The 8p23 Inversion Polymorphism Determines Local Recombination Heterogeneity across Human Populations

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Accepted: March 14, 2014

Abstract

For decades, chromosomal inversions have been regarded as fascinating evolutionary elements as they are expected to suppress recombination between chromosomes with opposite orientations, leading to the accumulation of genetic differences between the two configurations over time. Here, making use of publicly available population genotype data for the largest polymorphic inversion in the human genome (8p23-*inv*), we assessed whether this inhibitory effect of inversion rearrangements led to significant differences in the recombination landscape of two homologous DNA segments, with opposite orientation. Our analysis revealed that the accumulation of genetic differentiation is positively correlated with the variation in recombination profiles. The observed recombination dissimilarity between inversion types is consistent across all populations analyzed and surpasses the effects of geographic structure, suggesting that both structures (orientations) have been evolving independently over an extended period of time, despite being subjected to the very same demographic history. Aside this mainly independent evolution, we also identified a short segment (350 kb, $<$10% of the whole inversion) in the central region of the inversion where the genetic divergence between the two structural haplotypes is diminished. Although it is difficult to demonstrate it, this could be due to gene flow (possibly via double-crossing over events), which is consistent with the higher recombination rates surrounding this segment. This study demonstrates for the first time that chromosomal inversions influence the recombination landscape at a fine-scale and highlights the role of these rearrangements as drivers of genome evolution.

Key words: chromosomal inversion, recombination, population differentiation.

Introduction

Genetic recombination is one of the key evolutionary processes affecting variation throughout the genome. This process, generally mediated by homology, involves the exchange of genetic information between two homologous chromosomes (or between different albeit homologous regions of the same chromosome) (Faria and Navarro 2010), potentially disrupting the relationship between alleles at those loci and ensuring new allelic combinations.

Traditionally, recombination has been estimated using pedigree-based or sperm-typing methods, by directly counting the products of meiosis (Hubert et al. 1994; Brown et al. 1998). Given that such techniques are impracticable at a population level (Clark et al. 2010), recent years have witnessed the rise and improvement of statistical inferential approaches to indirectly detect recombination events at a genome wide scale, from population genetic data (Li and Stephens 2003; McVean et al. 2004; Auton and McVean 2007). In general, these methods rely on the assumption that linkage disequilibrium (LD; i.e., nonrandom association of alleles) is significantly reduced in regions that are exposed to recombination (Clark et al. 2010). Studies applying these alternative methods (Fearnhead et al. 2004; Li et al. 2006) validated the empirical evidence from the late 1990s (Lichten and Goldman 1995; Purandare and Patel 1997) that suggested an uneven distribution of recombination

along the genome. In other words, recombination appears to be clustered in specific genomic regions, now known as recombination hotspots. Such finding encouraged the emergence of fine-scale comparative analysis at multiple levels (Jensen-Seaman et al. 2004; Serre et al. 2005; Cheung et al. 2007) and it has become increasingly clear that, even though the global recombination landscape is largely conserved among humans, local recombination patterns are significantly heterogeneous between different present-day populations (Serre et al. 2005; Cheung et al. 2007; Keinan and Reich 2010; Fledel-Alon et al. 2011; Laayouni et al. 2011).

Interestingly, a particular type of chromosomal rearrangement—inversions—became subject of intense research in the last few years due to their negative effects on recombination (Hoffmann and Rieseberg 2008; Kirkpatrick 2010; Alves et al. 2012). Inversions are known to suppress recombination between differently oriented chromosomal segments, and it has been suggested that such rearrangements may play an important role shaping species divergence and evolution (Kirkpatrick and Barton 2006; Ayala et al. 2010). At present more than 1,000 inversions have been identified and validated in the human genome (Iafrate et al. 2004), but only a few have been studied at a population scale (Stefansson et al. 2005; Antonacci et al. 2009; Donnelly et al. 2010; Salm et al. 2012; Steinberg et al. 2012). In the following study, we focus on the human 8p23 region. This region harbors the largest polymorphic inversion known in the human genome (Antonacci et al. 2009)—a 4-Mb-long paracentric inversion that shows a strong clinal distribution in human populations, with frequencies varying between 80% (in Africa), 50% (in Europe), and 20% (in Asia) (Salm et al. 2012). Even though the 8p23 region harbors several candidate loci for natural selection (Pickrell et al. 2009), and genes related to autoimmune disorders (Deng and Tsao 2010), a model of neutral evolution shaped mostly by demographic factors has been suggested to explain its current distribution (Salm et al. 2012).

Here, we used this inversion polymorphism to study the evolution of recombination in a novel way. Taking advantage of the fact that this inversion is frequent in several human populations, our first aim is to quantify the distribution of recombination along the 4-Mb genomic segment and to determine whether the recombination landscape has evolved differently in the two chromosomal orientations. Although recombination is expected to be suppressed (or extremely rare) between heterokaryotypes (i.e., individuals heterozygous for the orientation), chromosomes with the same orientation should still be able to recombine freely across the region (Conrad and Hurles 2007). Indeed, chromosomal segments with opposing orientations may be seen as two different "subpopulations" subjected to the same demographic history while independently accumulating mutations and recombination events, leading to increasing divergence over time. By comparing the recombination patterns of inverted and noninverted chromosomes, we thus expect to gain insight on the evolution of recombination following a drastic chromosome rearrangement.

