Exploiting selection at linked sites to infer the rate and strength of adaptation

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Genomic data encode past evolutionary events and have the potential to reveal the strength, rate and biological drivers of adaptation. However, joint estimation of adaptation rate ($\alpha$) and adaptation strength remains challenging because evolutionary processes such as demography, linkage and non-neutral polymorphism can confound inference. Here, we exploit the influence of background selection to reduce the fixation rate of weakly beneficial alleles to jointly infer the strength and rate of adaptation. We develop a McDonald–Kreitman-based method to infer adaptation rate and strength, and estimate $\alpha = 0.135$ in human protein-coding sequences, 72% of which is contributed by weakly adaptive variants. We show that, in this adaptation regime, $\alpha$ is reduced -25% by linkage genome-wide. Moreover, we show that virus-interacting proteins undergo adaptation that is both stronger and nearly twice as frequent as the genome average ($\alpha = 0.224$, 56% due to strongly beneficial alleles). Our results suggest that, while most adaptation in human proteins is weakly beneficial, adaptation to viruses is often strongly beneficial. Our method provides a robust framework for estimation of adaptation rate and strength across species.

The relative importance of selection and drift in driving species diversification has been a matter of debate since the origins of evolutionary biology. In the earliest formulations of evolutionary theory, natural selection was proposed to be the predominant driver of differences among species1–2. Subsequent theorists argued that random genetic drift could be a more important contributor to species differences2–3, with random changes accumulating over evolutionary time due to reproductive isolation between populations. Although it is now clear that natural selection plays a substantial role in both diversification and constraint in many species4–10, considerable uncertainty remains regarding the relative importance of stochastic drift, mutation, selection and linkage, with no clear functional sites (DFE) of fixing mutations19. The critical idea behind each of these methods is to compare evidence for differentiation at alleles that confer fitness benefits (that is, processes that do not increase fitness) such as guanine and cytosine (GC)-biased gene conversion20 could also lead to an excess of non-synonymous differences between species, but only if these processes differentially affect synonymous and non-synonymous mutations.

Unfortunately, this elegant framework is susceptible to many biases, most notably driven by the presence of weakly deleterious polymorphism in the class $P_N$. Deleterious polymorphism effectively makes the test overly conservative, because deleterious alleles are unlikely ever to reach fixation and therefore lead to overestimation of the expected background rate of substitutions in the functional class. The idea was proposed to include only common polymorphic alleles (for example, alleles at frequency 15% or greater), which should remove many deleterious alleles12; however, this approach has been shown to provide conservative adaptation rate estimates in many contexts12. More recently, it was shown that even removal of all polymorphism <50% is insufficient to correct this bias,
especially when slightly deleterious mutations are common and the rate of adaptive evolution is high\(^2\). To mitigate this effect, an asymptotic implementation of the MK test, called aMK, was introduced. In this implementation, \(P_n\) in equation (1) is replaced by \(P_n(x) P_s(x)\) where \(P_n(x)\) and \(P_s(x)\) are the number of segregating non-synonymous and synonymous alleles at frequency \(x\), respectively\(^1\). An exponential curve is fit to the resulting \(a(x)\) function, which can be calculated for all values of \(x\) in the interval \((0,1)\) for a sample of sequenced chromosomes. The intercept of the best-fit exponential curve at \(x = 1\) is a good approximation for \(a\), as this effectively removes all slightly deleterious polymorphism at all frequencies. This approach was shown to be robust to both the underlying distribution of deleterious effects and recent demographic events\(^1\). The aMK test has inspired new approaches to inferring adaptation in mitochondrial genes\(^2\) and revealed a high rate of adaptation in proteins interacting with pathogens\(^2\).

While aMK extends the elegant MK framework for estimation of adaptation rate, it does not explicitly account for the possibility that beneficial alleles contribute to segregating polymorphism. It is unknown whether aMK is robust to the presence of weakly beneficial alleles, but there is reason to believe that beneficial alleles would be problematic because these are preferentially found at very high frequencies\(^3\), and thus their effect would not be eliminated by the asymptotic procedure. The recent emphasis on adaptation from standing variation\(^4\), and the reported evidence for weakly beneficial polymorphism in *Drosophila*\(^1\), suggest that robust methods for inferring adaptation strength over longer evolutionary time-scales are needed.

A key limitation of existing MK-based approaches is that they provide estimates of adaptation rate but not adaptation strength, and therefore it is not clear whether weakly beneficial mutations contribute substantially to the fixation process. The underlying processes driving weak and strong adaptation might differ, and the ability to separately estimate rates of weak and strong adaptation could provide insight into the biological drivers of adaptation. We hypothesized that such a method could be developed by exploiting the impact of background selection (BGS) on the fixation rate of weakly beneficial alleles. BGS removes neutral and weakly beneficial variation via linkage to deleterious loci\(^1\), while the fixation rate of strongly adaptive alleles is not substantially affected\(^1\). Given that the strength of BGS varies widely and predictably across the human genome\(^4\), a method that interrogates the rate of adaptation as a function of BGS might be able jointly to infer the rate and strength of adaptation.

