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Measuring the Efficiency of Purging by non-random Mating in Human Populations

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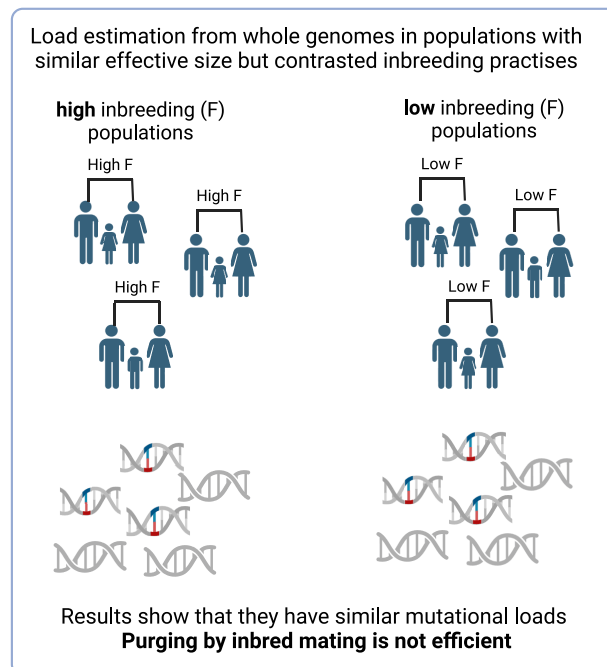
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Abstract

Human populations harbor a high concentration of deleterious genetic variants. Here, we tested the hypothesis that non-random mating practices affect the distribution of these variants, through exposure in the homozygous state, leading to their purging from the population gene pool. To do so, we produced whole-genome sequencing data for two pairs of Asian populations exhibiting different alliance rules and rates of inbreeding, but with similar effective population sizes. The results show that populations with higher rates of inbred matings do not purge deleterious variants more efficiently. Purging therefore has a low efficiency in human populations, and different mating practices lead to a similar mutational load.

Graphical abstract



Key words: purifying selection, kinship, mate choice, inbreeding, genomics, deleterious variants, mutational load.

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Introduction

Human populations worldwide harbor a high concentration of deleterious variants (Abecasis et al. 2010; Sulem et al. 2015). Each individual carries on average 85–115 loss-of-function (LOF) variants, and is homozygous for about 20–35 of them (Besenbacher et al. 2015; Exome Aggregation Consortium et al. 2016). The frequency spectrum of these deleterious variants is shifted toward low frequencies, with about 80% of LOF variants being exclusively encountered in single populations and about 20% of them being detected only in single individuals (Lupski et al. 2011; Abecasis et al. 2012). This spectrum suggests that purifying selection shapes the distribution of deleterious variants. There may exist differences in the efficiency of selection that reduce the burden of deleterious variants among human populations linked to their distinct demographic histories. This possibility has attracted considerable attention in recent years (Casals et al. 2013; Lim et al. 2014; Simons et al. 2014; Henn et al. 2015, 2016; Narasimhan et al. 2016; Simons and Sella 2016; Nakatsuka et al. 2017; Pedersen et al. 2017; Xue et al. 2017; Lopez et al. 2018). Most of these studies attempted to estimate the mutational load in each population (Morton et al. 1956). In practice, this load cannot be measured directly as we know little about the distribution of selection and dominance coefficients in the human genome. However, it can be approximated using a proxy. For instance, under the hypothesis that each variant acts additively (i.e. in the absence of dominance), the mean per-individual number of nonsynonymous derived variants has been shown to be directly related to the mutational load (Simons et al. 2014; Simons and Sella 2016). Using this proxy, contradictory results have been found. Thus, genomic data from Africans and non-Africans (Simons et al. 2014; Do et al. 2015; Lopez et al. 2018), hunter-gatherers and farmers (Lopez et al. 2018), Greenland Inuits and Europeans (Pedersen et al. 2017) respectively, were shown to exhibit similar amount of load. This is surprising as the efficiency of purifying selection is expected to depend on demography and to be weaker when the effective population size is small. It is possible that the duration of the demographic events (bottlenecks and expansions) that differ between these human populations may have been too short to have substantially affected this additive mutational load (Simons et al. 2014; Do et al. 2015; Lopez et al. 2018). In contrast, purifying selection was shown to be relaxed in isolated European populations (Xue et al. 2017) and Denisovans were demonstrated to have accumulated deleterious mutations at a significantly faster rate than present-day humans since their separation, leading to an increased mutational load in Denisovans (Do et al. 2015; Simons and Sella 2016). Using the same methodological approach, no increase in load was detected in Neanderthal compared to present-day humans (Simons and Sella 2016) (but also see Castellano et al. 2014; Harris and Nielsen 2016).

Although variants acting additively are expected to accumulate at higher rates in small populations compared to larger ones due to weaker purifying selection, variants acting recessively may be removed from smaller ones due to

exposure in the homozygous state. This phenomenon, known as purging by drift, is expected to only be efficient for highly deleterious variants and within a narrow range of effective population sizes (Glémin 2003). However, little evidence of purging by drift has been found in human populations, except possibly in the Finnish population, which experienced a severe founder effect. One study (Narasimhan et al. 2016) found a decrease in the number of LOF variants per genome in this population as compared to another European population (but see Lim et al. 2014).

Purging may also occur due to repeated matings between relatives (Charlesworth and Willis 2009). Purging by drift and purging by non-random mating are often conflated under the name of “purging by inbreeding” as they both increase homozygosity, exposing deleterious variants to selection (Glémin 2003). However, these two kinds of purging have distinct properties. Purging by non-random mating theoretically targets all deleterious variants, although its efficiency increases with the recessivity and deleteriousness of the variant (Hedrick 1994; Glémin 2003). Overall, purging by non-random mating is expected to be more efficient than purging by drift, but is limited for small population sizes. Purging by non-random mating is expected to be efficient even with a low level of inbreeding (Glémin 2003).

Theoretical studies indicate that the efficiency of purging by non-random mating depends on many other parameters, such as the level of interference among loci (the “Hill–Robertson” effect) which could reduce the independence and efficiency of purifying selection (Keller and Waller 2002), the dynamics of purging (slow inbreeding provides more opportunity for purging than fast inbreeding) (Frankham et al. 2001), the occurrence of reproductive compensation (Ober et al. 1999; Overall et al. 2002), and the reintroduction of deleterious variants through immigration (Keller and Waller 2002). In addition, the genetic architecture of inbreeding depression plays a significant role in determining the efficiency of purging: if inbreeding depression is mainly due to a small number of lethal variants, purging is likely to be efficient; if it is due to numerous variants with smaller effects, the efficiency of purging may be reduced and the residual inbreeding depression can remain high after purging (Keller and Waller 2002; Glémin 2003). Consequently, it is difficult to theoretically predict whether purging by non-random mating occurs in human populations. Experimental data from other species, including self-fertilizing plants and haplo-diploid insects, have shown that the efficiency of purging by non-random mating is variable between species and populations (Byers and Waller 1999; Henter 2003; Baldwin and Schoen 2019). High-depth next-generation sequencing data allows the direct characterization of the distribution of deleterious variants segregating in human populations, as well as the estimation of inbreeding coefficients capturing both remote and recent parental relatedness. Here, to assess whether purging by non-random mating is efficient in human populations, we focused on populations from Central Asia and Southeast Asia, two regions harboring a great diversity of alliance rules (Marchi et al. 2018; Ly et al. 2019). We selected two pairs of

populations, one from each of these regions. Within each pair, one population exhibits a significantly higher rate of genetic inbreeding than the other, but their effective population sizes and genetic diversity were similar (effective population size is a key parameter for the evolution of both neutral and deleterious diversity that must be controlled for). We produced whole-genome data at high coverage and used different estimators to test whether the population with higher inbreeding had a smaller mutational load, as expected if purging by non-random mating is efficient in human populations.

