# Recombination Gives a New Insight in the Effective Population Size and the History of the Old World Human Populations

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†Membership of the Genographic Consortium is provided in the Acknowledgments.

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Associate editor: Rasmus Nielsen

## Abstract

The information left by recombination in our genomes can be used to make inferences on our recent evolutionary history. Specifically, the number of past recombination events in a population sample is a function of its effective population size ($N_e$). We have applied a method, Identifying Recombination in Sequences (IRiS), to detect specific past recombination events in 30 Old World populations to infer their $N_e$. We have found that sub-Saharan African populations have an $N_e$ that is approximately four times greater than those of non-African populations and that outside of Africa, South Asian populations had the largest $N_e$. We also observe that the patterns of recombinational diversity of these populations correlate with distance out of Africa if that distance is measured along a path crossing South Arabia. No such correlation is found through a Sinai route, suggesting that anatomically modern humans first left Africa through the Bab-el-Mandeb strait rather than through present Egypt.

Key words: recombination, effective population size, Out of Africa.

The estimation of effective population size ($N_e$) in human evolution has been a subject of intense research in the recent past. The seminal papers by Takahata (reviewed in Kim et al. (2010)) established the highly cited figure of 10,000 individuals for the past human evolutionary history, which has been lately revised using either measures of heterozygosity (Kim et al. 2010; Laval et al. 2010) or of linkage disequilibrium (LD) (McEvoy et al. 2011; Hayes et al. 2003; Tenesa et al. 2007). Gene diversity estimates tend to give effective population sizes higher than 10,000, whereas LD-based estimates give lower numbers. Gene diversity estimates would reflect average $N_e$ for long periods of time, whereas LD depends to a greater extent on the $N_e$ in more recent times.

We have developed a method called IRiS (Identifying Recombination in Sequences) to detect specific past recombination events from extant sequences (Melé et al. 2010) based on a combinatorial algorithm (Parida et al. 2008, 2009). The algorithm yields which sequences are descendants of ancient recombination events, which sequences carry the ancestral patterns that were involved in the recombination event, and where the breakpoint is located in the genome. Here, we use IRiS to extract the number of recombinations present in diverse populations of the Old World and use this to estimate their $N_e$. Several aspects of this approach are novel: the detection of recombinations is not based on LD and the time distribution of the reconstructed recombinations is known (Melé et al. 2010). In our previous study (Melé et al. 2010), we showed that recent recombinations are detected by IRiS with greater sensitivity: 90% of the events detected by IRiS occurred after the out of Africa migration. Therefore, recombinations can be
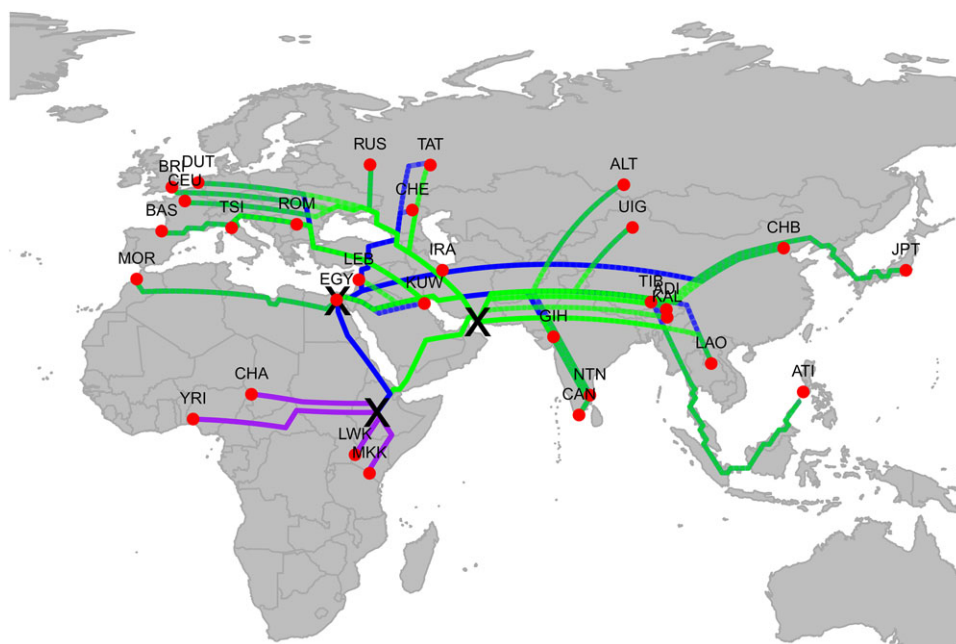
**Fig. 1.** Map of the Old World with the samples of the present study. The two tested routes out of Africa are marked: a route through the North of Egypt in blue and a South Arabian route in green. Wherever the two lines overlap, colors are added up. In purple, the putative path followed by sub-Saharan African populations. The X symbol marks a presumable origin (or a later stage) of AMH in Addis Ababa and the two locations in which AMH are forced to go through in their way out of Africa for each of the two possible routes (present Egypt or Iran).

