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Performance and limitations of linkage-disequilibrium-based methods for inferring the genomic landscape of recombination and detecting hotspots: a simulation study

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Abstract

Knowledge of recombination rate variation along the genome provides important insights into genome and phenotypic evolution. Population genomic approaches offer an attractive way to infer the population-scaled recombination rate $\rho=4N_er$ using the linkage disequilibrium information contained in DNA sequence polymorphism data. Such methods have been used in a broad range of plant and animal species to build genome-wide recombination maps. However, the reliability of these inferences has only been assessed under a restrictive set of conditions. Here, we evaluate the ability of one of the most widely used coalescent-based programs, *LDhelmet*, to infer a genomic landscape of recombination with the biological characteristics of a human-like landscape including hotspots. Using simulations, we specifically assessed the impact of methodological (sample size, phasing errors, block penalty) and evolutionary parameters (effective population size (N_e), demographic history, mutation to recombination rate ratio) on inferred map quality. We report reasonably good correlations between simulated and inferred landscapes, but point to limitations when it comes to detecting recombination hotspots. False positive and false negative hotspots considerably confound fine-scale patterns of inferred recombination under a wide range of conditions, particularly when N_e is small and the mutation/recombination rate ratio is low, to the extent that maps inferred from populations sharing the same recombination landscape appear uncorrelated. We thus address a message of caution for the users of these approaches, at least for genomes with complex recombination landscapes such as in humans.

Key words

Population-scaled recombination rate, LDhelmet, simulations, linkage disequilibrium, recombination hotspots

Introduction

Recombination is highly conserved among sexually reproducing species of eukaryotes. This fundamental mechanism of meiosis is essential for the proper segregation of homologous chromosomes during the reductional division. Recombination involves crossing over events (CO) that play a crucial evolutionary role by allowing genetic mixing and generating new combinations of alleles (Baudat and de Massy 2007; Cromie et al. 2001; Capilla et al. 2016). Measuring the rate at which recombination occurs and the magnitude of its variation along the genome has important implications for fundamental research in molecular biology and evolution (Stapley et al. 2017), but also for applied genomics such as genome-wide association studies (GWAS) (Morris et al. 2013; Hunter et al. 2016). Several approaches have been developed to reconstruct genome-wide recombination maps (reviewed in Peñalba and Wolf 2020). Cytological methods, like ChIP-seq, target protein-DNA complexes directly involved in the formation of double-strand breaks (DSB) and CO during meiosis (Pratto et al. 2014). Gamete typing methods analyse the meiotic products of a diploid individual (reviewed in (Carrington and Cullen 2004; Dréau et al. 2019; Sun et al. 2019). Methods based on pedigree analysis reconstruct the gametic phase from patterns of allele inheritance in bi-parental crosses (Lander et Green 1987; Kong et al. 2002; Kodama et al. 2014; Rastas 2017). All these approaches have the advantage of providing direct estimates of the recombination rate. However, by focusing on CO that occurred in a few individuals or families across one or a couple of generations, they remain intrinsically limited in resolution due to the small number of recombination events that occur per chromosome per generation (Clark et al. 2010; Peñalba and Wolf 2020).

Another type of approach uses genome sequence data from population samples to take advantage of the large number of recombination events that have occurred during the history of the considered population. Instead of directly observing crossover products, these methods detect the footprints left by historical recombination events on patterns of haplotype segregation and linkage disequilibrium (LD) (reviewed in Stumpf and McVean 2003). The recombination rate and its variation across the genome are inferred via coalescent-based analysis of DNA sequence polymorphism data (Chan et al. 2012; Kamm et al. 2016; Li and Stephens 2003.; McVean et al. 2004; Spence and Song 2019). The resulting LD maps have been widely used to evaluate the genomic impact of natural selection and admixture, and to perform genome-wide association studies (GWAS) (*e.g.* Chan et al. 2012; The International HapMap Consortium 2007). These approaches provide an accessible and attractive way of describing recombination landscapes - *i.e.* the variation of recombination rates along the

genome - particularly in non-model taxa where direct methods are often difficult to implement (Auton et al. 2012; 2013; Melamed-Bessudo et al. 2016; Shanfelter et al. 2019; Singhal et al. 2015; Shield et al., 2020).

Direct and indirect methods have revealed considerable variation in recombination rate at different scales along the genome, particularly in vertebrates. At a large scale (of the megabase order), recombination tends to be concentrated in subtelomeric regions compared to centromeric and centro-chromosomal regions, a pattern shared among many species of plants and animals (Auton et al. 2012; Melamed-Bessudo et al. 2016; Capilla et al. 2016; Danguy des Déserts et al. 2021; Haenel et al. 2018). At a finer scale (of the kilobase order), recombination events often cluster within small regions of about 2 kb, called recombination hotspots (Choi and Henderson 2015; Kim et al. 2007; Mancera et al. 2008; Myers et al. 2005; Singhal et al. 2015; Shanfelter et al. 2019; Schield et al., 2020). Two distinct regulatory systems of recombination hotspot location have been described to date, with major implications on the evolutionary dynamics of recombination landscapes. In passerine birds (Singhal et al. 2015), dogs (Axelsson et al. 2012; Auton et al. 2013) and some teleost fishes (Baker et al., 2017; Shanfelter et al., 2019), recombination hotspots tend to be found in CpG-islands / promoter-like regions, and are highly conserved between closely-related species (Singhal et al. 2015). In contrast, in humans (Myers et al. 2005; 2010), apes (Auton et al. 2012 ; Great Ape Genome Project 2016) and mice (Booker et al. 2017), hotspot location is directed by the PRDM9 protein, which binds specific DNA motifs and triggers DSBs (Oliver et al. 2009; Baudat et al. 2010; Myers et al. 2010; Parvanov et al. 2010; Grey et al. 2018). In these taxa, hotspots are mostly located away from genes (Auton et al., 2012; Baker et al. 2017), and show little or no conservation between closely related species (Myers et al. 2005, 2010; Auton et al. 2012; Booker et al. 2017) due to self-destruction by gene conversion and rapid turnover of PRDM9 alleles (Coop and Myers 2007; Lesecque et al. 2014; Latrille et al. 2017).

Population-based inference methods aim to infer the population recombination rate $\rho = 4N_e r$, r being the per generation, per bp recombination rate and N_e the effective population size (Stumpf et McVean 2003). The ρ parameter reflects the density of population recombination events that segregate in polymorphism data, integrated across time and lineages. Several programs have been developed for reconstructing LD-maps (reviewed in (Peñalba and Wolf 2020; including PHASE: Li and Stephens 2003, LDhat: McVean et al. 2004, LDhelmet: Chan et al. 2012, LDpop: Kamm et al. 2016, and pyrho: Spence and Song 2019), which use the theory of coalescence with recombination to model the complex genealogies of samples stored in the underlying ancestral recombination graph (Griffiths et al. 1997; Arenas,

2013). The most popular family of LD-based methods, comprising LDhat (McVean et al. 2004) and its improved version LDhelmet (Chan et al. 2012) (see a literature survey in Supplementary Figure S1), implement a pairwise composite likelihood method under a Bayesian framework using a reversible jump Markov Chain Monte Carlo (rjMCMC) algorithm. They have been used for building fine scale LD-based maps in a broad range of animal taxa including humans (McVean et al. 2004), dogs (Axselsson et al. 2012; Auton et al. 2013), fruit flies (Chan et al. 2012), finches (Singhal et al. 2015), honeybees (Wallberg et al. 2015), sticklebacks (Shanfelter et al. 2019) and rattlesnakes (Schield et al., 2020). In some species, inferred LD-based maps have been validated by comparison with recombination maps obtained using direct approaches, confirming their overall good quality (McVean et al. 2004; Chan et al. 2012; Singhal et al. 2015; Booker et al. 2017; Shanfelter et al. 2019). However, as genetic and LD-based maps greatly differ in their resolution (pedigree-based inference provide resolution of about 1 cM, while population-based methods can infer recombination events at the kilobase scale, Peñalba and Wolf 2020), such comparisons do not provide qualitative information on the reliability of inferred fine-scale variation and hotspots detection. Moreover, the heterogeneity of studies in terms of taxonomy, genetic diversity, demography, sample size, and software parameters, among other things makes it difficult to appreciate the performance and the possible weaknesses of LD-based methods. For these reasons, the reliability and conditions of application of LD-based methods are still poorly understood and need to be more thoroughly characterised, considering the growing importance of these tools.

The power and sensitivity of LDhat and LDhelmet have been tested by simulations aiming to evaluate the influence of switch error in haplotype phasing (Singhal et al. 2015; Booker et al. 2017), the amount of polymorphism, and the intensity of recombination hotspots (Singhal et al. 2015). These studies simulated simple recombination landscapes assuming either homogeneous recombination rates or a few, well-defined hotspots contrasting with a low-recombination background (McVean et al. 2004; Auton and McVean, 2007; Chan et al. 2012; Singhal et al. 2015; Booker et al. 2017; Shanfelter et al. 2019; Schield et al., 2020). Real recombination landscapes that were characterised with a fine-scale resolution such as in humans, however, appeared to be more complex and involve a continuous distribution of recombination hotspot density and intensity across genomic regions. This complexity has not been taken into account so far in benchmarking studies assessing the performance of LD-map reconstruction methods. We thus lack a comprehensive picture of the ability of these methods to properly recover the biological characteristics of human-like recombination landscapes interspersed with hotspots. In particular, the proportion of the inferred recombination hotspots that are correct, and the proportion of true hotpots that are missed,

have not yet been quantified under a biologically realistic scenario. These are crucial quantities to properly interpret and use reconstructed LD-maps in genomic research.