Here, we probe the performance of aMK when weakly beneficial alleles substantially contribute to segregating polymorphism, and we show that aMK underestimates \(a\) in this adaptation regime. We additionally show that when adaptation is weak, true \(a\) is predicted to vary substantially across the genome as a function of the strength of BGS. We exploit this signal of co-variation between \(a\) and BGS in the weak-adaptation regime to develop an approximate Bayesian computation (ABC) method, which we call ABC-MK, that separately infers the rate of adaptation for both weakly and strongly beneficial alleles. Both our approach and aMK rely on similar input data, but we use a model-based fitting procedure that directly accounts for BGS and weakly beneficial alleles. We apply our method to human genetic data to provide evidence that adaptation in humans is primarily weakly beneficial and varies as a function of BGS strength. Interestingly, adaptation rate estimates on virus-interacting proteins (VIPs) support a much higher rate of strong adaptation, suggesting that adaptation to viruses is both frequent and strongly fitness-increasing. We address seven potential sources of confounding, and discuss our results in light of recent research on adaptation in humans.

**Results**

**Estimates of \(a\) are conservative for weakly beneficial selection.** The aMK approach is known to converge to the true \(a\) at high frequency under the assumption that positively selected mutations make negligible contributions to the frequency spectrum\(^2\). This assumption is likely to be met when beneficial alleles confer large fitness benefits, because selective sweeps occur rapidly and beneficial alleles are rarely observed as polymorphic. However, when selection is predominantly weak, attaining a substantial \(a\) requires much higher mutation rates for beneficial alleles and longer average transit time to fixation, introducing the possibility that weakly beneficial alleles will contribute non-negligibly to the frequency spectrum.

To test whether aMK is sensitive to polymorphic weakly adaptive alleles, we used simulated polymorphism and divergence data to estimate the rate of adaptation using published aMK software\(^1\). In our simulations, we set the true value of \(a\) to 0.2 and varied the contribution of weakly and strongly beneficial alleles to the adaptation process (see Methods and Supplementary Information). When adaptation was due entirely to strongly adaptive alleles, the estimated value of \(a\) (\(\hat{a}\)) was close to the true value but slightly conservative (\(\hat{a} = 0.181 \pm 0.01\); Fig. 1a). As we increased the contribution of weakly beneficial alleles (\(a_w\)) to \(a\), estimates of \(a\) became increasingly conservative (\(\hat{a} = 0.144 \pm 0.01\) when \(a_w = 0.1\), and \(\hat{a} = 0.122 \pm 0.015\) when \(a_w = 0.2\); Fig. 1b,c). Removing polymorphism of frequency >0.5 has been suggested as an approach to account for potential biases induced by high-frequency-derived alleles, which could be mis-polarized in real datasets\(^2\). Restriction to alleles of frequency <0.5 produced similar (but conservative) estimates for all three models (\(\hat{a} = 0.14271, 0.14529\) and 0.14264, for \(a_w = 0.0, 0.1\) and 0.2, respectively), probably because the frequency spectrum is not strongly dependent on the rate of weakly beneficial alleles.
mutation for low-frequency alleles. Lastly, we performed a much larger parameter sweep across \( \alpha \) values and selection coefficients. We found that \( \alpha \) estimates became increasingly conservative as the proportion of weakly deleterious alleles increased, and as the strength of selection at beneficial alleles decreased (Supplementary Fig. 12a and Supplementary Information). Asymptotic-MK estimates of \( \alpha \) are only weakly dependent on the distribution of deleterious selection coefficients (Supplementary Fig. 12).

To better understand why parameter estimates decreased as the proportion of weakly adaptive alleles increased, we performed analytical calculations of \( \alpha(x) \) using diffusion theory\(^\text{25,26}\). Since we use large sample sizes in our analysis herein, we replace the terms \( P_0(x) \) and \( P_1(x) \) in \( \alpha(x) \) with \( \sum P_0(x) \) and \( \sum P_1(x) \) in our calculations, which trivially asymptote to the same values as the original formulation but are not strongly affected by sample size (see Supplementary Information). We find that the downward bias in estimates of \( \alpha \) is due to segregation of weakly adaptive alleles, and removal of these alleles from the simulated and calculated \( \alpha(x) \) curves restored the convergence of \( \alpha(x) \) to the true \( \alpha \) at high frequency (Fig. 1a–c, red curves). In real data, it is not possible to perfectly partition positively selected and deleterious polymorphic alleles. Hence, in later sections we focus on using the shape of the \( \alpha(x) \) curve to infer the strength and rate of adaptation under models that include linkage and complex demography.

Background selection reduces true \( \alpha \) when adaptation is weak.

We have shown that weakly beneficial alleles may impact aMK analyses by contributing to segregation of polymorphism. This presents an opportunity to study whether aMK estimates vary as a function of BGS strength. BGS, the action of linkage between deleterious alleles and neutral alleles, reduces genetic diversity in the human genome\(^\text{22}\) and affects neutral divergence rates\(^\text{23}\), and is predicted to decrease the fixation probability of weakly adaptive alleles\(^\text{24}\). Hence, we hypothesized that if adaptation is partially driven by weakly beneficial alleles in some species, BGS could play a role in modulating adaptation rate across the genome.