Materials and Methods

Genotype Data, Inference of Inversion Status, and Population Sets

Genotype data were obtained from the Stanford Human Genome Diversity Project (HGDP) website (http://www.hagsc.org/hgdp/, last accessed April 8, 2014) and subsequently stored as a single raw file using the Plink software (Purcell et al. 2007). Individuals were grouped according to continental origin, as in Salm et al. (2012), and four distinct groups were thus defined, Sub-Saharan Africa, Europe, Middle East, and Central South Asia. Altogether 1,447 single nucleotide polymorphisms (SNPs) were identified for the whole data set (685 individuals) with an average spacing of 3.1 kb. Note that the geographical groupings above are only used as practical units devised to achieve sufficient sample sizes. The PFIDO (Phase-Free Inversion Detection Operator) R package (Salm et al. 2012) was then used to infer the orientation of the 8p23 region. This package uses a database of genotypes for which the inversion profile is known and a statistical approach to then assign new multilocus genotypes to one of the three possible inversion statuses (i.e., two different homokaryotypes and one heterokaryotype). This step was independently applied to the four metapopulation groups as, in each region, different SNPs may be statistically associated with the inversion status. Moreover, as no single SNP can be used as proxy of the inversion status (i.e., no inversion marker has yet been identified), PFIDO was applied following the package recommendations on the entire SNP set.

Due to the low coverage of the HGDP SNP panel, we were unable to accurately predict the 8p23 orientation in the Sub-Saharan individuals with PFIDO. Given that the International HapMap Project (International HapMap Consortium 2003) comprises a larger density marker panel (>4,000 SNPs encompassing the region), we retrieved the available genotype data for the YRI population (Yoruba in Ibadan, Nigeria) from the project Phase II (release 23) and applied the same procedure as above. We were thus able to infer the 8p23 orientation for all YRI individuals and used them as our African group for the remaining of the study. To minimize any bias related to the source of the data for the African sample, we additionally obtained genotype information and inferred the 8p23 orientation in individuals from the Hapmap CEU population (Utah residents with Northern and Western European ancestry from the CEPH collection). These samples were merged to the HGDP European set once we confirmed that no bias could be identified (see below).

Table 1
Number of Individuals and Corresponding Inversion Status by Geographical Origin

Population	Data Source	Structural Orientation	
		Inverted	Standard
Yoruba in Ibada, Nigeria	Hapmap	43	16
Africa		43	16
French, France	HGDP	7	6
Sardinian, Italy	HGDP	8	6
Orcadian, GB	HGDP	8	2
Russian, Russia	HGDP	6	6
Italian, Italy	HGDP	7	2
Basque, Spain	HGDP	14	3
Adygei, Russia	HGDP	4	2
CEPH	Hapmap	17	8
Europe		71	35
Brahui, Pakistan	HGDP	2	7
Balochi, Pakistan	HGDP	2	5
Hazara, Pakistan	HGDP	1	8
Makrani, Pakistan	HGDP	1	7
Sindhi, Pakistan	HGDP	2	9
Pathan, Pakistan	HGDP	5	9
Kalash, Pakistan	HGDP	6	5
Burusho, Pakistan	HGDP	1	13
Uygur, China	HGDP	1	5
Central South ASIA		21	68
Druze, Israel	HGDP	12	11
Bedouin, Israel	HGDP	12	7
Palestinian, Israel	HGDP	10	11
Mozabite, Algeria	HGDP	17	1
Middle East		51	30
Total		186	149

Once the orientation was determined for the different groups, each was again split according to the inversion status. As our working set was mostly composed of unphased genotype data, heterokaryotypes were excluded from the analysis to avoid inaccurate recombination rate estimates. A list of the number of individuals used in this study is shown in table 1. Also, only SNPs identified within the HGDP data were considered for subsequent analysis, thus minimizing missing data. Finally, a principal component analysis (PCA) was conducted prior to the estimation of recombination rates to examine the consistency of the data. As figure 1 shows, all individuals clustered according to the inversion status and continental origin regardless of the data set used.

Recombination Rate Estimation

Estimates of recombination rate were obtained using the *rhomap* program distributed within the LDHat package (v2.2) (Auton and McVean 2007). LDHat uses a composite-likelihood scheme, where population-scaled recombination rates are estimated between each pair of consecutive SNPs.

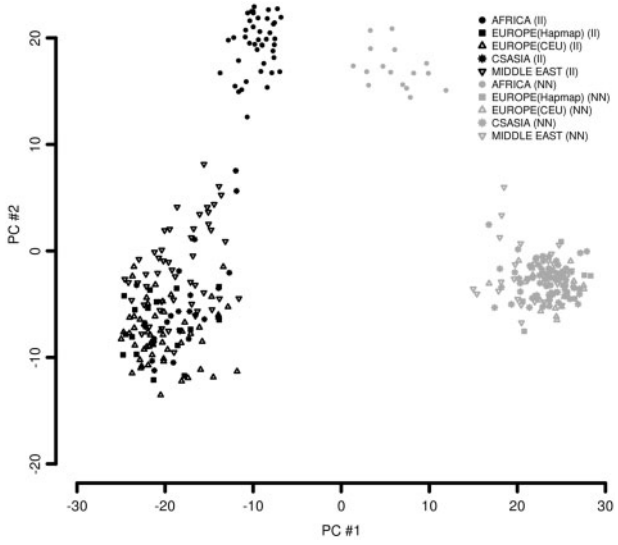

Fig. 1.—Global genetic stratification at the 8p23 region. PCA performed on HGDP ($n = 251$) and HapMap ($n = 84$) population genotype data. A total of 1,447 SNPs were used. Each dot corresponds to one individual, with distinct symbols representing geographical- and orientation-specific groups. The first principal component (i.e., horizontal axis) illustrates the strong genetic differentiation between the two main haplotypes (Inverted/Standard).