Results

Population Whole-Genome Sequencing Data

Based on our previous high-density genotype analyses (Marchi et al. 2018; Ly et al. 2019), we selected two pairs of populations exhibiting different alliance rules and inbreeding coefficients, but of similar effective population sizes. From Central Asia, we selected the Turkmen (TUR) and the Tadjik (TJE), with patrilineal and cognatic (bilateral) descent, respectively. From Southeast Asia, we selected the Tampuan (CTA) and the Pacoh (LPO), with matrilineal and patrilineal descent, respectively. The TUR population has a higher mean inbreeding coefficient than the TJE population (Marchi et al. 2018), and the Tampuan population has a higher mean inbreeding than the Pacoh population (Ly et al. 2019). We performed whole-genome sequencing at high coverage (mean depth 31.01) for 24 TUR individuals, 19 TJE individuals, 22 CTA individuals and 21 LPO individuals. We identified a total of 13,834,705 quality-filtered autosomal variants. Using this whole genome data, we confirmed that the level of genetic diversity within each pair of populations was similar (nucleotide diversity $\pi = 6.875 \times 10^{-4}$

and 6.734×10^{-4} for CTA and LPO, respectively, and 7.508×10^{-4} and 7.474×10^{-4} for TUR and the TJE, respectively; [supplementary fig. S1](#)). The difference between TUR and TJE was not statistically significant (Mann–Whitney test, P -value = 0.61). Although the difference between CTA and LPO was statistically significant (Mann–Whitney test, P -value = 8.2×10^{-3}), our previous analysis based on genome-wide genotyping data indicated that these two populations had a similar effective population size (Ly et al. 2019). The allele frequency distribution was similar between populations within each pair of populations, except for an excess of singletons in the CTA population as compared to the LPO population ([supplementary fig. S2](#)).

Genomic Inbreeding Profiles

We estimated the genomic inbreeding coefficient for each individual tested and inferred the most likely genealogical relationships between his/her parents (Fig. 1). As expected, and in line with our previous publications, the mean per-population inbreeding coefficient was significantly higher in CTA (0.024) than in LPO (0.004) (one-sided Mann–Whitney P -value = 4.812×10^{-5}), and significantly higher in TUR (0.020) than in TJE (0.001) (P -value = 2.151×10^{-4}). Similarly, the proportion of individuals inferred to be the offspring of first cousins or second cousins was higher in CTA (91%) than in LPO (48%) (one-sided proportion test, P -value = 2.912×10^{-3}) and higher in TUR (63%) than in TJE (16%) (P -value = 2.784×10^{-3}). In line with these findings, the mean total length of long runs of homozygosity (class C ROH)—contiguous regions of the genome where an individual is homozygous across all sites—was significantly higher in CTA (118 Mb) than in LPO (96 Mb) (one-sided Mann–Whitney test, P -value = 0.03). It was also higher (but not significantly so) in TUR (85 Mb) than in TJE (46 Mb)

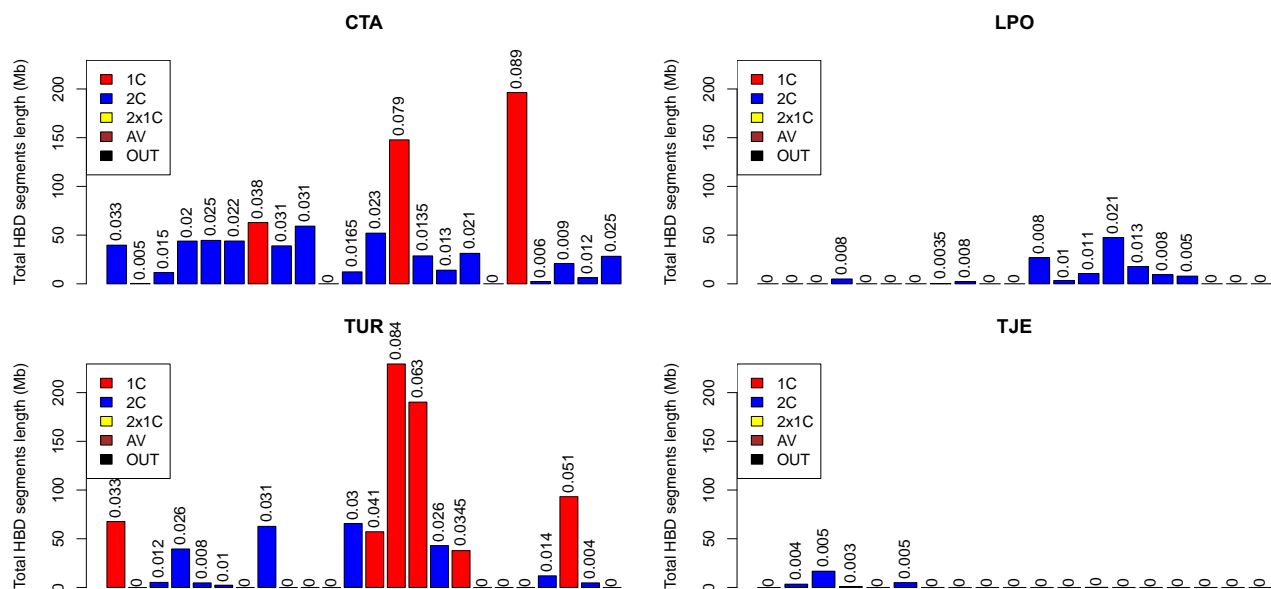


Fig. 1. Genetic inbreeding profiles. Inbreeding coefficient, total HBD length and inferred parental mating type for each individual from the four populations studied: TUR and TJE from Central Asia, CTA and LPO from Southeast Asia. Parental mating types: 1C, first cousin; 2C, second cousin; $2 \times 1C$, double 1C; AV, avuncular; OUT, outbred. Each column corresponds to 1 individual.