In this work, we specifically assessed the performance of the LDhelmet program to detect hotspots while assuming a biologically realistic recombination landscape. We evaluated the influence of methodological parameters including sample size, phasing errors and block penalty, the impact of the population demographic history including its long-term effective size and the occurrence of bottleneck and admixture events, and finally the effect of the mutation to recombination rate ratio. We also considered different definitions of a recombination hotspot relative to its background recombination rate, with the aim of improving the sensitivity of the analysis. We identified the conditions in which LD-based inferences can provide an accurate mapping of hotspots, and the parameters that negatively affect the sensitivity and specificity of their detection across biologically realistic recombination landscapes.

Results

Recombination landscape modelling

Five realistic, heterogeneous recombination landscapes (referred to as “underlying landscapes” throughout) of 1Mb length were built using the human genome high resolution map of meiotic DSB from Pratto et al. (2014). In order to mimic both broad and fine-scale variation in the recombination rate parameter “*r*”, the first and second half of each landscape were drawn from a gamma distribution with mean 1 cM/Mb and 3 cM/Mb, respectively, and parameters fitted from Pratto et al. (2014) (1-500,000bp: shape=rate=0.1328; 500,001pb-1Mb: shape=0.1598, rate=0.0532). Accordingly, the 5 recombination landscapes generated (Supplementary Figure S2) showed broad-scale differences in recombination peak intensity, with less elevated recombination peaks in the first half compared with the second half of each chromosome. At a fine scale, recombination was concentrated in numerous peaks resembling human recombination hotspots, with about 85% of the recombination concentrated in 15% of the genome. The map lengths in recombination units were about 0.02 Morgan (Supplementary Figure S2, S4).

Population-scaled recombination landscapes simulated under a constant effective population size (hereafter called “simulated landscapes”) were generated in 10 replicates for the five underlying landscapes, using coalescent simulations with a mutation rate μ=10^-8 and 4 combinations of sample sizes (SS=10 or 20) and effective population sizes (N_e=25,000

176 or 250,000) (Figure 1A, Supplementary Figure S3A). The map lengths of simulated
177 landscapes were a little shorter than the underlying landscapes (about 0.015-0.018 Morgan),
178 reflecting the occasional occurrence of more than one recombination event between two
179 adjacent SNPs during the simulated coalescent histories (Supplementary Figure S4). These
180 simulated landscapes were also highly correlated with the underlying landscapes for each
181 combination of parameters (Spearman's rank correlation > 0.8 using a 500bp resolution level),
182 showing that the coalescent history has not resulted in a substantial loss of information about
183 recombination rate variation across the underlying landscape. As expected from the $\theta=4N_e\mu$
184 values used in our simulations (θ = 0.001 and 0.01 for N_e = 25,000 and 250,000, respectively),
185 the SNPs density of the large N_e populations was about one order of magnitude higher than
186 for smaller N_e populations (Supplementary Figure S5).
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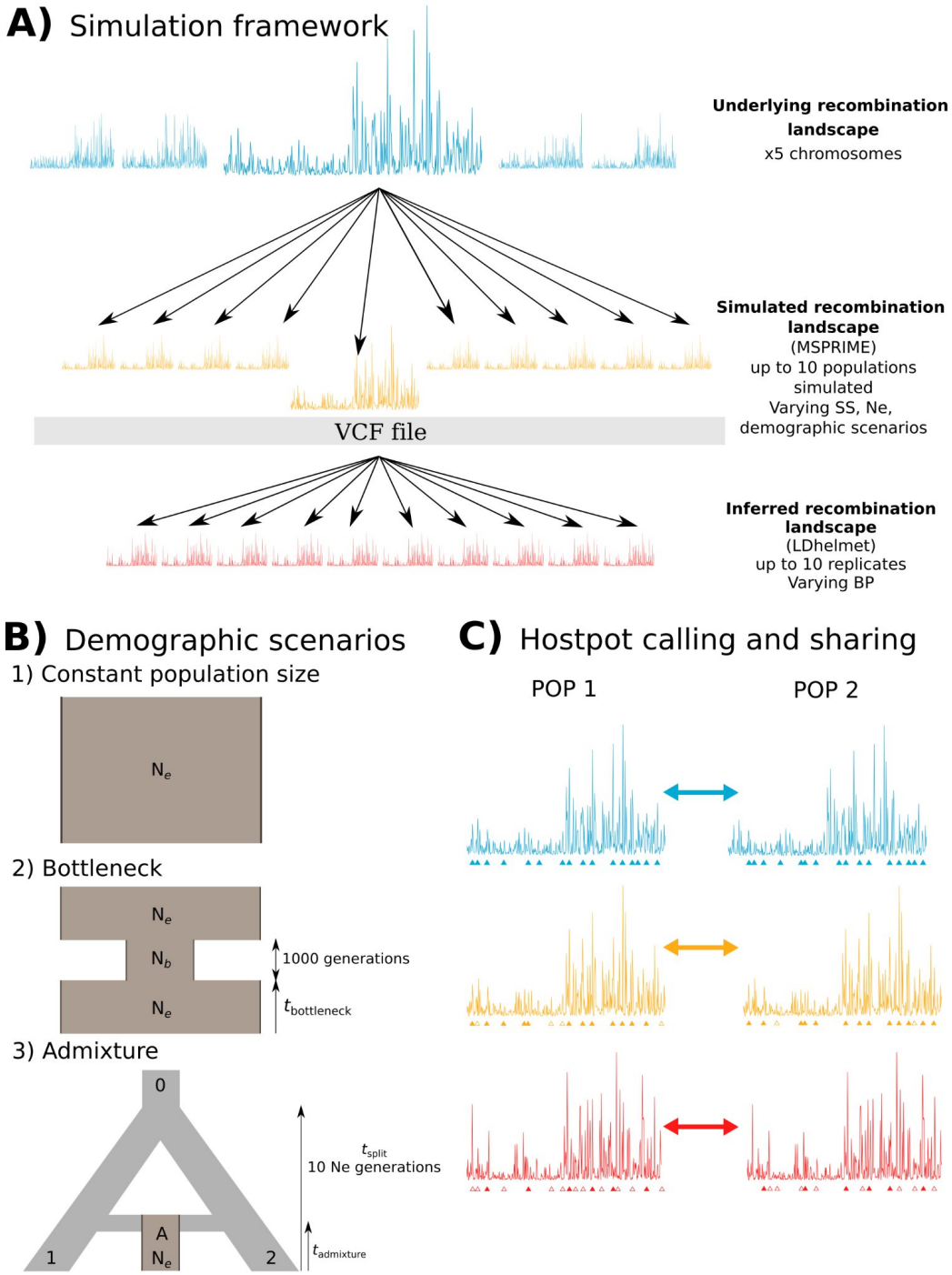

Figure 1. Simulation and hotspot calling protocols. **A)** Simulation framework. Five different underlying recombination landscapes were generated based on human empirical data. These five landscapes can either be considered as different regions from distinct chromosomes within a same species, or as orthologous regions of a same chromosome in different species. From these underlying landscapes, up to 10 recombination landscapes were simulated with MSPRIME 0.7.4 under various demographic scenarios, varying the effective population size (i.e. N_e=25,000, 100000, 250,000) and the sample size (i.e. SS=10, 20), to generate a VCF file for each simulated population. The VCF files were then used to infer the local population recombination rates using LDhelmet 1.19 setting the block penalty to BP = 5, 10 or 50. Up to

198 10 replicates per simulated population were analysed with LDhelmet. **B)** Demographic
199 scenarios. Three demographic scenarios were simulated with MSPRIME: 1) Constant
200 population size, varying the N_e of the simulated population; 2) Bottleneck event, varying the
201 age of the bottleneck (i.e. 500, 5000, 50000) and the N_e of the population during the bottleneck
202 (i.e. N_b=2500, 25,000), and setting the duration of the bottleneck to 1000 generations; 3)
203 Admixture event, varying the age of the admixture event (i.e. 500, 5000, 50000), and setting
204 the time of the split of the ancestral population into two populations 10*Ne generations ago.
205 **C)** Hotspot calling and sharing. Hotspots in the underlying (blue), simulated (orange) and
206 inferred (red) landscapes were defined as 2.5 kb-windows with a local recombination rate *X*
207 times as high as the averaged recombination rate of the 50 kb flanking regions. Several
208 threshold values were used to call the hotspots (i.e. *X*=2.5, 5, 10). The location of hotspots
209 was compared between populations (that share or not the same underlying landscape), to
210 compute the proportion of shared hotspots. Triangles below each landscape represent called
211 hotspots, with filled triangles indicating shared hotspots, and empty triangles hotspots that are
212 not shared with the compared landscape. In this example, the two compared populations
213 share the same underlying landscape, meaning that all the hotspots are shared between them
214 (all blue triangles are filled). The two simulated landscapes with independent coalescent
215 histories share most of these hotspots, while the two landscapes inferred with LDhelmet share
216 a smaller fraction of them, due to the additional inference step.
217