Independent runs of *rhomap* were carried out for all geographical- and orientation-specific groups for a total of 10,000,000 iterations with a burn-in of 100,000 iterations. Samples were taken every 5,000 iterations after the burn-in, with block and hotspot penalties set to zero. Given that 1) LDHat ignores nonpolymorphic positions, and 2) the HGDP panel is composed of SNPs that are not globally segregating as polymorphisms (i.e., some SNPs are monomorphic in certain populations), the comparison of the results for different populations requires that intervals be defined which will then be comparable. To do this, we adopted a similar approach to McVean et al. (2004) and the local recombination rates were "averaged" by summing all estimated values over nonoverlapping segments of 20 kb. This approach has the advantage of allowing a direct comparison of recombination estimates while maintaining a good resolution of the recombination landscape.

Recombination Dissimilarity and Genetic Differentiation between and within the Inversion Haplotypes

To determine whether the different structural haplotypes had similar recombination profiles, we compared the sets of rho values estimated in the previous section. Spearman rank correlation coefficients were obtained between each pair of structural haplotype (Inverted vs. Standard) within each geographical group using a 500-kb sliding-window approach (in

other words: 25 rho values were used to compute one correlation coefficient). These coefficients were then transformed into dissimilarity values by subtracting them from 1, as in Laayouni et al. (2011). In parallel, the same 500-kb blocks were used to estimate the differentiation between the two structural orientations. F_{st} values were computed using the Hierfstat R package (Goudet 2005). Finally, the obtained dissimilarity measures were compared with the corresponding F_{st} estimates. All statistical analyses were performed using the R software.

To further evaluate the variation in the distribution of recombination within the 8p23 region, we applied the same method and performed pairwise comparisons between geographical groups within each structural haplotype.

Results

Recombination Patterns along the 8p23 Region

Population-scaled recombination rates (4 Ner/kb) were inferred for a total of 335 individuals from eight distinct groups (according to inversion status and geographical origin) using the 1,447 SNPs identified. The cumulative plot of the proportion of recombination occurring in a given fraction of the sequence (fig. 2*A*) shows that there is an uneven distribution of recombination across the interval. Although such distribution is expected based on previous genome-wide recombination maps (McVean et al. 2004; Clark et al. 2010), it is interesting to note differences between the populations analyzed (see below).

Figure 2*B* shows the recombination profile of each group for the 8p23 region. Although there is a good overall agreement in the large-scale patterns of recombination (i.e., the strongest peaks are shared across all analyzed groups), significant differences in local recombination estimates are also observable, suggesting that we have sufficient power to detect fine-scale variation inside the region. Table 2 shows the mean recombination rate across all SNPs for each group. Significant differences in recombination rates between the groups were confirmed by a repeated measures analysis of variance (ANOVA) test ($P < 0.00001$). Interestingly, it appears that a significant part and perhaps most of the variation in the recombination landscape is associated with the chromosomal rearrangement. Indeed, a much stronger concordance can be observed between the profiles of individuals sharing the same chromosomal configuration (i.e., orientation) than between individuals sharing the same continental origin but having different orientations (fig. 2*B*). For instance, we can identify a peak around 9.5 Mb that is shared between all "standard individuals" but absent or much weaker in the inverted chromosomes. Another similar example can be found around 11.0 Mbp, where a relatively strong peak shared between all non-African inverted chromosomes is substantially weaker in the standard chromosomes.

Given that LD-based recombination estimates are influenced by the allele frequencies (hereafter, AFs) of the used markers, and that the ability to reliably resolve recombination events may become progressively weaker for SNPs showing low minor AFs (MAF) (Auton and McVean 2007; Laayouni et al. 2011), we next placed the estimated recombination rates for each SNP in five bins ordered according to increasing MAF. Note that each group was treated independently, as the AFs varied across populations and inversion status and, therefore, the same SNP will not necessarily fall in the same MAF bin for every group. We then performed a repeated measures ANOVA with the recombination estimates as the dependent variable and our results showed that, indeed, significantly lower recombination rates were found for SNPs with lower MAF ($P < 0.005$). However, the effect disappears once only SNPs with MAF > 0.1 are considered. A new repeated measures ANOVA excluding the local recombination estimates for SNPs with MAF < 0.1 was applied and the differences in recombination rates between the groups remained highly significant ($P < 0.00001$).