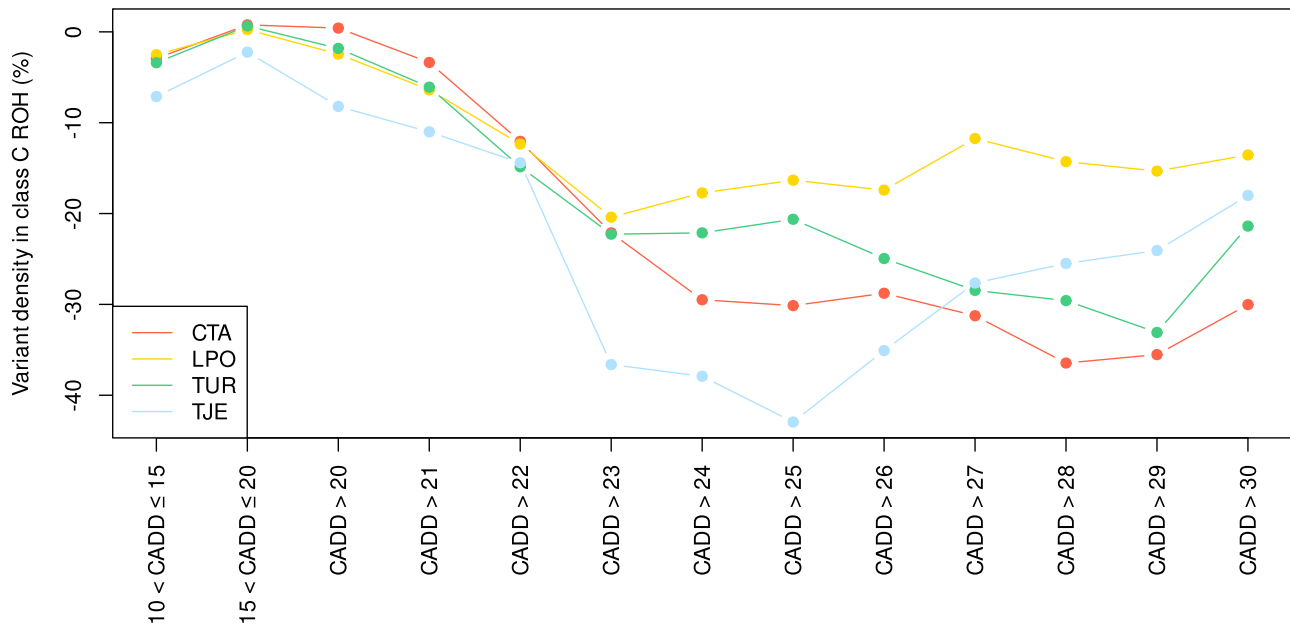


Fig. 2. Deleterious variant density in autozygous tracts (Class C ROH) relative to non-autozygous tracts. Variant density is reported as a percentage (%). Variants were stratified in several CADD classes reflecting the severity of the deleterious effect: neutral (below 10), moderate (10 to 15), large (15 to 20), and extreme (from above 20 to above 30 to assess the sensitivity of the statistics to this threshold). Populations: TUR and TJE from Central Asia, CTA and LPO from Southeast Asia.

(P -value = 0.16) (supplementary fig. S3). The distribution of segments inferred to be homozygous by descent (HBD), as identified using FSuite, was compared to the distribution of long ROH (i.e. class C ROH). Both approaches yielded similar results (supplementary fig. S4). In the following sections, long ROH segments are referred to as autozygous segments.

Testing the Efficiency of Purging by Non-Random Mating in Human Populations

We scored the deleteriousness of each variant using the Combined Annotation Dependent Depletion (CADD) framework, which integrates multiple annotations (combining conservation and functional information) into a single metric (Kircher et al. 2014). We checked that the variants with higher CADD scores had the strongest deleterious consequences on the protein, as predicted by ENSEMBL (supplementary fig. S5). We stratified variants into several classes reflecting the severity of the mutational effect: “neutral” (CADD score below 10), “moderate” (CADD score between 10 and 15), “large” (CADD score between 15 and 20), and “extreme”. For this final category, we progressively increased the threshold from CADD above 20 to CADD above 30, to explore the sensitivity of our estimates of mutational load to the threshold applied.

First, we compared the density of derived deleterious variants in autozygous regions (i.e. class C ROH) and in non-autozygous regions of the genome. Because the sampled individuals were healthy, recessive deleterious variants that led to early death or resulted in severe early-onset Mendelian disorders should be less frequent in autozygous tracts, where they are exposed to selection, than in

non-autozygous portions of the genome, where they are not exposed (Narasimhan et al. 2016). To make this estimation, we normalized the number of deleterious derived variants relative to the number of neutral derived variants (CADD < 10). We expected purging by non-random mating to target all deleterious variants, but to be more efficient for more deleterious variants (Hedrick 1994; Glémin 2003). In line with this hypothesis, our results showed that, whereas the density of variants with moderate and large deleterious effects (CADD below 20) is not reduced in autozygous tracts as compared to non-autozygous tracts, deleterious variants with CADD scores above 22 are depleted in autozygous regions compared to nonautozygous regions in all populations (Fig. 2). This is an indication of direct ongoing purging targeting a fraction of variants with extreme deleteriousness located in autozygous tracts. The intensity of this purging does not appear to be different between the populations with low and high inbreeding.

Second, we tested whether purging of these derived variants with extreme deleteriousness in autozygous tracts led to a lower mutational load in populations with high inbreeding as compared to populations with low inbreeding. To do so, for each population, we computed an estimator of the mutational load (supplementary table S1), defined as the mean number of derived variants per individual (Simons et al. 2014). We then estimated the between-population ratios of this number for variants with different deleteriousness scores (using the more inbred population as the numerator when calculating the ratio). Under the purging hypothesis, these ratios should be below 1, particularly for variants with extreme deleteriousness. However, our analysis revealed that these ratios were usually not significantly different

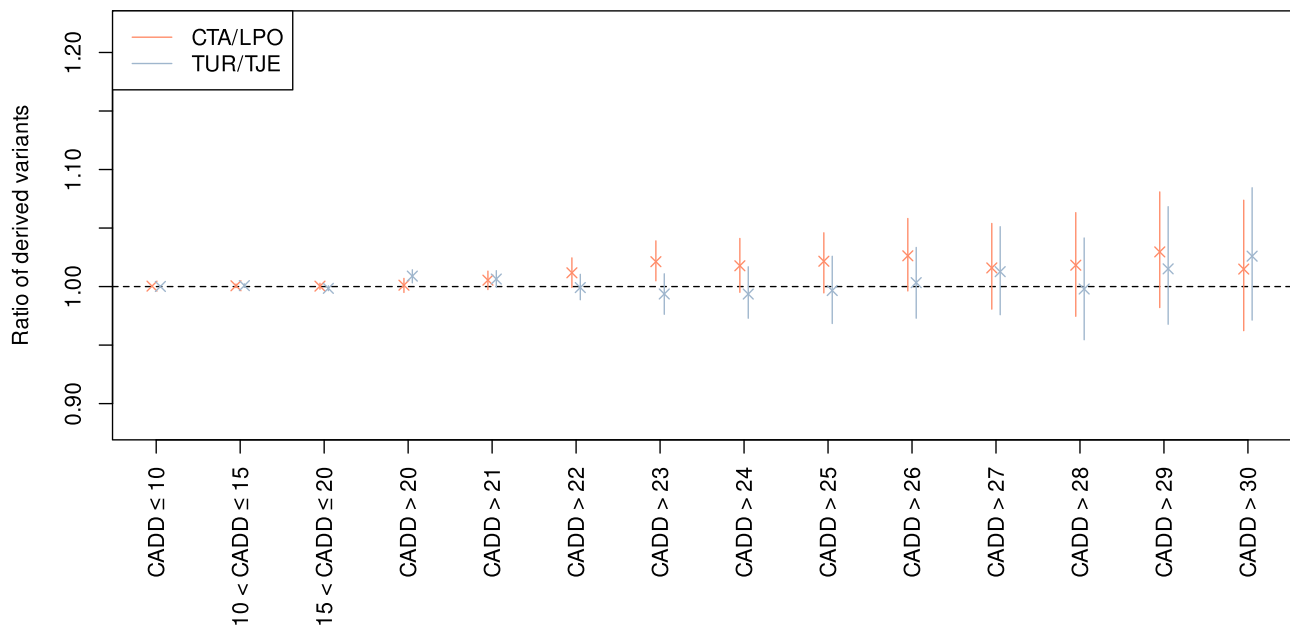


Fig. 3. Between-population ratios of the mean per-individual number of derived variants. Variants were stratified in several CADD classes reflecting the severity of the deleterious effect: neutral (below 10), moderate (10 to 15), large (15 to 20), and extreme (from above 20 to above 30 to assess the sensitivity of the statistics to this threshold). Errors bars represent the 95% confidence intervals obtained from 1,000 block-bootstrap resampling. Populations: TUR and TJE from Central Asia, CTA and LPO from Southeast Asia.

from 1 when comparing CTA to LPO, nor when comparing TUR to TJE; there was even a trend for these ratios to be higher than 1 (Fig. 3). Similar results were found when a slightly different definition of the same estimator was used, focusing on the number of derived variants seen in one population and not seen in the other (supplementary fig. S6), or using a drift-corrected version of this estimator, i.e. when the estimator was normalized relative to neutral variants (variants with $CADD < 10$) (supplementary fig. S7) (Do et al. 2015; Narasimhan et al. 2016). In addition, for each population, we computed the mean number of derived homozygous sites per individual (Simons et al. 2014) (supplementary table S2). Once again, the between-population ratios of this number failed to support the purging hypothesis (supplementary fig. S8). Finally, we estimated the number of lethal recessive variants per individual from the deficit of homozygous deleterious variants (Narasimhan et al. 2016). Indeed, homozygous genotypes that were depleted compared to the neutral expectation are anticipated to be incompatible with adult life, so they provide an estimate of the number of lethal equivalents per genome. This analysis confirmed that the numbers of recessive lethal variants are not significantly different between the populations with low and high inbreeding, the actual numbers depend on the deleteriousness threshold we considered (supplementary fig. S9).