218 ### *Methodological parameters affecting LDhelmet performance*

219 Population-scaled recombination rates (ρ) were inferred from the simulated
220 polymorphism datasets using the program LDhelmet (Chan et al. 2012) (referred to as
221 "inferred landscapes" throughout). The effect of sample size and landscape resolution level
222 were assessed for a constant effective population size (N_e=25,000) using 10 or 20 diploid
223 individuals (SS=10 or 20) and three block penalty (BP) values (BP=5, 10 or 50), which
224 inversely determine the number of allowed changes in ρ value within windows of 50
225 consecutive SNPs (Figure 1A, Supplementary Figure S3A). Underlying and simulated
226 landscapes were converted into population-scaled recombination rates ($\rho=4N_er$), and each
227 underlying, simulated and inferred maps was smoothed using 500bp (*i.e.* the underlying
228 landscape resolution level) and 2500bp windows (*i.e.* a resolution level better suited to the
229 SNP density in our low-N_e simulations). The 10 simulated and inferred replicates of each
230 SS/BP condition were averaged to perform landscapes comparisons. Overall, local
231 recombination rates tended to be overestimated by LDhelmet, no matter the value used for
232 SS and BP, but this was especially observed when the local ρ was either very low (ρ < 10^-4)
233 or very high (ρ > 10^-2) (Supplementary Figure S6, panels A-F). The mean inferred map
234 lengths calculated across replicates varied substantially among tested conditions (0.017-
235 0.125 M), reaching up to 6 times the length of simulated maps in low SS and BP conditions
236 (*i.e.* SS = 10, BP = 5, 10, Supplementary Figure S4, upper panel). A BP value of 50 produced

very smooth recombination maps, which did not capture fine-scale variation in recombination rate. By contrast, maps inferred with BP=5 or BP=10 were visually similar and better reflected the fine-scale variation of the underlying landscapes (Supplementary Figure S7A). Spearman rank correlation coefficient between the mean simulated and inferred landscapes was reduced when SS were small (Figure 2A). Replicate runs of LDhelmet showed a strong consistency, as revealed by elevated correlations among the 10 replicate landscapes inferred from the same simulated landscape, whatever the SS and BP values being tested (Spearman's rho > 0.89, Figure 2B).

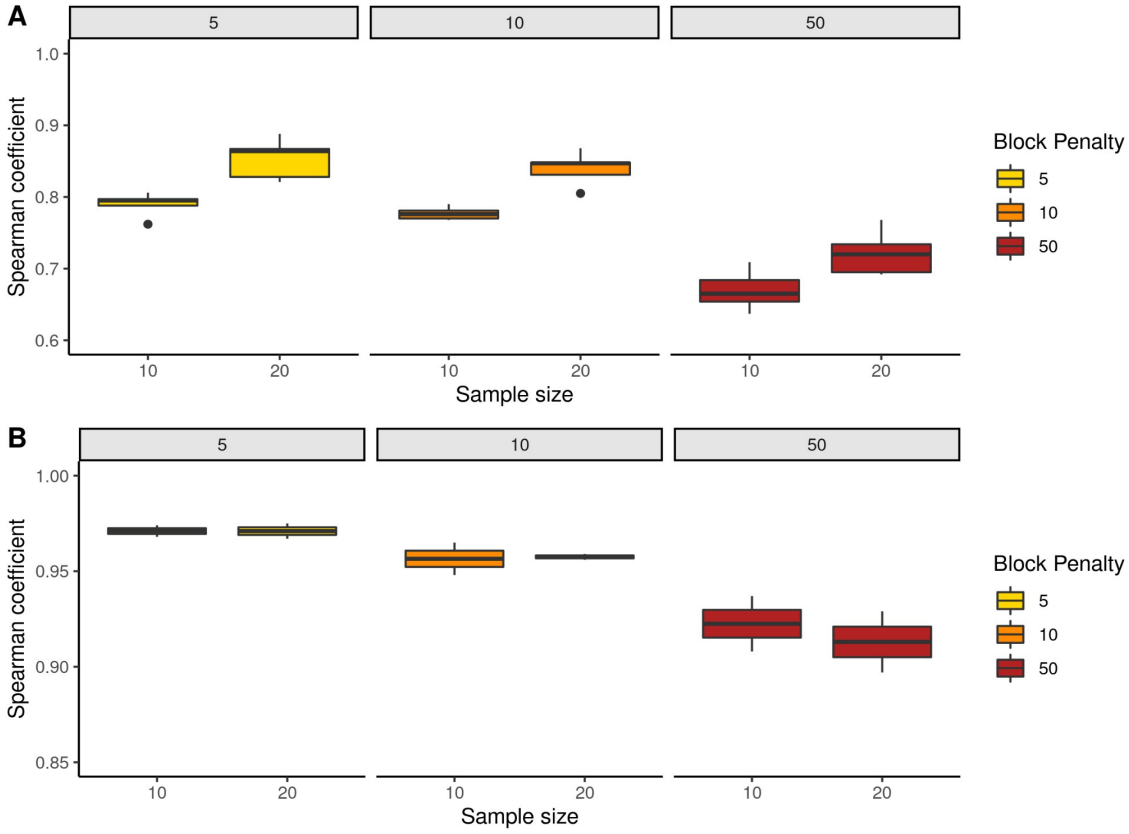

Figure 2. Performance (A), and repeatability (B) of LDhelmet as a function of the different parameters tested (i.e. N_e, SS, BP). The N_e of the simulated population is 25,000, the sample size is shown on the x axis (i.e. SS=10 or 20), and the LDhelmet BP values shown in color correspond to the different panels (i.e. BP=5, 10 or 50). **A)** Spearman correlation coefficients between the mean simulated and the mean inferred landscape calculated across the 10 replicates obtained from each of the five underlying landscapes (i.e. using simulation framework of Supplementary Figure S3A). **B)** Mean pairwise Spearman correlation coefficients calculated between the 10 replicates of inferred landscapes from simulated populations sharing the same underlying landscape (i.e. using simulation framework of Supplementary Figure S3B).

Recombination hotspots of the underlying, simulated and inferred landscapes were

called using three different threshold values commonly used in the literature (*i.e.* local recombination rate at least 2.5, 5 or 10 times higher than the background rate). True/False positives/negatives rates and discovery rates (TPR, FPR, TDR, FDR, TNR, FNR) were computed under each tested condition. The hotspot detection threshold ratio of 10 between the focal and flanking positions appeared too stringent and yielded a very small number of called hotpots (Supplementary Figure S8). Using a less conservative threshold ratio of 5, we detected 4 to 8 hotspots per Mb in the simulated landscapes, and 5 to 20 per Mb in the inferred landscapes. These numbers reached 40-50 and 20-50 per Mb, respectively, when a threshold of 2.5 was used. Irrespective of the chosen threshold, the number of inferred hotspots tended to be overestimated, notably when SS was small (Supplementary Figure S8). The 2.5 threshold was used for the remaining analysis as it reduced the variance in the number of called hotspots due to a higher call rate. The sensitivity (or TPR) of LDhelmet was medium, since depending on the SS and the BP used, between 29.4% and 52.7% of the simulated hotpots were inferred as such. The TPR was higher for small BP values (*i.e.* 5 or 10), but relatively insensitive to the SS value (Figure 3 left panel, Supplementary Figure S9A). The proportion of false hotspot calls (FDR, *i.e.* inferred hotspots corresponding to non-hotspot windows in the simulated maps) ranged between 25.6% and 52.9%, and was higher for SS = 10, without major differences between the BP values tested (Figure 3 right panel, Supplementary Figure S9B and C). No significant difference in the correlation between simulated and inferred landscapes was found between the first half of the chromosome with a mean r of 1 cM/Mb (referred to as the “cold” region) and the last half with a mean *r* of 3 cM/Mb (the “hot” region). This was also true for the TPR and the FDR, whatever the hotspot detection threshold used (*i.e.* 2.5 or 5) (Student test, $p > 0.05$).

