Repeated out-of-Africa expansions of *Helicobacter pylori* driven by replacement of deleterious mutations

Harry A. Thorpe[1&], Elise Tourrette[2&], Koji Yahara[3&], Filipa F. Vale[4], Siqi Liu[2], Mónica Oleastro[5], Teresa Alarcon[6], TsachiTsadok Perets[7], Saeid Latifi-Navid[8], Yoshio Yamaoka[9], Beatriz Martinez-Gonzalez[10], Ioannis Karayiannis[10], Timokratis Karamitros[10], Dionyssios N. Sgouras[10], Wael Elamin[11,12], Ben Pascoe[13], Samuel K. Sheppard[13], Jukka Ronkainen[14,15], Pertti Aro[16], Lars Engstrand[17], Lars Agreus[18], Sebastian Suerbaum[19, 20, 21§], Kaisa Thorell[22, 23§], Daniel Falush[2*§]

1 Institute of Basic Medical Sciences, University of Oslo, Oslo, harry.thorpe@medisin.uio.no
2 Centre for Microbes Development and Health, Institute Pasteur Shanghai. sqliu@ips.ac.cn, danielfalush@googlemail.com, elise.tourrette@gmail.com
3 Antimicrobial Resistance Research Center, National Institute of Infectious Diseases, Toyo, Japan, k-yahara@nih.go.jp
4 Pathogen Genome Bioinformatics and Computational Biology, Research Institute for Medicines (iMed-ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal vale.filipa@gmail.com
5 National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health Dr Ricardo Jorge, Lisboa, Portugal. Monica.Oleastro@insa.min-saude.pt
6 Department of Microbiology, Hospital Universitario La Princesa, Instituto de Investigación Sanitaria Princesa, Madrid, Spain, talarcon@helicobacterspain.com
7 Division of Gastroenterology, Rabin Medical Center, Petah Tikva, Israel, tzahipe1@clalit.org.il
8 Department of Biology, Faculty of Sciences, University of Mohaghegh Ardabili, Iran, s_latifi@uma.ac.ir
9 Department of Environmental and Preventive Medicine, Oita University Faculty of Medicine, Yufu, Oita, Japan yyamaoka@oita-u.ac.jp
10 Laboratory of Medical Microbiology, Hellenic Pasteur Institute, Greece, bmartinez@pasteur.gr, y.karayiannis@hotmail.com, tkaram@pasteur.gr, sgouras@pasteur.gr
11 G42 Healthcare; Abu Dhabi, wfmelamin@yahoo.com
12 Elrazi University; Khartoum; Sudan
13 The Milner Centre for Evolution, Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath, UK, bp467@bath.ac.uk, sks70@bath.ac.uk
14 Center for Life Course Health Research, University of Oulu;
15 Primary Health Care Center, Tornio, Finland jukka.ronkainen@fimnet.fi
16 Arokero Oy, Tornio, Finland. pertti.aro@fimnet.fi
17 Center for Translational Microbiome Research, Department for Microbiology, Tumor, and Cell Biology, Karolinska Institutet, Stockholm, Sweden, lars.engstrand@ki.se
18 Division of Family Medicine, Karolinska Institutet, Stockholm, Sweden, lars.agreus@ki.se

19 Department of Medical Microbiology and Hospital Epidemiology, Max von Pettenkofer Institute, Faculty of Medicine, LMU Munich, Munich, Germany, suerbaum@mvp.lmu.de
20 Department of Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Hannover, Germany
21 DZIF German Center for Infection Research, Hannover-Braunschweig and Munich Partner Sites, Germany
22 Institute of Biomedicine, Department of Infectious Diseases, University of Gothenburg, Gothenburg, Sweden. Kaisa.thorell@gu.se
23 Department of Clinical Microbiology, Sahlgrenska University Hospital, Gothenburg, Sweden
& Contributed equally.
§ Shared senior authors
* Corresponding author: Daniel Falush, danielfalush@googlemail.com

Abstract

Helicobacter pylori lives in the human stomach and has a population structure which resembles that of its host. However, *H. pylori* from Europe and the Middle East trace a substantially higher fraction of ancestry from modern African populations than the humans that carry them. We use a collection of Afro-Eurasian genomes to show that this African ancestry is due to at least three distinct admixture events. *H. pylori* from East Asia, which have undergone little admixture, have accumulated many more non-synonymous mutations than African strains. European and Middle Eastern bacteria have elevated African ancestry at the sites of these mutations compared to either non-segregating or synonymous sites. We use simulations to show demographic bottlenecks can lead to long-term segregation of deleterious mutations, despite high rates of homologous recombination, but that population fitness can be restored by migration of small numbers of bacteria from non-bottlenecked populations, leading to mosaic patterns of ancestry like that seen for *H. pylori*. We conclude that *H. pylori* have been able to spread repeatedly from Africa due to deleterious mutations accumulated during the original out-of-Africa bottleneck.

Main text

Introduction

Helicobacter pylori is the dominant bacterial member of the human stomach microbiota in infected individuals and is an aetiological agent in most cases of gastric cancer, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastroduodenal ulcer disease [1]. *H. pylori* causes chronic, decades-long infections and is often acquired within the household, limiting the rate of its diffusion through human populations in comparison with more readily transmissible pathogens [2]. Genetic variation in *H. pylori* genome sequences shows a phylogeographic pattern similar to that of its host, consistent with an inference that human and bacterial genes are often spread by the same migrations[3-5]. However, the *H. pylori* population found in Europe and other parts of Eurasia is admixed, with many strains having more than half of their DNA attributable to populations closely related to those prevalent in Africa [3,6-8]. There is evidence for several recent human migrations out of Africa[9], but together they have only contributed a small fraction of the ancestry of non-Africans. This discrepancy in ancestry proportions between the bacteria and their hosts implies that African *H. pylori* has been spread to Eurasia by movements of people that have left weaker signals in human DNA.

To understand why African *H. pylori* have contributed extensive ancestry within parts of Eurasia, we have assembled a collection of strains from Europe and the Middle East, from putative source populations in Africa as well as less-admixed strains in Asia. We infer a recent demographic history of the European and Middle Eastern strains that includes genetic drift, migration and admixture from external sources. We show that there have been at least three admixture events from African source populations that have each contributed substantial ancestry. By examining the distribution of non-synonymous

mutations in different populations, we conclude that there was a large and lasting increase in the frequency of segregating deleterious mutations during the out-of-Africa bottleneck associated with the initial spread of modern humans from Africa. When African *H. pylori* strains reached Eurasia due to later contact between humans, they, and the DNA they carried, had a fitness advantage and were able to spread. In the process, they reduced the mutational load in the newly admixed populations.

Repeated African Admixture into Europe and the Middle East

Based on fineSTRUCTURE clustering (Figure S1), we grouped European and Middle Eastern strains into four subpopulations named hspEuropeNEurope, hspEuropeCEurope, hspEuropeSWEurope and hspEuropeMiddleEast according to the locations they were most commonly isolated from. The first Europe in the name indicates they are subpopulations of hpEurope but for brevity we omit this part of the name in the rest of the manuscript. We investigated their sources of external ancestry by performing *in silico* chromosome painting (Figure 1A), using donor strains from three African populations (hspENEAfrica, hspCNEAfrica, hspAfrica1WAfrica) and two Asian populations (hpAsia2 and hspEAsia). Representative strains from these populations were selected from a larger collection on the basis that they showed little sign of recent admixture from other continents (Methods). The chromosome painting analysis supports previous findings that there is a North-South cline in the overall proportion of African ancestry and that hpAsia2 is a closer relative of the pre-admixture population than hspEAsia[3,7]. However, all isolates are painted with a substantial and largely consistent fraction of hspEAsia (with the hpAsia2:hspEAsia ratio varying from 1:1.74 for hspNEurope to 1:2.02 for hspMiddleEast), implying that hpAsia2 is not a close surrogate for the pre-admixture population.

To investigate the demographic history of admixture further, we measured genetic drift profiles separately for each ancestry component (Figure 1B). Specifically, we compared the painting profile of pairs of admixed individuals to identify regions of the genome in which they were painted by the same donor population. For these genomic regions, we recorded if they were painted by the same specific donor strain (Methods). High rates of painting by the same donor strain indicates shared genetic drift within that ancestry component. Genetic drift profiles for the hpAsia2 and hspEAsia ancestry components show a similar pattern across the four hpEurope subpopulations, as indicated by near identical pattern of colours for these two ancestry components in Figure 1B. The indistinguishable drift profiles provide evidence that within each hpEurope subpopulation, both components have been affected similarly by genetic drift. The simplest explanation is that these components are both being used to paint a single ancestry source that persisted in western Eurasia since the out-of-Africa bottleneck. Therefore, the data does not provide evidence for either ancient or more modern genetic contributions from the East.