Discussion

In this study, to assess whether purging by non-random mating is efficient in human populations, we analyzed whole-genome sequencing data to compare the mutational load in two pairs of Asian populations exhibiting different alliance rules and inbreeding coefficients. Specifically,

in Southeast Asia, the Tampuan (CTA) population—with matrilineal descent—was compared to the Pacoh (LPO) population—with patrilineal descent. In Central Asia, the Turkmen (TUR) population—with patrilineal descent—was compared to the Tadjiks (TJE) population—with cognatic (bilateral) descent. CTA enter into significantly more frequent inbred unions than LPO; similar observations can be made for TUR compared to TJE. Within each pair of populations, the level of genetic diversity was similar. We tested whether the two populations practicing more frequently inbred unions purged their deleterious mutations more efficiently than the two populations practising less frequently inbred unions. First, we confirmed that autozygous tracts are depleted in variants with extreme deleteriousness, which confirms direct ongoing purging targeting a fraction of those variants when exposed in the homozygous state. Because CTA and TUR have more autozygous tracts than LPO and TJE, respectively, such purging of autozygous tracts may result in a reduced mutational load in the two more inbred populations. To test this hypothesis, we computed five direct estimates of the mutational load: the mean per-individual number of derived variants (Simons and Sella 2016), the mean per-individual number of derived variants seen in one population and not seen in the other as well as a drift-corrected version of this estimator (Do et al. 2015; Narasimhan et al. 2016), the mean per-individual number of derived homozygous sites (Simons and Sella 2016), and the mean per-individual number of recessive lethal variants estimated from the deficit of homozygous deleterious variants (Narasimhan et al. 2016). None of these estimators were observed to be decreased in the populations with high inbreeding as compared to the populations with low inbreeding.

Consequently, while we confirmed that ongoing purging of autozygous tracts occurs for variants with extreme deleteriousness, such purging is insufficient to translate into a reduced mutational load in more inbred populations compared to less inbred populations. Altogether, these analyses suggest that purging by non-random mating does occur, but has a low efficiency in human populations.

As stressed in the introduction, many parameters influence the efficiency of purging and it is difficult to determine which are responsible for the low efficiency we detected in this study. The low efficiency observed may relate to the genetic architecture of inbreeding depression: if it is due to many variants with small effects (rather than a small number of lethal variants), the purging process is expected to have low efficiency (Glémin 2003). Indeed, if purging by non-random mating affects all deleterious variants, the magnitude of the reduction in frequency of these variants at equilibrium depends on the dominance coefficient (Glémin 2003). Based on equation 6 in Glémin (2003), we can express the Differential Purging Efficiency between the populations with high and low inbreeding as:

$$\text{DPE} = 1 - \frac{x_{\text{High-Inb}}}{x_{\text{Low-Inb}}}, \quad (1)$$

where $x_{\text{High-Inb}}$ and $x_{\text{Low-Inb}}$ are the expected frequencies of a deleterious variant at equilibrium in the populations with high and low inbreeding, respectively.

This reduces to:

$$\text{DPE} = \frac{(1-h)(F_{\text{High-Inb}} - F_{\text{Low-Inb}})}{h + (1-h)F_{\text{High-Inb}}}, \quad (2)$$

where h is the dominance coefficient and $F_{\text{High-Inb}}$ and $F_{\text{Low-Inb}}$ are the inbreeding coefficients in populations with high and low inbreeding, respectively (adapted from equation 7 in Glémin (2003)). The purging differential between the two populations, as a function of the dominance coefficient h , exhibits a hyperbolic shape asymptotically approaching 0 as h tends toward 1 (supplementary fig. S10). For both pairs of populations, a plateau is reached from a dominance value around $h = 0.2$. Using $h = 0.25$, a widely accepted dominance value for weakly selected alleles (Manna et al. 2011), a purging differential of less than 0.06 is obtained. Thus, non-random mating in the more inbred population (CTA or TUR) would only result in a 6% reduction in frequency compared to the less inbred populations (LPO or TJE). This slight reduction in variant frequency cannot be confidently identified given the sample sizes in our dataset. Conversely, for $h = 0.02$, a dominance value typical of major effect variants (Manna et al. 2011), a 45% frequency reduction is predicted. Consequently, the lack of evidence found for differences in purging efficiency between the two populations may indicate that h values are too high for most deleterious variants detected in our samples to differ in frequency between the populations compared. Given the inferred negative relationship between dominance coefficients and selection coefficients (Agrawal and Whitlock 2011;

Manna et al. 2011), this result suggests that the inbreeding depression in the populations studied may be due to many variants having small deleterious effects.

Another possibility for our lack of evidence of purging could be that the kinship systems studied here are not sufficiently ancient and stable for the purging process to have occurred. In an infinite population, the change in frequency of a deleterious allele in one generation of selection is $\Delta x \approx -ax$, with $a = s(h + (1-h)F)$, where s and h , respectively, are the selection and dominance coefficients of the deleterious variant, and F is the inbreeding coefficient of the population (Gillespie 2004). Thus, the time required to reach a new equilibrium under mutational and selective pressures after an increase in inbreeding level is of the order of $1/a$. As an example, we plotted the time needed to reach the equilibrium in a population with $F = 0.024$, as in the CTA (supplementary fig. S11), for different h and s values. For variants with weak effects ($h = 0.25$ and $s = 0.01$), to get halfway to the new equilibrium would take 259 generations ($\sim 6,500$ years with a generation time of 25 years), and for variants with severe deleterious effects ($h = 0.02$ and $s = 0.25$), it would take 64 generations ($\sim 1,600$ years). Genetic data suggest that the kinship structures of the populations studied are ancient. Several reports indicate that traits associated with family structure and kinship are highly conserved over generations (for example, Guglielmino et al. 1995). In addition, Y chromosome diversity is decreased in the patrilineal LPO population and mitochondrial diversity is decreased in the matrilineal CTA population, suggesting stable patriliney and matriliney, respectively (Ly et al. 2018). Matriliney has been shown to be associated with increased consanguinity as a result of the matrilineal puzzle (a man has to conciliate his loyalties to his conjugal and to his natal kin, Ly et al. 2019). In addition, Y chromosome data suggest that the patrilineal descent of the TUR may date from 2,600 years ago (Marchi et al. 2017). At the worldwide level, Y chromosome data suggest that patrilineal systems may be even older, dating from up to or exceeding 5,000 years (Zeng et al. 2018; Guyon et al. 2024). TUR individuals are not allowed to marry within their own patrilineage but may still marry a cousin on the maternal side from a different lineage. The evidence that these kinship structures are stable and ancient suggests that we should have seen at least some differences between the more and less inbred populations for the variants with severe deleterious effects. As no such difference was observed, either inbreeding depression is mainly due to variants with small effects, or the kinship structures are not as ancient as we believe; in any case, not sufficiently ancient for the purging of variants with large effects to have occurred. Alternatively, reproductive compensation (Ober et al. 1999; Overall et al. 2002) or the reintroduction of deleterious variants through immigration (Keller and Waller 2002) could also have reduced the efficiency of purging by non-random mating.