used as recent genetic markers and they can potentially help to make inferences on the most recent events of human evolutionary history, such as the estimation of population-specific $N_e$. In fact, most of the reconstructed recombinations are population-specific (93.1%).

The dataset was taken from a large survey of 1250 SNPs or single-nucleotide polymorphisms (Javed A, Melé M, Marc Pybus, Zalloua P, Haber M, Comas D, Netea MG, Balanovsky O, Balanovska E, Jin L, Yang Y, Arunkumar G, Pitchappan R, Bertranpetit J, Calafell F, Parida L, The Genographic Consortium, unpublished data) (supplementary table S1, Supplementary Material online) belonging to five gene-free regions of the X chromosome spanning 2 Mb genotyped in 1240 males from 30 Old World populations (fig. 1, supplementary table S1, Supplementary Material online). High uniform SNP coverage was necessary to detect as many recombination events as possible and therefore a customized genotyping array was used. By choosing only male samples, we could overcome the uncertainty associated with phasing haplotypes. Finally, regions known to contain genes were avoided in order to eschew the possible confounding effects of natural selection. Further details about region selection and genotyping can be found in the supplementary text, Supplementary Material online.

We used the expression $\rho = 3N_e r$ (Hudson 1987) to infer $N_e$ which is analogous to the $\theta = 4N_e\mu$ formula (Nei 1987) for recombination and the X chromosome, where rho ($\rho$) is the population recombination parameter and $r$ is the recombination rate. In a similar way in which the number of segregating sites can be used to infer theta ($\theta$) (Nei 1987), the number of recombinations can be used to calculate rho by means of the equation $\rho = 3N_e r$ (3 is for the

X chromosome), where $\rho = \frac{R}{\sum_{i=1}^{n-1}\frac{1}{i}}$, where $R$ is the number of recombinations inferred for each population and $n$ is the number of sequences analyzed.