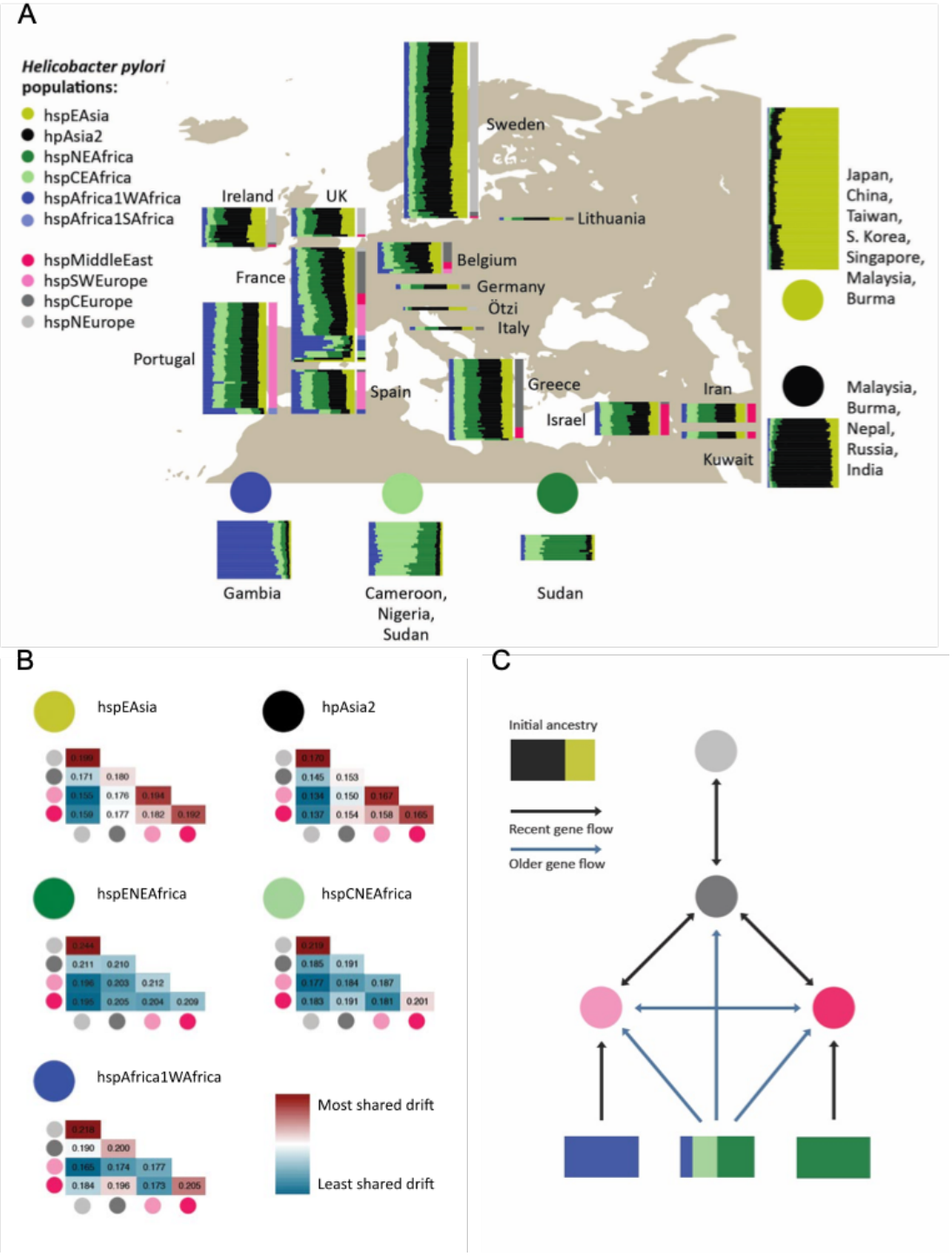

Figure 1 *Ancestry and migration history of hpEurope isolates. (A) Painting profiles of hpEurope isolates and their putative ancestral populations from Africa and Asia showing proportion of each genome (horizontal bar) painted by each of 5 ancestral donor populations (circles). HpEurope isolates are grouped by country of isolation, with bars to the right indicating the H. pylori population each strain is assigned to. The representative isolates from each donor population are grouped by population, with countries of isolation listed for each group. (B) Genetic drift profiles for hpEurope subpopulations, shown separately for each ancestry component. (C) Schematic summarizing the migration and admixture history of the hpEurope subpopulations.*

In addition to the North-South cline, there is also an East-West ancestry cline in the source of African admixture (Figure 1A), with distinct drift patterns for each African component in the four hpEurope subpopulations (Figure 1B). Strains from hspSWEurope have the highest fractions of hspAfrica1WAfrica and this ancestry component shows low levels of drift, implying that this subpopulation has undergone recent admixture from strains closely related to those currently found in West Africa. Furthermore, there are strains from Spain, Portugal and France assigned to hspAfrica1 subpopulations and a strain in Portugal with an intermediate ancestry profile suggesting they have arrived within the last few human generations. Strains from the hspMiddleEast subpopulation have the highest fraction from hspENEAfrica and the lowest levels of drift in this component. These two populations have therefore received genetic material from Africa after the initial gene flow that has introduced ancestry across the continent. These patterns imply that there have been at least 3 separate admixture events involving distinct populations of African bacteria (Figure 1C).

The drift components also provide evidence about local migration. hspMiddleEast and hspSWEurope have high levels of shared drift in both Asian components, implying that prior to admixture, these two populations were closely related. hspNEurope has high subpopulation-specific drift in all ancestry components, showing that it has undergone recent genetic drift, while hspCEurope has almost none in any component, suggesting that it has been a hub for migration between populations.

The genome of a strain colonizing the Tirolean iceman, Ötzi, has been inferred to be a nearly pure representative of the pre-admixture population based on more limited data, which was interpreted as evidence that most of the admixture took place in the 5,300 years since his death [7]. In our fineSTRUCTURE analysis, the Ötzi genome clusters with hspNEurope isolates from Ireland and Sweden with the lowest African ancestry, one of which has the same non-African ancestry proportion as Ötzi in the chromosome painting, showing that the Ötzi genome in fact falls within modern variation in ancestry proportions in Europe. We interpret this as evidence that substantial African ancestry had already been introduced into Europe when Ötzi lived but that ancestry proportions in particular locations have changed substantially in subsequent millennia.

Overall, the chromosome painting results show that, in addition to contemporary migrations that have introduced *H. pylori* with atypical profiles into countries such as Ireland, Portugal, France and Spain (Figure 1A), *H. pylori* have spread out of Africa at least three times (Figure 1C). Each of these migrations is sufficiently old that the DNA has been absorbed into the local gene pools, leading to a high degree of uniformity in ancestry profiles for most isolates in individual locations (Figure 1A). At least one of the early admixture events was shared between the four subpopulations, spanning Europe and the Middle East, and left traces in

Ötzi's genome, while later ones, labelled as "recent" in Figure 1C had foci in South Europe and the Middle East respectively. Gene flow between the regional subpopulations has affected all ancestry components but has not been sufficient to homogenize ancestry proportions across the continent.

Evidence for a role of deleterious mutations in the repeated expansion of bacteria from Africa.

The ability of *H. pylori* of African origin to spread effectively in non-African populations on multiple independent occasions is unexpected, since the resident bacteria will have had an opportunity to adapt to local conditions. One potential explanation is that deleterious mutations accumulated in genomes of strains carried by the early waves of modern humans that spread from Africa. Demographic bottlenecks associated with these migrations have been sufficient to leave an imprint on neutral genetic variation within the human genome, which indicate a reduction in effective population size in the ancestry of non-African humans around 50,000 years ago, followed by more recent expansion[10].

