Recent Selection Changes in Human Genes under Long-Term Balancing Selection

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Abstract

Balancing selection is an important evolutionary force that maintains genetic and phenotypic diversity in populations. Most studies in humans have focused on long-standing balancing selection, which persists over long periods of time and is generally shared across populations. But balanced polymorphisms can also promote fast adaptation, especially when the environment changes. To better understand the role of previously balanced alleles in novel adaptations, we analyzed in detail four loci as case examples of this mechanism. These loci show hallmark signatures of long-term balancing selection in African populations, but not in Eurasian populations. The disparity between populations is due to changes in allele frequencies, with intermediate frequency alleles in Africans (likely due to balancing selection) segregating instead at low- or high-derived allele frequency in Eurasia. We explicitly tested the support for different evolutionary models with an approximate Bayesian computation approach and show that the patterns in *PKDREJ*, *SDR39U1*, and *ZNF473* are best explained by recent changes in selective pressure in certain populations. Specifically, we infer that alleles previously under long-term balancing selection, or alleles linked to them, were recently targeted by positive selection in Eurasian populations. Balancing selection thus likely served as a source of functional alleles that mediated subsequent adaptations to novel environments.

Key words: **natural selection, environmental changes, out-of-Africa.**

Introduction

Natural selection drives the adaptation of populations to their environment (Darwin and Wallace 1858). Balancing selection maintains advantageous polymorphisms in populations and, as a consequence, it increases genetic diversity. This is in contrast to the reduction in diversity that results from favoring the single, most advantageous allele via positive or purifying selection. Mechanisms of balancing selection include overdominance (Allison 1956), frequency-dependent selection (Wright 1939), fluctuating selection (Gillespie 1978), and pleiotropy (Gendzekhadze et al. 2009), although when selection is old the genetic signatures of all these types of selection can be similar (Andrés 2011; Key, Teixeira, et al. 2014). The first signature is an excess of polymorphic over divergent sites. Old selection maintains the advantageous polymorphism and linked neutral polymorphisms longer than expected under neutrality (Wiuf et al. 2004; Charlesworth 2006). This results in an unusual accumulation of polymorphisms that is typically reflected in a local excess of diversity over divergence (Hudson et al. 1987), and the intensity of this signature depends mostly on the age of the balanced polymorphism (Charlesworth 2006). The second signature is a shift in allele frequencies. When a frequency equilibrium (an allele frequency that maximizes fitness in the population) exists, balancing selection maintains the selected polymorphism close to the frequency equilibrium; neutral variants also accumulate at a similar frequency due to linkage, shifting the local distribution of allele frequencies (the site frequency spectrum, SFS) toward the frequency equilibrium (Andrés 2011). For instance, if the frequency equilibrium is 0.5, the SFS is expected to show a shift toward intermediate frequency alleles close to 0.5.

Balancing selection can act for long periods of time. Some polymorphisms persist for millions of years and can even be shared among species as trans-species polymorphisms, which exist in humans (Loisel et al. 2006; Ségurel et al. 2012; Leffler et al. 2013; Teixeira et al. 2015) but are rare (Asthana et al. 2005). Most balanced polymorphisms are present in single species, with the catalog of human candidate targets of balancing selection (Andrés et al. 2009; DeGiorgio et al. 2014; Rasmussen et al. 2014) far surpassing the catalog of trans-species polymorphisms. This is because selection is rarely old and constant enough (for more than 6 My) to create trans-species polymorphisms. Within species, targets of balancing selection are classically assumed to be shared across populations, with unusually low F_{ST} values flagging such cases (Schierup et al. 2000; Bamshad and Wooding 2003; Key, Teixeira, et al. 2014). This builds on the reasonable expectation that selection that has maintained a polymorphism for millions of years (and is thus detectable on the patterns of

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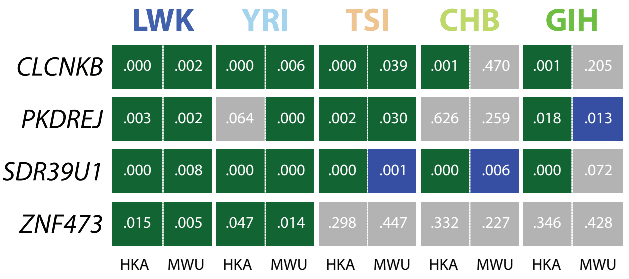

	LWK		YRI		TSI		CHB		GIH	
CLCNKB	.000	.002	.000	.006	.000	.039	.001	.470	.001	.205
PKDREJ	.003	.002	.064	.000	.002	.030	.626	.259	.018	.013
SDR39U1	.000	.008	.000	.000	.000	.001	.000	.006	.000	.072
ZNF473	.015	.005	.047	.014	.298	.447	.332	.227	.346	.428
	HKA	MWU	HKA	MWU	HKA	MWU	HKA	MWU	HKA	MWU

Fig. 1. *P* values of the neutrality tests: HKA and MWU. The cells are colored according to the 5% significance threshold: Green for balancing selection with excess of diversity (HKA) or intermediate frequency alleles (MWU); blue for positive or negative selection, with excess of low-frequency alleles (MWU). For the results of all genes, see supplementary figure S2, Supplementary Material online.

linked variation) is likely shared across populations. Yet this is not necessarily the case.

For example, there are several loci where signals of long-term balancing selection are detected in human populations of African origin, but not in populations outside of Africa (Andrés et al. 2009; DeGiorgio et al. 2014). This is an unexpected observation because the balanced polymorphisms are old and predate the out-of-Africa migration, and it raises the question of whether these population differences are explained by drift alone (e.g., during and after the out-of-Africa migration, characterized by population bottlenecks and expansions) or by changes in the selective pressure, outside of Africa, on previously balanced loci. Changes in selective pressure might be reasonable because, while the ancestors of modern humans have lived in Africa for millions of years (long adapting to the environment), the colonization of the rest of the world happened only in the last 50,000 years (Gravel et al. 2011). These migrating human populations encountered new environments, and they experienced novel, local adaptations (Cavalli-Sforza 1966; Lewontin and Krakauer 1973; Akey et al. 2004; Coop et al. 2009; Pickrell et al. 2009; Fumagalli et al. 2011) or changes in the strength of selection (Key, Peter, et al. 2014).

Here we aim to explore a model of adaptation where balancing selection turned into positive selection. We purposely focus on a small number of genes in order to perform detailed analyses and computationally intensive inferences that allow us to distinguish between competing models of adaptation. We selected a number of genes previously identified (Andrés et al. 2009) as showing African-specific signatures of balancing selection, as they are prime candidates for having experienced shifts in selective pressure outside of Africa. We analyzed six human populations and confirmed both the African signatures of long-term balancing selection and the absence of these signatures in Eurasians. We investigated the probability that population differences are due to demography or to changes in selective regime, showing that a model where selection changed after the out-of-Africa migration favoring an existing or linked new variant best explains the patterns of genetic variation in three genes. This reveals a shift in selective pressure in previously balanced loci that created genetic differences among human populations.

Results

Genes with Signatures of Balancing Selection in Africa

We initially investigated 14 genes (supplementary table S1, Supplementary Material online) previously shown to have significant signatures of long-term balancing selection in Africa only (Andrés et al. 2009). In addition, we analyzed 49 "control" loci (old, processed pseudogenes) as our proxy for neutrality (see Materials and Methods and supplementary table S1, Supplementary Material online). We produced a combination of Sanger and Illumina-derived sequence data for a total of near 230 kb, and obtained high-quality polymorphism data in the coding and adjacent noncoding regions of these genes from five human populations (each $N = 30$): Yoruba (YRI) and Luhya (LWK) from Africa, Toscani (TSI) from Europe, and Gujarati (GIH) and Han Chinese (CHB) from Asia. We identified signatures of balancing selection with two neutrality tests: Hudson–Kreitman–Aguadé (HKA) (Hudson et al. 1987) and Mann–Whitney *U* (MWU) (Nielsen et al. 2009). These tests detect departures from the neutral expectation in the density of polymorphisms and in the SFS, respectively (see Materials and Methods), and significant signatures for both tests are expected only under long-term balancing selection. At the 5% *P* value cut-off, four genes (*CLCNKB* [chloride channel, voltage-sensitive kb], *PKDREJ* [polycystic kidney disease and receptor for egg jelly], *SDR39U1* [short-chain dehydrogenases/reductases family 39U member 1], and *ZNF473* [zinc finger protein 473]) show both significant excess of polymorphism and significant shifts toward intermediate-frequency alleles in African populations (fig. 1). We note that these signatures are not due to mapping errors or partial duplications (supplementary mate rial section 2, Supplementary Material online). All four genes thus display strong signatures of balancing selection in both African populations and, conservatively, we focused only on these for the remainder of the study.

Outside of Africa, two of the four genes (*CLCNKB* and *PKDREJ*) display significant signatures of long-term balancing selection in the European TSI, but no gene shows similar signatures in the Asian populations (GIH and CHB). The differences between African and non-African populations are not surprising because these genes were originally selected for their discordant signatures among human groups (Andrés et al. 2009). But they confirm that these loci are adequate for our purposes.