To better understand how BGS might affect aMK inference in the presence of weakly beneficial alleles, we performed analytical calculations and simulations of \( \alpha(x) \) with various levels of BGS. We set \( \alpha = 0.2 \) in the absence of BGS, and then performed simulations while fixing the rate of adaptive mutations and changing the level of BGS (ranging from \( \frac{d}{c} = 0.4 \) to \( 1.0 \), where \( \pi \) is neutral nucleotide diversity as compared to the neutral diversity in the absence of linked selection, \( \pi_0 \)). We find that when adaptation is strong, BGS has a modest effect on \( \alpha(x) \) and the true value of \( \alpha \) (Fig. 2a,c), mostly driven by an increase in the rate of fixation of deleterious alleles (Supplementary Fig. 2e). When adaptation is weak, BGS removes a substantial portion of weakly adaptive alleles and precludes these from fixing, resulting in much stronger dependence of \( \alpha(x) \) on BGS and a substantial reduction in the true value of \( \alpha \) (Fig. 2b,d and Supplementary Fig. 2c). Similar to the previous section, estimates of \( \alpha \) were conservative across all models but the underestimation was much more pronounced for weak adaptation (Fig. 2c,d).

Human adaptation rate is shaped by linked selection. Our modeling results show that \( \alpha \) is likely to be underestimated when weakly beneficial alleles contribute substantially to the frequency spectrum, and that background selection may reduce adaptation rate when fitness benefits of adaptive alleles are small. Since BGS is thought to drive broad-scale patterns of diversity across the human genome\(^\text{14}\), we hypothesized that directly accounting for the action of BGS on adaptation rate could provide new insights into the evolutionary mechanisms driving adaptation. Moreover, the fact that weak adaptation is strongly affected by BGS, while strong adaptation is not, suggests that strong and weak adaptation could be differentiated in genomic data by comparing regions of differing BGS strength (from \( \frac{d}{c} = 0.2 \) to \( \frac{d}{c} = 1 \)). We therefore designed an ABC-based method to infer \( \alpha \) while accounting for both BGS and weakly beneficial alleles.

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**Fig. 2 |** The effect of BGS on \( \alpha \). a, \( \alpha(x) \) is plotted as a function of derived allele count \( (x) \) for various background selection \( (\frac{d}{c}) \) values. Adaptive alleles are strongly beneficial \( (2N_s = 500) \) (a) or are weakly beneficial \( (2N_s = 10) \) (b). The lines represent analytical approximations, while the points represent the results of stochastic simulations. The dashed lines at \( \alpha = 0.2 \) represent the true rate of adaptation in the absence of BGS. c, d, True (dark colours) and estimated (light colours) \( \alpha \) for each of the corresponding models in a,b which corresponds to strong adaptation \( (2N_s = 500) \) (c) or weak adaptation \( (2N_s = 10) \) (d). Estimates of \( \alpha \) were made using existing asymptotic-MK software\(^\text{24}\), and the error bars correspond to 95% confidence intervals reported by the software. For each parameter combination, we used \( 2 \times 10^5 \) independent simulations of \( 10^3 \) coding base pairs each.
Fig. 3 | Adaptation rate and strength estimates for human genomic data. a, Posterior distribution of $\alpha_W$, $\alpha_S$, and $\alpha = \alpha_W + \alpha_S$ as inferred by application of our ABC approach to 661 samples of African ancestry from TGP phase3. b, $\alpha(x)$ as a function of derived allele frequency (DAF) for genomic data (black points) plotted along with the mean posterior estimate from our model (orange line) and 99% confidence interval (grey envelope), as obtained by an independent set of simulations using posterior parameter estimates. c, Inferred posterior distribution of $\alpha$ as a function of BGS strength in the human genome. d, Mean posterior estimates of $\alpha_W$ as determined by separate fitting of the model to alleles from each independent background selection strength bin. A linear model fit to the data (green line) supported statistically significant co-variation between $\alpha/\alpha_W$ and $\alpha_S$ ($P = 0.0343$). The black dashed line shows the predicted change in $\alpha_W$ as a function of $\alpha/\alpha_W$, given the mean estimate of $\alpha_S$.

We applied our inference procedure (ABC-MK) to empirical $\alpha(x)$ data computed from human genomes obtained from the Thousand Genomes Project (TGP) for all 661 samples with African ancestry. We find strong posterior support for a substantial component of $\alpha$ driven by weakly beneficial alleles ($\hat{\alpha}_x \approx 0.097$; see Fig. 3a and see Table 1 for area of 95% highest posterior density), as well as posterior support for a smaller component of $\alpha$ from strongly beneficial alleles ($\hat{\alpha}_W = 0.041$). We estimate that total $\hat{\alpha} = 0.135$, nearly twice the estimate obtained with the same dataset using the original aMK approach ($\hat{\alpha} = 0.076$, see Supplementary Information; we note that while our estimate is similar to previous estimates, we used a much larger set of genes in our inference and hence the estimates are not directly comparable). In addition to rates of positive selection, our approach provides estimates of negative selection strength. We find support for mean strength of negative selection of $2N_s \approx -220$ (Supplementary Fig. 9C), which is consistent with recent studies using large sample sizes but weaker than earlier estimates using small samples.