Population genetic theory [11] and evidence from experimental systems [12] has shown that demographic bottlenecks can lead to reduction in average fitness through processes such as fixation of deleterious mutations of small effect or the stochastic loss of the fittest genomes (Muller's ratchet [13,14]). However, although the out-of-Africa bottlenecks have had measurable impact on the pattern of segregating mutations within human populations [15], there is less evidence that individuals from non-African populations have accumulated a larger burden of non-synonymous mutations when measured relative to an outgroup[16]. *H. pylori* shows much higher rates of genetic differentiation between geographical regions [4] than its host [10], which most likely reflects transmission bottlenecks during spread from person to person as well as the expansion of fit clones. Consequently, demographic bottlenecks that have had modest fitness consequences for humans can potentially have more substantial effects on the bacteria they carry.

Bacteria from the hspEAsia and hpAsia2 populations, which have been through the bottleneck have a higher number of deleterious mutations segregating between strains (Figure 2A) and have higher dN/dS to the *H. acinonychis* outgroup (Figure 2B, S2) than do the three African populations with comparable synonymous divergence levels dS. dN/dS for the three African populations range between 0.137 and 0.138, with Asian populations having values of 0.140 and 0.141. This corresponds to an average of 574 extra non-synonymous mutations in the Asian populations or 1 mutation for every 3 genes. dN/dS values within populations vary between 0.123-0.133 for African populations and 0.163-0.169 for the Asian ones. This difference corresponds to an average of 1216 non-synonymous differences between pairs of strains or about 2/3 of a SNP per gene. These results show that the larger number of differences in dN/dS to the outgroup in the Asian

populations reflect a larger number of segregating non-synonymous variants and thus cannot simply be attributed to fixation of mutations during the out-of-Africa bottleneck.

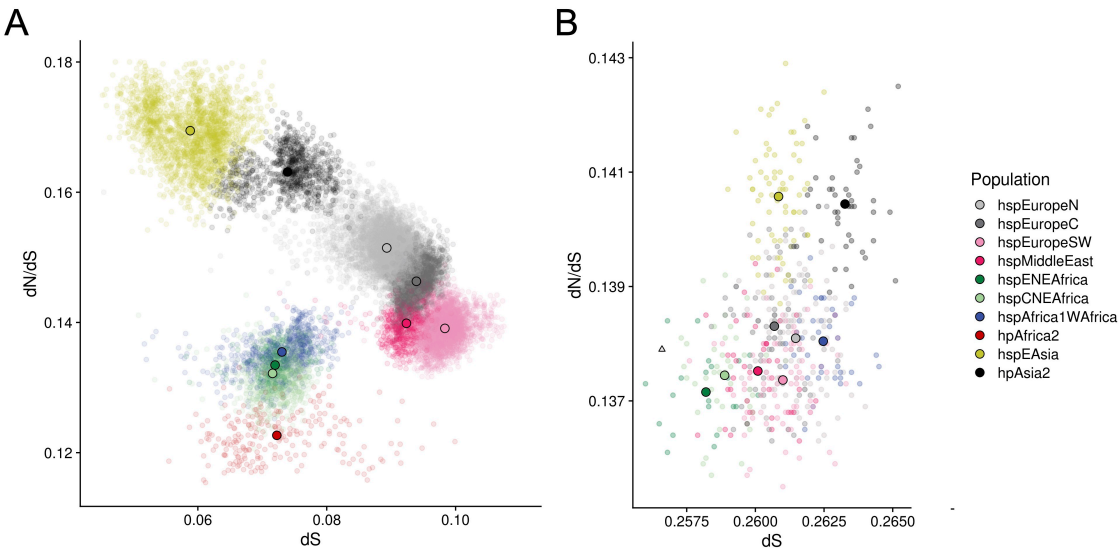

Figure 2 *Within and between population divergence. (A) Within population dN/dS (y axis) plotted against dS (x axis). Semi opaque points show pairwise distances; solid points indicate population means.* ***(B)*** *dN/dS calculated to the H. acinonychis outgroup plotted against dS for isolates (semi opaque points) and populations (solid points), excluding hpAfrica2 isolates. The triangle indicates the genome from Ötzi*

We also see evidence for amelioration of the mutational load by admixture and selection within hpEurope subpopulations. Bacteria from hpEurope subpopulations have dN/dS values with the *H. acinonychis* outgroup that are intermediate between African and Asian populations but are lower (0.137-0.138) than would be predicted if they were random mixtures of African and Asian genomes with the proportions estimated by chromosome painting in Figure 1 (0.139-0.140). However, this mutation deficit is not on its own compelling evidence for a direct benefit of admixture, since it might instead reflect differences in dN/dS between the population that existed in Europe prior to admixture and the Asian populations that are used as surrogates for this ancestry in this analysis.

More specific evidence that admixture has purged deleterious non-synonymous mutations is provided by tabulating the effect of mutations that accumulated in African and Asian populations on the ancestry of admixed bacteria in Europe and the Middle East (Figure 3). For each position in the alignment, we calculated a mutation score, which is the difference between African and Asian strains in the proportion of nucleotides that differed *from H. acinonychis* (with equal weight given to each subpopulation, see methods). We then investigated whether there is variation in overall Asian ancestry proportion (hpAsia2+hspEAsia in the chromosome painting) associated with this score. Most sites are

non-polymorphic and have a mutation score of 0.0 which is therefore used as a baseline and compared independently to positive and negative scoring sites. To allow for correlations between adjacent sites, statistical significance of the regression was assessed using a gene-by-gene jackknife (methods).

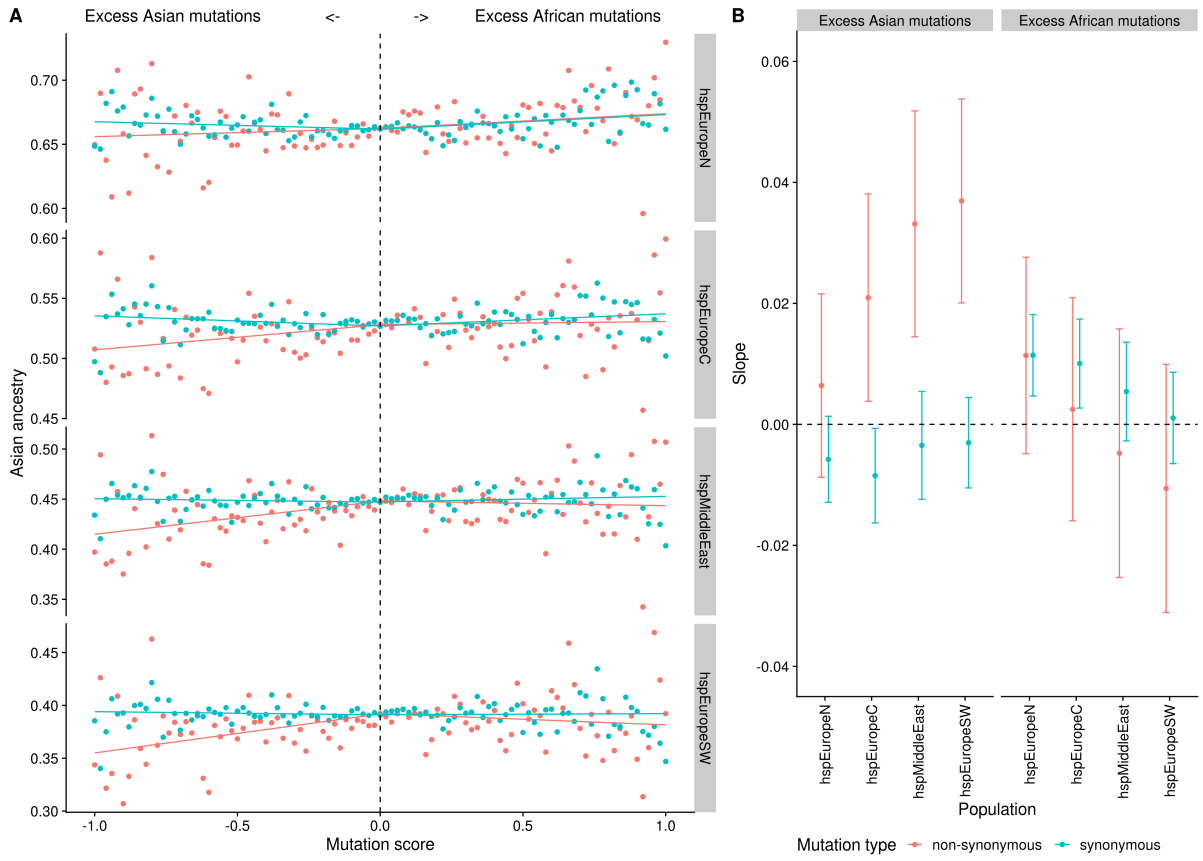

Figure 3 *Genetic ancestry of hpEurope subpopulations as a function of mutation score. (A) average ancestry in chromosome painting analyses plotted against mutation score (mutation frequency in African population minus mutation frequency in Asian population) in bins of 0.02. Regression lines are calculated separately for positive and negative mutation scores (B, C) regression slopes with 95% confidence intervals estimated using a gene-by-gene jackknife for excess Asian and excess African mutations respectively.*