Influence of the Inversion Rearrangement on Recombination Patterns

Given that genome-wide studies (Keinan and Reich 2010; Laayouni et al. 2011) have argued that the amount of recombination variation might be positively correlated with the genetic distance found between populations, we next asked whether there was a relationship between the recombination dissimilarity and the genetic distance (as measured by F_{st}) between the structural pairs for each geographical group (fig. 3). A statistically significant positive association was found ($r^2 = 0.27$, $P < 0.0015$) indicating that the genetic divergence between the haplotypes is correlated with the observed dissimilarity in the recombination patterns across the region. Although these results were obtained from recombination rates estimated at SNPs showing MAF > 0.1, a similar, but less robust, significant positive association is also detected when the global set is used (Supplementary fig. S1, Supplementary Material online; $r^2 = 0.11$, $P < 0.05$). In addition, the association is robust to sample size change as the obtained results persisted when all geographical- and orientation-specific groups had equal sample size (Supplementary fig. S2, Supplementary Material online; $r^2 = 0.1495$, $P < 0.015$).

The same method was then applied to test whether population differences within each major haplotype could also account for some of the heterogeneity in the estimated patterns of recombination. Both sets (i.e., inverted and standard) were analyzed independently and pairwise comparisons were performed between population pairs. The results are shown in figure 4. Here, a much less clear relationship was found suggesting that perhaps the limited degree of divergence, for this genomic region, between the populations under study may be insufficient to produce clear departures between the

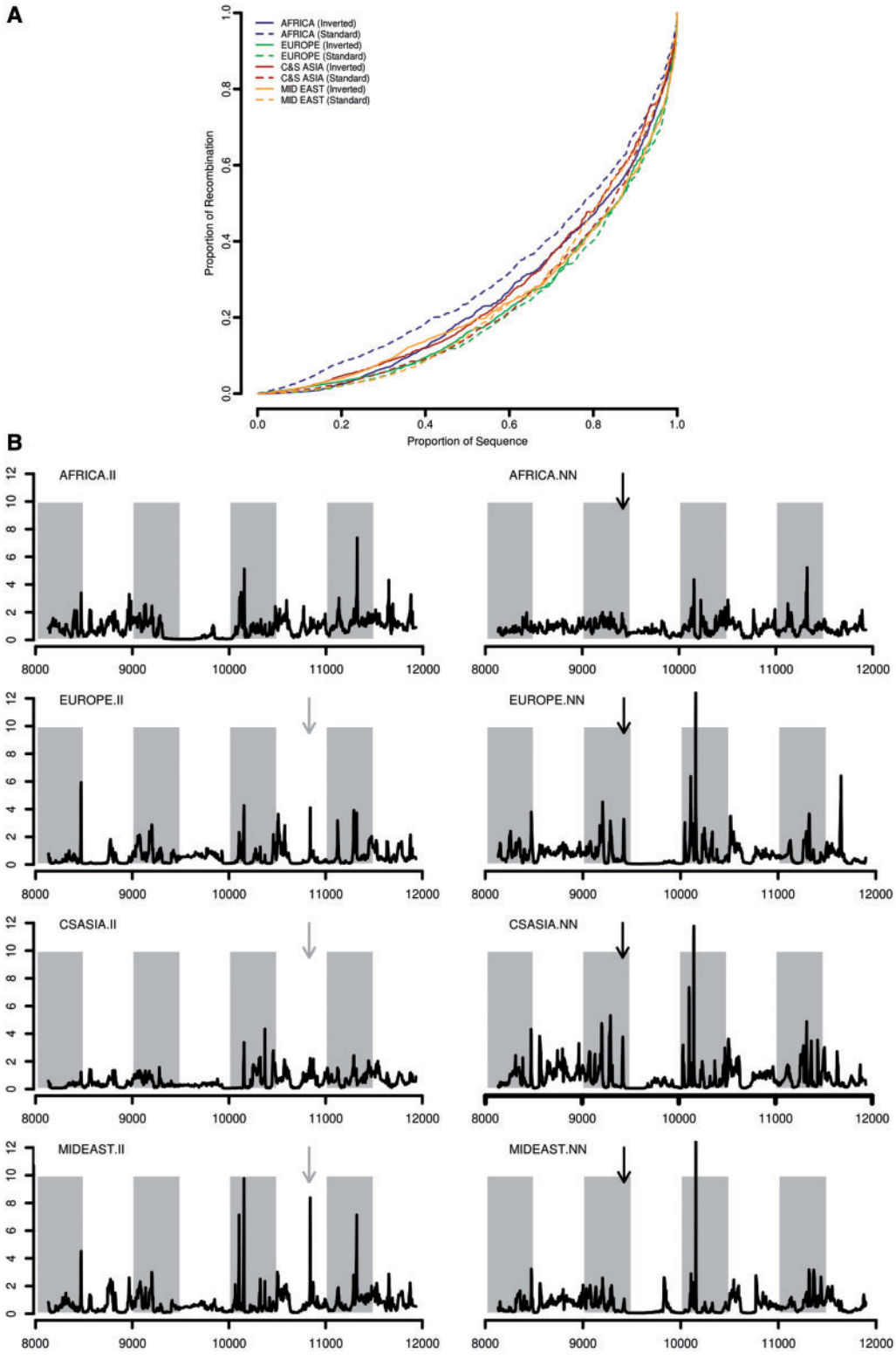

Fig. 2.—Distribution of recombination rate along the 8p23 inversion. (*A*) Uneven distribution of recombination within the 8p23 region: The cumulative plot illustrates the proportion of recombination occurring in a given portion of the sequence. Recombination estimates have been sorted in increasing order of intensity. (*B*) Recombination estimates for each (geographical and orientation specific) group obtained from *LdHat*. The left and right panels show the results for the Inverted (II) and Noninverted (NN) subtypes, respectively. Gray bins of 500 kb are displayed to ease comparisons between the different groups along the region. Drawn arrows denote recombination events that appear to be unique (or much more frequent) in one of the two structural haplotypes.