In addition to estimation of evolutionary parameters, we sought to better understand how BGS might impact adaptation rates across the genome. We resampled parameter values from our posterior estimates of each parameter, and ran a new set of forward simulations using these parameter values. We then calculated $\alpha$ as a function of BGS in our simulations. We find that $\alpha$ co-varies strongly with BGS, with $\alpha$ in the lowest BGS bins being 33% of $\alpha$ in the highest bins (Fig. 3c). Integrating across the whole genome, our results suggest that human adaptation rate in coding regions is reduced by approximately 25% by BGS (Supplementary Fig. 9d). To confirm that these model projections are supported by the underlying data, we split the genome into BGS bins and separately estimated adaptation rate in each bin. Although these estimates are substantially noisier than our inference on the full dataset, we find that the rate of adaptation due to weakly beneficial alleles decreases as a function of BGS strength in accordance with the model predictions (Fig. 3d). In contrast, estimates of the mean strength of negative selection against non-synonymous mutations did not co-vary with BGS strength (Supplementary Fig. 20). Lastly, to validate that our model recapitulates $\alpha(x)$ values that we observe in real data, we also used our independent forward simulations to re-compute $\alpha(x)$. We find that our model is in close agreement with observed data across the majority of the frequency spectrum. The model and data deviate at high frequency, but both are within sampling uncertainty (Fig. 3b, grey envelope).

Previous research has shown that VIPs have undergone faster rates of adaptation than the genome background. However, the strength of selection acting on these genes is unknown and, given our BGS results, it is plausible that the higher rate of adaptation in VIPs is driven by lower overall background selection at VIPs rather than increased selection pressure for adaptation. In contrast, if pathogens have imposed large fitness costs on humans it is possible that VIPs would support both higher adaptation rates and greater adaptation strength. We ran our method while restricting it to an expanded set of 4,066 VIPs for which the divergence and polymorphism data were available. We found evidence for strikingly higher adaptation rates in VIPs than the genome background ($\xi = 0.224$) and a much larger contribution from strongly adaptive alleles ($\hat{\alpha}_S = 0.126$; Fig. 4). The higher value of $\alpha$ for VIPs cannot be explained by BGS, because VIPs undergo slightly stronger BGS than average genes; the mean BGS strength at VIPs is 0.574 as compared to 0.629 for all genes (in units of $\pi/\xi_s$). Taking $\alpha_S = 0.126$ as a point estimate for the rate of strongly beneficial substitutions in VIPs and $\alpha_S = 0.041$ genome-wide, we estimate that 61% of all strongly
beneficial substitutions occurred in VIPs (Table 1). Moreover, we estimate that the posterior probability that \( \alpha \) is greater in VIPs than non-VIPs is 99.97%, while the posterior probability that \( \alpha_\beta \) is greater in VIPs is 88.9% (Fig. 4c). Bootstrap samples of non-VIPs (1,000 replicates) resulted in no \( \alpha_\beta \) estimates as high as those obtained from VIPs (Supplementary Fig. 19). These results are concordant with the \( \alpha(x) \) summary statistics for VIPs, which had larger values at high-frequency alleles than non-VIPs (Fig. 4d). Interestingly, \( \alpha(x) \) is lower for VIPs than non-VIPs at low frequency, suggesting increased overall levels of conservation among VIPs (see also Supplementary Fig. 9, where we find support for stronger negative selection against non-synonymous mutations in VIPs).

**Discussion**

A long-running debate in evolutionary biology has concerned the relative importance of drift and selection in determining the rate of diversification among species\(^{3,4,43}\). While previous studies have shown that there is a substantial signal of adaptation in *Drosophila*\(^2\), estimates of adaptation rate in humans are much lower\(^1\). Here, we extended the classic MK framework to account for weakly beneficial alleles, and we provide evidence for a high rate of weakly adaptive mutation in humans. We show that a state-of-the-art approach to adaptation rate estimation that does not account for beneficial polymorphism provides conservative estimates of \( \alpha \) (\( \tilde{\alpha} = 0.076 \) for these data)\(^{25}\), while our method nearly doubles the estimated human adaptation rate (to \( \tilde{\alpha} = 0.135 \)). Most of the adaptation signal that we detected was due to weakly beneficial alleles. Interestingly, VIPs supported a much higher rate of adaptation than the genome background (\( \tilde{\alpha} = 0.226 \)), especially for strongly beneficial substitutions (\( \tilde{\alpha}_S = 0.126 \) compared to \( \tilde{\alpha}_S = 0.041 \) genome-wide). Our results provide an evolutionary mechanism that partially explains the apparently low observed rate of human adaptation in previous studies, and extends support for viruses as a major driver of adaptation in humans\(^{31}\).