H. pylori shows little evidence for codon usage bias[17], so most synonymous mutations should be approximately neutral and can therefore be used as a control. A negative mutation score at non-synonymous sites leads to a deficit in Asian ancestry, either in comparison with non-polymorphic sites or with synonymous sites with the same scores. The strongest regression slopes are estimated for the two Southern populations, and the weakest estimated for hspNEurope (Figure 3A,B). By contrast, excess mutations in African strains do not have a detectable effect on ancestry in any of the four hpEurope subpopulations, with regression slopes statistically indistinguishable from those found for synonymous positions. Thus, admixed bacteria have avoided non-synonymous mutations

that accumulated in populations that have been through the out-of-Africa bottleneck, but not those that have not. We infer that a proportion of these non-synonymous mutations are deleterious and have been selected against in the admixed population.

We investigated whether specific genes were enriched for African ancestry. There is substantial variation amongst genes in the average African ancestry proportion with strong correlations between proportions in the four hpEurope subpopulations (Figure S3), which is consistent with much of the ancestry being due to a single shared admixture event. However, there is no clear differences between COG categories in average ancestry proportion (Figure S4), making it difficult to come to conclusions about selection on classes of genes.

To show that selection on deleterious mutations is a plausible explanation for the patterns that were observed, we performed simulations of bacterial populations evolving under deleterious mutations and homologous recombination of short tracts (Figure 4, Figure S5,S6). These simulations showed that at high recombination rates, demographic bottlenecks could generate long-term increases in the number of deleterious mutations segregating in the population, especially at high recombination rates (Figure 4B, Figure S6C,D), and in dN/dS measured relative to an outgroup (Figure 4C, Figure S6E,F). These patterns qualitatively match those seen in the data.

In our simulations, the average fitness of the bottlenecked population underwent the largest decrease at intermediate recombination rates (Figure 4A, Figure S6A,B) and in this simulation, the bottlenecked population was susceptible to invasion from strains from the non-bottlenecked population, leading to the generation of highly mosaic genomes (Figure 4E). As migrant DNA spread through the population, mutations from the bottlenecked population that were deleterious fell faster in frequency than mutations at neutral sites (Figure 4D), showing that recombination and selection acted together to reduce the mutational burden of the admixed population.

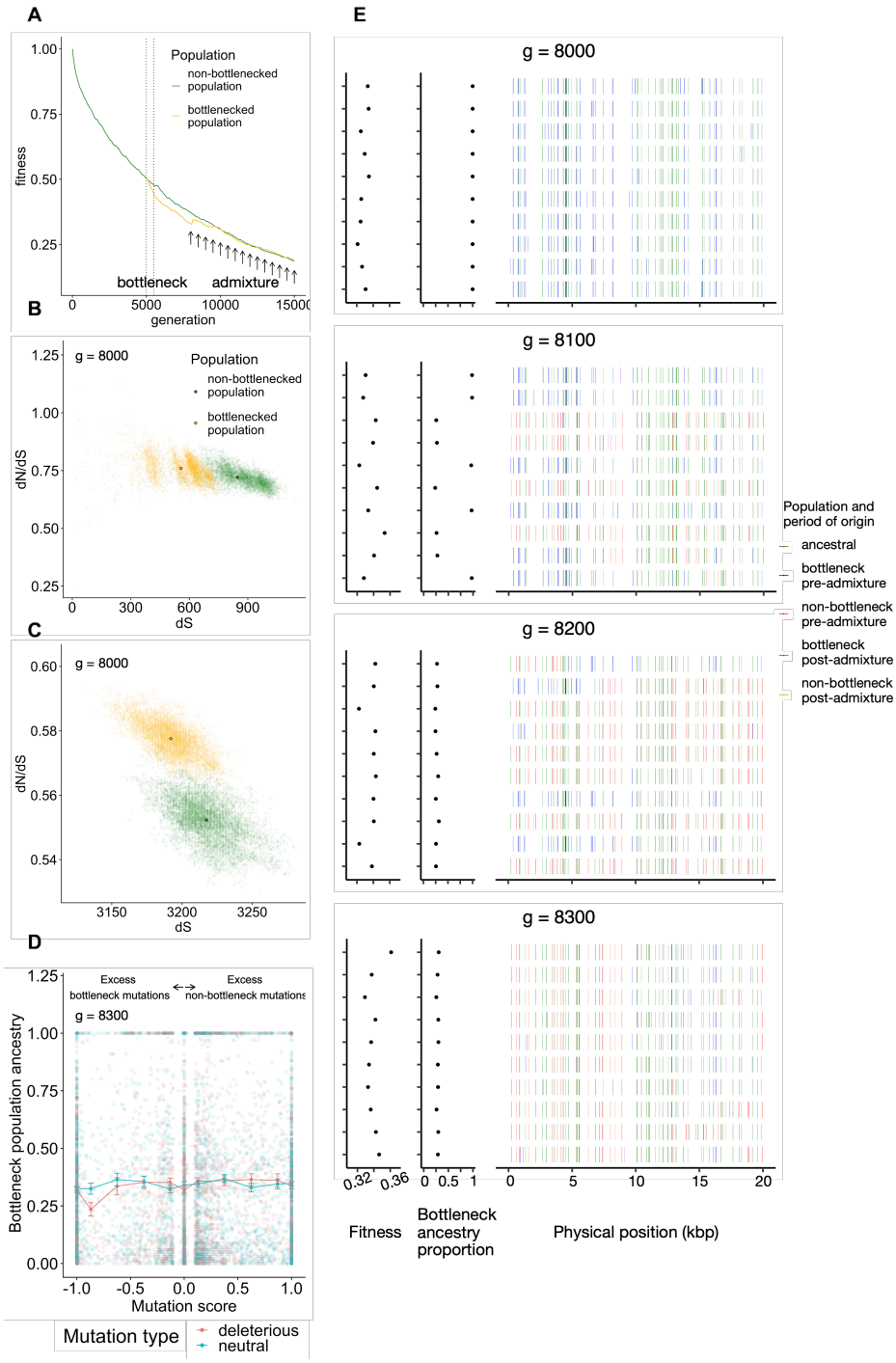

Figure 4. *Simulations of populations under bottlenecks and admixture. (A)Population average fitness shown during the generations of the simulation, with bottleneck starting at generation 5000 and admixture at generation 8000, with each arrow corresponding to the migration of a single strain. Within population dN/dS (B) and dN/dS calculated to the ancestor (C) measured at generation 8000. Semi opaque points show pairwise distances; solid points indicate population means. (D) Average bottleneck population ancestry, at generation 8300 plotted against mutation score (frequency in the non-bottleneck population minus the frequency in the bottleneck population before the admixture begins). (E) Ancestry painting in a sample of genomes from the bottleneck population, in the generations subsequent to admixture. Only the first 20kb of each genome is shown.*

Discussion

Deleterious mutations provide a compelling explanation for the repeated spread of African *H. pylori* into other continents, as shown by the good qualitative match between our simulation results and the pattern of diversity within contemporary *H. pylori* populations. First, we find elevated dN/dS values in Asian populations, consistent with a higher load of deleterious mutations accumulating during the out-of-Africa bottleneck. Secondly, our simulations show that rare migrants from non-bottlenecked populations can spread and generate highly mosaic genomes, as observed in Europe and the Middle East. Third we show that ancestry from these invading lineages is higher in regions of the genome where non-synonymous mutations are segregating within the bottlenecked populations, implying that selection has acted to purge these mutations during admixture.

Many of the details concerning the spread of African ancestry remain to be elucidated. The initial admixture event(s) left traces in the *H. pylori* genome of the Tirolian Iceman Otzi, which has an ancestry profile like that found in modern day samples in Ireland and Sweden but has less African ancestry than in modern genomes from Southern Europe. Therefore, the first admixture event occurred more than 5300 years ago but the average level of African ancestry has increased substantially in the last few millennia.