Table 2
Mean Recombination Rate across the 8p23 for the Different Groups

	Inverted	Standard
Africa	0.979	0.920
Europe	0.551	0.781
CS Asia	0.573	0.829
Mid East	0.745	0.647

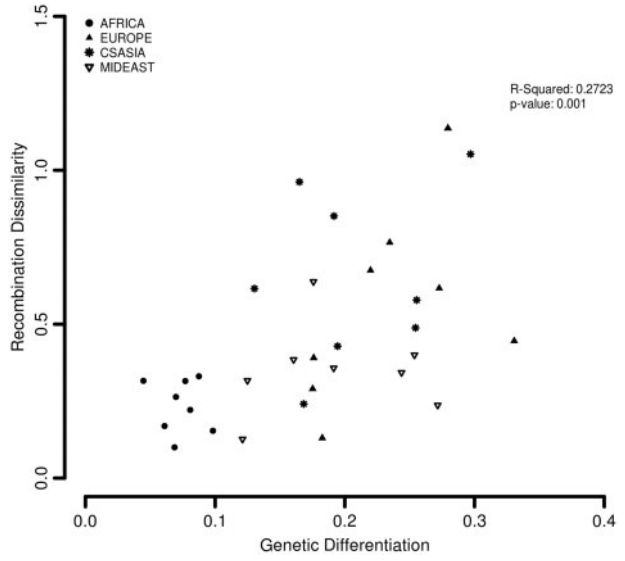

Fig. 3.—Relationship between dissimilarity in recombination patterns and genetic differentiation between inversion types. The figure illustrates the relationship between the dissimilarity observed in the patterns of recombination and the F_{st} values between the two major haplotypes for SNPs with MAF > 0.1 ($P < 0.0015$). The different symbols represent population-specific comparisons. Also, each plotted value represents the relationship observed in a genomic window of 500 kb. In total, eight nonoverlapping windows per population are shown. Comparisons between each chromosomal form were independently performed for each population.

estimated recombination patterns. Indeed, only for "Standard" chromosomes is the recombination dissimilarity positively associated with the genetic differentiation ($r^2 = 0.3246$, $P < 0.0001$) and this effect is mainly driven by the differences between African and non-African chromosomes (fig. 5).

Gene Flow within the 8p23 Region

Although theory predicts that recombination should be prohibited between inverted regions (Hoffmann and Rieseberg 2008; Kirkpatrick 2010; Faria and Navarro 2010; Alves et al. 2012), it has been proposed that limited gene flow may have occurred between the two major haplotypes at 8p23. Using inferential methods to ancestral sequence reconstruction, Salm et al. (2012) found individual genomes bearing interspersed runs of distinct ancestry (i.e., "Inverted"-ancestry and Standard-ancestry) and concluded that double-recombination events have, to some extent, homogenized the genetic diversity of the region. We, therefore, examined whether similar signals could be identified in our data. Interestingly, a 350-kb segment encompassing the center of the inversion showed significantly lower levels of diversity ($\pi = 5.2 \times 10^{-5}$) (fig. 6*A*) that overlapped with a region of deflated F_{st} ($F_{st} = 0.11$), when compared with the average diversity over the whole interval ($\pi = 12.5 \times 10^{-5}$; $F_{st} = 0.17$). Moreover, this segment is flanked by regions with signals of higher recombination activity (i.e., putative hotspots), which are shared between the two chromosomal forms. In order to explore this effect in greater detail we performed a PCA with SNPs located within the portion showing lower divergence between inverted and standard haplotypes, and for comparison, in two flanking regions (5′ and 3′). When SNPs located in the central segment of the 8p23 inversion were analyzed, no clear segregation of inverted and standard chromosomes was observed. In contrast, a much cleaner structured environment with only a slight overlap was obtained when including SNPs within each of the flanking regions (fig. 6*B*).

Discussion

Inversions have long been regarded as privileged systems to study major evolutionary processes, potentially playing a significant role in species divergence. By preventing gene flow between two different structural types, these rearrangements are thought to allow the accumulation of mutations, representing an initial step toward chromosomal differentiation that may ultimately lead to speciation (see Alves et al. [2012] for a detailed review). Here, we took advantage of the coexistence of two groups of structurally distinct chromosomes and assessed how inverted rearrangements influence the evolutionary trajectory of the affected genomic region.

In agreement with what has been described in genome-wide surveys comparing the recombination profiles of different human populations (McVean et al. 2004; Clark et al. 2010; Keinan and Reich 2010; Fledel-Alon et al. 2011; Laayouni et al. 2011), we found evidence supporting a strong correlation between recombination dissimilarities and genetic divergence. Our results indicate that the presence of the rearrangement largely contributed to the accumulation of distinct mutation and recombination events between inversion types, which resulted in extended local recombination heterogeneity within the 8p23 segment.