Extensive simulations were performed in order to assess whether the number of recombinations detected by IRiS could be used as a proxy to infer the effective population size both at populations under equilibrium and under different demographic models. In total, 14 demographic models were tested, three under equilibrium differing in their $N_e$, and 11 based on the calibrated demography published in Schaffner et al. (2005) in which simulations are performed such that they resemble empirical human data of three populations. Demographic models differed either in the $N_e$ of the different populations or else in the time of the split between populations (see supplementary fig. S1 and supplementary materials and methods, including supplementary table S3, Supplementary Material online). Results show first, that under equilibrium, estimates of $N_e$ obtained based on the number of recombinations inferred by IRiS correspond to the value of $N_e$ set in the simulations (supplementary table S2, Supplementary Material online). Second, we show that under a human-like demography in which three different populations are modeled, differences in $N_e$ between populations will be detected by IRiS as a proportional increase in the number of recombinations detected (supplementary table S3, Supplementary Material online). Third, it is shown that the time of the split between European and Asians or the time set for the out of Africa in the simulations does not affect our results. Thus, the method is robust for the proposed goals.
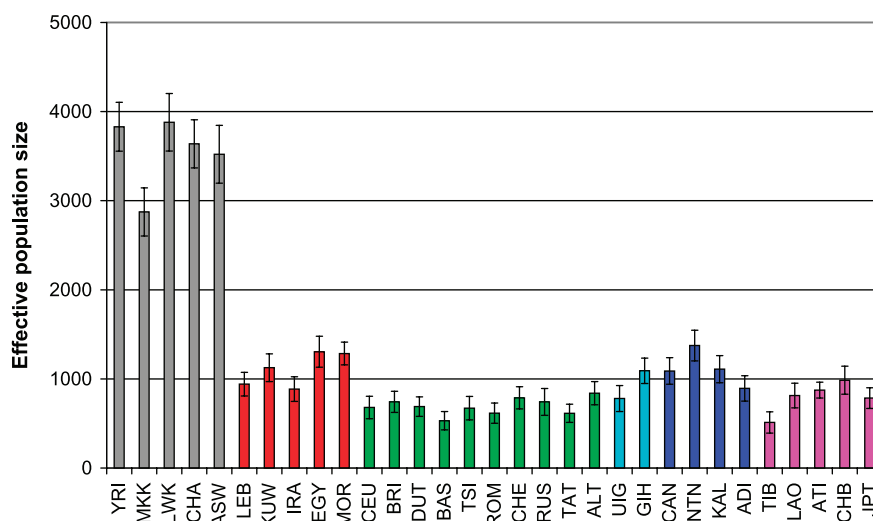
**Fig. 2.** Inferred effective population sizes from the number of recombinations detected and the corresponding sampling standard deviations calculated based on the 100 permuted datasets. Population abbreviations as in supplementary table S4, Supplementary Material online.

We then extracted the number of recombinations per population with the IRiS method run on 100 permuted datasets with 18 chromosomes per population. The average number of recombinations detected per population was multiplied by the corresponding sensitivity of the method (7.7%) assessed by simulations (see supplementary material, Supplementary Material online) at a recombination rate of $1.8 \times 10^{-8}$. Sensitivity was not affected by SNP diversity (Spearman's $r=-0.161$; $P=0.111$) and therefore, the same value could be applied for all populations.

Estimates of $N_e$ for each of the populations based on the 100 permutations and the corresponding standard deviations are given in supplementary table S4, Supplementary Material online and plotted in figure 2. As expected, results consistently show that Sub-Saharan Africans have much higher $N_e$ than all other populations; values are roughly 4-fold larger or, in absolute terms, of ~4,000 for African populations and of ~1,000 for the rest. This result is in line with the low values obtained with LD-based estimates (Tenesa et al. 2007; Laval et al. 2010) and the ~2.5 times higher African effective sizes found from genetic diversity estimates (Laval et al. 2010) and from LD (Hayes et al. 2003; Tenesa et al. 2007). On the other hand, the estimation of $N_e$ is subject to uncertainties that may carry into the inference of $N_e$ as observed in the simulation analysis, but if significant differences in $N_e$ exist between populations, they will be recovered by our method.

For the first time, we provide specific effective sizes for a wide range of Old World populations in relative and absolute values (supplementary table S4, Supplementary Material online) and a number of interesting patterns are revealed. The populations with the largest sizes other than Sub-Saharan Africans are North Africans (Moroccans and Egyptians) due to their known Sub-Saharan admixture (Krings et al. 1999; Bosch et al. 2001; Brakez et al. 2001). Outside of Africa, the largest $N_e$ is found in South Asia; only recently, the high internal diversity of Indian populations is being appreciated (Xing et al. 2010). Europeans and East

Asians have similar $N_e$. Tibetans and Basques showed the lowest values, a direct measure of small population size and isolation.