H. pylori of different origins can mix together and recombine within extended families[18]. Over time, extensive ongoing contacts between populations within Europe would be expected to homogenize *H. pylori* ancestry proportions both within and between locations. However, we lack quantitative information on transmission dynamics that might allow us to estimate mixture dates based on the properties of the ancestry clines we observe.

An intriguing question is why some populations appear to have been resistant to invasion by DNA from African *H. pylori*. For example, within East Asia, most strains appear to come from the hpEAsia population, with very little evidence of admixture. Lack of contact with Africans does not seem a sufficient explanation, given the large number of documented contacts between East Asians and other Eurasian populations within the last several thousand years. The high burden of *H. pylori* related gastric disease in the region is notorious and hpEAsia bacteria are known for distinctive variants at virulence associated loci including *cagA* and *vacA*[19]. It is possible that strains from this population have acquired a suite of adaptations that allows them to outcompete invading bacteria despite the large mutation load within their genomes. This raises the possibility that some of the non-synonymous mutations that rose to high frequency during the bottleneck may have allowed rapid adaptation, in other words a form of evolution by shifting balance[20].

H. pylori seems to be an outlier amongst bacteria in many features of its biology including its slow rate of spread between human populations and its high mutation and recombination rates. Our results suggest that recombination may save strains from rapid mutational

meltdown but that deleterious mutations persist within populations, with the effects of bottlenecks enduring for millennia. The unusual properties of *H. pylori* make it a powerful model system for understanding how deleterious mutations interact with demographic processes and adaptive ones to mold diversity within natural populations.

Methods

Dataset collection

A dataset of 716 *Helicobacter pylori* whole-genome sequences was assembled consisting of 213 newly sequenced isolates from Europe, Asia and Africa (supplementary table 1) and selected publicly available genomes (supplementary table 2).

Genome sequencing

Genomes new for this study were sequenced at five different centres, Karolinska Institute, Sweden (KI), Hannover Medical School, Hannover, Germany (MHH), Hellenic Pasteur Institute, Greece (HPI), Oita University, Japan (OiU), and University of Bath, UK (UBa), see supplementary table 1.

Genomic DNA from strains marked with KI in supplementary table 1 was extracted using DNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's guidelines for Gram-negative bacteria. Sequencing libraries were prepared using the TruSeq Nano kit (Illumina, San Diego, CA, USA) and sequenced on the MiSeq platform, v3 chemistry, using 300 bp paired end mode.

Genomic DNA from strains marked MHH was isolated from *Helicobacter pylori* strains after 24h culture on *Helicobacter pylori* selective agar (in-house recipe) with the Genomic Tip 100/G (Qiagen, Hilden, Germany). Nextera XT libraries were generated and sequenced in three different runs on MiSeq 2x300bp paired (Illumina, San Diego, CA, USA), as recommended by the manufacturer. All quantification steps of gDNA and NGS libraries were done with Qubit dsDNA HS Assay Kit (Invitrogen, ThermoFisher Scientific, Carlsbad, CA, USA).

For strains marked with HPI adapter-compatible DNA was prepared using Ion Xpress™ Plus Fragment Library Kit and enzymatically fragmented for 5-12 minutes, resulting in a median fragment size of 350-450 bp and the libraries were prepared using the Ion Plus Fragment Library Kit. The resulting 400 bp insert libraries were used to prepare enriched, template-positive Ion PGM™ Hi-Q™ View Ion Sphere Particles (ISPs) with the Ion OneTouch™ 2 System. 850-flows sequencing was performed using the Ion PGM™ Hi-Q™ View Sequencing Kit with the Ion 318™ Chip Kit v2.

For genomes marked with UBa, genomic DNA was quantified using a NanoDrop spectrophotometer, as well as the Quant-iT DNA Assay Kit (Life Technologies, Paisley, UK) before sequencing. High-throughput genome sequencing was performed using a HiSeq 2500 machine (Illumina, San Diego, CA, USA).

Genomic DNA from strains marked with OiU was extracted using DNeasy Blood & Tissue kit (QIAGEN, Hilden, Germany). DNA concentration was measured using QuantusTM Fluorometer (Promega). High-throughput genome sequencing was performed either on Hiseq 2000 (2 × 100 or 2 × 150 paired-end reads) or Miseq (2 × 300 paired-end reads) sequencer (Illumina, San Diego, CA) following the manufacturer 's instruction.

Primary bioinformatics analysis

For the KI genomes the raw sequencing reads were quality trimmed and filtered using TrimGalore! (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) applying a minimum q-score of 30, and *de novo* assembled using SPAdes [21] with the –careful option. Contigs with very low coverage and that were shorter than 500 bp were discarded prior to annotation.

For the MHH genomes quality filtering was done with Trimmomatic version 0.36 [22] and the assemblies were performed with SPAdes genome assembler v. 3.9.0 and resulted in number of all contigs from 26 up to 117. Assemblies were quality controlled with QUAST[23].

For the HPI genomes quality control of raw sequencing reads was performed using FASTQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc). Unbiased *de novo* assembly was performed using SPAdes genome assembler v.3.5.0in default mode.

For the UBa genomes, raw sequencing reads were quality trimmed and filtered using Trimmomatic version 0.33 and the 100-bp short read paired-end data was assembled using the *de novo* assembly algorithm Velvet version 1.2.08[24]. The VelvetOptimiser script (version 2.2.4) was run for all odd k-mer values from 21 to 99. The minimum output contig size was set to 200 bp with default settings, and the scaffolding option was disabled.

For the OiU genomes, Trimmomatic v0.35 was used to remove adapter sequences and low-quality bases from raw short reads data. Trimmed reads were then de novo assembled to produce contigs using SPAdes genome assembler v3.12.0 with the "-careful" option to reduce mismatches in the assembly. The minimum contig length was set to 200 bp.

Annotation of both newly sequenced draft genomes and publicly available sequences was performed using the prokka annotation pipeline v 1.12 [25] using the most recent version of the 26695 genome [26] as primary annotation source.

Genome size and contig/scaffold number was collected from the prokka annotation output using the MultiQC tool [27] and collected into Supplementary table 3. All newly sequenced genomes were submitted to GenBank under BioProject PRJNA479414.
All strains, their population designations and their role in the respective analyses is shown in Supplementary table 4.

Sequence comparison and alignment:
All isolates were mapped to the 26695 genome (NC000915.1) using the Snippy software version 3.2-dev (https://github.com/tseemann/snippy). The resulting core genome, which was collected with the same tool, contained 287 746 core SNPs from 979 771 variant sites.

FineSTRUCTURE
We inferred population structure among the strains based on the genome-wide haplotype data of the reference-based alignment to 26695 described above, using the chromosome painting and fineSTRUCTURE [28] according to a procedure of our preceding study that applied them to *H. pylori* genome[29]. Briefly, we used ChromoPainter (version 0.04) to infer chunks of DNA donated from a donor to a recipient for each recipient haplotype and summarized the results into a "co-ancestry matrix" which contains the number of recombination-derived chunks from each donor to each recipient individual. We then ran fineSTRUCTURE (version 0.02) for 100,000 iterations of both the burn-in and Markov chain Monte Carlo (MCMC) chain in order to conduct clustering of individuals based on the co-ancestry matrix.

Choice of donor and recipient strains and chromosome painting
D-statistics were calculated for strains assigned to each of the five ancestral populations hspEAsia, hpAsia2, hspCNEAfrica, hspENEAfrica and hspAfrica1WAfrica. D-statistics were calculated using popstats (https://github.com/pontussk/popstats) and specifying individual A as SouthAfrica7 (hpAfrica2), individual B as GAM260Bi (hspAfrica1WAfrica), individual Y as F227 (hspEAsia) and individual X. In this comparison, negative D-statistics imply more African ancestry in the strain designated as individual X than in F227. D-statistics values can be found in Supplementary Table 5 and Supplementary Table 6.

A subset of strains from each of the five ancestral populations were chosen as donors to get groups of similar size and were selected based on the fineSTRUCTURE analysis to get good representativeness over the donor populations. Some hpAsia2 strains showed signs of elevated African admixture based on negative D-statistics values and these strains were not selected as donors.