The genetic differences found between the two major haplotypes (i.e., Inverted and Standard) surpassed the differentiation found at the population level (i.e., geographical stratification), suggesting that both orientations have been around throughout most human evolutionary history (note the range of F_{st} values in figs. 3 and 4). Indeed, a recent

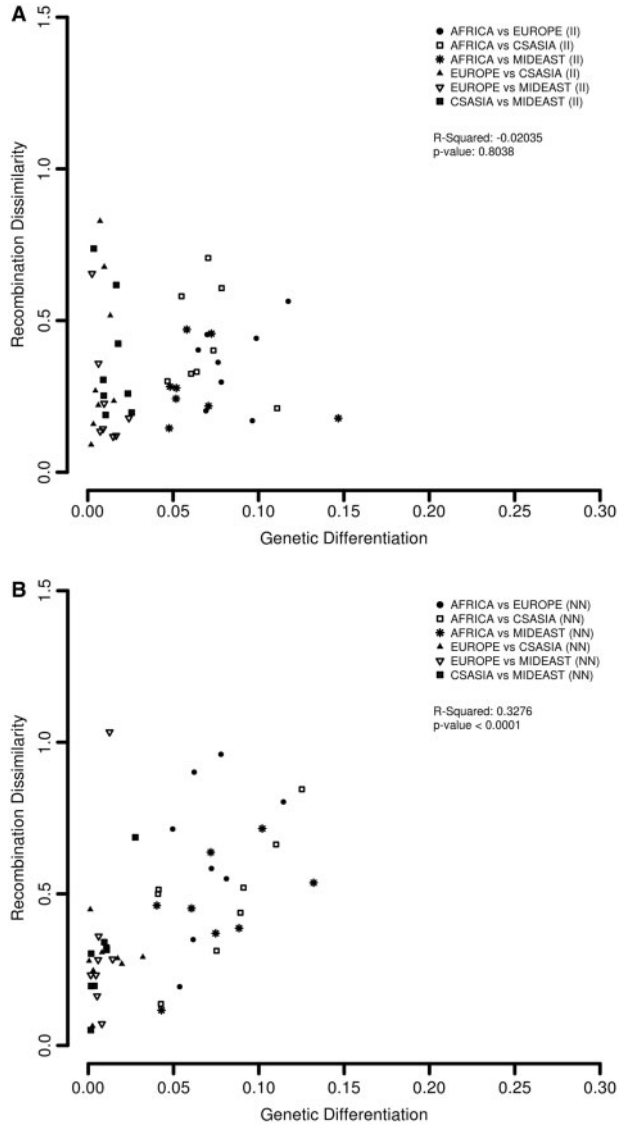

Fig. 4.—Relationship between dissimilarity in recombination patterns and genetic differentiation between geographical regions. Dissimilarity in recombination rate and F_{st} values based on six pairwise comparisons between all geographical regions within the (*A*) Inverted (II) haplotype and (*B*) Standard (NN) haplotype.

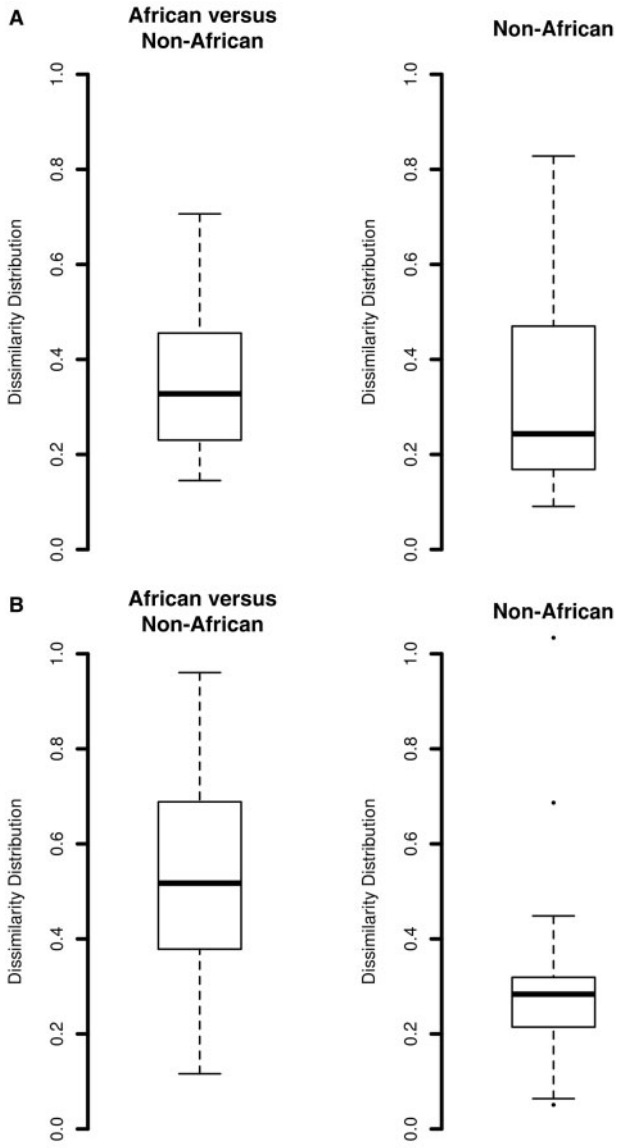

Fig. 5.—Asymmetry in recombination dissimilarity scores between African and non-African groups. Boxplots displaying the asymmetry in the distribution of recombination dissimilarities between African versus non-African groups for the (*A*) Inverted and (*B*) Standard haplotypes. For each figure, the "African versus non-African" boxplot represents the distribution of dissimilarity scores observed when comparing the African set versus all other groups, whereas the "Non-African" boxplot represents the distribution of dissimilarity scores observed between all non-African sets.

study found that the inversion may have occurred as a single event in the human lineage somewhere around 200–600 ka (Salm et al. 2012) (i.e., before modern human emergence) with the inhibition of recombination leading to the formation of two highly divergent haplotype families segregating within populations (Antonacci et al. 2009; Salm et al. 2012). The clear differentiation between the two configurations in our PCA further supports the hypothesis of a single very ancient inversion event.