We further investigated the geographic variation of both SNPs and recombinations to understand the general pattern of genetic variation and population history. In order to compare patterns of diversity across populations, we used Nei's nucleotide diversity statistic (Nei 1987) to calculate diversity using either SNP allele frequencies (SNP diversity) or population frequencies of each recombination event (recombination diversity). We provide a geographic framework to these values by plotting them against the geographic distance of each population to Eastern Africa, the presumed place of origin of modern humans (Quintana-Murci et al. 1999; Tishkoff et al. 2009) if leaving Africa through the north of Egypt. Note that the relative differences in the distance between non sub-Saharan populations to a putative origin would not change even if an origin in more Central or Southern in Africa was considered (Liu et al. 2006; Betti et al. 2009; Henn et al. 2011) (see fig. 1). Geographic distances were calculated as in Prugnolle et al. (2005) in which the shortest landmass path between a specific point of origin and our 30 populations can be calculated based on graph theory.

As expected, SNP diversity was found to be highly correlated with geographical distance with East Africa (Spearman's $r=-0.596$; $P=0.00064$) (supplementary fig. S2, Supplementary Material online) (Prugnolle et al. 2005; Ramachandran et al. 2005; Li et al. 2008), even if sub-Saharan African samples were removed ($r=-0.441$, $P=0.024$). With recombination diversity, however, the correlation that is found is lower ($r=-0.391$; $P=0.033$) and vanishes if African samples are removed ($r=-0.077$; $P=0.71$) (fig. 3A). African populations show significantly higher recombination diversity than any other population (Mann–Whitney U test; $P=0.0015$), in a proportion that goes to a 4- or 5-fold higher diversity than the mean for non-Africans; European populations
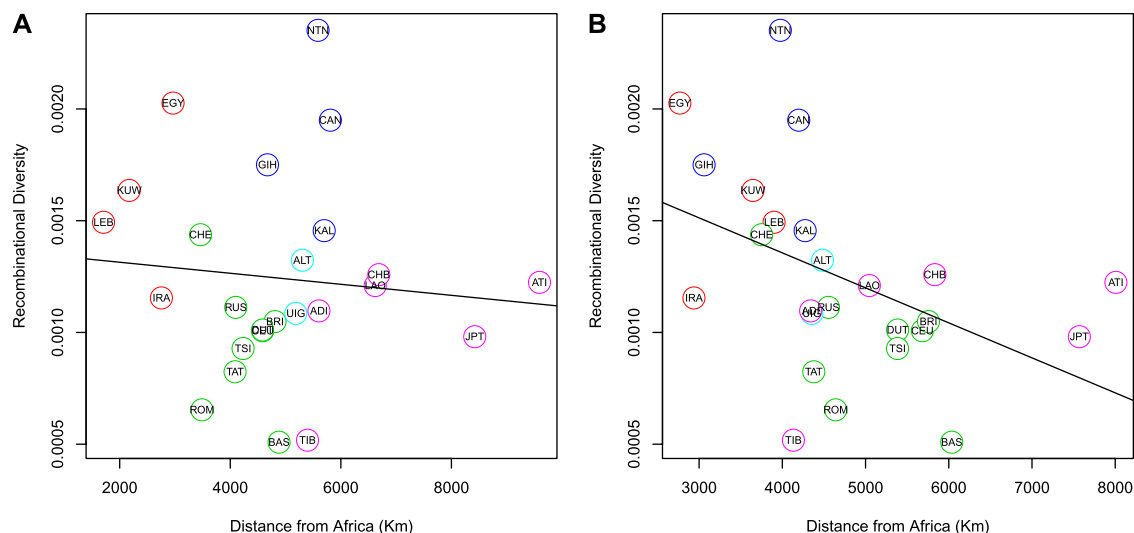
**Fig. 3.** Correlation between recombinational diversity and geographic distance from East Africa (in kilometers). (A) Following the North of Egypt route. (B) Following the South Arabian route. Populations are color-coded by continent as in figure 2.