It has long been known that recombination could, in principle, affect the evolutionary trajectories of both beneficial and deleterious alleles\(^{3,4,43}\), and studies in *Drosophila*\(^{45,46}\) and dogs\(^{47}\) have provided evidence for the effect of recombination on divergence and load. Despite the expectation that recombination could have a strong effect on adaptation in humans, studies have differed on how recombination affects human divergence and polymorphism. One human genomic study explored the ratio \( D_N / D_S \) as a function of recombination rate, and found no evidence for an effect of recombination on divergence rate\(^\text{64}\). Our results may partially explain why \( D_N / D_S \) does not fully capture the effect of recombination on divergence in humans. As BGS increases in strength, the rate of accumulation of deleterious alleles increases while the rate of fixation of weakly adaptive alleles decreases. These two effects partially offset each other, which should reduce the sensitivity of \( D_N / D_S \) as a tool in detecting the effect of recombination on divergence. A more recent study

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**Table 1 | Datasets and corresponding adaptation rates**

| Dataset          | NS   | SYN  | \( \tilde{\alpha} \) | \( \tilde{\alpha}_W \) | \( \tilde{\alpha}_S \) |
|------------------|------|------|----------------------|----------------------|----------------------|
| Whole-genome     | 29,925 | 38,135 | 0.135 (0.096,0.17) | 0.097 (0.0,0.21) | 0.041 (0.0,0.13) |
| VIPs             | 6,249  | 10,309 | 0.224 (0.17,0.28) | 0.098 (0.0,0.24) | 0.126 (0.018,0.26) |
| Non-VIPs         | 23,676 | 27,826 | 0.12 (0.09,0.15)  | 0.077 (0.01,0.13) | 0.042 (0.0,0.09)  |

Estimated \( \alpha \) values represent the mean of posterior distribution. NS and SYN represent the number of non-synonymous and synonymous fixations, respectively. Values in parentheses represent the area of 95% highest posterior density.
provided evidence that recombination affects the accumulation of deleterious polymorphic alleles\textsuperscript{29}, but did not provide detailed information about the effect of recombination on adaptation. Our results are consistent with the idea that weakly deleterious alleles are predicted to segregate at higher frequencies in regions under strong BGS, and we additionally show that BGS affects the accumulation of weakly beneficial alleles in humans.

While classic MK approaches estimate only the rate of adaptation, our method extends the MK framework to provide information on both the rate and strength of selection. While previous approaches used to estimate the strength of adaptation have either focused on the dip in diversity near sweeping alleles\textsuperscript{1,4,6,10} or directly inferred the DFE from the frequency spectrum\textsuperscript{10}, our approach capitalizes on an orthogonal signal of the reduction in fixation rate of weakly beneficial alleles induced by selection at linked sites. We developed an ABC method to capture this signal, but less computationally intensive methods could also be used—for example, the original aMK approach could be applied in bins of BGS strength. If a substantial proportion of adaptation is due to weakly beneficial alleles, such an analysis should result in a strong correlation between BGS strength and (potentially conservative) $\alpha$ estimates. However, it should be noted that cryptic co-variation between gene functions (such as VIPs) and BGS strength could confound such inferences.

We supposed that the main effects of linked selection in humans are due to background selection, but in principle genetic draft could drive similar patterns. Draft is expected to substantially reduce genetic diversity when sweeps occur frequently, and can impede the fixation of linked beneficial alleles\textsuperscript{31,32}. Previous work has also shown that strong draft can alter the fixation rate and frequency spectra of neutral and deleterious alleles\textsuperscript{32}. We performed simulations of strong draft in 1-MB flanking sequences surrounding a gene evolving under natural selection, and tested the magnitude of the deviation from theoretical predictions under a model of background selection alone. Consistent with previous work, we observed that draft increases the fixation rate of deleterious alleles and thereby decreases $\alpha$ (ref. \textsuperscript{32}). However, the effect on $\alpha(x)$ is only modest at the frequencies that we used in our inference procedure (that is, $<75\%$), even when the strength and rate of positive selection were much higher than we and others have inferred in humans (although there is a modest deviation around 75% frequency, the highest frequency we used in our inference; Supplementary Fig. 4c,d). This implies that draft due to selected sites outside genes would have to be much stronger than that due to positive selection inside exons to drive the effects that we infer in the human genome. We note that it is likely that in species undergoing both strong, frequent sweeps and BGS (for example, \textit{Drosophila}—see ref. \textsuperscript{31}), draft will contribute to the removal of weakly beneficial polymorphism.

Selection has left many imprints on the human genome, with studies reporting signatures of selective sweeps\textsuperscript{25,32}, soft sweeps\textsuperscript{33}, background selection\textsuperscript{31}, negative selection\textsuperscript{30,42} and polygenic adaptation\textsuperscript{34}. Nevertheless, considerable uncertainty remains about the relative importance of these evolutionary mechanisms, especially in regard to the rate and strength of positive selection. Recent work has suggested that the contrasting adaptation rate estimates of previous studies\textsuperscript{1,12} can be reconciled by arguing that most adaptation signals in humans are consistent with adaptation from standing variation\textsuperscript{43}. Our results show that the frequency spectra and patterns of divergence are also consistent with the idea that many adaptive alleles segregate for much longer than is expected for a classic sweep, and hence also help to reconcile the results of previous studies.

In addition to determining the rate, strength and mechanisms of adaptation, there is an ongoing effort to find the biological processes most important for driving adaptation. Previous work has shown that viruses are a critical driver of adaptation in mammals\textsuperscript{25}, but the strength of the fitness advantages associated with resistance to (or tolerance of) infection remains unclear. Our approach clarifies that strongly adaptive fixed differences are also enriched, approximately threefold, in VIPs relative to non-VIPs. In contrast, weak adaptation rate was not substantially different between VIPs and non-VIPs, suggesting that weak adaptation may proceed through mechanisms that are shared across proteins regardless of function (for example, optimization of stability). While we have focused on VIPs here due to the expected fitness burdens associated with infection, in future research our approach could be used to investigate adaptation in any group of genes, or extended to partitioning of genes into strong and weak adaptation classes.