It is, therefore, not unexpected that the rearrangement exerts a stronger effect on the variation of the recombination patterns than population structure. Early migrations of modern humans (i.e., Out of Africa) are believed to have started approximately 100 ka (Jobling et al. 2004) with complex spatial demographic phenomena (e.g., expansions, contractions, admixture events) being particularly responsible for much of the variation identified between present-day human populations. This variation has induced fine-scale differences

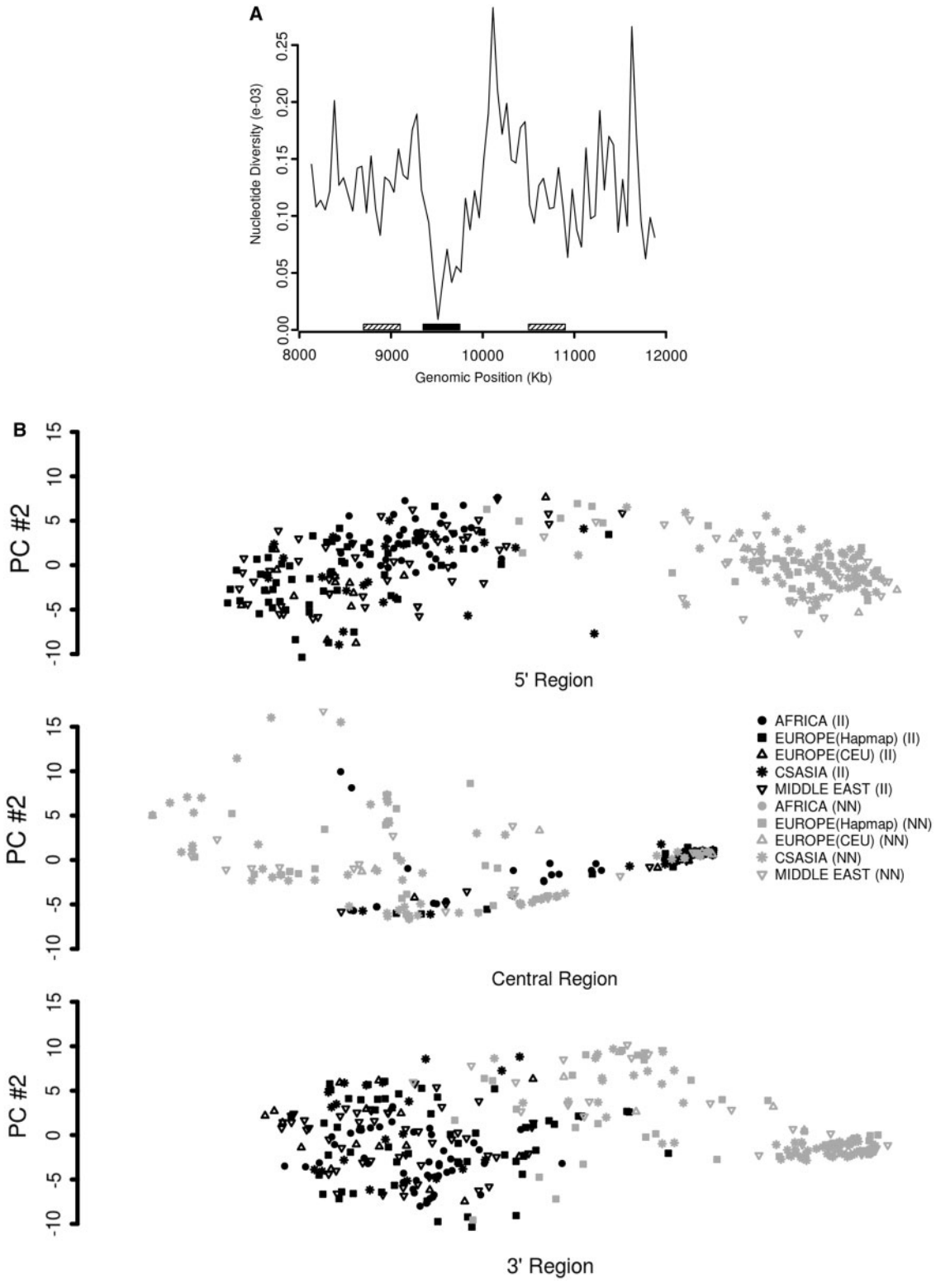

Fig. 6.—Evidence of gene flow within the 8p23 region. (*A*) Nucleotide diversity across the 8p23 region. The central filled rectangle highlights the region of reduced diversity; dashed rectangles represent the flanking regions (5′ and 3′) randomly picked for comparison (see text). (*B*) PCA on three nonoverlapping regions within the 8p23 (see left plot). The top and bottom plots represent the distribution of individual genotypes for the 5′ and 3′ regions used for comparison. The center plot shows the distribution of individual genotypes in the region of reduced diversity. Each dot represents one individual, with distinct symbols representing the geographical- and orientation-specific groups.