Excess of Polymorphism

As discussed above, all four genes have unexpectedly high levels of polymorphism in African populations (HKA test, in fig. 1). To better understand the distribution of single nucleotide polymorphisms (SNPs) in the genomic region, we extended the analysis to a larger genomic region (400,000 bp centered on each gene) and computed, in sliding windows, the ratio of "polymorphism to divergence" (PtoD, the number of SNPs divided by the number of substitutions to the chimpanzee genome). PtoD is thus a measure of diversity that controls for local heterogeneity in mutation rate and grows with older local coalescent times (Hudson et al. 1987;

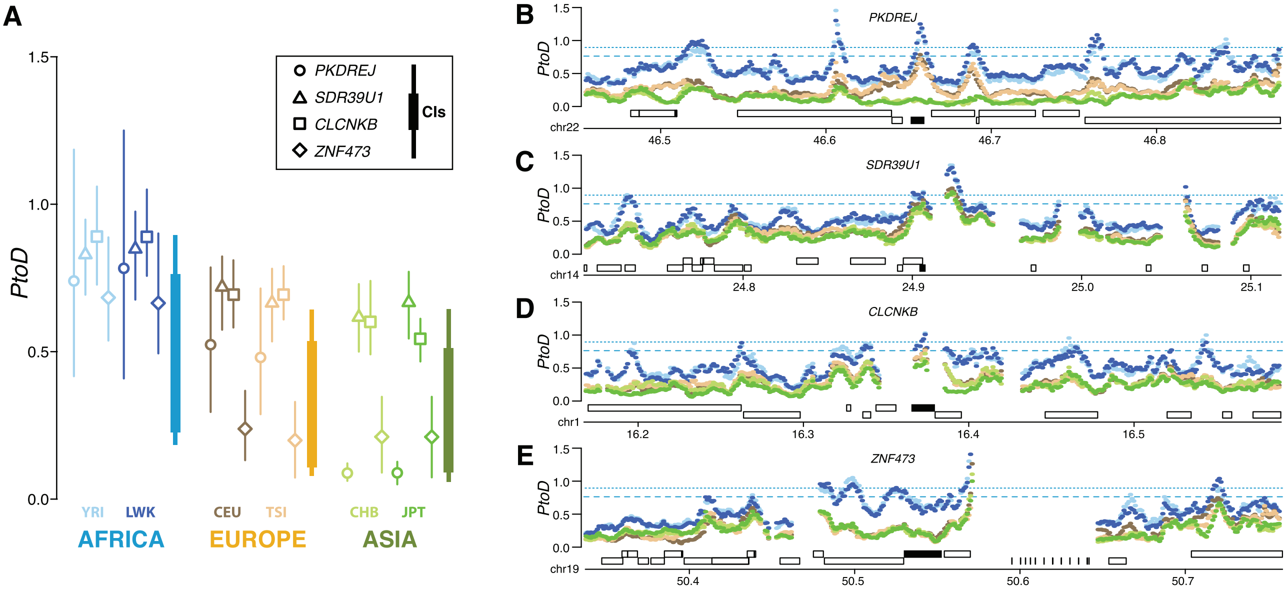

Fig. 2. PtoD in 1000 Genomes populations. We performed the analysis in windows of 10,000 bp sliding by 100 bp. Windows with more than 40% of the sequence not passing our quality filters were excluded. (*A*) The ranges of PtoD (*y*-axis) across all windows in each gene are shown as vertical lines, with the gene symbol placed in the average PtoD. For each continent, we also show the expectation under neutrality as the 95% and 99% CIs (thicker and thinner vertical lines, respectively), calculated from 10,000 neutral simulations of the human demography (Gravel et al. 2011) using 1×10^{-8} per site per generation as average mutation and recombination rates. (*B–E*) PtoD along 400,000 bp region of the chromosome (*x*-axis) centered on each candidate gene. The dots are colored according to population (as in *A*); the dotted and dashed blue lines mark the 95% and 99% CIs of expected PtoD for Africans (they are a conservative representation in non-Africans, which have lower levels of genetic diversity). The rectangles on the *x*-axis represent genes in positive (above) and negative (below) orientation, with the candidate genes in black.

McDonald 1998). PtoD was computed in six populations from the 1000 Genomes (see Materials and Methods), and compared with values generated via neutral coalescent simulations (fig. 2). As expected, all genes show high SNP density in Africans (fig. 2A), although the excess does not reach significance in *ZNF473*. Non-African populations also show overall high mean PtoD, with the exception of *PKDREJ* in East Asians and *ZNF473* in TSI, CHB, and JPT, which show significantly low PtoD mean values (fig. 2A).

When PtoD is investigated along each genomic region (fig. 2B–E), in African populations all genes contain peaks of PtoD above the 95% confidence interval (CI) of the neutral expectation, with *PKDREJ*, *SDR39U1*, and *CLCNKB* having peaks above the 99% CI. The highest local PtoD peak always falls within or very close to the gene, except in *ZNF473* (fig. 2E). In non-African populations, when peaks of high diversity exist they overlap those in Africans (fig. 2B–*E*). The observed reduction of diversity in *PKDREJ* for Asians (fig. 2B) is also in agreement with a previous study (Pickrell et al. 2009).

Alleles at Intermediate Frequency in Africa and at Low or High Frequency Out-of-Africa

We next investigated the distribution of derived allele frequencies in our set of four genes. The density of alleles at intermediate frequency is higher in Africans than in non-Africans (supplementary figs. S5 and S6, Supplementary Material online). To compare the SFS across populations, we used the joint SFS, which shows the frequency of every polymorphic allele in two populations (fig. 3A). In the neutral control regions, allele frequencies correlate well among populations: Pearson's correlation coefficient $r^2 = 0.96$ in the comparison between two African populations and $r^2 > 0.81$ in the comparisons between African and non-African populations (table 1).

In the four genes, allele frequencies are also similar between the two African populations (Pearson's correlation $r^2 = 0.94$; table 1) and among all non-African populations (Pearson's $r^2 > 0.89$; table 1 and supplementary fig. S7B, Supplementary Material online). In fact, the correlation between pairs of non-African populations is 4–10% higher in the four genes than in the controls (a significant difference, all Fisher *r*-to-*z* transformation two-tailed $P < 0.001$), and it is also 8% higher for these alleles than for non-genic alleles in the 1000 Genomes data set (supplementary table S4, Supplementary Material online). When we contrast African and non-African populations (fig. 3B), though, the correlation in allele frequencies between any African and any non-African population is significantly lower in the four genes than in the neutral controls (all Fisher *r*-to-*z* transformation two-tailed $P < 0.005$). This corresponds to a 6–26% weaker correlation between Africans and non-Africans in the four genes than in the neutral controls (in the 1000 Genomes data set this correlation is 30–35% weaker, while genome-wide there is no difference between genic and non-genic alleles; supplementary table S4, Supplementary Material online).

To understand the basis of these population differences, we focus on the alleles at intermediate frequency in Africa, which are most interesting from the standpoint of balancing selection. We define alleles with derived allele frequency $0.20 \leq \text{DAF} \leq 0.80$ in Africa as "intermediate in Africa alleles" (*iA-alleles*). We then ask what proportion of them has very low ($\text{DAF} \leq 0.05$) or very high ($\text{DAF} \geq 0.95$) frequency in

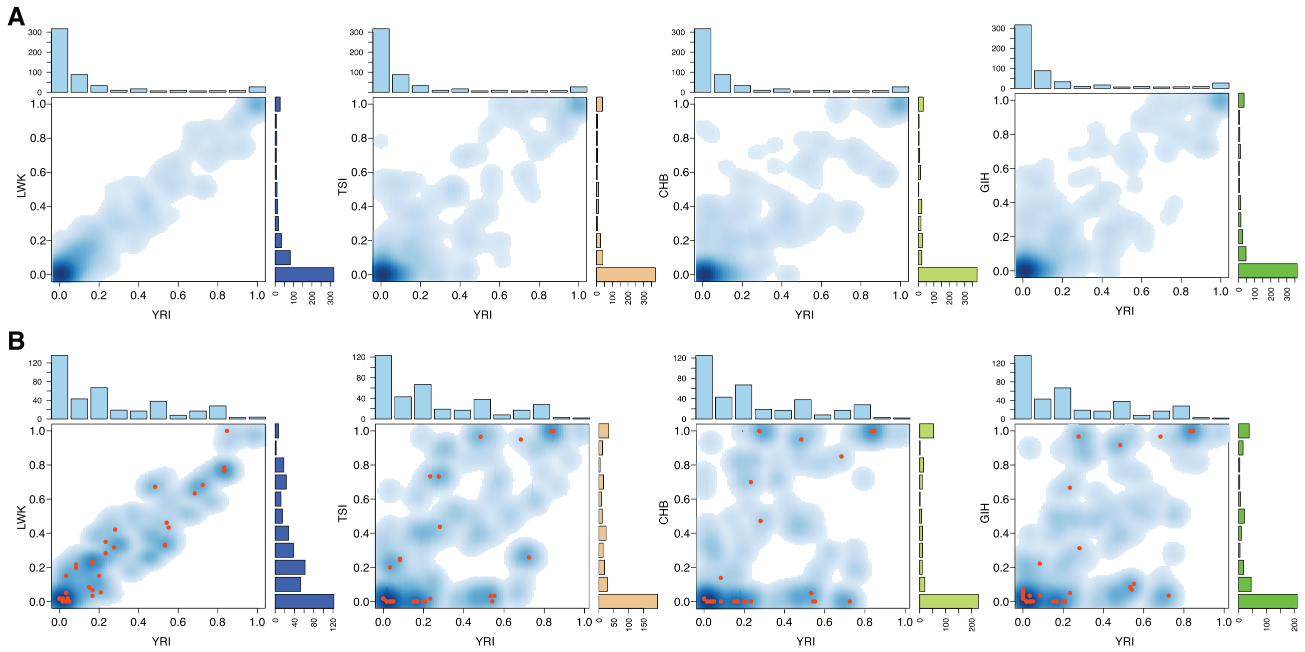

Fig. 3. Two-dimensional SFS. (*A*) SNPs from the control regions and (*B*) SNPs from the four candidate genes combined, where red dots are nonsynonymous SNPs. The histograms on the top and right side of the scatterplot are the SFS for the *x* and *y* population. The representation of the scatter plot is colored according to the SNP density. Because the SFS in each population includes sites that are monomorphic but segregate in the other population, the excess of intermediate frequencies in the candidate genes is not as evident as in classical SFS plots (see supplementary fig. S5, Supplementary Material online, for the one-dimensional SFS for each population and supplementary fig. S6, Supplementary Material online, for the SFS for each gene). Supplementary figure S7, Supplementary Material online, shows the other pairwise population comparisons, which are very similar.