The model that we fit to human data does an excellent job of recapitulating the observed patterns in the TGP data, but we were concerned that several possible confounding factors could have influenced our results. We showed that seven confounding factors (ancestral mis-polarization\textsuperscript{30}, demographic model mis-specification\textsuperscript{30}, BGS model mis-specification, co-variation of BGS and sequence conservation, GC-biased gene conversion\textsuperscript{30}, selection on synonymous alleles\textsuperscript{31} and mis-specification of strongly and/or weakly beneficial selection coefficients) are unlikely to have substantially influenced the results (see Supplementary Information), but it should be noted that the adaptive process in our model is exceedingly simple and it is very likely that the evolutionary processes driving diversification are much more complex. We supposed that adaptation proceeds in two categories, weak and strong selection, each of which is described by a single selection coefficient. In reality, adaptive alleles are likely to have selection coefficients drawn from a broad distribution, and adaptation is likely to proceed by a variety of mechanisms, including sweeps\textsuperscript{31}, polygenic adaptation\textsuperscript{30} and selection from standing variation\textsuperscript{31}. While our results show that BGS shapes adaptation rate across the genome, our method does not differentiate among adaptation mechanisms. We expect that future research will further clarify the relative importance of various selection mechanisms in shaping genomic patterns of diversity in the genomes of humans and other organisms\textsuperscript{31,32}.

Our method is flexible in that it could be applied to any species for which both divergence/polyorphism data and estimates of background selection strength are available. As with the original aMK approach, we showed that the $\alpha$ estimates we obtained are not highly sensitive to recent demographic uncertainty. Our approach may therefore be effective in providing more accurate estimates of adaptation rate in non-model species. Despite recent advances, the evolutionary mechanisms that shape genetic diversity across species (which could include linked selection, population size and/or population demography) remain the subject of debate\textsuperscript{11,12,15}. Future work using and extending our method, which accounts for the effect of weakly beneficial alleles on adaptation rate estimates, could help to resolve this open question.

**Methods**

**Divergence and polymorphism data.** We retrieved the number of polymorphic sites and their allele frequencies in human coding sequences, as well as the number of human-specific fixed substitutions in coding sequences since divergence with chimpanzees. Fixed substitutions were identified by parsimony based on alignments of human (hg19 assembly), chimpanzee (panTro4 assembly) and orangutan (ponAbe2 assembly) coding sequences. Human coding sequences from Ensembl v.73 (ref. \textsuperscript{19}) were blatted\textsuperscript{61} on the panTro4 and ponAbe2 assemblies and the best corresponding hits were blatted back on the hg19 human assembly to finally identify human–chimp–orangutan best reciprocal orthologous hits. We used the Blat-fine option to ensure that even short exons at the edge of coding sequences would be included in the hits. We further used a Blat protein–minidentity threshold of 60%. The corresponding human, chimpanzee and orangutan coding sequences were then aligned with the PRANKs\textsuperscript{40} coding sequence evolution model\textsuperscript{31} after removal of codons containing undefined positions.

For each human coding gene in Ensembl we considered all possible protein-coding isoforms and aligned each isoform individually among human, chimpanzee and orangutan. The numbers of polymorphic or divergent sites are therefore the numbers over all possible isoforms of a human gene (however, the same polymorphic or divergent site present in multiple isoforms was counted only once). If a polymorphic or divergent site was synonymous in an isoform but non-synonymous in another isoform, we counted that as a single non-synonymous
polymorphic or divergent site. Only fixed divergent sites were included, meaning that substitutions still polymorphic in humans were not counted as divergent. The derived allele frequency of polymorphic sites herein corresponds to the frequency across all African populations from TGP phase 3, which comprises 661 individuals spread across seven different subpopulations\(^1\). Allele frequencies were extracted from vcf files provided by the TGP for the phase 3 data. In total, 17,740 human–chimpanzee–orangutan orthologues were included in the analysis. Supplementary Data Table 1 provides the number of synonymous and non-synonymous polymorphic or divergent sites for each of these 17,740 orthologues, as well as the allelic frequencies of the polymorphic sites. Polymorphic sites were counted only if they overlapped those parts of human coding sequences that were aligned with chimpanzee and orangutan coding sequences. The ancestral and derived allele frequencies were based on the ancestral alleles inferred by TGP phase 3 and available in the previously mentioned vcf files\(^1\).

