to the correct ancestral population(s) and to estimate its specific contribution(s); (2) Several types of genetic markers that evolve at different rates have been discovered on the Y-chromosome (Santos & Tyler-Smith, 1996; de Knijff, 2000; YCC, 2002), allowing study of evolutionary phenomena that occur at different tempos.

In order to confirm our previous results of no differentiation between Brazil and Portugal and within Brazil (our null hypothesis) and to partially discriminate between the possibilities exposed above, we studied different classes of Y polymorphic markers with higher mutation rates than the UEPs, namely the αh polymorphic system and microsatellites. As indicated in Figure 1, the inclusion of the αh system allowed a finer resolution of the Y-chromosome phylogeny, providing useful phylogeographic information about the likely origin of Brazilian Y-chromosomes. The simplest αh types (II, III and IV) were the most frequent ones in both Brazilians and Portuguese and observed in different UEP haplogroup backgrounds, indicating their known recurrent nature. The αh II and αh III chromosomes were observed in five [P*(xR1a*, R1b3f, Q3), Y*, R1a*, J, R1b3] and four [P*(xR1a*, R1b3f, Q3), Y*, J, O2b] different UEP haplogroups respectively (Table 1). On the other hand, the more complex unique event-origin alphoid types IX, XII, XVI and LI, were, as expected, restricted to a single UEP haplogroups: E3a (αh IX and LI) and E3* (αh types XVI and XII). This means that probably the identification of complex αh types could preclude typing of UEPs.

In both Brazilians and the Portuguese, UEP haplogroup E3* could be divided into three

phylogeographically relevant UEP–αh haplogroups through the typing of the αh system: E3*-V, E3*-XII and E3*-XVI (Table 1; Figure 1). Their origins can be traced back by comparison with some of our previous population surveys (F.R. Santos & C. Tyler-Smith, unpublished data). Haplogroup E3*-αhXVI is typical of North Africans and Arabs and its presence in both Brazilians and Portuguese indicates gene flow with those populations, as is historically well-known. On the other hand, haplotype E3*-αhXII is characteristic of Eastern European populations, having been seen in 14% of Germans, 15% of Hungarians, and 23% of Slovakian gypsies (F.R. Santos & C. Tyler-Smith, unpublished data). One third of the Portuguese E3* chromosomes in our sample belonged to αh type XII, and thus seems to indicate an Eastern European origin. Gitanos, one of the three main tribal groups of the gypsies, have inhabited the Iberian peninsula for centuries, and the presence of the UEP–αh haplogroup E3*-XII very likely is originated from them. In Brazil, the UEP–αh haplogroup E3*-XII was seen in the South and Southeast and its immediate origin could be Portuguese, German or even East European. It is worth noting that no instance of αhXII was observed in the North and Northeast Brazil. The UEP–αh haplogroup E3*-V was also seen in both Portugal and Brazil, but it has a less well defined geographical origin, although it has been detected in most of Jews bearing the YAP marker (F.R. Santos & C. Tyler-Smith, unpublished data).

The typing of the αh system unraveled new and interesting phylogeographic information for the Y chromosomes currently observed in Brazil and Portugal but did not allow us to reject our null hypotheses of no genetic differentiation among the four geographical regions of Brazil, or between Brazil and Portugal. Nevertheless, the UEP–αh compound haplotypes showed a tendency for higher intrapopulation diversity in the Brazilian regions (especially for the South region) in comparison to Portugal although these differences did not remain statistically significant after applying the Bonferroni correction for multiple comparisons.

Similarly to what has been observed with the Y-UEPs (Carvalho-Silva et al., 2001) and with the αh system (present work), the typing of six Y microsatellites revealed no among population diversity but different from the previous slower evolving markers the microsatellites did allow to discriminate higher intrapopulation diversity in all Brazilian regions (when compared to Portugal), being the South of Brazil the most diverse one. Therefore, we have experimental evidence that historically documented immigrations of Europeans from different origins to the South of Brazil have significantly increased the genetic diversity of the Y-chromosome population of 'white' Southern Brazilians. On the other hand, there is no compelling evidence for that in the rest of the country. Although the Northern, Northeastern and Southeastern samples show higher mean values of genetic diversity when compared with the Portuguese sample, the differences are not statistically significant.

In respect to the lack of Y-chromosome genetic differentiation among Brazilian geographical regions, there could be some speculations in order to tentatively explain this scenario. First, all Brazilian regions could have been subjected to about the same immigration waves of males from Europe, Africa and Asia since its discovery in 1500. Historical records however, show that there were different numbers of male immigrants (predominantly Europeans in the South and Africans in the Northeast; and many East Asians in both South and Southeast) but subsequent internal migration of males in Brazil could have caused the homogenization that we are observing now, i.e. no significant differences among regions in the frequency of Y chromosomes, not matter which marker we survey. However, we are dealing with a subset of Brazilians ('whites') which have been already shown to be mostly from European origin from the Y chromosome point of view (Carvalho-Silva et al., 2001). Thus, the homogenization observed among populations must be explained mainly by Y chromosomes derived from Europeans as well as the intra-population diversity differences. The Brazilian populations show higher UEP–αh and Y-μsat diversities than Portugal (excepting NE region for the UEP–αh comparison), much likely due to the multiple European sources of male immigrants to distinct regions of Brazil. Particularly, the South region received a very diverse import of European immigrants (Italians, Germans, Polish, Eastern Europeans) and shows slightly but significantly higher within population variability when compared to Portugal. A recent study (Callegari-Jacques et al., 2003), using several autosomal microsatellites, has

shown a north–south trend of increasing European heritage which supports our observation.

Our results corroborate the idea that to reach further informativeness at the phylogeographic level is better to use a combination of slow and fast evolving markers. Population genetic structure is a mosaic of molecular footprints left by the evolutionary fate of polymorphisms that mutate at distinct paces. To investigate recent population history is needed detailed information that can only be rescued by the combined use of these markers, if not, precious information can pass undetected.

Acknowledgements

We specially thank to the donors of samples, which allowed this study to be carried out. This research was supported by Grants from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–Brazil), and FCT of Portugal (POCTI/42510/ANT/2001). FRS, SDJP and DRC-S were supported by a fellowship from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico–Brazil).

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