A limitation of this study is that, although the TUR and CTA have a rate of inbreeding that is particularly high among present-day human populations (63% and 91% of individuals being the offspring of first and second cousins in these two populations, respectively), they do not enter

into unions between first-degree relatives. It is therefore unclear whether our conclusions of low purging efficiency would hold for a population where first-degree unions are common. Since such unions are forbidden in most, if not all, human populations (Pasternak 1976; Fox 1983; Wolf 2014), this question is difficult to answer. We used data from the 1 000 Genome Project to test whether our conclusions hold when focusing on other human populations with high inbreeding. We found no significant correlation between the mutational load (based on LOF variants computed by Narasimham et al. (2016)) and the inbreeding coefficients computed by Gazal et al. (2015) (supplementary fig. S12, Spearman $\rho = 0.21$, P -value = 0.41). Under the purging hypothesis, the population with the highest inbreeding coefficients should have a lower mutational load. Note that this analysis should be considered with caution since the effective sizes of these populations are different. A particular strength of our study was that it compared populations with different inbreeding practices but of similar effective population sizes. Indeed, effective population size is a key parameter when monitoring changes of neutral and deleterious diversity and must be controlled for when assessing the efficiency of purging by non-random mating. Another study, where effective population size was not controlled for, compared Middle Eastern populations (with a high rate of inbred mating) to European populations and found similar frequencies of deleterious variants among these populations. This result also suggests that purging by inbred mating has a low efficiency (Scott et al. 2016) (however the fact that the authors selected healthy individuals from families brought to medical attention may tend to overestimate the mutational load in the Middle Eastern populations). An analysis focused on a UK-based population of Pakistani heritage, which exhibits one of the highest inbreeding levels among present-day human populations (Narasimhan et al. 2016), reported a lower density of LOF variants within autozygous tracts compared with non-autozygous tracts. This result indicates direct ongoing purging by non-random mating of a fraction of LOF variants. However, it also showed that a typical individual from the population studied carries 1.6 recessive LOF lethal equivalents. This number is of the same order as previous estimates based on epidemiological data for worldwide human populations (Morton et al. 1956; Bittles and Neel 1994) and corroborates the estimates presented here. This concordance between populations exhibiting contrasted inbreeding practices suggests that the purging process is ongoing but incomplete and has a low efficiency. Epidemiological data from highly inbred royal families such as the Habsburgs provide some evidence of purging over time. However, the overall high inbreeding load (3.937 lethal equivalents) confirms that purging by non-random mating has a low efficiency (Ceballos and Álvarez 2013). Finally, our results are also in line with those of Szpiech et al. (2013), demonstrating that long ROH are enriched in deleterious variants in the homozygous state, supporting the low efficiency of purging by non-random mating. It should be noted that, in our study, the use of the CADD score, which groups together conservation and functional

information into a single deleteriousness score, did reveal ongoing purging for the variants with the highest deleteriousness scores (CADD > 23) (Fig. 2). This effect remained unseen in Szpiech et al. (2013).

Altogether, these genetic data demonstrate that purging by non-random mating has a low efficiency in human populations and that different mating practices do not lead to different mutational loads. Thus, the hypothesis that deleterious variants may be purged through consanguineous mating is not supported. Consequently, we expect to observe inbreeding depression in individuals born from inbred mating (Hedrick and Garcia-Dorado 2016). Recent studies have tested this assumption in humans, by assessing the relationships between the inbreeding coefficient at the individual level and variations in a wide range of phenotypic traits of biomedical importance across multicohort studies including populations where close kin mating occurs (The BioBank Japan Project et al. 2015; Clark et al. 2019). Inbreeding coefficients were measured from ROH, as in our study, which captures the realized inbreeding level rather than the expected inbreeding. Links between these inbreeding coefficients and a wide range of phenotypic traits such as height, weight, forced expiratory volumes and fertility were confirmed. Thus, offspring of first cousins have a 55% decreased chance of having children, are 1.2 cm shorter and have a lung expiratory capacity that is 140 mL lower (The BioBank Japan Project et al. 2015; Ceballos et al. 2018; Clark et al. 2019). These results should be considered with care because of the potential socio-economic confounding factors of consanguinity (Bittles and Black 2010). Attempts to correct for such confounding factors—by evaluating the consequences of different realized inbreeding levels between siblings or by assessing the influence of ROH in cohorts of different continental origins and with different homozygosity levels—confirmed the reported deleterious consequence of inbreeding (The BioBank Japan Project et al. 2015; Clark et al. 2019; Malawsky et al. 2023). Inbreeding depression is also found in natural populations of vertebrates (e.g. Stoffel et al. 2021).

To sum up, in this study we tested the efficiency of purging by non-random mating in human populations using an original study design, comparing estimates of mutational loads based on whole-genome sequencing in populations with similar effective population sizes but different rates of inbred mating. Our results showed that purging by non-random mating has low efficiency and that different mating practices do not lead to different mutational loads. Thus, the hypothesis that deleterious variants may be purged through consanguineous mating is not supported. While future studies replicating this experimental design in other socio-cultural contexts would be useful, these results corroborate previous observations showing that the contrasted demographic histories of human populations have little impact on their mutational load (Simons and Sella 2016), although some exceptions, such as isolated European populations (Xue et al. 2017) have been reported. Altogether, the mutational load appears to have a low sensitivity to variations in demographic histories and to the non-random mating practices characterizing human populations.

Materials and Methods

Populations Studied

Two pairs of populations were selected based on ethno-demographic data collected during several field expeditions and on genotype data from previous studies. Populations were defined as groups of individuals belonging to the same ethnic group, based on the self-reported spoken native language. In Central Asia, Tadjiks (TJE) are sedentary farmers with cognatic (bilateral) descent, whereas TUR are nomadic herders with patrilineal descent. TUR was shown to have a higher inbreeding coefficient than TJE (Marchi et al. 2018). In Southeast Asia, the Tampuan (CTA) population has matrilineal descent and the Pacoh (LPO) population has patrilineal descent. Both are slash-and-burn rice farmers living in the low mountains of Cambodia and Laos. The CTA population has a higher genetic inbreeding coefficient than the LPO population as a result of the matrilineal puzzle (Ly et al. 2019).

Written informed consent was obtained from all participants, and our sampling and analyses were conducted in line with ethical principles according to a protocol approved by the Museum National d'Histoire Naturelle (France), the Uzbek Academy of Sciences (Uzbekistan), the National Ethics Committees for Health Research in Cambodia and Laos as well as by Comité Opérationnel pour l'Éthique (CNRS, France).

Genome Sequencing, Mapping and Variant Calling

DNA samples extracted from blood and saliva ($n = 86$) were prepared for whole-genome sequencing by the Centre National de Recherche en Génomique Humaine (CNRGH, CEA). After quality control, genomic DNA (1.1 μg) was used to prepare a library using the Illumina TruSeq DNA PCR-Free Library Preparation Kit, according to the manufacturer's instructions. After normalization and quality control, qualified libraries were sequenced on a HiSeqX5 platform from Illumina (Illumina Inc., CA, USA), as paired-end 150-bp reads. One HiSeqX5 flow cell lane was produced for each sample, providing an average sequencing depth of $30\times$ for each sample. Sequence quality metrics were assessed throughout the sequencing run, and FASTQ files were generated for each sample. In line with genome analysis toolkit (GATK) best practices, read pairs from FASTQ files were first mapped to the human reference genome (GRCh37 + decoy) using the bwa-mem program (<http://bio-bwa.sourceforge.net/>). Reads duplicating the start position of another read were marked as duplicates and eliminated from bam files by the Sambamba program (<http://lomeriteiter.github.io/sambamba/docs/sambamba-view.html>). We used GATK version 3.5 (Van der Auwera and O'Connor 2020) for base quality score recalibration (BaseRecalibrator, BQSR), insertion/deletion (indel) realignment (IndelRealigner), and single nucleotide polymorphism (SNP) and Indel discovery (Haplotype Caller) for each sample. A joint callset from the four populations was obtained using GATK version 4.2, applying the GATK best practices workflow. Individual variant files were combined with

Genotype genomic variant call format and filtered for variant quality score recalibration. Indels, SNPs located on the sex chromosomes, multiallelic sites, and sites with missing data for subsequent analyses were deleted.