Model-based simulations and calculations. We tested the robustness of the aMK approach to the presence of weakly beneficial alleles using simulation and theory. We simulated simultaneous negative and positive selection in coding sequences using model-based forward simulations under a range of scenarios\(^6\). We supposed that non-synonymous alleles are under selection while synonymous \(\alpha_S\) coefficients from a gamma distribution inferred from human sequence data\(^40\), is a model output and not a model input. We drew deleterious selection \(\alpha\) and advantageous alleles that result in the desired \(\alpha_S\) due to weakly beneficial mutations (2\(^{\text{Exome project African American samples}}\), with varying levels of background selection at a coding locus under a demographic model inferred from NHLBI demography\(^65\). We sampled parameters from previous distributions corresponding to the shape and scale of deleterious selection coefficients (assumed to be gamma distributed) and the rate of mutation of weakly and strongly beneficial mutations. We performed forward simulations\(^6\) of simultaneous negative and positive selection at a coding locus under a demographic model inferred from NHLBI Exome project African American samples\(^6\), with varying levels of background selection from \(\alpha = 0.2\) to \(\alpha = 1.0\) and the sampled parameter values. We then calculated \(\alpha(x)\) using these simulated data, sampling alleles from the simulations such that the distribution of BGS values in the simulation matches that in the empirical data as calculated by a previous study\(^6\). We used \(\alpha(x)\) values at a subset of frequencies \(x\) as summary statistics in ABC (specifically, at derived allele counts 1, 2, 5, 10, 20, 50, 100, 200, 500 and 1,000 in a sample of 1,322 chromosomes). To improve efficiency, we employed a resampling-based approach that allowed us to query many parameter values using the same set of forward simulations (see Supplementary Information).

We tested our approach by estimating parameter values (population-scaled mutation rates \(\theta\), \(\theta_e\) and the parameters of a gamma distribution controlling negative selection strength) and quantities of interest \((\alpha_{\text{mean}}, \alpha_{\text{median}}, \alpha)\) from simulated data. We found that the method produces highly accurate estimates for most inferred parameters and \(\alpha\) values (including \(\alpha_{\text{mean}}, \alpha_{\text{median}}\) and total \(\alpha\); Supplementary Fig. 6). Some parameter values (particularly those corresponding to the DFE) over deleterious alleles and mutation rates of beneficial alleles) were somewhat noisily inferred. We found that estimations of \(\alpha\) were not very sensitive to various types of model misspecification (see Supplementary Information, ‘Robustness of parameter estimates’), but \(\alpha_{\text{mean}}\) and \(\alpha_{\text{median}}\) were modestly affected by mis-specification of the demographic model or the DFE of alleles driving BGS. We term our approach ABC-MK.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Supplemental Data Table 1 is provided on the publisher's website. The data that we used to parameterize our model are also available online at https://github.com/uricchio/mktest. Column 1 in Supplementary Data Table 1 are as follows: 1, Ensembl coding gene identification; 2, number of non-synonymous polymorphic sites; 3, respective derived allele frequencies of these sites separated by common; 4, number of synonymous polymorphic sites; 5, frequency-derived allele frequencies of these sites; 6, number of fixed non-synonymous substitutions on the human branch; and 7, number of fixed synonymous substitutions on the human branch.

Code availability
The code that we used to parameterize our model is freely available online at https://github.com/uricchio/mktest.