Table 1. Correlation of Allele Frequencies between Populations in Genes and Controls.

	LWK	YRI	TSI	CHB	GIH
LWK	—	0.96	0.87	0.84	0.86
YRI	0.94	—	0.85	0.81	0.83
TSI	0.81	0.71	—	0.86	0.95
CHB	0.73	0.60	0.89	—	0.88
GIH	0.78	0.68	0.96	0.95	—

Note.—The values above and below the diagonal show the correlation coefficients (as Pearson's r^2) for SNPs in the control and the four candidate genes, respectively. All values are highly significant ($P < 1 \times 10^{-6}$). In each pairwise comparison, we consider only sites that are polymorphic in at least one of the two populations.

non-Africans; we call these "intermediate in Africa different Out-of-Africa alleles" (*iAdO-alleles*). In control regions, on average only 18.4% of *iA-alleles* are *iAdO-alleles*; in the four genes this proportion is 52.8% (table 2). This corresponds to an average 3.3-fold increase in *iAdO-alleles* in the genes when compared with neutral regions (table 2), a significant enrichment (all exact binomial tests $P < 0.008$). Therefore, the four genes are enriched in alleles at intermediate frequency in Africa but at high or low frequency outside of Africa. Many of these SNPs are among the most differentiated alleles between African and non-African populations in the 1000 Genomes data set, although only SNPs in *PKDREJ* remain significant after accounting for their intermediate frequency in Africa (fig. 4 and supplementary table S5, fig. S9, and section 3, Supplementary Material online). Importantly, these SNPs also drive the reduced allele frequency correlation among populations (fig. 3 and fig. 5).

Table 2. Proportion and Number of *iA-alleles* and *iAdO-alleles*.

		$0.20 \leq$ DAF ≤ 0.80		$0.25 \leq$ DAF ≤ 0.75	
Genes		YRI (155)	LWK (199)	YRI (131)	LWK (144)
	TSI	0.406 (63)	0.482 (96)	0.420 (55)	0.375 (54)
	CHB	0.677 (105)	0.714 (142)	0.671 (88)	0.639 (92)
	GIH	0.400 (62)	0.487 (97)	0.374 (49)	0.354 (51)
Controls		YRI (74)	LWK (71)	YRI (58)	LWK (66)
	TSI	0.297 (22)	0.254 (18)	0.310 (18)	0.258 (17)
	CHB	0.149 (11)	0.127 (9)	0.138 (8)	0.091 (6)
	GIH	0.149 (11)	0.127 (9)	0.138 (8)	0.106 (7)
Genes/Controls[a]		2.872	3.792	2.978	3.941

Note.—DAF is defined as "intermediate" in Africans according to two different criteria. In both cases, the table shows the proportion of sites with frequency defined as low (DAF $\leq$ 0.05) or high (DAF $\geq$ 0.95) in non-Africans. All values of the "Genes" are significantly higher than those of the Controls (all exact binomial tests $P < 0.008$). The numbers in parenthesis correspond to the number of *iA-alleles* in Africans and *iAdO-alleles* in non-Africans.

[a] The values correspond to the mean of the ratio Genes/Controls across the three populations (TSI, CHB, and GIH). Note that the values are very similar with both criteria used to define intermediate allele frequencies.

The Model of Selection

In our set of four genes the double signature of balancing selection in Africans (with increased diversity and intermediate-frequency alleles) indicates long-term balancing selection. The incomplete signature of balancing selection outside of Africa, with excess of polymorphism yet absence of intermediate-frequency alleles, suggests a possible change in the frequency of the balanced polymorphism(s) and linked variation in these populations.

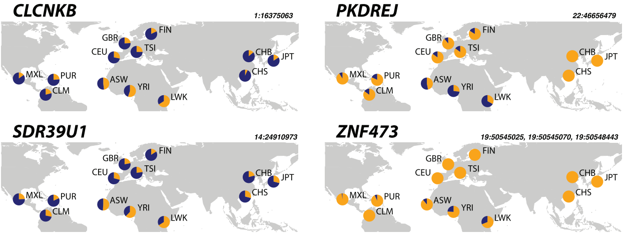

FIG. 4. Allele frequency of the most differentiated nonsynonymous *iAdO-alleles* (one per gene) in the 1000 Genomes populations. The blue and orange portions of the pie charts represent the ancestral and derived alleles, respectively. The SNP names (as "chromosome:position") are on the top right of each plot. The patterns are similar for all other nonsynonymous *iAdO-alleles* (supplementary table S5 and fig. S9, Supplementary Material online).

Genetic drift or a change in selective forces might explain this unexpected observation. In what follows, we aimed to infer the selective history of these genes and the possible causes for the different signatures between African and non-African populations. To do this, we modeled five evolutionary scenarios that include one African, one European, and one East-Asian population (fig. 6A) and performed an approximate Bayesian computation (ABC) analysis (Beaumont et al. 2002).

Because signatures of balancing selection are clear in Africa, we keep the selective history in Africans identical in all models: A balanced polymorphism arose in the ancestor of all human populations and selection acted continuously in Africa by maintaining the balanced polymorphism at approximately 0.5 (see Materials and Methods). For simplicity, balancing selection is simulated with overdominance, which here is also appropriate to simulate other mechanisms of long-term balancing selection that leave patterns of diversity that are compatible with those observed in Africa (e.g., frequency-dependent selection that favors intermediate-frequency alleles or mild fluctuating selection that maintains polymorphisms for long periods of time; see Discussion and supplementary material section 8, Supplementary Material online, on the likelihood of other types of balancing selection).

Because all non-African populations show similarly incomplete signatures of selection and a higher correlation in allele frequencies compared with controls (fig. 2 and table 1), we kept the selective history identical in the two non-African populations (supplementary material section 4.4, Supplementary Material online). In order to model the changes outside of Africa, we considered five scenarios (fig. 6A):

(1) Balancing to Balancing (B-B), in which balancing selection continued acting after the out-of-Africa migration in non-Africans.
(2) Balancing to Neutrality (B-N), in which balancing selection stopped acting after the out-of-Africa migration, and the gene evolved neutrally in non-Africans.
(3) Balancing to Positive (B-P), in which balancing selection stopped acting after the out-of-Africa migration, and the gene evolved under different types of natural selection in non-Africans as follows:
    i. Balancing to Positive on standing variation (B-Psv): One of the two alleles of the balanced polymorphism became directionally (positively) selected in non-Africans right after the out-of-Africa migration. This is a model of positive directional selection acting on a previously balanced allele, which is similar to a soft sweep or selection on standing variation (sv). It also closely models positive selection on an intermediate-frequency allele that is closely linked to the balanced polymorphism.
    ii. Balancing to Positive on de novo mutation (B-Pdn): The balanced polymorphism became neutral, and a de novo (dn) advantageous mutation appeared in the ancestors of non-Africans right after the out-of-Africa migration, and immediately became directionally (positively) selected.
    iii. Balancing to Positive due to change in frequency equilibrium (B-Pcfe): The frequency equilibrium of the balanced polymorphism changed from ~0.50 to 0.07 in non-Africans right after the out-of-Africa migration, and so the allele was subject to selection to change its allele frequency. We chose the value of 7% because it yielded similar summary statistics to the B-Psv model, and we sought to discriminate between these two models. Because the change is recent this model also mimics changes in other types of balancing selection (e.g., recent changes in long-term mild fluctuating selection driving a selected allele to very high frequency). For simplicity, we consider this also a Balancing-to-Positive selection model.

In summary, we have a model B-B where selection did not change outside of Africa, one model B-N where selection stopped acting outside of Africa, and three B-P models where outside of Africa selection favored the increase in allele frequency of a new or existing variant.

We used an ABC framework (Beaumont et al. 2002) to infer the posterior probability of each of these models given the data. ABC is a useful tool that allows probabilistic model testing and parameter estimation when calculating the model's likelihood function is not feasible (Beaumont et al. 2002; Bertorelle et al. 2010). In short, the ABC procedure is based on running several thousand simulations (160,000 in our analyses) under each model, with the relevant evolutionary parameters being drawn from a prior distribution. The simulated genetic data are then summarized in a number of summary statistics (see Materials and Methods and supplementary material section 4, Supplementary Material online) and simulations producing statistics that resemble best those of real data are selected to estimate the posterior probability of each model (Beaumont 2008).