Genetic Diversity Estimators

Nucleotide diversity was computed for each population using VCFtools version 0.1.16 (Danecek et al. 2011) using the "window-pi" option with a window size of 1 Mbp. The unfolded Site Frequency Spectrum (SFS) was computed for each population using in-house software. To obtain comparable SFS between populations with different sample sizes, we down-sampled all populations to 19 samples (the smallest sample size in our dataset) and repeated this resampling 1,000 times to determine the confidence intervals for each allele frequency.

Variant Annotation

The ancestral and derived allele state of each variant was inferred using a 6-primate alignment following the 1,000 Genomes Project methodology (Auton et al. 2015). We scored the deleteriousness of each variant using the CADD version 1.6 framework (Kircher et al. 2014), which integrates multiple annotations (combining conservation and functional information) into one metric. We stratified the variants into several classes reflecting the severity of the mutational effect: "neutral" (CADD score below 10), "moderate" (CADD score between 10 and 15), "large" (CADD score between 15 and 20) and "extreme" (CADD higher than 20). In the latter class, CADD scores below 30 mostly corresponded to missense, splice acceptor/donor and codon initiator variants, whereas CADD scores above 30 were mostly stop variants (supplementary fig S5). As part of our analyses, we analyzed the sensitivity of this extreme CADD score threshold by progressively increasing its value from 20 to 30.

Estimation of Genetic Inbreeding

For each population, we used FSuite v1.0.4 (Gazal et al. 2014) to estimate the inbreeding coefficient for each individual and the HBD segments, as well as the parents' mating type (Leutenegger et al. 2003, 2011). We used the joint Hap Map Phase II Build GRCh37 genetic recombination maps (Frazer et al. 2007) and performed computations on 100 submaps per population.

We identified ROH in the genome of all individuals from each population independently with GARLIC (Szpiech et al. 2017), using the weighted logarithm of the odds (wLOD) scheme, a genotyping error rate of 1×10^{-3} (probably an overestimate), the same recombination maps as for the FSuite analyses, and windows sizes ranging from 30 SNPs to 100 SNPs in 10-SNP increments. We only considered results obtained using a window size of 50 SNPs, which was the largest providing a bimodal wLOD score distribution and a sensitive wLOD score cutoff for all populations. GARLIC uses a Gaussian Mixture Model (GMM) to determine length boundaries for short, medium, and

long ROHs (Pemberton et al. 2012), which are computed on a per-population basis. To obtain comparable ROH classes between pairs of populations, we performed the GMM step separately for the two sets of paired populations: using all the ROHs found in any CTA or LPO samples, and all the ROHs found in any TUR or TJE samples. This procedure ensures that class lengths are comparable between populations within each pair.

Evaluating the Mutational Load

Various estimators of mutational load based on individual whole-genome data were calculated. Firstly, we compared the load inside and outside autozygous regions for each population. For each individual, and for each CADD class, we counted the number of derived variants inside autozygous regions (i.e. class C ROH) and outside ROH of any class. These numbers were calculated for each individual as $N_{\text{variants}} = N_{\text{heterozygous genotypes}} + 2 \times N_{\text{derived homozygous genotypes}}$. Then, we computed the ratio of these numbers (averaged over all individuals from the same population) and normalized this ratio relative to the same ratio computed for neutral variants (CADD < 10).

Secondly, we computed an estimator of the mutational load defined, for each population, as the mean number of derived variants per individual (Simons et al. 2014). Then, we computed the between-population ratio of this number.

We also computed $R_{X/Y}$ and $R'_{X/Y}$ statistics, as introduced by Do et al. (2015). These are slightly different definitions of the same estimator. For $R_{X/Y}$, we calculated the mean per-individual number of derived variants seen in one population but not the other and vice versa, and then we computed the ratio between these two numbers. $R'_{X/Y}$ is the drift-corrected version of this ratio, normalized relative to the same ratio computed for neutral variants (CADD < 10).

Thirdly, we computed an estimator of mutational load defined as the mean per-individual homozygous derived sites, as proposed by Simons et al. (Simons et al. 2014).

For the estimators described above, confidence intervals were calculated using bootstrapping (performed within each pair of populations) by splitting the SNP data into 1,000 blocks and resampling with replacement 1,000 times. This approach allowed us to take into account the variance introduced by demographic processes (Gravel 2016; Simons and Sella 2016).

Finally, we computed the number of lethal equivalents per genome using the approach described by Narasimhan et al. (2016), based on the suppression of deleterious homozygous genotypes. In each population, for each derived allele frequency, we computed the average number of homozygous genotypes per individual for both deleterious variants (increasing the CADD threshold progressively from above 20 to above 30) and for neutral variants (CADD < 10). Then, for each CADD class, we computed the number of homozygous genotypes that were depleted compared to the neutral expectation. These variants are

expected to be incompatible with adult life and provide an estimate of the number of lethal equivalents per genome. We obtained a genome-wide estimate of this deficit by computing the weighted sum of deficits over the derived allele frequencies.

Supplementary Material

Supplementary material is available at *Molecular Biology and Evolution* online.

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Data Availability

The data underlying this article will be shared on reasonable request to Evelyne Heyer, Raphaëlle Chaix and Romain Laurent. Codes are provided on github (https://github.com/romain-laurent/genetic_load_project).

References

- Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, Gibbs RA, Hurles ME, McVean GA. A map of human genome variation from population-scale sequencing. *Nature*. 2010;**467**(7319): 1061–1073. <https://doi.org/10.1038/nature09534>.
- Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA. An integrated map of genetic variation from 1,092 human genomes. *Nature*. 2012;**491**(7422):56–65. <https://doi.org/10.1038/nature11632>.
- Agrawal AF, Whitlock MC. Inferences about the distribution of dominance drawn from yeast gene knockout data. *Genetics*. 2011;**187**(2):553–566. <https://doi.org/10.1534/genetics.110.124560>.
- Auton A, Abecasis GR, Altshuler DM, Durbin RM, Bentley DR, Chakravarti A, Clark AG, Donnelly P, Eichler EE, Flicek P, et al.