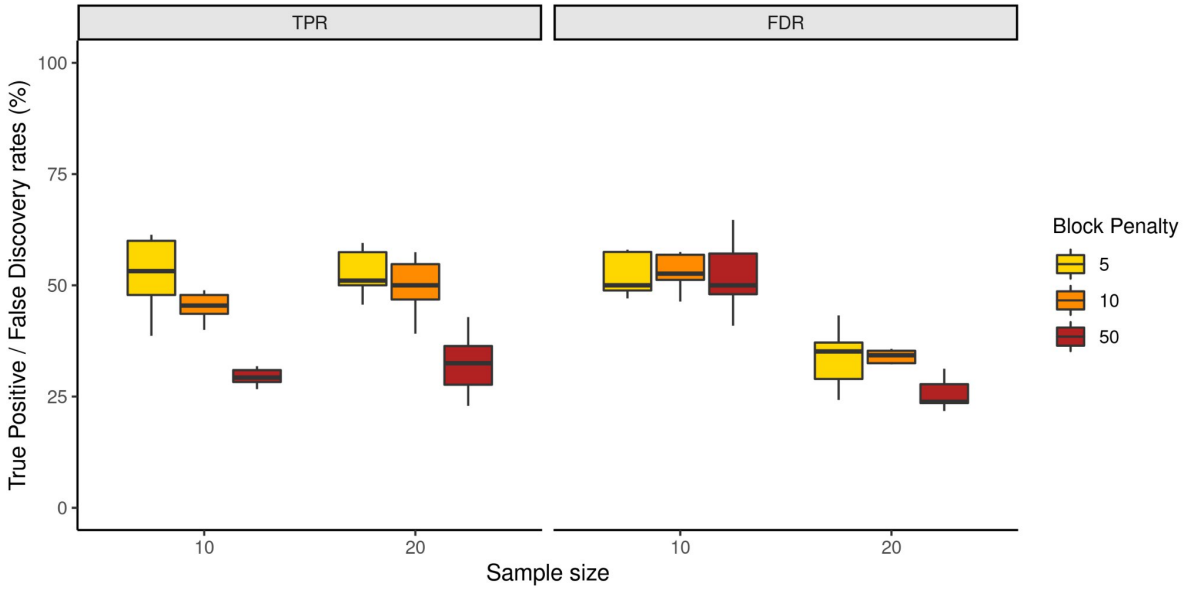

Figure 3. Hotspot detection. True positive (TPR, left panel) and false discovery (FDR, right panel) rates of inferred, as compared to simulated hotspots, called using a detection threshold of 2.5, for different sets of methodological parameters. The sample size parameter is shown on the x axis (i.e. 10 or 20), the block penalty (i.e. 5, 10, 50) is shown in color, and the N_e of the populations simulated is 25,000. See full results in Supplementary Figure S9.

To assess the impact of phasing errors on LDhelmet performance, the phase information was first removed from the whole VCF of the simulated landscapes for two replicate populations simulated with a constant Ne of 250,000 and a SS of 20. Statistical phasing performed with Shapeit 4.2.2 (Delaneau et al. 2019) resulted in a 6.7% average phasing error. We then introduced random phasing errors in the simulated VCFs with rates ranging from 2 to 10%, before inferring ρ with a BP value of 5. As the phasing error rate increased, Spearman rank correlation coefficients between simulated and inferred landscapes slightly decreased below the value obtained with perfectly phased data (Supplementary Figure S10A). Hotspot calling performance assessed with TPR and FDR was also negatively impacted by phasing errors (Supplementary Figure S10B).

Demographic and evolutionary parameters affecting LDhelmet performance

Methodological parameters were then set to SS=20 individuals and BP=5 - a trade-off optimising the balance between TPR and FDR - to focus on the effect of N_e on the quality of the LD inferences. When the simulated effective population size was large (i.e. 250,000, as compared to 25,000), the inferred map length was closer to the expected value of 0.02 M (Supplementary Figure S4, lower panels) and the local recombination rate tended to be less

overestimated (Supplementary Figure S6, panels G-L). A larger N_e also significantly increased the correlation between simulated and inferred landscapes (Figure 4A, Supplementary Figure S11), increased the TPR and decreased the FDR (Figure 4B, C).

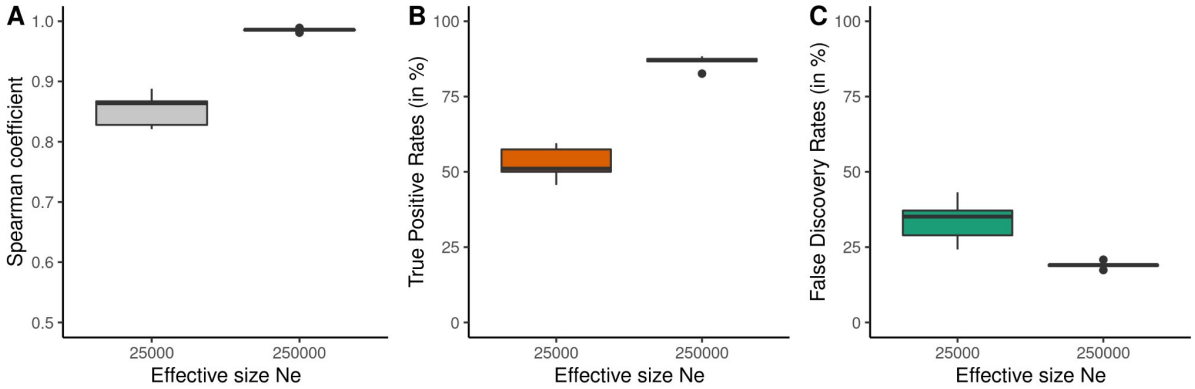

Figure 4. Effect of the effective population size parameter (Ne) on recombination rate inference and hotspot detection. **A)** Spearman correlation coefficients between simulated and inferred landscapes as a function of N_e (SS = 20, BP = 5). **B)** and **C)** True positive (TP) and false discovery (FD) rates of inferred, as compared to simulated hotspots, called using a detection threshold of 2.5, as a function of N_e (SS = 20, BP = 5). See full results in Supplementary Figure S9 and S11.

We then assessed the impact of non-equilibrium demographic histories. Populations undergoing bottleneck or admixture events of various ages were simulated, with the N_e of the ancestral and present-day populations set to 250,000, the SS to 20, and making other demographic parameters vary (*i.e.* t_b, N_b t_a, Figure 1B, see Materials and Methods). These demographic scenarios generally had a negative impact on the quality of the reconstructions (using a BP of 5), as compared to constant-size populations (Figure 5). The correlation between simulated and inferred maps decreased with the strength of the bottleneck (i.e. with lower N_b), and with the recentness of bottleneck and admixture events (Figure 4A, B). In the same way, TPR and FDR were degraded as compared to constant-size population scenarios (Figure 4C, D), particularly for young events.

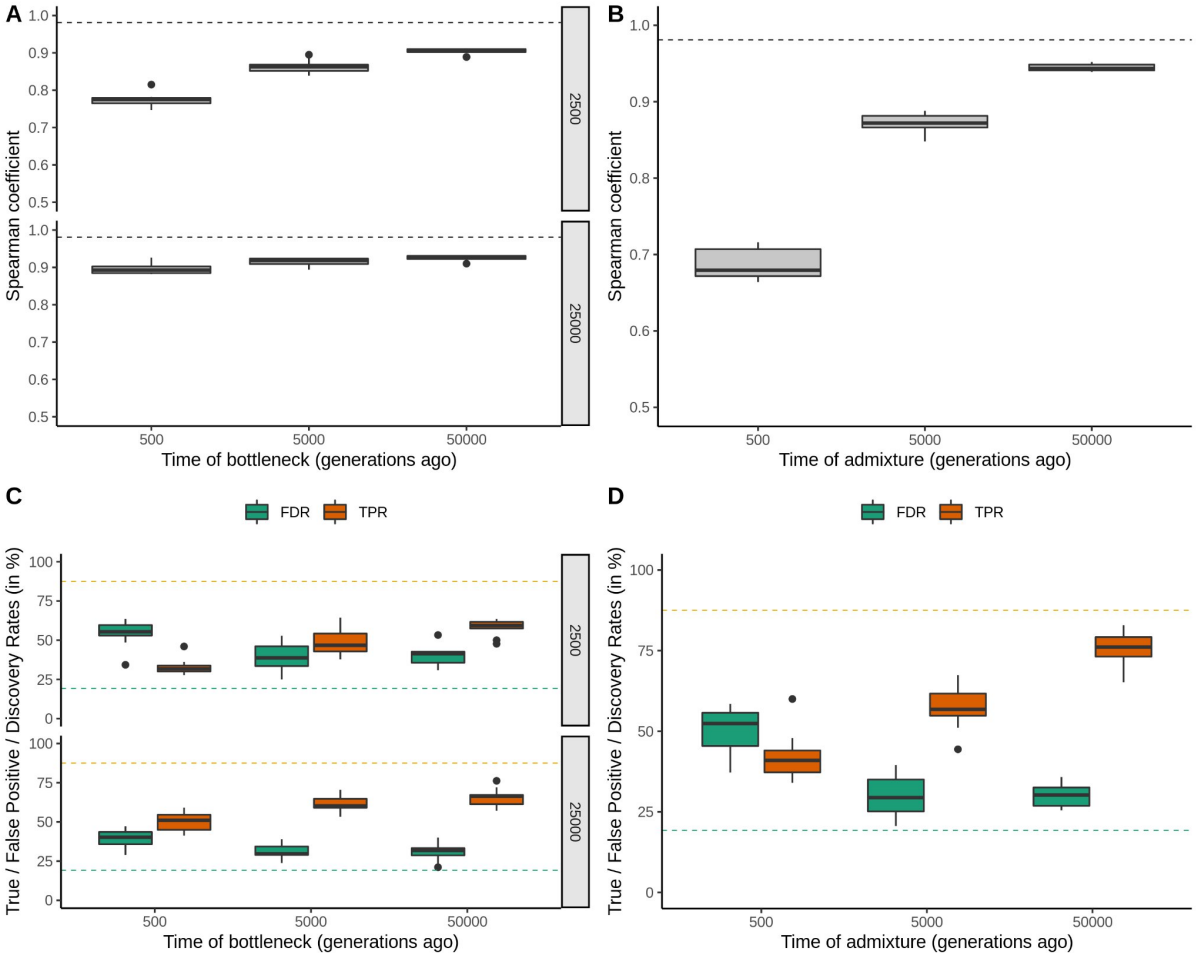

Figure 5. Influence of bottleneck (left panels A and C) and admixture (right panels B and D) events on recombination rate inference and hotspot detection. Spearman correlation coefficients between simulated and inferred landscapes are shown as a function of the age of the bottleneck event and the strength of the bottleneck (i.e. N_b, the N_e value during the 1000 generations of the bottleneck) (**A**), and as a function of the age of the admixture event (**B**). True positive (TP, in orange) and false discovery (FD, in green) rates of hotspots called using a detection threshold of 2.5, as a function of the time of the bottleneck event and the strength of the bottleneck (**C**), and as a function of the time of the admixture event (**D**). Dashed lines correspond to averaged Spearman's rho, TPR and FDR values in populations that did not experience bottleneck or admixture events.