We conducted chromosome painting of 646 recipient strains (belonging to hspNEurope, hspCEurope, hspSWEurope, hspMiddleEast, hspENEAfrica, hspEAsia, hspCNEAfrica, hspAfrica1WAfrica, hpAsia2, and hpAfrica2). For this purpose, we used ChromoPainterV2 software [28]. For each recipient population, we calculated site-by-site average copying

probability from each of the five donor populations. Gene-by-gene averages were also calculated by averaging of the sites in each gene for the 790 genes in the alignment. Regressions between gene-by-gene averages in different hpEurope subpopulation were calculated using the aq.plot() function in R.

Shared drift estimation

We used the chromosome painting analysis to investigate shared genetic drift profiles, within and between hpEurope subpopulations with different ancestry components. For each donor population used in the painting we tabulated the number of sites in the genome that each pair of recipient individuals used the same donor individual in the painting as well as the proportion of sites in which they copied from the same donor population (same individual or different individual). These two numbers were summed up for recipient individuals from particular combinations of hpEurope subpopulation pairs with the ratio giving the estimate of shared drift.

dN/dS calculations

From the fineSTRUCTURE analysis, a sub-dataset was collected consisting of the European strains assigned to the hpEurope populations, together with a representative selection of strains from the ancestral populations hpAfrica2, hspAfrica1WAfrica, hspCNEAfrica, hspENEAfrica, hpAsia2, and hspEAsia. The *H. acinonychis* genome was added to this dataset to provide an outgroup. For a detailed list of which strains that were included in these analyses, see columns in table supplementary tables 1 and 2.dN/dS was estimated pairwise between these strains from core genome alignments using the method of Yang and Nielson[30], as implemented in Paml v4.7.

To calculate the numbers of excess mutations observed in the Asian populations, the mean number of non-synonymous mutations was calculated for African and Asian populations, and the difference was then multiplied by a correction factor of 1.37 to account for the loss of some coding sites in the core genome alignment (1.11Mb) compared with the reference genome (1.52Mb).

Ancestry and mutation score analysis

Using *H. acinonychis* as a reference, non-synonymous and synonymous mutations were called against representative strains from the African and Asian populations. For this analysis the ancestral populations were combined to reduce the ancestry components to either African (hpAfrica) or Asian (hpAsia). For each site, the frequencies of these mutations within each ancestral population were calculated to give a score between 0 and 1. These scores were then combined by subtracting the hpAsia scores from the hpAfrica scores to give a score between -1 and 1, where -1 = fixed in hpAsia, absent in hpAfrica, 0 = equal frequencies in hpAsia and hpAfrica, 1 = absent in hpAsia, fixed in hpAfrica. Thus, these

scores represent the mutational load for each site in the ancestral populations. We then combined these site by site scores with the site by site chromosome painting data for the European populations so that each site had an estimate of ancestry component from both Asia and Africa, and a mutational score representing the mutational load in the ancestral populations.

We then computed linear regressions of hpAsian ancestry against mutation score for each European population, and we did this for the positive (excess African) and negative (excess Asian) mutation scores separately. To confirm that the regression slopes were not driven by a small number of outlier genes we conducted a gene-by-gene jackknife by repeating the regressions but removing all the sites from a single gene each time. From this distribution of slope values we calculated the pseudovalues as pseudovalue=slope-((n-1)*(j_slope-slope)). We then calculated the mean and confidence limits of these pseudovalues as mean(pseudovalues) ± quantile(p=0.05/2, df=n-1) * se(pseudovalues).All data manipulation and statistical analysis was performed in R 3.6.1. Packages from the 'tidyverse' collection were used extensively, and ggplot2 was used for plotting.

Simulation of bacterial populations evolving under deleterious mutation pressure.
We used SLiM v3 [31] to simulate the accumulation of deleterious mutations in bacterial populations. Each bacterial genome was a single haploid chromosome of length 1.6 Mb, with a mutation rate per site of $5x10^{-7}$. Half of the mutations are neutral, with the other half being deleterious with six different selection coefficients (-5e-3, -2e-3, -1e-3, -5e-4,-2e-4, -1e-4), each accounting for 1/12 of the mutations. There is no back mutation. Mutations have a multiplicative effect on fitness.
At the beginning of the simulation, there is no variation in the population. A single population of size 10,000 evolves for 5000 generations. 1000 of the strains are moved into a second population, which retains this size for 500 generations before expanding to size 10,000. From generation 8000 onwards, migration from the non-bottleneck to the bottleneck populations happens at a rate of one strains every 500 generations. The simulations are completed after 15000 total generations. Once every 100 generations, the full genome (mutations present in each strain, their position, selection coefficient) is saved. Every generation, the number of mutations per strain, the fitness of each strain, the population size and the number of segregating mutations and substitutions are saved. Simulation output was analysed using R. For each population we calculated its average fitness, its fitness variance, its dN/dS to the ancestor, using the number of mutations per strains as the differences with the ancestor and the within population dN/dS, using the difference between pair of strains of the same population. The SLiM code is reproduced in supplementary material.

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Supplementary Figures:

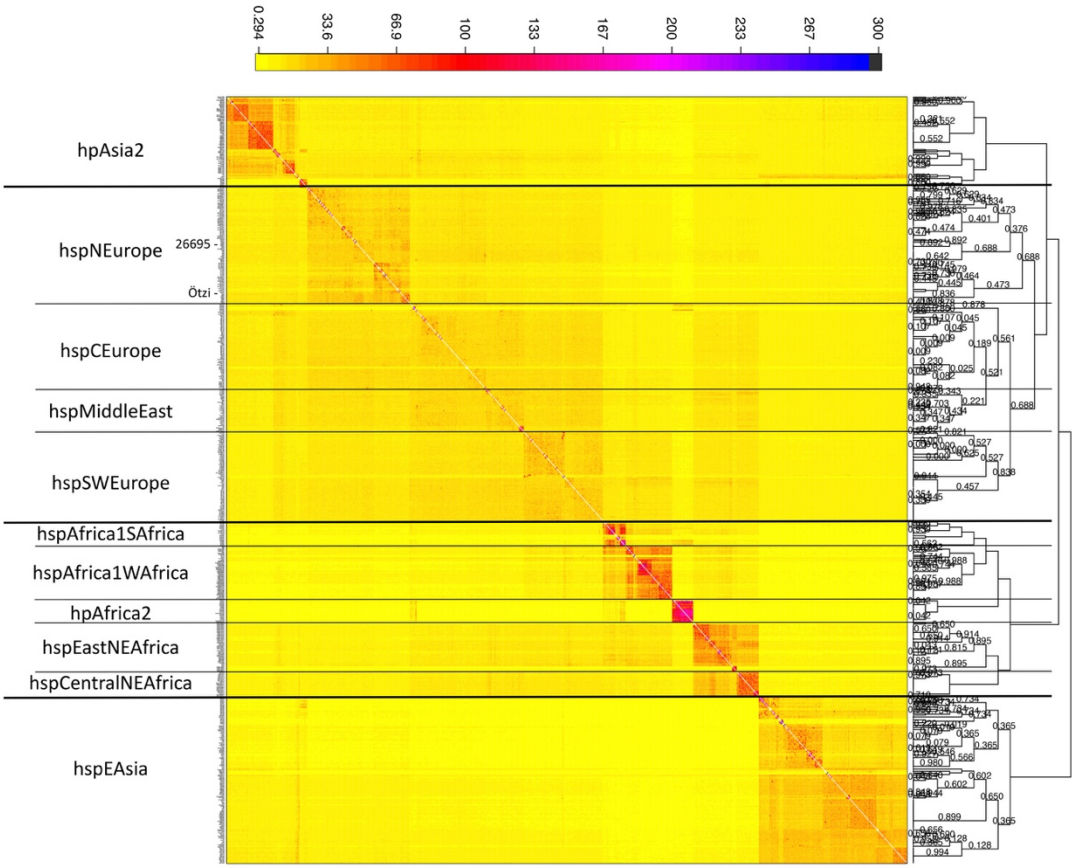

Figure S1 *heatmap showing populations inferred by fineSTRUCTURE. For each individual the number of chunks donated by other individuals are shown according to the colour scale at*

the top. Note position of Otzi within hpsNEurope. Tree on right shows hierarchical clustering of fineSTRUCTURE populations.