- A global reference for human genetic variation. *Nature*. 2015;**526**(7571):68–74. <https://doi.org/10.1038/nature15393>.
- Baldwin SJ, Schoen DJ. Inbreeding depression is difficult to purge in self-incompatible populations of *Leavenworthia alabamica*. *New Phytol*. 2019;**224**(3):1330–1338. <https://doi.org/10.1111/nph.15963>.
- Besenbacher S, Liu S, Izarzugaza JMG, Grove J, Belling K, Bork-Jensen J, Huang S, Als TD, Li S, Yadav R, et al. Novel variation and de novo mutation rates in population-wide de novo assembled Danish trios. *Nat Commun*. 2015;**6**(1):5969. <https://doi.org/10.1038/ncomms6969>.
- Bittles AH, Black ML. Consanguineous marriage and human evolution. *Annu Rev Anthropol*. 2010;**39**(1):193–207. <https://doi.org/10.1146/annurev.anthro.012809.105051>.
- Bittles AH, Neel J V. The costs of human inbreeding and their implications for variations at the DNA level. *Nature*. 1994;**1**:638–653. <https://doi.org/10.1038/ng1094-117>.
- Byers DL, Waller DM. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annu Rev Ecol Syst*. 1999;**30**(1):479–513. <https://doi.org/10.1146/annurev.ecolsys.30.1.479>.
- Casals F, Hodgkinson A, Hussin J, Idaghdour Y, Bruat V, de Maillard T, Grenier JC, Gbeha E, Hamdan FF, Girard S, et al. Whole-exome sequencing reveals a rapid change in the frequency of rare functional variants in a founding population of humans. *PLoS Genet*. 2013;**10**(2):e1004260. <https://doi.org/10.1371/journal.pgen.1003815>.
- Castellano S, Parra G, Sánchez-Quinto FA, Racimo F, Kuhlwilm M, Kircher M, Sawyer S, Fu Q, Heinze A, Nickel B, et al. Patterns of coding variation in the complete exomes of three Neandertals. *Proc Natl Acad Sci USA*. 2014;**111**(18):6666–6671. <https://doi.org/10.1073/pnas.1405138111>.
- Ceballos FC, Álvarez G. Royal dynasties as human inbreeding laboratories: The Habsburgs. *Heredity (Edinb)*. 2013;**111**(2):114–121. <https://doi.org/10.1038/hdy.2013.25>.
- Ceballos FC, Joshi PK, Clark DW, Ramsay M, Wilson JF. Runs of homozygosity: windows into population history and trait architecture. *Nat Rev Genet*. 2018;**19**(4):220–234. <https://doi.org/10.1038/nrg.2017.109>.
- Charlesworth D, Willis JH. The genetics of inbreeding depression. *Nat Rev Genet*. 2009;**10**(11):783–796. <https://doi.org/10.1038/nrg2664>.
- Clark DW, Okada Y, Moore KHS, Mason D, Pirastu N, Gandin I, Mattsson H, Barnes CLK, Lin K, Zhao JH, et al. Associations of autozygosity with a broad range of human phenotypes. *Nat Commun*. 2019;**10**(1):4957. <https://doi.org/10.1038/s41467-019-12283-6>.
- Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, et al. The variant call format and VCFtools. *Bioinformatics*. 2011;**27**(15):2156–2158. <https://doi.org/10.1093/bioinformatics/btr330>.
- Do R, Balick D, Li H, Adzhubei I, Sunyaev S, Reich D. No evidence that selection has been less effective at removing deleterious mutations in Europeans than in Africans. *Nat Genet*. 2015;**47**(2):126–131. <https://doi.org/10.1038/ng.3186>.
- Fox R. *Kinship and marriage: an anthropological perspective*. Cambridge (NY): Cambridge University Press; 1983.
- Frankham R, Gilligan DM, Morris D, Briscoe DA. Inbreeding and extinction: effects of purging. *Conserv Genet*. 2001;**2**(3):279–284. <https://doi.org/10.1023/A:1012299230482>.
- Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, Belmont JW, Boudreau A, Hardenbol P, Leal SM, et al. A second generation human haplotype map of over 3.1 million SNPs. *Nature*. 2007;**449**(7164):851–861. <https://doi.org/10.1038/nature06258>.
- Gazal S, Sahbatou M, Babron M-C, Génin E, Leutenegger A-L. FSuite: exploiting inbreeding in dense SNP chip and exome data. *Bioinformatics*. 2014;**30**(13):1940–1941. <https://doi.org/10.1093/bioinformatics/btu149>.
- Gazal S, Sahbatou M, Babron M-C, Génin E, Leutenegger A-L. High level of inbreeding in final phase of 1000 Genomes Project. *Sci Rep*. 2015;**5**(1):17453. <https://doi.org/10.1038/srep17453>.
- Gillespie JH. *Population Genetics: A Concise Guide*. Johns Hopkins University Press; 2004.
- Glémin S. How are deleterious mutations purged? Drift versus non-random mating. *Evolution*. 2003;**57**(12):2678–2687. <https://doi.org/10.1111/j.0014-3820.2003.tb01512.x>.
- Gravel S. When is selection effective? *Genetics*. 2016;**203**(1):451–462. <https://doi.org/10.1534/genetics.115.184630>.
- Guglielmino CR, Viganotti C, Hewlett B, Cavalli-Sforza LL. Cultural variation in Africa: role of mechanisms of transmission and adaptation. *Proc Natl Acad Sci USA*. 1995;**92**(16):7585–7589. <https://doi.org/10.1073/pnas.92.16.7585>.
- Guyon L, Guez J, Toupance B, Heyer E, Chaix R. Patrilineal segmentary systems provide a peaceful explanation for the post-Neolithic Y-chromosome bottleneck. *Nat Commun*. 2024;**15**(1):3243. <https://doi.org/10.1038/s41467-024-47618-5>.
- Harris K, Nielsen R. The genetic cost of neanderthal introgression. *Genetics*. 2016;**203**(2):881–891. <https://doi.org/10.1534/genetics.116.186890>.
- Hedrick PW. Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity (Edinb)*. 1994;**73**(4):363–372. <https://doi.org/10.1038/hdy.1994.183>.
- Hedrick PW, Garcia-Dorado A. Understanding inbreeding depression, purging, and genetic rescue. *Trends Ecol Evol (Amst)*. 2016;**31**(12):940–952. <https://doi.org/10.1016/j.tree.2016.09.005>.
- Henn BM, Botigué LR, Bustamante CD, Clark AG, Gravel S. Estimating the mutation load in human genomes. *Nat Rev Genet*. 2015;**16**(6):333–343. <https://doi.org/10.1038/nrg3931>.
- Henn BM, Botigué LR, Peischl S, Dupanloup I, Lipatov M, Maples BK, Martin AR, Musharoff S, Cann H, Snyder MP, et al. Distance from sub-Saharan Africa predicts mutational load in diverse human genomes. *Proc Natl Acad Sci USA*. 2016;**113**:E440–E449.
- Henter HJ. Inbreeding depression and haplodiploidy: experimental measures in a parasitoid and comparisons across diploid and haplodiploid insect taxa. *Evolution*. 2003;**57**(8):1793–1803. <https://doi.org/10.1111/j.0014-3820.2003.tb00587.x>.
- The BioBank Japan Project, Joshi PK, Esko T, Mattsson H, Eklund N, Gandin I, Nutile T, Jackson AU, Schurmann C, Smith AV, et al. Directional dominance on stature and cognition in diverse human populations. *Nature*. 2015;**523**(7561):459–462. <https://doi.org/10.1038/nature14618>.
- Keller LF, Waller DM. Inbreeding effects in wild populations. *Trends Ecol Evolut*. 2002;**17**(5):19–23. [https://doi.org/10.1016/S0169-5347\(02\)02489-8](https://doi.org/10.1016/S0169-5347(02)02489-8).
- Kircher M, Witten DM, Jain P, O’roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet*. 2014;**46**(3):310–315. <https://doi.org/10.1038/ng.2892>.
- Exome Aggregation Consortium, Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O’Donnell-Luria AH, Ware JS, Hill AJ, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;**536**(7616):285–291. <https://doi.org/10.1038/nature19057>.
- Leutenegger A-L, Prum B, Génin E, Verny C, Lemainque A, Clerget-Darpoux F, Thompson EA. Estimation of the inbreeding coefficient through use of genomic data. *Am J Hum Genet*. 2003;**73**(3):516–523. <https://doi.org/10.1086/378207>.
- Leutenegger A-L, Sahbatou M, Gazal S, Cann H, Génin E. Consanguinity around the world: what do the genomic data of the HGDP-CEPH diversity panel tell us? *Eur J Hum Genet*. 2011;**19**(5):583–587. <https://doi.org/10.1038/ejhg.2010.205>.
- Lim ET, Würtz P, Havulinna AS, Palta P, Tukiainen T, Rehnström K, Esko T, Mägi R, Inouye M, Lappalainen T, et al. Distribution and medical impact of loss-of-function variants in the Finnish founder population. *PLoS Genet*. 2014;**10**(7):e1004494. <https://doi.org/10.1371/journal.pgen.1004494>.
- Lopez M, Kousathanas A, Quach H, Harmant C, Mougouma-Daouda P, Hombert JM, Froment A, Perry GH, Barreiro LB, Verdu P, et al. The demographic history and mutational load of African hunter-gatherers and farmers. *Nat Ecol Evolut*. 2018;**2**(4):721–730. <https://doi.org/10.1038/s41559-018-0496-4>.