The influence of species-specific evolutionary parameters such as the mutation and recombination rates was assessed by generating coalescent simulations under two additional underlying landscapes using a ten times higher (*i.e.* 20 cM/Mb) and a ten times lower (*i.e.* 0.2 cM/Mb) average recombination rate, and three different mutation rates (*i.e.* 10^-9, 10^-8 and 10^-7). The μ/r ratio under these 6 simulated conditions thus equalled 0.1, 1 or 10. For all conditions, Spearman's rank correlation between the mean simulated and the mean inferred landscapes was greater than 0.9, except when μ equalled 10^-9 (Spearman's rho ≃0.7,

Supplementary Table S1). An increased *μ*/*r* ratio improved the ability to detect hotspots when *r* was fixed to 10^-8, with a higher TPR (up to >80%) and a lower FDR (<5%) when *μ* increased (prop.test, p-value < 0.05, Figure 6, threshold 5 in Supplementary Figure S12). The *μ*/*r* ratio did not affect the performances the same way when *μ* was fixed to 10^-8: a *μ*/*r* ratio of 10 (r = 10^-9) yielded lower TPR (< 60%) and higher FDR (> 25%) than a ratio of 1 or 0.1, although these trends were not significant (prop.test, p-value > 0.05, Figure 6, threshold 5 in Supplementary Figure S12).

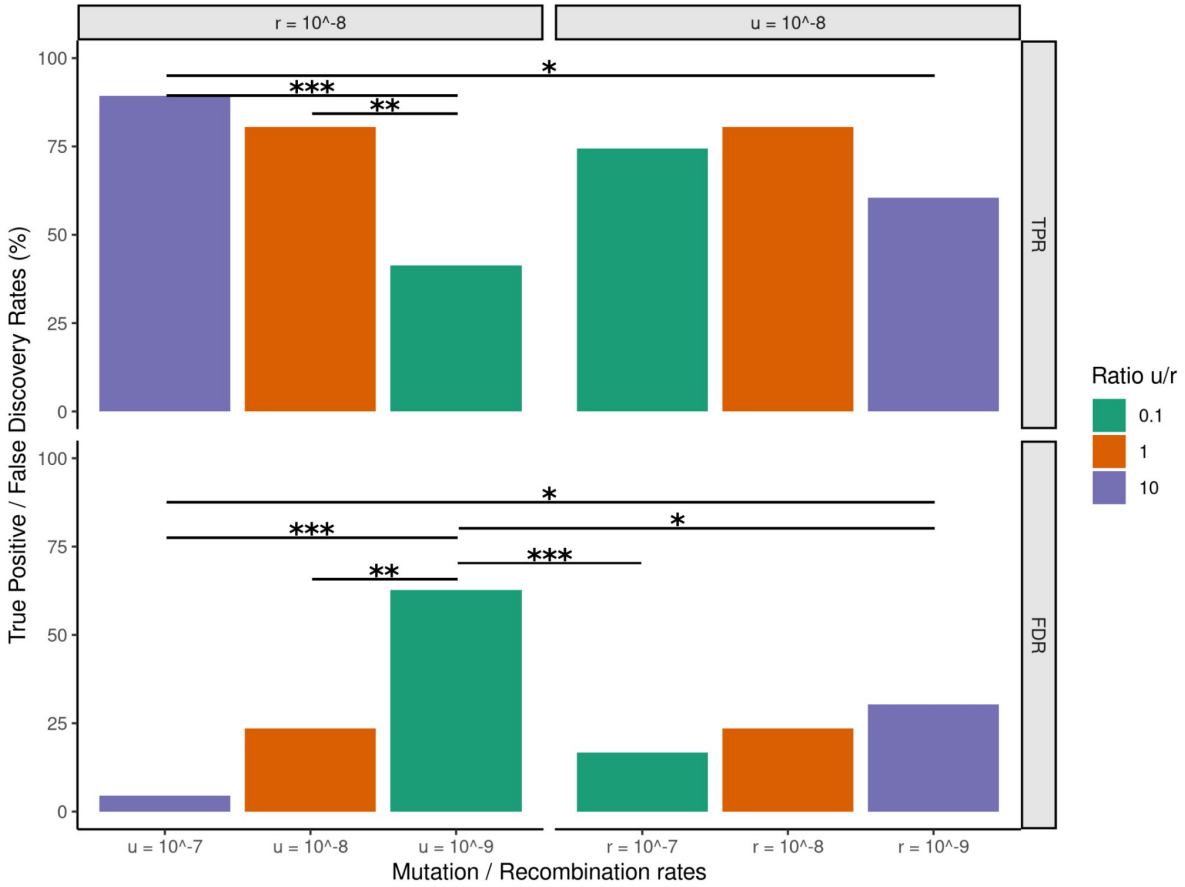

Figure 6. Influence of the u/r ratio on hotspot detection. True positive (TP, upper panel) and false discovery (FD, lower panel) rates of hotspots called using a detection threshold of 2.5. The x axis indicates values of u for *r*=10^-8 (left panels), and values of *r* for *μ*=10^-8 (right panels). Colors correspond to different values of the *μ*/*r* ratio. Asterisks show significant differences in percentages between comparisons (* prop.test p-value<0.05, **<0.01, ***<0.001).

Hotspot sharing between populations with different versus identical underlying recombination landscapes

As expected for a comparison between two populations simulated with different underlying recombination landscapes, the mean linear correlation (R^2 coefficient) between the corresponding inferred landscapes was low, between 0.012 and 0.084, and similar to the R^2 between the simulated landscapes (0.012-0.017) (Supplementary Table S2). A low percentage of shared hotspots (around 8% with a calling threshold of 2.5) occurred by chance between populations simulated with distinct underlying landscapes, with a SS of 20. Roughly similar proportions of shared hotspots were found between the corresponding inferred landscapes (with BP = 5), although these proportions were slightly overestimated (Figure 7, see Supplementary Table S2 to see all conditions). A minority of the shared inferred hotspots were TP, indicating that a non-zero fraction of truly shared hotspots is expected to be found between species with different biological recombination landscapes.

Then we compared simulated populations sharing the same underlying landscape, in order to check the ability of LDhelmet to recover similar recombination rates between populations with independent coalescence histories. The correlations between simulated landscapes were generally high for both low (R^2>0.7) and large N_e (R^2>0.9) conditions, but the correlations between inferred landscapes were much lower, with R^2< 0.3 and <0.75 for N_e = 25,000 and 250,000, respectively (Supplementary Table S2). The proportion of shared hotspots called with a threshold of 2.5 followed the same trend: it was high between simulated landscapes (>80%) and much lower between inferred landscapes (<50%) (Figure 7, Supplementary Table S2). Thus, one can expect LDhelmet to detect a moderate to low fraction of shared hotpots even between species truly sharing a common recombination landscape, depending on population size and sample size (and also hotpot definition).

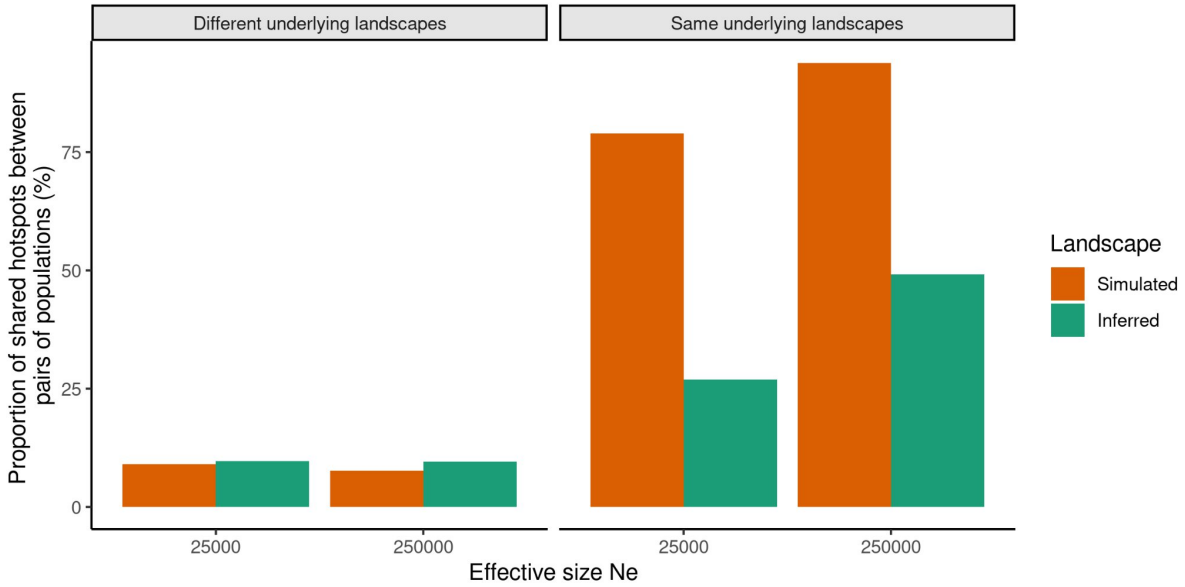

Figure 7. Expected and observed hotspot sharing between populations with different (left panel, following simulation framework from Supplementary Figure S3 A)) or identical (right panel, following simulation framework from Supplementary Figure S3 B) underlying landscapes. Mean proportion of shared hotspots between pairs of simulated (expected proportion, orange bars) and pairs of inferred (observed proportion, green bars) recombination landscapes as a function of N_e (i.e. 25,000 and 250,000, x axis). Only shown here are simulations with SS = 20, BP = 5, hotspots detection threshold = 2.5. The proportion of shared simulated and inferred hotspots for all combinations of parameters and for populations sharing or not the same underlying landscape are shown in Supplementary Table S2.