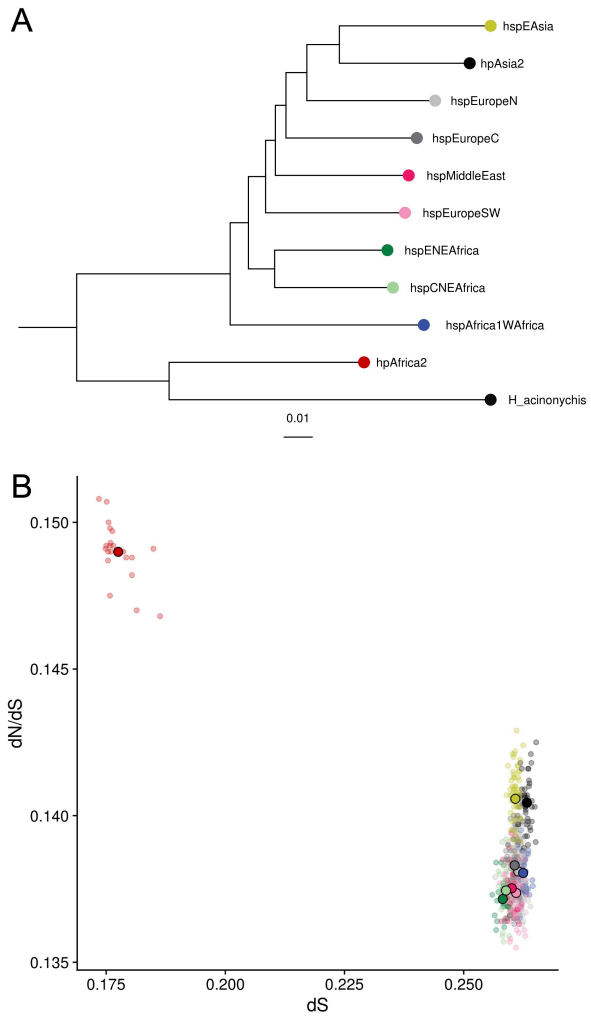

Figure S2 Relationships amongst populations. (A) Neighbor joining tree based on genetic distances between populations. (B) dN/dS plotted against dS to H. acinonychis outroups for all populations, including Africa2.

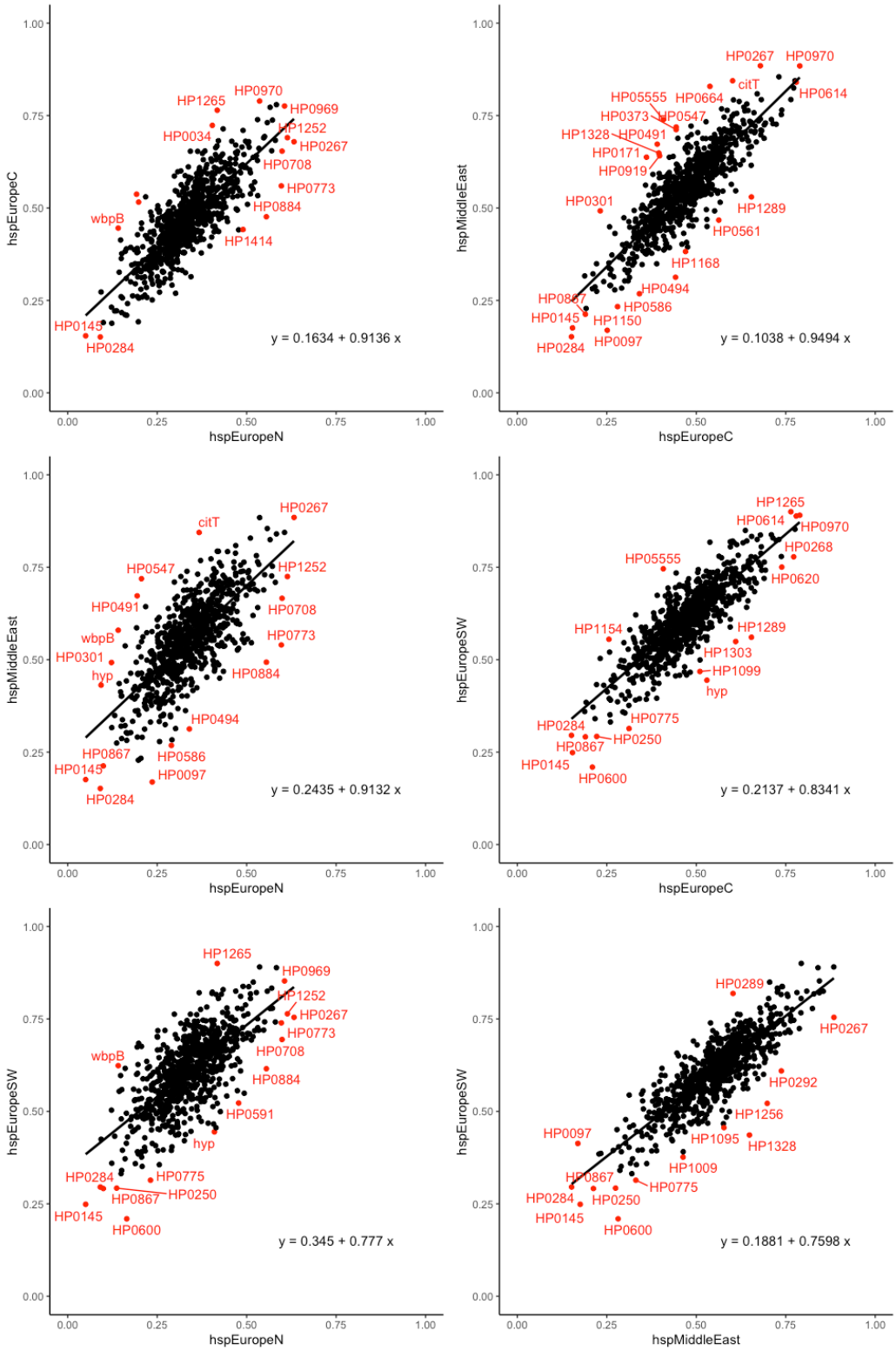

Figure S3 *Average African ancestry proportions of genes.*

Each panel shows relationship between the proportion of ancestry assigned to Africa in the chromosome painting for each gene for pairs of hpEurope subpopulations. Outliers from the regression slope are calculated based on robust Mahalanobis distances using the R function aq.plot(data, quan =1,alpha =0.015).

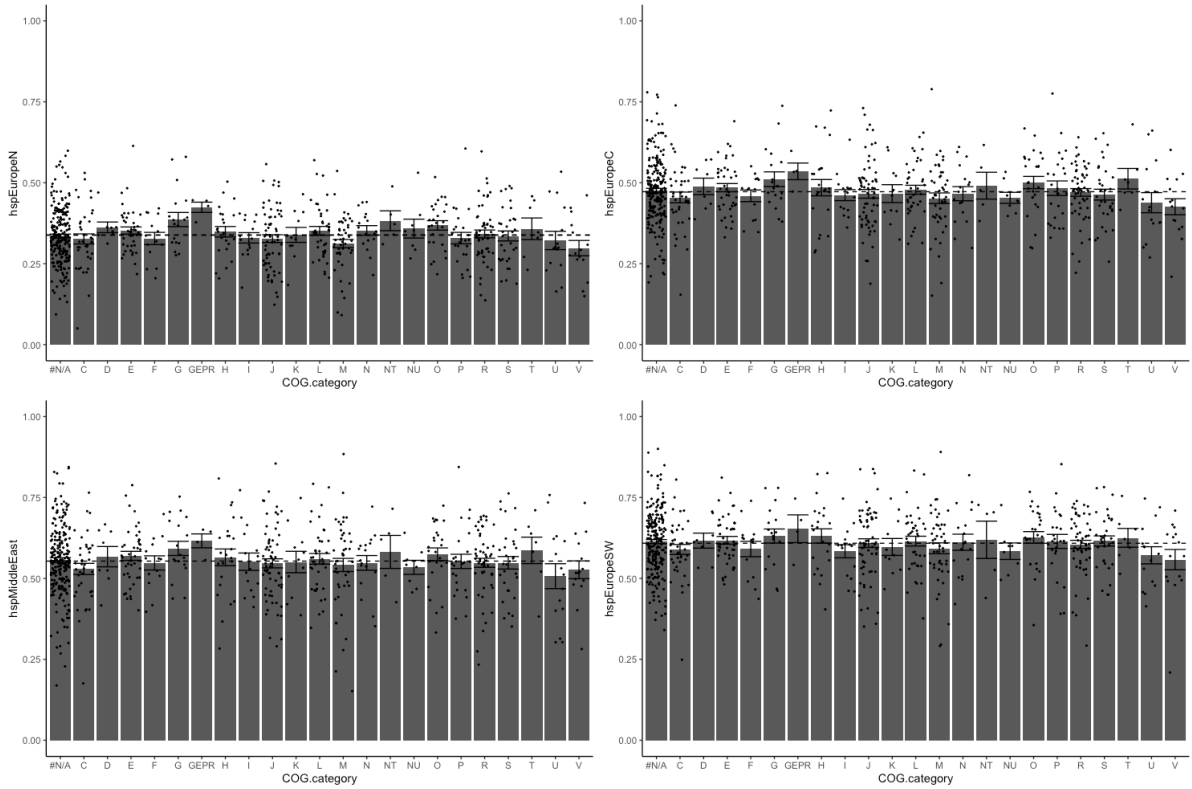

Figure S4 *Average African ancestry proportion of genes in particular COG category. Calculated separately for each hpEurope subpopulation. Whiskers show standard error of the average for each category.*