Our main goal is to distinguish B-B, B-N and B-P models. To account for the presence of three B-P models, we run the ABC model selection with two different approaches. The first ABC model selection approach was carried out in two hierarchical steps. We first compared the three B-P models (B-Pcfe, B-Psv, and B-Pdn) and performed an ABC model

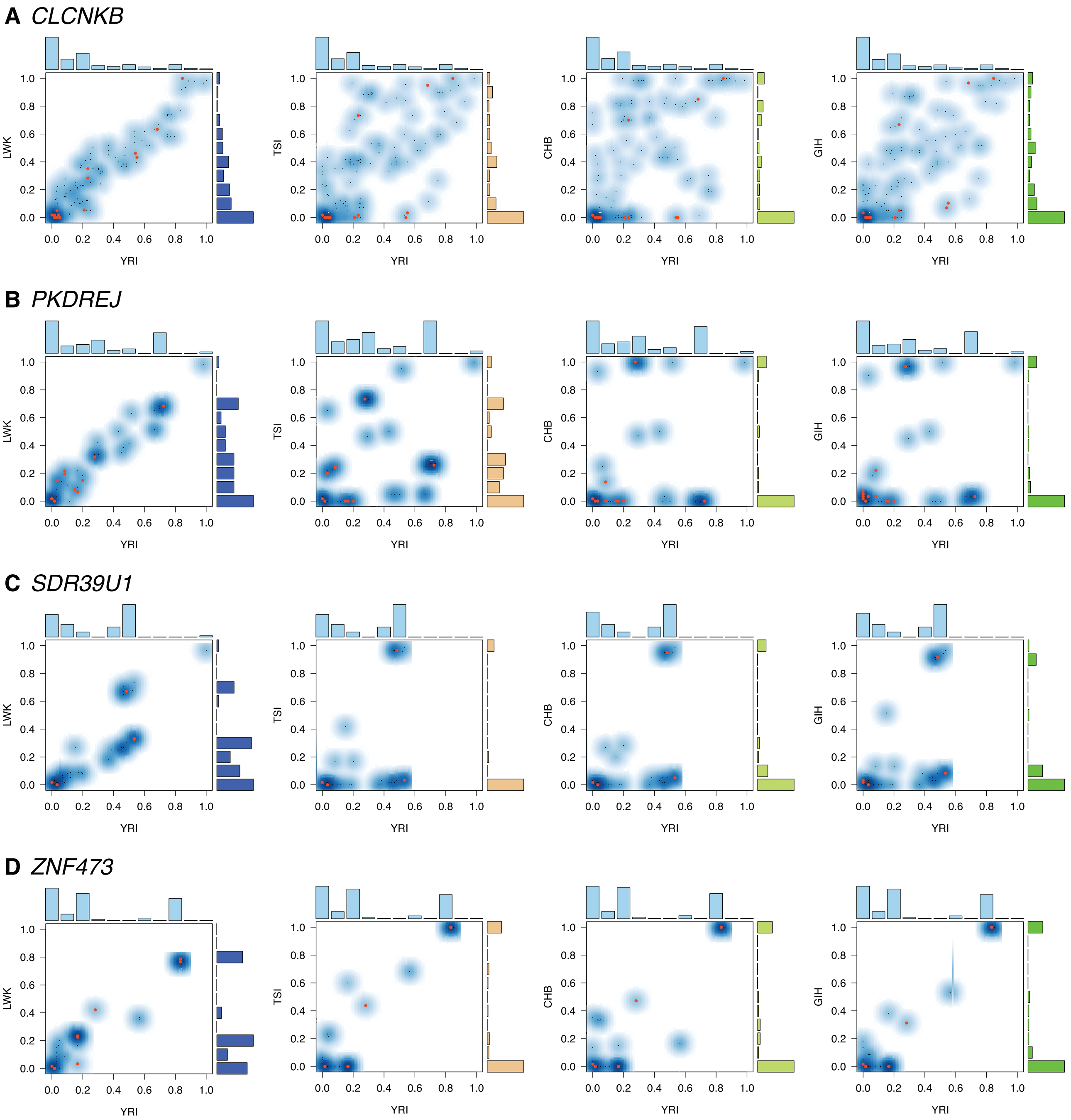

FIG. 5. Two-dimensional SFS for each of the candidate genes. See figure 3 for more details and supplementary figure S8, Supplementary Material online for other pairwise comparisons.

choice analysis to select the model with the highest posterior probability. Then the chosen B-P model was compared with the other two models (B-B and B-N) via a second ABC model choice analysis (supplemental material section 4.4, Supplementary Material online).

We determined the accuracy of our model choice inferences calculating the true and false positive rates using 1,000 simulations as Pseudo-Observed-Data for each model (supplementary material section 4.4.2, Supplementary Material online, for the full procedure). The results (supplementary tables S7 and S8, Supplementary Material online) indicate that the true positive rate is good for model B-B (81%), moderate for the three B-P models (on average 63%), and weak for B-N (47%). The false positive rate is relatively high (roughly 12%) and very similar for the three models. Therefore, our analysis is somehow biased in favor of the B-B model. When we compare the three B-P models, the true positive rate is low for each of them (all lower than 47%) although the false positive rate is quite low as well (<8%). This is not surprising given that the tested scenarios are similar and difficult to differentiate by a set of summary statistics (supplementary fig. S13B, Supplementary Material online). Nevertheless, as stated above, we focused on the distinction among three main models (B-B, B-N, and B-P)

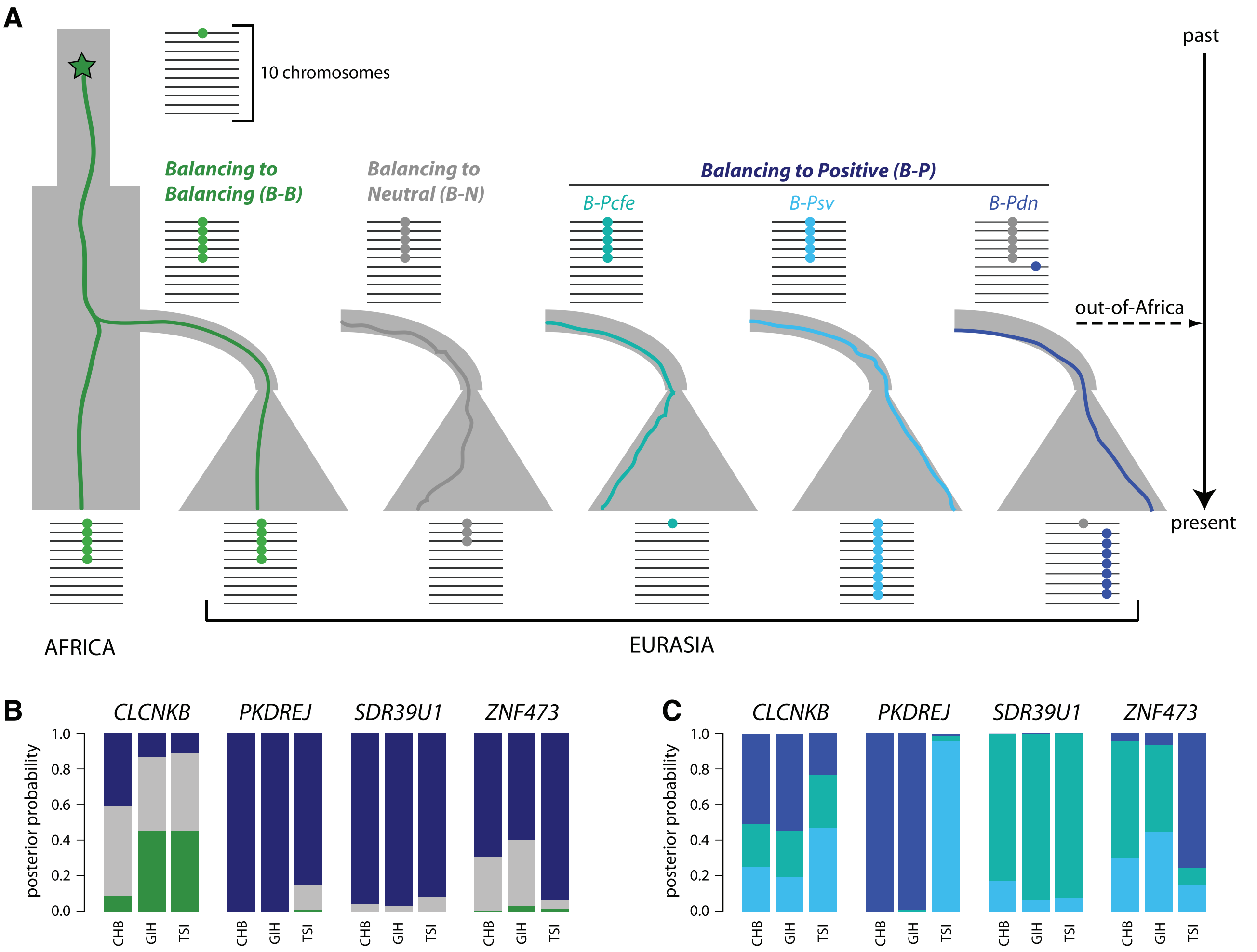

Fig. 6. Evolutionary models and ABC results. (*A*) An overdominant balanced polymorphism (green dot) that arose Tbs generation ago (green star) increases to intermediate frequency (~50%) and is maintained at that frequency in African populations for all models. To illustrate the behavior of the balanced polymorphism in Eurasia in each model, we represent in horizontal lines a population of ten chromosomes at different times. The colored vertical lines illustrate one sample of the possible allele frequency trajectories (with derived allele frequency on the *x*-axis). We refer to the Results and Materials and Methods sections for a detailed description of the models. (*B*) Posterior probabilities of the hierarchical ABC approach when one B-P model (dark blue) was tested against the B-B (green) and B-N (gray) models. (*C*) Posterior probabilities of each of the three B-P models: B-Pcfe (aquamarine), B-Psv (light blue), and B-Pdn (blue). Supplementary figure S14, Supplementary Material online, shows very similar results when using the ABC approach that compares all five models together.

and give little emphasis to the distinction among the three B-P models (fig. 6C).