in recombination patterns between populations, with multiple lines of evidence now suggesting that recombination is a rapidly evolving process partially controlled by the surrounding DNA sequence (Baudat et al. 2010; Parvanov et al. 2010). Recent studies using genome wide data have focused on the recombination heterogeneity accumulated at shorter timescales (i.e., separation of human populations) (Keinan and Reich 2010; Fledel-Alon et al. 2011; Laayouni et al. 2011). Given that the inversion event predates human expansions (Salm et al. 2012), our results are not only consistent with these previous findings but they also extend the analysis into a greater time depth and therefore into a genomic region of increased evolutionary significance.

Despite the overall genetic distinctiveness of the two major haplotypes, we also identified a short region of weaker differentiation at the center of the inversion. Although this could be caused by other factors (e.g., stochasticity in the mutation process), it supports previous claims of moderate gene flow between inversion-types throughout the evolution of this genomic region (Salm et al. 2012). Indeed, genetic exchange between inverted arrangements may be possible via double cross-over events in inversion loops, with the probability of recombination increasing with physical distance from the inversion breakpoints (Navarro et al. 1997; Faria and Navarro 2010; Stevison et al. 2011). Given the size of the 8p23-*inv*, it is surely plausible that double cross-over events may have occurred within inversion heterozygotes.

As 1) our primary goal was to evaluate the recombination heterogeneity within the 8p23 segment, and 2) the SNP density was below optimal for an accurate high resolution inference (<1/kb), we intentionally avoided to characterize the precise location of recombination hotspots. Hotspots are defined as regions showing enriched recombination rate by several orders of magnitude and have been repeatedly associated with a 13-bp sequence motif that is specifically recognized by PRDM9, a rapidly evolving protein believed to be involved in speciation in mammals (Baudat et al. 2010; Parvanov et al. 2010). Although the connection between PRDM9 and recombination hotspots is not perfect (Myers et al. 2008; Berg et al. 2010), approximately 40% of hotspots in the human genome contain this motif which remains, so far, one of the very few known determinants of meiotic recombination. Interestingly, in a recent comparative study of fixed inverted differences between the genomes of humans and chimpanzees, Farré et al. (2013) have proposed that the lower recombination activity observed within inverted segments was linked to a lower density of PRDM9 binding motifs found within these regions, when compared with collinear regions on the same chromosome. In our analysis, and likely due to the short evolutionary timescale that this rearrangement represents, we have found no depletion of PRDM9 motifs within the inverted segment (54.35 motifs/Mb) when compared with collinear regions (44.42 motifs/Mb) in the entire chromosome 8. Nevertheless, it will be interesting to test with deep sequenced data whether the differences in recombination profiles between inverted and standard chromosomes may be partly explained by sequence variants within PRDM9 motifs.

In conclusion, while confirming that recombination is likely suppressed in inverted regions (i.e., recombination is almost entirely restricted to chromosomes oriented in the same direction) in global terms, our work showed that fine-scale recombination patterns are evolving differently between chromosomal forms, highlighting the role of inversions as evolutionary significant elements acting at intraspecific level. Also, we provided evidence that this effect is robust to differences in the proportion of inverted to standard chromosomes in a population as the trend was shared by several geographical regions where the two haplotypes segregate at considerably different frequencies. This work will therefore contribute to a better understanding of recombination heterogeneity at a population level and reinforce the need to extend these studies to other known inverted regions on the human genome in order to obtain a more comprehensive and meaningful human recombination map. As information on the architectural plasticity of the human genome continues to accumulate (MacDonald et al. 2013), future studies should also consider the implications of these rearrangements in genome-wide selection scans, given that the long-range LD patterns usually manifested within chromosomal inversions may generate signals that could be confounded with selection. As demonstrated here, controlling for inversion-type may help circumvent these limitations. Moreover, our work suggests a new research line devoted to the unveiling of the sequences internal to the inversions that allow for double recombination, and thus overcoming the meiotic problems associated with this rearrangement.

Supplementary Material

Supplementary figures S1 and S2 are available at *Genome Biology and Evolution* online (http://www.gbe.oxfordjournals.org/).are available.

Acknowledgments

The authors are thankful to the PCG group members at the IGC for useful discussion on data analysis. They would also like to acknowledge two anonymous reviewers for their comments and suggestions that greatly improved the manuscript. IPATIMUP is an Associate Laboratory of the Portuguese Ministry Education and Science and is partially supported by FCT, the Portuguese Foundation for Science and Technology. J.M.A. is the recipient of a doctoral fellowship from FCT (SFRH/BD/51004/2010). L.C. is partly funded by the Laboratoire d'Excellence (LABEX) entitled TULIP (ANR-10-LABX-41) and by the FCT project (PTDC/BIA-BIC/4476/2012). A.M.L. is the recipient of a postdoctoral fellowship from FCT (SFRH/BPD/73366/2010).

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Associate editor: Partha Majumder