Populations simulated with the same underlying landscape that underwent a bottleneck or an admixture event also showed reduced hotspot sharing between inferred landscapes, as compared to truly shared hotspots between simulated landscapes. The proportion of shared hotspots between pairs of simulated landscapes was similar to constant-size populations and did not vary substantially according to the time or the strength of the demographic event, while the proportion of shared inferred hotspots decreased for younger events. This proportion was overall lower than for constant-size populations, but it sometimes reached, or even exceeded, the constant-N_e reference when events were ancient (Supplementary Figure S13 G, H).

417 # Discussion

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419 ## *Inferred LD-maps should be interpreted with caution*

420 Inference methods based on linkage disequilibrium provide an attractive way to
421 characterise genomic recombination landscapes from sequence data. As such, they promise
422 to become increasingly popular in empirical studies of eukaryotes. However, their ability to
423 accurately reproduce real recombination landscapes has not been specifically evaluated. It
424 should be recalled, however, that LD-based recombination maps are inferences, not
425 observations; biases and uncertainty must be quantified and taken into account when it comes
426 to interpreting the results. Here, we modelled the biological characteristics of a particularly
427 well-documented recombination landscape, that of humans, as a basis for assessing the
428 impact of methodological and species-specific demographic and evolutionary parameters on
429 the performance of the LDhelmet method. Our results send a message of caution regarding
430 the reliability of reconstructed recombination maps and hotspot location.

431 Indeed, we show that the recombination landscapes inferred with LDhelmet differ from
432 real landscapes, sometimes substantially, with Spearman rank correlation between simulated
433 and inferred 2.5 kb windows sometimes getting as low as 0.7 (Figure 2A, Supplementary
434 Figure S11A). Hotspot detection is a particularly tricky and error-prone task: while up to 85%
435 of true hotspots can be detected in the most favourable situations (N_e = 250,000, SS = 20, BP
436 = 5, threshold = 2.5), the FDR ranged from 19% to 82% (Figure 3, Figure 4B and C,
437 Supplementary Figure S9) according to the type of data and parameters used, meaning that
438 in many cases a majority of the detected hotspots are incorrect calls. These discrepancies
439 lead to a reduction in the apparent overlap in hotspot location between species/populations
440 with identical recombination landscapes, while in turn inflating apparent hotspot sharing in
441 populations with divergent landscapes (Figure 7, supplementary Table S2). These results
442 were obtained with recombination landscapes of simulated populations with constant effective
443 sizes, and with perfectly phased data. In reality, empirical data needs to be phased, and the
444 phasing process can be prone to errors. Our analyses suggest that the typical error rate of
445 statistical phasing methods such as Shapeit4 is relatively low (~ 6.7% in our simulations) and
446 only marginally affects the performance of LDhelmet in terms of sensitivity and hotspot sharing
447 (Supplementary Figure S10), which is quite reassuring. Moreover, studied populations often
448 hide complex demographic histories that are known to impact the power to correctly infer
449 recombination rates (Dapper and Payseur 2018, Samuk and Nook 2021). We showed that
450 recent bottleneck and admixture events tend to decorrelate simulated and inferred
451 landscapes, decrease the TPR and increase the FDR, thus increasing the difficulty to call

452 shared hotspots between populations sharing the same underlying landscape (Figure 5,
453 Supplementary Figure S13). The significant impact of non-equilibrium demographic histories
454 illustrated by our simulations provides additional motivation to characterise these histories in
455 comparative studies of recombination landscapes. These results also provide a qualitative
456 assessment of the impact of linked selection, whose effects may be similar to a local reduction
457 in N_e (i.e. purifying selection), or to the maintenance of anciently diverged alleles (i.e. balanced
458 selection, possibly involving structural variants such as chromosome inversions). If neglected,
459 these effects might mislead biological interpretations regarding the evolutionary conservation
460 of recombination maps.

461 In a study of the short time-scale dynamics of recombination landscapes based on
462 LDhelmet, Shanfelter et al. (2019) found only 15% of shared hotspots between two recently-
463 diverged populations of threespine stickleback. A greater overlap in hotspot location was *a*
464 *priori* expected given that this species lacks a functional PRDM9 protein, which is responsible
465 for the rapid turnover of recombination landscapes in mammals (Axelsson et al. 2012; Paigen
466 and Petkov 2018). The authors suggested that a new mechanism of recombination hotspot
467 regulation, different from the two already described in the literature, might be at play in this
468 teleost species. In the light of our results, however, one cannot exclude that the strong
469 divergence between the two reconstructed landscapes is due to a lack of power of the method
470 in the first place. While the sample size of both fish populations was at least 20 individuals, θ
471 was about 0.002, similar to our simulated conditions with a low N_e. Under these conditions, a
472 high FDR and a low proportion of shared hotspots can be expected even if the true underlying
473 maps are identical (Supplementary Figure S9, Supplementary Table S2).

474 It should be recalled that real data sets typically carry less signal and more noise than
475 simulated data sets, meaning that our assessment of the reliability of LDhelmet might be an
476 overoptimistic one. In particular, our data sets are immune from sequencing errors or mapping
477 errors, all of which presumably make the problem of recombination map inference an even
478 harder one.

479

480 ***Guidelines for population-based inference of recombination maps***

481 Our study revealed that whatever the parameters used, the inference of recombination
482 rates by LDhelmet is more reliable for species with large as compared to small effective
483 population size (Figure 4, 7, Supplementary Figure S9, S11). This might be expected since
484 long-term N_e determines the amount of nucleotide diversity ($\theta=4N_e\mu$, Watterson 1975), so that
485 a higher N_e results in a higher SNP density and a finer scale characterization of the
486 recombination rate variation along the genome. Moreover, a higher effective size greatly

487 corrects the general tendency of LDhelmet to overestimate the ρ value, especially for low and
488 high recombination rates (Supplementary Figure S6, Singhal et al. 2015; Booker et al. 2017).
489 Thus, when studying species with heterogeneous effective population sizes in nature, it is
490 recommended to select populations with the largest N_e, for which genetic diversity is greater.
491 The question is then: how to obtain a good-quality recombination map when dealing with low
492 N_e species? The sampling effort also determines, to a lesser extent, the polymorphism level
493 of the dataset (Supplementary Figure S5), improving the accuracy of the inference (Figure 2,
494 3 Supplementary Figure S9, S11). A sample size of 20 is recommended based on our
495 simulations. Moreover, as previously mentioned (Chan et al. 2012; Singhal et al. 2015), the
496 block penalty parameter of LDhelmet, which determines the resolution level of the inferred
497 landscape, also influences the length of the inferred map (*i.e.* a higher BP tends to mitigate
498 the tendency of LDhelmet to overestimate the map length) and the number of detected
499 hotspots (Supplementary Figure S4, S8). A small BP, that allows more fine-scale changes in
500 the inferred ρ value, should be used to detect recombination hotspots. The ability of LDhelmet
501 to faithfully reflect the fine-scale variation of real recombination landscapes is of great
502 importance when it comes to detecting recombination hotspots. To this purpose, the threshold
503 used to decide which region is defined as a "hotspot" is a key parameter that determines the
504 level of detection stringency. If the chosen value is not appropriate, LDhelmet will detect false
505 positives while also missing true hotspots (Supplementary Figure S9). This threshold should
506 thus be adapted to the species studied, using a less stringent threshold in species with lower
507 genome-wide average recombination rate.
508 Other intrinsic biological variables influence the ability to produce a faithful
509 recombination map, such as the μ/r ratio, which in part determines the power to measure ρ at
510 a fine-scale. The among-species variations in genome-average recombination rate r is well
511 documented, ranging from 0.01 to 100 cM/Mb in animals and plants, with vertebrate taxa
512 displaying an average r around 1 cM/Mb (Stapley et al. 2017). As previously mentioned, high
513 and low recombination rates tend to be overestimated by LDhelmet, thus the average r of the
514 studied species is obviously a key parameter to account for. The mutation rate μ also has a
515 key impact on the performance of LDhelmet, since ancestral recombination events can only
516 be detected if properly tagged by flanking mutations. The variation in μ across taxa, and
517 consequently the ratio of μ/r, are much less well known than the variation in r. This ratio, which
518 does not depend on the effective size of the population, is about 1 in humans, which means
519 that two recombination events are separated by one mutation on average. A ratio in favour of
520 mutations ($\mu/r > 1$) will improve the signal, increasing the TP rate and reducing the FD rate
521 (Figure 6, Supplementary Figure S12). But ultimately the performance of LDhelmet is