- Lupski JR, Belmont JW, Boerwinkle E, Gibbs RA. Clan genomics and the complex architecture of human disease. *Cell*. 2011;**147**(1):32–43. <https://doi.org/10.1016/j.cell.2011.09.008>.
- Ly G, Alard B, Laurent R, Lafosse S, Toupance B, Monidarin C, Diffloth G, Bourdier F, Evrard O, Pavard S, et al. Residence rule flexibility and descent groups dynamics shape uniparental genetic diversities in South East Asia. *Am J Phys Anthropol*. 2018;**165**(3):480–491. <https://doi.org/10.1002/ajpa.23374>.
- Ly G, Laurent R, Lafosse S, Monidarin C, Diffloth G, Bourdier F, Evrard O, Toupance B, Pavard S, Chaix R. From matrimonial practices to genetic diversity in Southeast Asian populations: the signature of the matrilineal puzzle. *Philos Trans R Soc B Biol Sci*. 2019;**374**(1780):20180434. <https://doi.org/10.1098/rstb.2018.0434>.
- Malawsky DS, van Walree E, Jacobs BM, Heng TH, Huang QQ, Sabir AH, Rahman S, Sharif SM, Khan A, Mirkov MU, et al. Influence of autozygosity on common disease risk across the phenotypic spectrum. *Cell*. 2023;**186**(21):4514–4527. <https://doi.org/10.1016/j.cell.2023.08.028>.
- Manna F, Martin G, Lenormand T. Fitness landscapes: an alternative theory for the dominance of mutation. *Genetics*. 2011;**189**(3):923–937. <https://doi.org/10.1534/genetics.111.132944>.
- Marchi N, Hegay T, Mennecier P, Georges M, Laurent R, Whitten M, Endicott P, Aldashev A, Dorzhu C, Nasyrova F, et al. Sex-specific genetic diversity is shaped by cultural factors in Inner Asian human populations. *Am J Phys Anthropol*. 2017:1–14. <https://doi.org/10.1002/ajpa.23210>.
- Marchi N, Mennecier P, Georges M, Lafosse S, Hegay T, Dorzhu C, Chichlo B, Ségurel L, Heyer E. Close inbreeding and low genetic diversity in inner Asian human populations despite geographical exogamy. *Sci Rep*. 2018;**8**(1):1–10. <https://doi.org/10.1038/s41598-018-27047-3>.
- Morton NE, Crow JF, Muller H. An estimate of the mutational damage in man from data on consanguineous marriages. *Proc Natl Acad Sci USA*. 1956;**42**(11):855–863. <https://doi.org/10.1073/pnas.42.11.855>.
- Nakatsuka N, Moorjani P, Rai N, Sarkar B, Tandon A, Patterson N, Bhavani GS, Girisha KM, Mustak MS, Srinivasan S, et al. The promise of discovering population-specific disease-associated genes in South Asia. *Nat Genet*. 2017;**49**(9):1403–1407. <https://doi.org/10.1038/ng.3917>.
- Narasimhan VM, Hunt KA, Mason D, Baker CL, Karczewski KJ, Barnes MR, Barnett AH, Bates C, Bellary S, Bockett NA, et al. Health and population effects of rare gene knockouts in adult humans with related parents. *Science*. 2016;**352**(6284):474–477. <https://doi.org/10.1126/science.aac8624>.
- Ober C, Hyslop T, Hauck WW. Inbreeding effects on fertility in humans: evidence for reproductive compensation. *Am J Hum Genet*. 1999;**64**(1):225–231. <https://doi.org/10.1086/302198>.
- Overall ADJ, Ahmad M, Nichols RA. The effect of reproductive compensation on recessive disorders within consanguineous human populations. *Heredity (Edinb)*. 2002;**88**(6):474–479. <https://doi.org/10.1038/sj.hdy.6800090>.
- Pasternak B. *Introduction to kinship and social organization*. Englewood Cliffs, NJ: Prentice-Hall; 1976.
- Pedersen CET, Lohmueller KE, Grarup N, Bjerregaard P, Hansen T, Siegmund HR, Moltke I, Albrechtsen A. The effect of an extreme and prolonged population bottleneck on patterns of deleterious variation: insights from the Greenlandic Inuit. *Genetics*. 2017;**205**(2):787–801. <https://doi.org/10.1534/genetics.116.193821>.
- Pemberton TJ, Absher D, Feldman MW, Myers RM, Rosenberg NA, Li JZ. Genomic patterns of homozygosity in worldwide human populations. *Am J Hum Genet*. 2012;**91**(2):275–292. <https://doi.org/10.1016/j.ajhg.2012.06.014>.
- Scott EM, Hales A, Itan Y, Spencer EG, He Y, Azab MA, Gabriel SB, Belkadi A, Boisson B, Abel L, et al. Characterization of Greater Middle Eastern genetic variation for enhanced disease gene discovery. *Nat Genet*. 2016;**48**(9):1071–1076. <https://doi.org/10.1038/ng.3592>.
- Simons YB, Sella G. The impact of recent population history on the deleterious mutation load in humans and close evolutionary relatives. *Curr Opin Genet Dev*. 2016;**41**:150–158. <https://doi.org/10.1016/j.gde.2016.09.006>.
- Simons YB, Turchin MC, Pritchard JK, Sella G. The deleterious mutation load is insensitive to recent population history. *Nat Genet*. 2014;**46**(3):220–224. <https://doi.org/10.1038/ng.2896>.
- Stoffel MA, Johnston SE, Pilkington JG, Pemberton JM. Genetic architecture and lifetime dynamics of inbreeding depression in a wild mammal. *Nat Commun*. 2021;**12**(1):2972. <https://doi.org/10.1038/s41467-021-23222-9>.
- Sulem P, Helgason H, Oddson A, Stefansson H, Gudjonsson SA, Zink F, Hjartarson E, Sigurdsson GT, Adalbjorg J, Aslaug J, et al. Identification of a large set of rare complete human knockouts. *Nat Genet*. 2015;**47**(5):448–452. <https://doi.org/10.1038/ng.3243>.
- Szpiech ZA, Blant A, Pemberton TJ. GARLIC: genomic autozygosity regions likelihood-based inference and classification. *Bioinformatics*. 2017;**33**(13):2059–2062. <https://doi.org/10.1093/bioinformatics/btx102>.
- Szpiech ZA, Xu J, Pemberton TJ, Peng W, Zöllner S, Rosenberg NA, Li JZ. Long runs of homozygosity are enriched for deleterious variation. *Am J Hum Genet*. 2013;**93**(1):90–102. <https://doi.org/10.1016/j.ajhg.2013.05.003>.
- Van der Auwera GA, O'Connor BD. *Genomics in the cloud: using Docker, GATK, and WDL in Terra*. 1st ed. O'Reilly Media; 2020.
- Wolf AP. *Incest avoidance and the incest taboos: two aspects of human nature*. Stanford, CA: Stanford Briefs, an imprint of Stanford University Press; 2014.
- Xue Y, Mezzavilla M, Haber M, McCarthy S, Chen Y, Narasimhan V, Gilly A, Ayub Q, Colonna V, Southam L, et al. Enrichment of low-frequency functional variants revealed by whole-genome sequencing of multiple isolated European populations. *Nat Commun*. 2017;**8**(1):1–7. <https://doi.org/10.1038/ncomms15927>.
- Zeng TC, Aw AJ, Feldman MW. Cultural hitchhiking and competition between patrilineal kin groups explain the post-Neolithic Y-chromosome bottleneck. *Nat Commun*. 2018;**9**(1):2077. <https://doi.org/10.1038/s41467-018-04375-6>.