Figure 6B shows the results of this model selection approach for each gene and population. For *PKDREJ*, *SDR39U1*, and *ZNF473*, the B-P model has the highest support consistently in all populations; the B-B and B-N models have no and minor support, respectively. For *CLCNKB*, an ambiguous picture emerges, with modest posterior probabilities favoring the B-B and B-N models. This ABC analysis thus provides little support for stable balancing selection or neutrality outside of Africa in *PKDREJ*, *SDR39U1*, and *ZNF473*, suggesting instead a change in selective pressure as the most likely scenario.

In order to identify potential bias in our estimates due to the hierarchical procedure of selection within the three B-P models, we performed a second model selection with a different approach, which consists in comparing simultaneously the five models (B-B, B-N, B-Pcfe, B-Pssv, and B-Pdn). Given that the three B-P models (B-Pcfe, B-Pssv, and B-Pdn) produced similar results, we assigned a prior probability of 1/3 for the B-B and B-N models, and of 1/9 for each B-P model. A single ABC model choice analysis was then run to obtain the posterior probability of each of these five models. The results of this approach are extremely similar to those of the hierarchical approach (supplementary fig. S14, Supplementary Material online) and also support the B-P model for *PKDREJ*, *SDR39U1*, and *ZNF473* and show inconclusive results for *CLCNKB*.

The joint SFS was not explicitly considered in the ABC analysis, but it clearly displays (fig. 5 and supplementary fig. S8, Supplementary Material online) the differences between *CLCNKB* (with many alleles in the diagonal of the joint SFS of African vs. non-African populations) and *PKDREJ*, *SDR39U1*, and *ZNF473*, all with a virtually empty diagonal in the joint SFS.

Haplotypes and Populations

The relationships among haplotypes in these three genes showed, as expected, higher diversity in Africa than outside

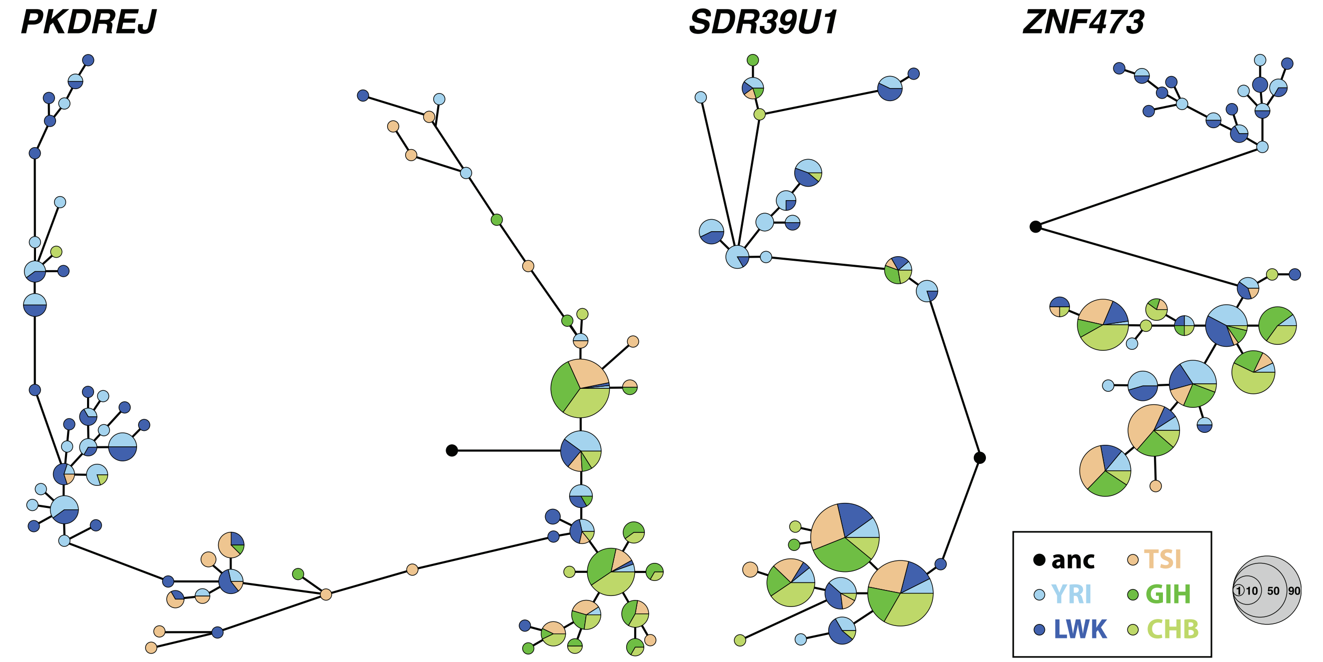

FIG. 7. Haplotype networks. The circles are proportional to the number of haplotypes, with colors representing populations. The length of the branch between two haplotypes is proportional to the number of differences. SNPs with a global count lower than six were removed to reduce complexity. The networks were generated using the function "haploNet" from the R-package "pegas" (Paradis 2010) and are cladistic trees (Templeton et al. 1992) which do not allow reticulations.

of Africa. Nevertheless, this is not due to a general loss of low-frequency polymorphisms as in other genomic regions: In all three genes we observe the complete or nearly complete loss of one haplotype lineage outside of Africa (fig. 7). This agrees well with a model where a set of haplotypes increased rapidly in frequency in populations outside of Africa, putatively due to linkage to a variant that is advantageous in non-African populations.

The Functional Effect of *iAdO-Alleles*

No SNP in these four genes has been associated with diseases or phenotypes in genome-wide association studies (Welter et al. 2014). But the *iAdO-alleles* are prime candidates to be the targets of changing selective pressures, so we investigated their putative functional consequences. As a group, the 102 *iAdO-alleles* have significantly higher *C*-scores for deleteriousness (Kircher et al. 2014) than expected given *C*-scores in the rest of the genome (supplementary fig. S15B, Supplementary Material online). This is not the case when only non-synonymous *iAdO-alleles* are considered (supplementary fig. S15A, Supplementary Material online), suggesting that the potential functional effect is due to regulation. In fact, the set of *iAdO-alleles* show a significant enrichment ($P < 0.001$) in high scores for regulatory features (supplementary table S9 and fig. S16, Supplementary Material online) as described in *RegulomeDB* (Boyle et al. 2012). For example, 31% of *iAdO-alleles* are predicted with high confidence to affect DNA–protein binding and are associated with changes in gene expression (i.e., mapped to an eQTL; Boyle et al. 2012). Less than 3% of SNPs fall in these functional annotated categories when we randomly sample three genes in the genome (supplementary fig. S16, Supplementary Material online), so this is an unusual enrichment in functional alleles that suggests a possible effect in gene regulation of the observed allele frequency differences. For more details on these analyses, see supplementary material section 7, Supplementary Material online.

The Balanced Alleles through the Out-of-Africa Bottleneck

It is theoretically possible that a change in selective pressure is a direct consequence of demography if, for example, the balanced polymorphism is lost during a bottleneck and selection can no longer act. This is more likely in non-African populations, which experienced a severe out-of-Africa bottleneck (Gravel et al. 2011). The possibility is included in our simulations, but we wanted to formally ask how often we expect it to happen.

The fixation of a balanced allele only due to the increased drift produced by a demographic event is an unlikely scenario, at least for the parameters we considered (i.e., overdominance with selection coefficient ranging from 0.01% to 10%). In none of the simulations under stable balancing selection (model B-B) was the balanced polymorphism lost. In the scenario with neutrality in non-African populations (model B-N), the probability of fixation of the balanced polymorphism after the out-of-Africa bottleneck is also low according to our simulations: 11% in Europeans, 15% in Asians, and 7% in both Europeans and Asians. This should be considered as a conservative upper bound as we did not allow in the simulations migration between Africans and non-Africans (supplementary material section 4.1, Supplementary Material online), eliminating chance of reintroduction in non-Africans of the lost allele.

Discussion

Humans are a young and quite homogeneous species, with substantial genetic and phenotypic similarity among populations (Rosenberg et al. 2002). In fact, although humans inhabit a wide variety of environments, they colonized areas outside of the African continent only in the last 50,000 years (Gravel et al. 2011). These migrating populations adapted to their new habitats biologically and/or culturally (Richerson and Boyd 2008; Coop et al. 2009), and these local adaptations undoubtedly explain some of the phenotypic differences that exist among human groups today. Because the rate of new mutations is low in humans (Scally and Durbin 2012; Fu et al. 2014), mostly due to our low effective population size (Lynch 2010, 2011), it is likely that these novel adaptations are largely mediated by selection on previously existing variation (Pritchard et al. 2010; Messer and Petrov 2013). The classical definition of positive selection from standing variation considers that alleles segregate neutrally (or nearly neutrally) before becoming advantageous upon environmental change (Innan and Kim 2004; Przeworski et al. 2005; Pennings and Hermisson 2006; Messer and Petrov 2013). However, experimental evolution on yeast has shown that these alleles usually have significant fitness effects (often deleterious) before changes in the environment turn them advantageous (Hietpas et al. 2013). It is indeed logical that the environment will rarely determine whether an allele has functional and phenotypic consequences that affect fitness. Most likely, environmental shifts will instead modify the magnitude and perhaps the direction of the fitness effect of a given mutation.