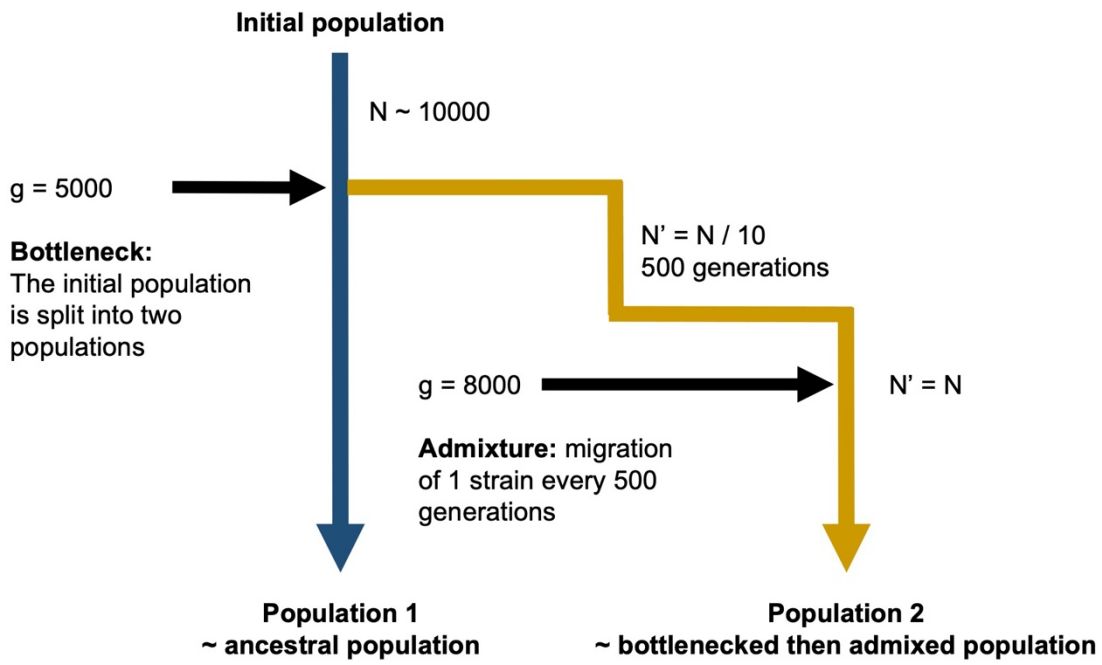

Figure S5 *The different steps of the simulation process.*
Parameters values: N = 10000; genome length = 1.6 Mbp; mutation rate = $5x10^{-7}$ per bp; deleterious mutations: s = (-0.005, -0.002, -0.001, -0.0005, -0.0002, -0.0001); 50% of the mutations (the other 50% are neutral mutations). Look at different recombination levels: clonal reproduction (import size per generation = 0bp), intermediate recombination levels (import size per generation = 500bp and 5000bp) and nearly free recombination (import size per generation = 50000bp).

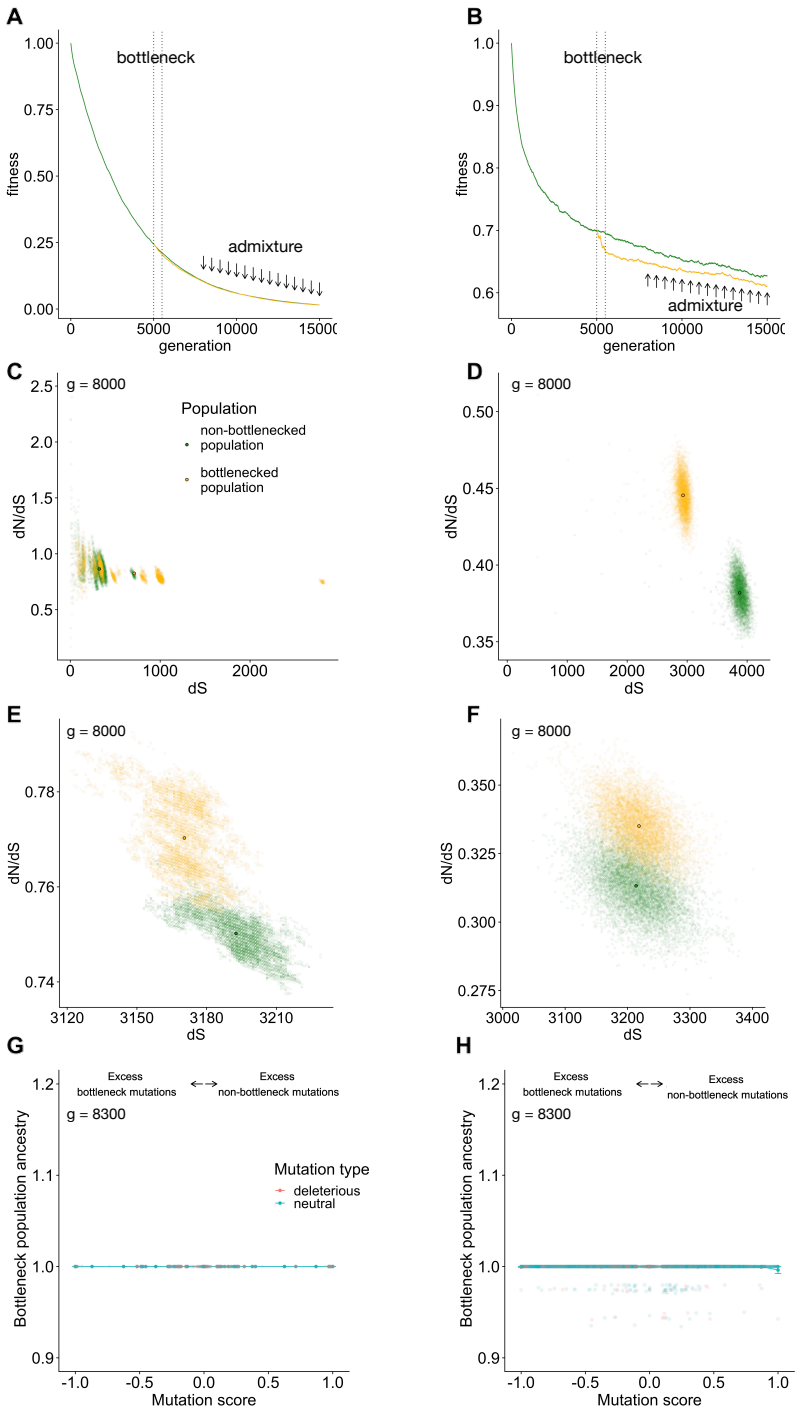

Figure S6 *The effect of different recombination rates.*
(A-B) Within population dN/dS (y axis) plotted against dS (x axis). Semi opaque points show pairwise distances; solid points indicate population means. (C-D) dN/dS calculated to the ancestor plotted against dS for isolates (semi opaque points) and populations (solid points). (E-F) Average bottleneck population ancestry, after the first migration event, plotted against mutation score (frequency in the non-bottleneck population minus the frequency in the bottleneck population before the admixture begins). (G-H) Average fitness over the generations, under no recombination (A,C,E,G) and high recombination (B,D,F,H), the segment represents the bottleneck and the arrow signals when the admixture begins.

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