522 conditioned by *r*, as low *r* values provide less power to detect the recombination events, even
523 with μ/r = 10. Thus, the mutation to recombination rate ratio is crucial to build a non-biased
524 recombination map. When studying a species for which it appears that this ratio is not
525 favourable, a high rate of false positive hotspots is expected from the inferred population
526 recombination landscape (Figure 6), making it difficult to compare maps between closely
527 related species in a meaningful way.
528

529 ***Limitations***

530 The aim of our study was to determine the limits of LD-based methods in inferring
531 biologically realistic recombination landscapes. For this purpose, we used the Pratto et al.
532 (2014) ChIP-seq DMC1 data set to build human-like recombination landscapes including both
533 broad and fine scale variation, reflected by the presence of numerous recombination hotspots
534 of different intensities (Supplementary Figure S2, Myers et al. 2005, 2006; Pratto et al. 2014).
535 We therefore assumed that the distribution of DSB reflects the distribution of crossing overs,
536 which is not true for sure. For instance, hotspots were here placed without taking into account
537 the existence of genomic features that correlate with the recombination rate, such as genes
538 and promoter-like regions, GC-rich regions, CpG islands, and polymorphic regions, which can
539 explain why a very intense and narrow hotspot is never found within a region of near zero
540 recombination. The sensibility of LD-based methods with respect to this architecture was not
541 tested. We did not take into account the effect of gene conversion on the dissipation of LD in
542 high-recombining regions. While recent methods aim to distinguish between crossing-over
543 (CO) and non-crossing-over (NCO) events (heRho, Setter et al. 2022), they do not (yet)
544 account for the small-scale heterogeneity of recombination rates, and so are not really
545 applicable when it comes to differentiate hotspots and NCO. Our simulated data were perfectly
546 polarised, without missing or low-quality genotypes, which can't be the case when dealing
547 with empirical data. We simulated phasing errors in order to assess the robustness of
548 LDHelmet to this problem. However, we estimated the phasing error rate of Shapeit4 from our
549 simulated data which lack most of the biases found in empirical data, thus probably
550 underestimating the typical phasing error of this method.

551 Finally, we don't know if these simulated landscapes are representative of the diversity
552 of recombination landscapes that exist in the living world (*i.e.* PRDM9-dependent vs
553 independent landscapes, hotspot-free landscapes…). Indeed, it is likely that the high
554 complexity of the human recombination landscape is not a universal feature in the animal
555 kingdom. Singhal et al. (2015) used LDhelmet for building the recombination map in two
556 species of birds, the zebra finch and the long-tailed finch, that lack a full-length PRDM9 gene

557 copy and diverged about 2.9 Myr. The sample size for both populations was about 20
558 individuals, and θ (~ 0.01) was about ten times higher than in apes or the threespine
559 stickleback (Shanfelter et al. 2019), thus corresponding to our high N_e simulation conditions.
560 Singhal et al. (2015) found 73% of shared hotspots between the two finch species, which is
561 a higher rate of hotspot sharing than in any of the scenarios we simulated. The median
562 estimated recombination rate was 0.14 cM/Mb in both species of finch, which is seven times
563 lower than the genome-wide average recombination rate in humans (about 1 cM/Mb, Jensen-
564 Seaman et al. 2004). Combined with the strong polymorphism in those species, we may
565 suppose that birds possess less complex recombination landscapes than humans or
566 compared to what we simulated, which might explain why LDhelmet recovered such a high
567 percentage of shared hotspots in this study.
568
569

570 ## Conclusion

571
572 In the past few years, we have seen a growing interest in recombination rate estimation
573 in functional and evolutionary genomics. Indirect, LD-based approaches raise methodological
574 challenges that are addressed by sophisticated methods such as LDhat or LDhelmet, the
575 reliability of which is still poorly characterised. Our study provides guidance to users of these
576 methods based on the characteristics of their species, and calls for caution when it comes to
577 interpreting fine-scale differences in recombination rates between species. Extending this
578 approach to a more diverse set of underlying recombination landscapes would help
579 characterise further the reliability of these methods and their range of applicability across data
580 sets and taxa.
581
582

583 ## Materials and Methods

584
585 Our approach separately considers three different layers of information that are
586 involved in the study of recombination landscapes (Figure 1). The first layer that we call the
587 "underlying" recombination landscape corresponds to the true biological distribution of
588 recombination rate (*r*) across the considered genome. We here used experimental
589 measurements from human studies to model and generate the "underlying" landscapes. The
590 second layer, the population recombination landscape, describes the genomic location of
591 recombination events that occurred during the history of the sample. We used coalescent

592 simulations to produce these population recombination landscapes, thereafter called
593 "simulated" landscapes. Simulated landscapes differ from the underlying landscape due to
594 the stochasticity of the coalescent process, which is inversely proportional to N_e. The third
595 layer, called the "inferred" landscape, corresponds to the output of LDhelmet, *i.e.* an estimate
596 of the population recombination rate between adjacent SNPs. In total we generated five
597 independent replicates of underlying landscapes, and for each of them up to 10 simulated and
598 inferred landscapes under various demographic scenarios (Figure 1, Supplementary Figure
599 S3A).
600

601 ***Underlying landscapes***

602 Underlying recombination landscapes were first generated to reproduce the features
603 of the human recombination landscape. These include large-scale variation in the mean
604 background recombination rate and fine-scale variation reflecting the presence of hotspots
605 with varied intensities. Meiotic DSB are the major determinant of crossing over (CO) location
606 along the genome (Li et al. 2019; Pratto et al. 2014). We used the high-resolution map of
607 meiotic DSB obtained using ChIP-seq DMC1 in 5 non-related human genomes (Pratto et al.
608 2014) to define the genome-wide distribution of recombination rates in our simulations. The
609 five individuals analysed in Pratto et al. (2014) carried different PRDM9 genotypes totalizing
610 about 40,000 hotspots per individual, with distinct genotypes having different sets of DSB
611 hotspots. For each individual, a gamma distribution was fitted to the empirical distribution of
612 hotspot intensity measured by ChIP-seq DMC1 with the R package *figdistribplus* (Delignette-
613 Muller and Dutang 2015). Extreme ChIP-Seq intensity values (>500) lying above the 97.5th
614 quantile and likely representing technical artefacts were removed. Remaining values were
615 rescaled to 0-100, so as to transform ChIP-Seq intensity values into quantities reflecting the
616 range of recombination rates reported in cM/Mb across the human genome (McVean et al.
617 2004, 2005). This conversion assumed a linear relation between DMC1 activity and CO
618 frequency (Pratto et al. 2014). We then removed null values and replaced them with small but
619 non-null values (0.001), so that the genome-wide mean recombination rate equaled a target
620 average (*e.g.* 1 cM/Mb). A Gamma distribution was fitted to these transformed empirical values
621 separately for each of the 5 individuals, before averaging shape and scale parameters across
622 individuals. Targeted genome-wide average value was set to either 1 cM/Mb or 3 cM/Mb,
623 respectively reflecting the average centro-chromosomal and subtelomeric rates in humans.
624 Underlying landscapes of 1 Mb length were built by randomly drawing independent
625 recombination rate values from the fitted distribution and assigning these to non-overlapping
626 windows of 500 pb. Values in the first 500 kb were drawn from a distribution of mean 1 cM/Mb,

627 while values in the last 500 kb were drawn from a distribution of mean 3 cM/Mb. Our approach
628 thus mimics both the large scale variation in recombination rate existing in humans (Nachman,
629 2002; Myers et al. 2005; Buard and de Massy 2007; Pratto et al. 2014) and the nearly absence
630 of recombination events outside hotspots (96% of CO occur in hotspots in mice, Pratto et al.
631 2014; Li et al. 2019). In total, 5 underlying landscapes were generated (mean r = 2 cM/Mb),
632 which can be considered as independent replicates driven from the same distribution (*i.e.*
633 regions from different chromosomes of the same species, or orthologous chromosome region
634 from closely related species).
635