Therefore variants that have been under balancing selection with a significant (and likely complex) effect on fitness are prime candidates to be affected by selection from standing variation. In addition, loci that contain balanced polymorphisms accumulate a high number of additional variants, some of which are not neutral and may later become advantageous. Given the demographic history of humans, alleles under long-term balancing selection in Africa (or functional, linked alleles) could have contributed to recent human local adaptation.

We investigate this possibility by exploring in detail four genes in humans, and their patterns suggest that this might be the case. The four genes show hallmark signatures of long-term balancing selection in Africa that combines an excess of polymorphism and an excess of intermediate-frequency alleles in both African groups. These patterns are expected under balancing selection with frequency equilibrium around 0.5, such as overdominance (with similar fitness of both homozygotes) or frequency-dependent selection (with favored frequency close to 0.5). Moderately fluctuating selection (with a selected allele varying mildly in frequency around 0.5) lacks a frequency equilibrium but it could produce similar patterns. Strongly fluctuating selection and negative frequency-dependent selection would likely leave different genetic signatures (lacking the excess of polymorphism, the excess of intermediate-frequency alleles, or both; supplementary material section 8, Supplementary Material online). We thus focused on the mechanisms that best predict the patterns observed in Africa. Importantly, these three mechanisms would result in modest differences between populations if selection remained unchanged.

Nevertheless, these four genes show extremely different patterns in Europe and/or Asia, with an absence of the hallmark double signature of balancing selection. It is interesting that the levels of diversity are overall high in non-African populations, and the main difference between African and non-African populations is in the distribution of allele frequencies, which in non-Africans lacks the excess of intermediate-frequency alleles observed in Africans. Correlation in allele frequency between Africans and non-Africans is weaker than in neutral regions, and a substantial amount of alleles present at intermediate frequency in Africans segregate at low or high frequency in populations outside of Africa. These signatures, combined with the incomplete signatures of balancing selection outside of Africa, are compatible with a recent change in selective pressure, which would have changed the haplotype landscape and shifted allele frequencies but not wiped out (quite yet) segregating alleles. The strong correlation in allele frequencies among the non-African populations (stronger than in neutral controls) suggests that the similarities between Europeans and Asians are due to their shared demographic and selective histories.

We formally tested this hypothesis by considering different evolutionary scenarios and conclude that a model with changes in selection outside of Africa (where selection favored an existing or new mutation) best explains the data for three genes: *PKDREJ*, *SDR39U1*, and *ZNF473*. For these genes a model of continuous balancing selection (B-B) or change to neutrality (B-N) has little support. For a fourth gene *CLCNKB*, results are less conclusive. This could be the result of a reduction in the strength of selection without a change in the selective regime, a possibility that we did not consider. We note that the power of the ABC analysis to distinguish among the three main models is moderate because these scenarios do not produce strikingly different signatures on top of the pre-existing signatures of long-standing balancing selection. This is, *per se*, a challenging exercise. Still, our main limitation is a bias toward the B-B model (continuous, unchanged balancing selection outside of Africa), which shows extremely weak support in the three genes that have a robust result.

A change from balancing to positive selection seems most likely given our observations, although the change in selective regime could in principle be more complex. For example, the locus might experience drastic changes in the frequency equilibrium (as in our B-Pcfe model) or changes in previously mild fluctuating selection such that the selected allele reaches very low or high frequency in Eurasian populations (while keeping similar, intermediate-frequency alleles in the two African groups). In all cases, one allele increases fast in frequency due to changes in selective pressure (supplementary material section 8, Supplementary Material online).

Table 3. Summary of Population Genetics Results for Eurasian Populations and Biological Features of the Four Genes.

Population	Statistics	CLCNKB	PKDREJ	SDR39U1	ZNF473
TSI	PtoD	bal	bal	bal	–*
	SFS	bal	bal	pos/neg	–
	ABC	B-B	B-P	B-P	B-P
GIH	PtoD	bal	bal	bal	–
	SFS	–	pos/neg	–	–
	ABC	B-B	B-P	B-P	B-P
CHB	PtoD	bal	–*	bal	–*
	SFS	–	–	pos/neg	–
	ABC	B-N	B-P	B-P	B-P

Note.—SFS, *PtoD*: summary of the evidence provided by the neutrality tests (MWU for SFS and HKA for PtoD from fig. 1). "bal" is for balancing selection, "pos/neg" for positive or negative selection (in the specific case of SFS, it refers to excess of low-frequency variants but not high-frequency derived), "–" stands for neutrality, and "–*" indicates evidence of positive selection in the 1000 Genomes data (fig. 2) but not for the neutrality tests carried on our data (fig. 1). ABC: Type of model supported in the ABC model choice (see main text and fig. 6 for more details).

The Candidate Genes and Their SNPs

In this section, we summarize the results for each gene (table 3) and provide further information about the genes and their SNPs.

CLCNKB shows strong evidence of long-term balancing selection in virtually all analyses, although the two Asian populations (CHB and GIH) do not show an excess of intermediate-frequency alleles (fig. 1). The ABC does not clearly favor one model across populations. Therefore, it is unclear whether selection remained stable or if it weakened outside of Africa, and we have no convincing evidence of a change in selective pressure in particular human groups.

The remaining three genes show instead clear evidence of a change in selective pressure outside of Africa.

PKDREJ encodes a protein known to play a role in fertilization by generating a Ca^{2+} transporting channel that is directly involved in initiating the acrosome reaction of the sperm (Butscheid et al. 2006). Its highest expression is in testis (Kissopoulou et al. 2013) and mice knockout spermatozoa are detected within the egg/cumulus complex later than the wild type (Sutton et al. 2008). Hamm et al. (2007) showed evidence of rapid, adaptive evolution of *PKDREJ* in primates (i.e., high divergence), a pattern commonly observed in fertilization proteins in mammals (Swanson et al. 2003). In humans, we observe evidence for long-standing balancing selection in African populations, in agreement with the signatures observed in African Americans by Hamm et al. (2007). But we also detect strong evidence for a change in selective pressure in non-African populations. Signatures of balancing selection are absent in Asian populations (fig. 1) and the ABC analysis supports model B-P as the most likely model in all non-African populations. As mentioned above this agrees with this locus having a classical signature of recent positive selection in Asians (Pickrell et al. 2009), and it highlights the complexity of the evolutionary forces acting on *PKDREJ*.

SDR39U1 encodes a putative nicotinamide adenine dinucleotide phosphate-dependent oxidoreductase protein. Although little is known about its function, the RNA expression of the gene is ubiquitous (Kissopoulou et al. 2013). The gene shows clear signatures of long-term balancing selection in Africans and very different signatures in all Eurasians, where some populations even show classical signatures of positive selection such as an excess of low-frequency alleles. In agreement with these patterns, the ABC results strongly favor a change in selective pressure involving B-P models in all non-African populations (fig. 6B and C).

ZNF473 encodes for a protein involved in histone 3′-end pre-mRNA processing. *ZNF473* associates with U7 small nuclear ribonucleo protein, which mutated in Xenopus blocks histone pre-mRNA processing and disrupt oogenesis (Dominski et al. 2002). Like *PKDREJ*, *ZNF473* is more expressed in testis than in other tissues (Kissopoulou et al. 2013). Despite showing signatures of long-term balancing selection in both African populations, non-African populations lack any signature of balancing selection, including excess of diversity (fig. 1). In addition, the ABC analysis supports the model B-P (fig. 6B and C). Together these results suggest that *ZNF473* has experienced drastic changes in selection outside of Africa, probably involving positive selection, although the pre-existing signatures of long-term balancing selection hide classical signatures of a selective sweep. We note that the highest PtoD peaks fall up- and downstream of *ZNF473* (fig. 2E) so we cannot discard that regulatory elements or neighboring genes are the targets of natural selection (supplementary material section 6, Supplementary Material online).

Conclusion

In conclusion, our study suggests that balancing selection can create reservoirs of genetic variants that mediate later adaptation. We focused on a number of genes to define this mechanism, but additional cases likely exist in the human genome. Ultimately, these represent events of positive selection on standing variation or soft sweeps, selective events that are notably difficult to identify with classical population genetics methods (Innan and Kim 2004; Przeworski et al. 2005; Pennings and Hermisson 2006) unless selection is recent and very strong (Albrechtsen et al. 2010; Peter et al. 2012; Messer and Petrov 2013; Ferrer-Admetlla et al. 2014). We expect that investigating shifts in selection of previously balanced alleles will help refine the catalog of loci that have contributed to recent adaptation of humans to their local environments.

Materials and Methods

Samples and Populations

We analyzed a total of 150 HapMap samples from five populations (30 individuals per population): YRI from Nigeria, LWK from Kenya, TSI from Italy, GIH from India, and CHB from Beijing. The DNA was purchased from Coriell Cell Repositories. In addition, for analyses where we need empirical genome-wide distributions or longer genomic regions, we also analyzed six populations from the 1000 Genomes phase 1 data (1000 Genomes Project Consortium et al. 2012): Two Africans (YRI and LWK), two Europeans (CEU and TSI), and two East Asians (CHB and JPT).