show similar diversity values as East Asian populations, whereas Indian populations showed significantly more diversity than Europeans (Mann–Whitney $U$ test; $P = 0.0055$) and East Asians (Mann–Whitney $U$ test; $P = 0.011$). Finally, Moroccans appeared as clear outliers in both analyses, especially in the SNP diversity estimate (supplementary fig. S2, Supplementary Material online) due to their high levels of sub-Saharan African admixture. This compromised the geography-based analysis and, therefore, they were removed from the subsequent analyses.

The present results stress the wide differences between Sub-Saharan Africans and the rest of the Old World populations and point to a special role for South Asia (India) in the Out of Africa expansion of modern humans. Unfortunately, the density of sampled population within sub-Saharan Africa does not allow discussing the place of origin of anatomically modern humans. Recently, evidence has been published for the expansion of anatomically modern humans throughout Asia through a single and fast route, most likely via a Southern coastal path through India and onward into Southeast Asia and Australasia (Macaulay et al. 2005; Thangaraj et al. 2005; Mellars 2006; Armitage et al. 2011). Previous studies on microsatellites (Liu et al. 2006) and morphological variation (Betti et al. 2009) among others show the strong patterns left by the out of Africa settlement of Eurasia, a pattern that can be refined using the present recombination analysis.

Given that recombination diversity was not correlated with the distance from East Africa following a route through Northern Egypt, we assessed whether a route going through the Bab-el-Mandeb Strait (South Arabian route) as proposed by Mellars (2006) could better explain the relationship between recombination diversity and distance from East Africa. Interestingly, the correlation when considering only non sub-Saharan African samples became significant ($r = -0.569$, $P = 0.0029$) (fig. 3B) and, as expected,

it became stronger when taking into account sub-Saharan Africans ($r = -0.723$, $P = 9 \times 10^{-6}$). In order to assess how much more robust the South Arabian route was compared with the Northern Egypt route, we performed a bootstrap analysis in which the populations used in the correlation were randomly sampled with replacement. Our results showed that 95.24% of the times, the South Arabian route had higher $r^2$ values than the North Egypt route.

Whereas a route through South Arabia explained better the patterns of recombination diversity, it did not for SNP diversity values (which have a slightly lower $r = -0.570$, $P = 0.0012$). These differences may be explained by two different factors. SNP diversity calculations may be affected by the ascertainment bias toward high frequency alleles in Europeans typical in HapMap2 SNPs (Clark et al. 2005), whereas recombination diversity estimates are more robust to this kind of bias (Melé et al. 2010). And the two approaches may reflect processes taking place in different time frames with the recombination-based analysis being more sensitive to more recent events.

Finally, given the higher $N_e$ values for the Indian populations, it is tempting to speculate whether our results point toward India as having had a major role in a maturation phase prior to the expansion of modern humans to the whole of Eurasia as suggested by Atkinson et al. (2008) (see supplementary fig. S3, Supplementary Material online). The correlation between recombination diversity and geographic distance of Eurasians from South Asia ($r = -0.532$; $P = 0.0016$) (supplementary fig. S4, Supplementary Material online) is similar to the correlation between recombination diversity and geographic distance from Iran (South Arabian route) ($r = -0.569$, $P = 0.0029$) and therefore we cannot draw any conclusion at this end. Nonetheless, the higher $N_e$ values present in South India compared with the East Asians do give support to the recent Southern coastal path (Macaulay et al. 2005; Thangaraj et al. 2005; Mellars 2006;

Armitage et al. 2011) proposed for the colonization of East Eurasia through India and onward into Southeast Asia and Australasia.

## Supplementary Material

Supplementary tables S1–S7, figures S1–S4, materials and methods, and material are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

## Acknowledgments

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