636 ***Simulated landscapes***

637 For each of the 5 underlying landscapes, 10 simulated landscapes were generated via
638 coalescent simulations using the program MSPRIME (v0.7.4, Kelleher et al. 2016), varying
639 the constant effective population size (N_e= 25,000 or 250,000) and the sample size (SS=10
640 or 20) and setting the mutation rate to μ = 10^-8. These sets of simulation parameters were
641 combined with three values of the Block Penalty (BP) parameter of the LDhelmet program
642 (see below), resulting in twelve conditions tested. For each combination of parameters, ten
643 population samples were simulated, to generate independent replicates of the coalescent
644 history (Figure 1A, Supplementary Figure S3).
645 Populations undergoing bottleneck and admixture events were also simulated with
646 MSPRIME, using one of the five underlying landscapes. The sample size parameter was set
647 to 20, and the N_e of the ancestral and present-day population was set to 250,000. The
648 simulated bottleneck scenarios varied according to the timing of the bottleneck event (t_b = 500,
649 5,000, 50,000 generations ago) and the N_e of the population during the bottleneck (N_b = 2,500,
650 25,000). The duration of the bottleneck was fixed to 1,000 generations. The admixture
651 scenarios varied in terms of the timing of the admixture event between the derived populations
652 1 and 2 (t_a = 500, 5,000, 50,000 generations ago). The time of split of the ancestral population
653 (0) into two derived population 1 and 2 (t_{split}) was set to 10*N_e generations. For each bottleneck
654 and admixture scenario, ten replicated populations were simulated (Figure 1A,B).
655 A VCF file was generated with MSPRIME for each simulated population (Figure 1A,
656 Supplementary Figure S3), which contains the genotypes of variants that segregate in the
657 population sample consisting of 2n sequences (with n being the number of diploid samples)
658 following the given underlying recombination landscape.
659 The impact of phasing errors on the inference of recombination rates was also
660 assessed. Two replicate VCFs, simulated using the same underlying landscape, were
661 manually dephased and then phased using Shapeit 4.2.2 (Delaneau et al., 2019). The phasing

662 error rate was computed using the --switch-error option of VCFtools 0.1.17 (Danecek et al.
663 2011 ; using the original phased VCF as a reference). Phasing error rates of 2, 4, 6, 8 and
664 10% between heterozygous positions of the two original phased VCFs were then randomly
665 generated, to produce five independent replicate VCF for each phasing error rate value.
666

667 ***Inferred landscapes***

668 Recombination rates were estimated for each of the simulated samples with LDhelmet
669 (v1.19, Chan et al. 2012, Figure 1). Briefly, LDhemet uses phased sequence data to infer the
670 ρ parameter locally, using likelihood computation between pairs of SNPs and then averaging
671 over 50 consecutive variants to obtain a composite likelihood. The ρ parameter is inferred with
672 a reversible-jump Markov Chain Monte Carlo algorithm using a step function applied to every
673 window of 50 consecutive SNPs and determined by three parameters: the number of change-
674 points, the locations of changes, and the recombination rate value of each constant fragment
675 between two changes. We used VCFtools 0.1.17 (Danecek et al. 2011) and the vcf2fasta
676 function of vcflib (https://github.com/vcflib/vcflib) to convert the SNP data obtained from
677 MSPRIME simulations into the input format to LDhelmet, consisting of FASTA sequences of
678 each individual haplotype. Ancestral states defined as the reference allele of each variant
679 were also used as inputs. Each simulated replicate was analysed with LDhelmet using the
680 following parameters. The haplotype configuration files were created with the find_conf
681 function using the recommended window size of 50 SNPs. The likelihood look-up tables were
682 created with the table_gen function using the recommended grid for the population
683 recombination rate (ρ/pb) (*i.e.* ρ from 0 to 10 by increments of 0.1, then from 10 to 100 by
684 increments of 1), and with the Watterson $\theta = 4N_e\mu$ parameter corresponding to the condition
685 analysed. The Padé files were created using 11 Padé coefficients as recommended. The
686 Monte Carlo Markov chain was run for 1 million iterations with a burn-in period of 100,000 and
687 a window size of 50 SNPs. An important parameter to LDhelmet is the block penalty (BP),
688 which determines the number of change-points, and thus the variance of the inferred
689 recombination rates at a fine scale (*i.e.* smaller block penalty values correspond to a lower
690 penalty for background rate changes, and thus generate more heterogeneous recombination
691 landscapes). For each simulated combination of N_e and SS for populations with constant size,
692 the block penalty was set to either 5, 10 or 50, and for the simulated populations undergoing
693 bottleneck or admixture event, as for the ”phase-error” datasets, the block penalty used was
694 set to 5. Finally, the population recombination rates between each SNP pair were extracted
695 with the post_to_text function, and were reported in $\rho=4N_er$ per pb unit.
696 The reliability of the inferences was evaluated in various ways. For each combination

697 of N_e, SS, BP parameters and demographic scenarios simulated, the inferred, simulated and
698 underlying landscapes were compared, in order to assess the ability of LDhelmet to reliably
699 infer the true biological landscape (Figure 1, Supplementary Figure S3A, and see below
700 Hotspot detection and Statistical Analysis). In order to evaluate the convergence of LDhelmet
701 inferences across replicate runs, LDhelmet was run 10 times, for the 12 combinations of the
702 parameters N_e, SS and BP, on two independently simulated VCF files from constant-size
703 populations sharing the same underlying landscape (Figure 1A, Supplementary Figure S3B).
704 Finally, the inferred recombination landscapes of pairs of populations sharing the same
705 underlying landscape were compared in order to assess the reproducibility of the LDhelmet
706 inference, *i.e.*, the expected variance between inferred maps in the absence of underlying
707 biological variation (Figure 1, Supplementary Figure S3B).
708

709 ### *Variation in the μ/r ratio*

710 To explore the influence of variation in mutation and recombination rates on the
711 inference of recombination maps, two additional underlying landscapes were generated using
712 the same procedure, this time targeting a ten times higher (i.e. 20 cM/Mb) or ten times lower
713 (0.2 cM/Mb) mean recombination rate. Then, using one of the 5 underlying landscapes ($r \sim$
714 10^-8 M/pb) and the 2 newly generated landscapes with mean r = 10^-7 and 10^-9 M/pb,
715 respectively, sets of simulations were run with a μ/r ratio of 0.1, 1 and 10. This was achieved
716 by fixing μ to either 10^-9, 10^-8 or 10^-7, while keeping a fixed N_e = 100000 and SS = 20
717 (Supplementary Table S1). For each of the tested combinations of μ and r, 10 populations
718 were simulated. These simulated landscapes were inferred with LDhelmet, using a block
719 penalty of 5.
720

721 ### *Hotspot detection*

722 Underlying and simulated landscapes were first converted into population
723 recombination rate landscapes by scaling them by $4N_e$. Underlying, simulated and inferred
724 landscapes were then smoothed at a 500 bp and 2500 bp resolution using the Python package
725 *scipy.stats*. The former corresponds to the underlying landscape resolution, and the latter to
726 a trade off between the density of segregating sites and the resolution often used in the
727 literature. For the different combinations of N_e, SS and BP of the constant-size populations
728 simulated with the 5 underlying landscapes, a mean simulated landscape and a mean inferred
729 landscape were generated by averaging recombination rates across replicates.
730 Recombination hotspots of the underlying, simulated and inferred landscapes were
731 called by comparing local vs surrounding recombination rates at each genomic window. A

732 hotspot was defined as a window of 2.5 kb with an average recombination rate either 2.5, 5
733 or 10 times higher than the 50 kb flanking regions (excluding the focal window). Hotspot
734 locations were then compared among landscapes using the same threshold values (Figure
735 1C)
736

737 ### *Statistical analyses*

738 Statistical analyses were run with R 4.0.3. The length of underlying, simulated and
739 inferred maps (*L*) was calculated at the 2.5 kb resolution using the formula:

740 $$L = \sum \quad (\rho \times win)/4Ne,$$

741 with ρ the population-scaled recombination rate, *win* the window size resolution used to
742 smooth the maps in bp, and N_e the effective size of the simulated population. Several
743 measures of the sensitivity, specificity, reliability, and repeatability of LDhelmet were
744 computed, using the mean simulated and inferred landscapes of the constant-size
745 populations, and replicates of simulated and inferred landscapes of the populations that
746 underwent bottleneck of admixture events. Spearman rank correlation coefficients were
747 calculated between the underlying and the corresponding simulated landscapes, between the
748 simulated and inferred landscapes, and pairwise Spearman coefficients among the 10
749 replicates inferred from the two simulated populations sharing the same underlying landscape.
750 True/false positive rates (TPR = TP/(TP+FN) ; FPR = FP/(FP+TN)), true/false negative rates
751 (TNR = TN/(TN+FP) ; FNR = FN/(FN/(TP+FN)), and true/false discovery rates (TDR =
752 TP/(TP+FP) ; FDR = FP/(TP+FP)) were calculated by comparing the simulated and inferred
753 landscapes. The mean pairwise linear correlation (R^2) and the proportion of shared hotspots
754 was calculated between the 5 underlying landscapes, and for each condition simulated with
755 a constant population size scenario, and for the three threshold values tested (*i.e.* 2.5, 5 and
756 10) between the simulated and inferred landscapes from the 5 different underlying landscapes,
757 as well as between the pairs of populations sharing the same underlying landscape.
758 The statistical analyses were performed using home-made R scripts available upon
759 request.
760
761

762 ## Data, script and code availability

763 The underlying landscapes, the main scripts used to generate the underlying
764 landscapes, run the simulations under the various demographic scenarios, infer the simulated
765 landscapes with LDhelmet, and call hotspots from the landscapes can be found at
766 https://doi.org/10.5281/zenodo.7657101. The Singularity container recipe built to run the

simulations is available at: https://doi.org/10.5281/zenodo.7657199. This recipe contains the installation command lines of the required programs, the scripts used for the simulations, and the five underlying landscapes used in our study. More scripts and data are available upon request.

Supplementary information

Supplementary Materials, containing Supplementary Figures and Tables are available at https://doi.org/10.5281/zenodo.7544555.

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Conflict of interest disclosure

The authors declare no conflict of interest.

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