Targeted Regions

We investigated 14 genes (supplementary table S1, Supplementary Material online). Four genes were reported by Andrés et al. (2009) as having signatures of long-term balancing selection in African Americans (significant departures from neutral expectations in two neutrality tests) and clearly lacking these signatures in European Americans, with $P > 0.2$ in at least one neutrality test. Because that analysis was performed in a potentially admixed group, we included ten additional genes where the signatures of balancing selection did not reach significance in African Americans and were absent in European Americans (Andrés et al. 2009).

We used Sanger and Illumina sequencing technologies to sequence the coding region and adjacent non-coding region of all target genes (supplementary table S1, Supplementary Material online). We also used 49 control regions described previously as a proxy for neutrality (Andrés et al. 2010). These regions are unlinked, ancient processed pseudogenes, which are distant from genes, do not overlap functional elements, and have GC content similar to coding genes and thus serve as adequate proxy for neutrality.

Together with Illumina and Sanger sequences, we analyzed a total of 230,452 bp (supplementary table S1, Supplementary Material online), and after stringent quality filters (supplementary material section 1.3, Supplementary Material online) we retrieved a total of 1,708 and 1,109 high-quality biallelic SNPs for Illumina and Sanger technologies, respectively. Supplementary table S3, Supplementary Material online, reports for each gene and population the number of segregating sites and fixed differences relative to the chimpanzee genome (PanTro3).

Population Genetics Analyses

We compared the patterns of the region of interest (each gene) with neutral regions (the 49 controls) with two neutrality tests, and assessed significance with neutral simulations. We thus determined how unusual the patterns of our genes are by comparing them both with neutral regions of the genome and with expectations under neutrality (Andrés et al. 2009).

The first neutrality test is a modified version of the MWU test that detects departures of the SFS in each gene when compared with the neutral regions (Nielsen et al. 2009). In particular, we compared the folded SFS of each gene with the folded SFS of all control regions with two MWU tests, one to detect an excess of low-frequency alleles and one to detect an excess of intermediate-frequency alleles. The second neutrality test is the HKA test to identify excess of polymorphic over divergent sites in a region of interest (Hudson et al. 1987). Specifically, we compare the ratio of polymorphisms over substitutions of each gene with that of the control regions. All tests were performed per population using in-house perl scripts (Andrés et al. 2010).

The significance of the neutrality tests was assessed by comparing the results of each gene with 10,000 coalescent simulations performed with *ms* (Hudson 2002), conditioning the simulations on the observed number of variable sites (i.e., SNPs and fixed differences) and the average recombination rate of the gene (Kong et al. 2010). The simulations were run under a state-of-the-art demographic model for human populations (Gravel et al. 2011) which depicts the demographic history of three populations: Africans, Europeans, and Asians. Therefore, we simulate the YRI and LWK populations with the African model, the TSI with the European, and the CHB and GIH with the Asian. The split time of human and chimpanzee was fit to the number of fixed differences observed in the 49 control regions. For all analyses, we considered the chimpanzee genome (PanTro3) to calculate the number of fixed differences. We use these tests to identify genes with population-specific signatures of balancing selection (present only in the African populations).

ABC Analyses

An ABC framework (Beaumont et al. 2002) was used to infer the most likely evolutionary model. We used 160,000 simulations for each of the five evolutionary scenarios to model changes in selective pressure after the out-of-Africa migration. In all models an overdominant balanced polymorphism arises Tbs generations ago, and is maintained until present-day in African populations. This balanced polymorphism has a selection coefficient (Sbs, drawn from a uniform prior distribution) and a dominance coefficient (h) fixed to 25.5 in order to achieve a frequency equilibrium of 0.51. This frequency equilibrium cannot be exactly 0.50 because the fitness model in "SLiM" (Messer 2013) (one of the two programs used to produce the simulations, see below and supplementary material section 4.1, Supplementary Material online) does not allow the two homozygous to have exactly the same fitness (Gillespie 1978). We refer the reader to the Discussion section and supplementary material section 8, Supplementary Material online, for a discussion on other mechanisms of balancing selection.

The five models differ in the selective regime of the non-African populations, where selection changes right after the out-of-Africa migration (for a full description of the models, see Results and fig. 6A). The following parameters were drawn from uniform prior distributions: Mutation rate, $\mu = U(1 \times 10^{-8}, 4 \times 10^{-8}$ per site per generation); recombination rate, $\rho = U(0, 4 \times 10^{-8}$ per site per generation); time since balancing selection, Tbs $= U$(40,000, 240,000 generations); selection coefficient of the balanced polymorphism, Sbs $= U(0.0001, 0.1)$; selection of the de novo advantageous mutation in model B-Pdn, Sps $= \log U(0.0001, 0.01)$. Other parameters were identical to those in the neutral simulations used in the neutrality tests; the only exception is that we did not allow migration (see later in the paragraph). The divergence time between human and chimpanzee was set to 6.5 My, that is, 260,000 generations considering a generation time of 25 years.

Given the complexity of the models and the limitations of current simulation software, we combined strategies of coalescent and forward simulations. Specifically, we used the coalescent simulator msms (Ewing and Hermisson 2010) to generate the genetic data until the time of the out-of-Africa migration. We then used the forward simulator SLiM (Messer 2013), which can model more complex scenarios, to simulate the evolution of all populations after the out-of-Africa event (supplementary fig. S10, Supplementary Material online). However, due to limitations in SLiM we did not include migration between populations because it can produce the coexistence of different types of natural selection in a population (see supplementary material section 4.3, Supplementary Material online, for more details). Supplementary figure S10, Supplementary Material online, illustrates the simulated models and their demographic parameters.

We considered the 27 summary statistics described in supplementary table S6, Supplementary Material online; we calculated them using "msstats" package from "Libsequence" (Thornton 2003) and in-house scripts in R-language (R Core Team 2013) and selected a subset of 16 informative summary statistics that show only moderate correlation (Pearson's $r^2 < 0.8$) and together give the greatest power of discrimination among the models (supplementary material section 4.2, Supplementary Material online). We corrected F_{ST} (Weir and Cockerham 1984) to take into account the absence of migration in our simulations (supplementary material section 4.3, Supplementary Material online). The model selection analysis (supplementary material section 4.4, Supplementary Material online) was performed independently for Europeans and Asians using the logistic regression approach (Beaumont 2008) and retaining 50,000 simulations out of 480,000.

Supplementary Material

Supplementary tables S1–S9, figures S1–S16, and sections 1–8 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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References

1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA. 2012. An integrated map of genetic variation from 1,092 human genomes. *Nature* 491:56–65.

Akey JM, Eberle MA, Rieder MJ, Carlson CS, Shriver MD, Nickerson DA, Kruglyak L. 2004. Population history and natural selection shape patterns of genetic variation in 132 genes. *PLoS Biol*. 2:e286

Albrechtsen A, Moltke I, Nielsen R. 2010. Natural selection and the distribution of identity-by-descent in the human genome. *Genetics* 186:295–308.

Allison AC. 1956. The sickle-cell and haemoglobin C genes in some African populations. *Ann Hum Genet*. 21:67–89.

Andrés AM. 2011. Balancing selection in the human genome. In: Encyclopedia of life sciences. Chichester (United Kingdom): John Wiley & Sons, Ltd.

Andrés AM, Dennis MY, Kretzschmar WW, Cannons JL, Lee-Lin SQ, Hurle B, NISC Comparative Sequencing Program, Schwartzberg PL, Williamson SH, Bustamante CD, et al. 2010. Balancing selection maintains a form of *ERAP2* that undergoes nonsense-mediated decay and affects antigen presentation. *PLoS Genet*. 6:e1001157.

Andrés AM, Hubisz MJ, Indap A, Torgerson DG, Degenhardt JD, Boyko AR, Gutenkunst RN, White TJ, Green ED, Bustamante CD, et al. 2009. Targets of balancing selection in the human genome. *Mol Biol Evol*. 26:2755–2764.

Asthana S, Schmidt S, Sunyaev S. 2005. A limited role for balancing selection. *Trends Genet*. 21:30–32.

Bamshad M, Wooding SP. 2003. Signatures of natural selection in the human genome. *Nat Rev Genet*. 4:99–111.

Beaumont MA. 2008. Joint determination of topology, divergence time, and immigration in population trees. In: C Renfrew, S Matsumura, P Forster, editors. Simulation, genetics and human prehistory. McDonald Institute Monographs. Cambridge: McDonald Institute for Archaeological Research. p. 134–1541.

Beaumont MA, Zhang W, Balding DJ. 2002. Approximate Bayesian computation in population genetics. *Genetics* 162:2025–2035.

Bertorelle G, Benazzo A, Mona S. 2010. ABC as a flexible framework to estimate demography over space and time: some cons, many pros. *Mol Ecol*. 19:2609–2625.

Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M, Karczewski KJ, Park J, Hitz BC, Weng S, et al. 2012. Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res*. 22:1790–1797.

Butscheid Y, Chubanov V, Steger K, Meyer D, Dietrich A, Gudermann T. 2006. Polycystic kidney disease and receptor for egg jelly is a plasma membrane protein of mouse sperm head. *Mol Reprod Dev*. 73:350–360.

Cavalli-Sforza LL. 1966. Population structure and human evolution. *Proc R Soc Lond B Biol Sci*. 164:362–379.

Charlesworth D. 2006. Balancing selection and its effects on sequences in nearby genome regions. *PLoS Genet*. 2:e64.

Coop G, Pickrell JK, Novembre J, Kudaravalli S, Li J, Absher D, Myers RM, Cavalli-Sforza LL, Feldman MW, Pritchard JK. 2009. The role of geography in human adaptation. *PLoS Genet*. 5:e1000500.

Darwin C, Wallace A. 1858. On the tendency of species to form varieties; and on the perpetuation of varieties and species by natural means of selection. *J Proc Linn Soc Lond Zool*. 3:45–62.

DeGiorgio M, Lohmueller KE, Nielsen R. 2014. A model-based approach for identifying signatures of ancient balancing selection in genetic data. *PLoS Genet*. 10:e1004561.

Dominski Z, Erkmann JA, Yang X, Sànchez R, Marzluff WF. 2002. A novel zinc finger protein is associated with U7 snRNP and interacts with the stem-loop binding protein in the histone pre-mRNP to stimulate 3′-end processing. *Genes Dev*. 16:58–71.

Ewing G, Hermisson J. 2010. MSMS: a coalescent simulation program including recombination, demographic structure and selection at a single locus. *Bioinformatics* 26:2064–2065.

Ferrer-Admetlla A, Liang M, Korneliussen T, Nielsen R. 2014. On detecting incomplete soft or hard selective sweeps using haplotype structure. *Mol Biol Evol*. 31:1275–1291.

Fu Q, Li H, Moorjani P, Jay F, Slepchenko SM, Bondarev AA, Johnson PLF, Aximu-Petri A, Prüfer K, de Filippo C, et al. 2014. Genome sequence of a 45,000-year-old modern human from western Siberia. *Nature* 514:445–449.

Fumagalli M, Sironi M, Pozzoli U, Ferrer-Admettla A, Pattini L, Nielsen R. 2011. Signatures of environmental genetic adaptation pinpoint pathogens as the main selective pressure through human evolution. *PLoS Genet*. 7:e1002355.

Gendzekhadze K, Norman PJ, Abi-Rached L, Graef T, Moesta AK, Layrisse Z, Parham P. 2009. Co-evolution of KIR2DL3 with HLA-C in a human population retaining minimal essential diversity of KIR and HLA class I ligands. *Proc Natl Acad Sci U S A*. 106:18692–18697.

Gillespie JH. 1978. A general model to account for enzyme variation in natural populations. V. The SAS–CFF model. *Theor Popul Biol*. 14:1–45.

Gravel S, Henn BM, Gutenkunst RN, Indap AR, Marth GT, Clark AG, Yu F, Gibbs RA, 1000 Genomes Project, Bustamante CD. 2011. Demographic history and rare allele sharing among human populations. *Proc Natl Acad Sci U S A*. 108:11983–11988.

Hamm D, Mautz BS, Wolfner MF, Aquadro CF, Swanson WJ. 2007. Evidence of amino acid diversity-enhancing selection within humans and among primates at the candidate sperm-receptor gene *PKDREJ*. *Am J Hum Genet*. 81:44–52.

Hietpas RT, Bank C, Jensen JD, Bolon DNA. 2013. Shifting fitness landscapes in response to altered environments. *Evolution* 67:3512–3522.

Hudson RR. 2002. Generating samples under a Wright-Fisher neutral model of genetic variation. *Bioinformatics* 18:337–338.

Hudson RR, Kreitman M, Aguadé M. 1987. A test of neutral molecular evolution based on nucleotide data. *Genetics* 116:153–159.

Innan H, Kim Y. 2004. Pattern of polymorphism after strong artificial selection in a domestication event. *Proc Natl Acad Sci U S A*. 101:10667–10672.

Key FM, Peter B, Dennis MY, Huerta-Sánchez E, Tang W, Prokunina-Olsson L, Nielsen R, Andrés AM. 2014. Selection on a variant associated with improved viral clearance drives local, adaptive pseudogenization of interferon lambda 4 (*IFNL4*). *PLoS Genet*. 10:e1004681.

Key FM, Teixeira JC, de Filippo C, Andrés AM. 2014. Advantageous diversity maintained by balancing selection in humans. *Curr Opin Genet Dev*. 29:45–51.

Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. 2014. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet*. 46:310–315.

Kissopoulou A, Jonasson J, Lindahl TL, Osman A. 2013. Next generation sequencing analysis of human platelet PolyA+ mRNAs and rRNA-depleted total RNA. *PLoS One* 8:e81809.

Kong A, Thorleifsson G, Gudbjartsson DF, Masson G, Sigurdsson A, Jonasdottir A, Walters GB, Jonasdottir A, Gylfason A, Kristinsson KT, et al. 2010. Fine-scale recombination rate differences between sexes, populations and individuals. *Nature* 467:1099–1103.

Leffler EM, Gao Z, Pfeifer S, Ségurel L, Auton A, Venn O, Bowden R, Bontrop R, Wall JD, Sella G, et al. 2013. Multiple instances of ancient balancing selection shared between humans and chimpanzees. *Science* 339:1578–1582.

Lewontin RC, Krakauer J. 1973. Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. *Genetics* 74:175–195.

Loisel DA, Rockman MV, Wray GA, Altmann J, Alberts SC. 2006. Ancient polymorphism and functional variation in the primate MHC-DQA1 5′ *cis*-regulatory region. *Proc Natl Acad Sci U S A*. 103:16331–16336.

Lynch M. 2010. Evolution of the mutation rate. *Trends Genet*. 26:345–352.

Lynch M. 2011. The lower bound to the evolution of mutation rates. *Genome Biol Evol*. 3:1107–1118.

McDonald JH. 1998. Improved tests for heterogeneity across a region of DNA sequence in the ratio of polymorphism to divergence. *Mol Biol Evol*. 15:377–384.

Messer PW. 2013. SLiM: simulating evolution with selection and linkage. *Genetics* 194:1037–1039.

Messer PW, Petrov DA. 2013. Population genomics of rapid adaptation by soft selective sweeps. *Trends Ecol Evol*. 28:659–669.

Nielsen R, Hubisz MJ, Hellmann I, Torgerson D, Andrés AM, Albrechtsen A, Gutenkunst R, Adams MD, Cargill M, Boyko A, et al. 2009. Darwinian and demographic forces affecting human protein coding genes. *Genome Res*. 19:838–849.

Paradis E. 2010. pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics* 26:419–420.

Pennings PS, Hermisson J. 2006. Soft sweeps II—molecular population genetics of adaptation from recurrent mutation or migration. *Mol Biol Evol*. 23:1076–1084.

Peter BM, Huerta-Sanchez E, Nielsen R. 2012. Distinguishing between selective sweeps from standing variation and from a de novo mutation. *PLoS Genet*. 8:e1003011.

Pickrell JK, Coop G, Novembre J, Kudaravalli S, Li JZ, Absher D, Srinivasan BS, Barsh GS, Myers RM, Feldman MW, et al. 2009. Signals of recent positive selection in a worldwide sample of human populations. *Genome Res*. 19:826–837.

Pritchard JK, Pickrell JK, Coop G. 2010. The genetics of human adaptation: hard sweeps, soft sweeps, and polygenic adaptation. *Curr Biol*. 20:R208–R215.

Przeworski M, Coop G, Wall JD. 2005. The signature of positive selection on standing genetic variation. *Evolution* 59:2312–2323.

R Core Team. 2013. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing.

Rasmussen MD, Hubisz MJ, Gronau I, Siepel A. 2014. Genome-wide inference of ancestral recombination graphs. *PLoS Genet*. 10:e1004342.

Richerson PJ, Boyd R. 2008. Not by genes alone: how culture transformed human evolution. Chicago (IL): University of Chicago Press.

Rosenberg NA, Pritchard JK, Weber JL, Cann HM, Kidd KK, Zhivotovsky LA, Feldman MW. 2002. Genetic structure of human populations. *Science* 298:2381–2385.

Scally A, Durbin R. 2012. Revising the human mutation rate: implications for understanding human evolution. *Nat Rev Genet*. 13:745–753.

Schierup MH, Charlesworth D, Vekemans X. 2000. The effect of hitch-hiking on genes linked to a balanced polymorphism in a subdivided population. *Genet Res*. 76:63–73.

Ségurel L, Thompson EE, Flutre T, Lovstad J, Venkat A, Margulis SW, Moyse J, Ross S, Gamble K, Sella G, et al. 2012. The ABO blood group is a trans-species polymorphism in primates. *Proc Natl Acad Sci U S A*. 109:18493–18498.

Sutton KA, Jungnickel MK, Florman HM. 2008. A polycystin-1 controls postcopulatory reproductive selection in mice. *Proc Natl Acad Sci U S A*. 105:8661–8666.

Swanson WJ, Nielsen R, Yang Q. 2003. Pervasive adaptive evolution in mammalian fertilization proteins. *Mol Biol Evol*. 20:18–20.

Teixeira JC, de Filippo C, Weihmann A, Meneu JR, Racimo F, Dannemann M, Nickel B, Fischer A, Halbwax M, Andre C, et al. 2015. Long-term balancing selection in *LAD1* maintains a missense trans-species polymorphism in humans, chimpanzees and bonobos. *Mol Biol Evol*. 32:1186–96.

Templeton AR, Crandall KA, Sing CF. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132:619–633.

Thornton K. 2003. Libsequence: a C++ class library for evolutionary genetic analysis. *Bioinformatics* 19:2325–2327.

Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370.

Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, Klemm A, Flicek P, Manolio T, Hindorff L, et al. 2014. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res*. 42:D1001–D1006.

Wiuf C, Zhao K, Innan H, Nordborg M. 2004. The probability and chromosomal extent of trans-specific polymorphism. *Genetics* 168:2363–2372.

Wright S. 1939. The distribution of self-sterility alleles in populations. *Genetics* 24:538–552.