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References
1. Darwin, C. On the Origin of Species (Murray, 1859).
2. Wallace, A. R. Darwinism: an exposition of the theory of natural selection with some of its applications (MacMillan & Co., 1889).
3. Wright, S. On the roles of directed and random changes in gene frequency in the genetics of populations. Evolution 2, 279–294 (1948).
4. Kimura, M. et al. Evolutionary rate at the molecular level. Nature 217, 628–628 (1968).
5. Ohba, T. Slightly deleterious mutant substitutions in evolution. Nature 246, 96 (1973).
6. Kimura, M. Preponderance of synonymous changes as evidence for the neutral theory of molecular evolution. Nature 267, 275 (1977).
7. McDonald, J. H. & Kreitman, M. Adaptive protein evolution at the ADH locus in Drosophila. Nature 351, 652 (1991).
8. Fay, J. C., Woyckoff, G. J. & Wu, C.-I. Testing the neutral theory of molecular evolution with genomic data from Drosophila. Nature 415, 1024 (2002).
9. Pollard, K. S., Hubisz, M. J., Rosenberg, K. R. & Siepel, A. Detection of nonneutral substitution rates on mammalian phylogenies. Genome Res. 20, 110–112 (2010).
10. Charlesworth, B. & Charlesworth, D. Neutral variation in the context of selection. Mol. Biol. Evol. 35, 1359–1361 (2018).
11. Corbett-Detig, R. B., Hartl, D. L. & Sackton, T. B. Natural selection constrains neutral diversity across a wide range of species. PLoS Biol. 13, e1002112 (2015).
12. Cocker, G. Does linked selection explain the narrow range of genetic diversity across species? Preprint at bioRxiv https://doi.org/10.1101/042598 (2016).
13. Kern, A. D. & Hahn, M. W. The neutral theory in light of natural selection. Mol. Biol. Evol. 35, 1366–1371 (2018).
14. Jensen, J. D. et al. The importance of the neutral theory in 1968 and 50 years on: a response to Kern and Hahn 2018. Evolution 73, 111–114 (2019).
15. Leffler, E. M. et al. Revisiting an old riddle: what determines genetic diversity levels within species? PLoS Biol. 10, e1001388 (2012).
16. Galtier, N. Adaptive protein evolution in animals and the effective population size hypothesis. PLoS Genet. 12, e1005774 (2016).
17. Smith, N. G. C. & Eyre-Walker, A. Adaptive protein evolution in Drosophila. Nature 415, 1022 (2002).
18. Sawyer, S. A. & Hartl, D. L. Population genetics of polymorphism and divergence. Genetics 132, 1161–1176 (1992).
19. Tataru, P., Mollion, M., Glémin, S. & Bataillon, T. Inference of distribution of fitness effects and proportion of adaptive substitutions from polymorphism data. J. Evol. Biol. 20, 1113–1119 (2007).
20. Ratnakumar, A. et al. Detecting positive selection within genomes: the problem of biased gene conversion. Philos. Trans. R. Soc. London B 365, 2571–2580 (2010).
21. Fay, J. C., Woyckoff, G. J. & Wu, C.-I. Positive and negative selection on the human genome. Genetics 158, 1227–1234 (2001).
22. Eyre-Walker, A. & Keightley, P. D. Estimating the rate of adaptive molecular evolution in the presence of slightly deleterious mutations and population size change. Mol. Biol. Evol. 26, 2097–2108 (2009).
23. Messer, P. W. & Petrov, D. A. Frequent adaptation and the McDonald–Kreitman test. Proc. Natl Acad. Sci. USA 110, 8615–8620 (2013).
24. James, J. E., Pignseau, G. & Eyre-Walker, A. The rate of adaptive evolution in animal mitochondria. Mol. Ecol. 25, 67–78 (2016).
25. Enard, D., Cai, L., Gwennap, C. & Petrov, D. A. Viruses are a dominant driver of protein adaptation in mammals. eLife 5, e12469 (2016).
26. Pritchard, J. K., Pickrell, J. K. & Coop, G. The genetics of human adaptation: hard sweeps, soft sweeps, and polygenic adaptation. Curr. Biol. 20, R208–R215 (2010).
27. Messer, P. W. & Petrov, D. A. Population genomics of rapid adaptation by soft selective sweeps. Trends Ecol. Evol. 28, 659–669 (2013).
28. Berg, J. J. & Coop, G. A population genetic signal of polygenic adaptation. Mol. Biol. Evol. 30, 1009412 (2013).
29. Schrider, D. R. & Kern, A. D. Soft sweeps are the dominant mode of adaptation in the human genome. Mol. Biol. Evol. 34, 1863–1877 (2017).
30. Uricchio, L. H., Kitano, H. C., Gusev, A. & Zaitlen, N. A. An evolutionary compass for detecting signals of polygenic selection and mutational bias. Evol. Lett. 3, 69–79 (2019).
31. Elbashir, E. et al. A genomewide map of the effects of linked selection in Drosophila. PLoS Genet. 12, e1006130 (2016).
32. Charlesworth, B., Morgan, M. T. & Charlesworth, D. The effect of deleterious mutations on neutral molecular variation. Genetics 134, 1289–1303 (1993).
33. Barton, N. H. Linkage and the limits to natural selection. Genetics 140, 821–841 (1995).
34. McVicker, G., Gordon, D., Davis, C. & Green, P. Widespread genomic signatures of natural selection in hominid evolution. PLoS Genet. 5, e1000471 (2009).
35. Haller, B. C. & Messer, P. W. asymptoticMK: a web-based tool for the asymptotic McDonald–Kreitman test. G3 (Bethesda) 7, 1569–1575 (2017).
36. Evans, S. N., Shvets, Y. & Slatkin, M. Non-equilibrium theory of the allele frequency spectrum. Theor. Popul. Biol. 71, 109–119 (2007).
37. Kimura, M. Diffusion models in population genetics. J. Appl. Probab. 1, 177–232 (1964).
38. Phung, T. N., Huber, C. D. & Lohmueller, K. E. Determining the effect of natural selection on linked neutral divergence across species. PLoS Genet. 12, e1006199 (2016).
39. Consortium TGP et al. A global reference for human genetic variation. Nature 526, 68 (2015).
40. Boyko, A. R. et al. Assessing the evolutionary impact of amino acid mutations in the human genome. PLoS Genet. 4, e1000083 (2008).
41. Kim, B. Y., Huber, C. D. & Lohmueller, K. E. Inference of the distribution of selection coefficients for new nonsynonymous mutations using large samples. Genetics 206, 345–361 (2017).
42. Eyre-Walker, A., Woolfit, M. & Phelps, T. The distribution of fitness effects of new deleterious amino acid mutations in humans. Genetics 173, 891–900 (2006).
43. Hill, W. G. & Robertson, A. The effect of linkage on limits to artificial selection. Genet. Res. 8, 269–294 (1966).
44. Smith, J. M. & Haigh, J. The hitch-hiking effect of a favourable gene. Genet. Res. 23, 23–35 (1974).
45. Macpherson, J. M., Sella, G., Davis, J. C. & Petrov, D. A. Genomewide spatial correspondence between nonsynonymous divergence and neutral polymorphism reveals extensive adaptation in Drosophila. Genetics 177, 2083–2099 (2007).
46. Castellano, D., Coronado-Zamora, M., Campos, J. L., Barbadilla, A. & Eyre-Walker, A. Adaptive evolution is substantially impeded by Hill–Robertson interference in Drosophila. Mol. Biol. Evol. 33, 442–455 (2015).
47. Marsden, C. D. et al. Bottlenecks and selective sweeps during domestication correspond to nonsynonymous divergence in Drosophila. Mol. Biol. Evol. 33, 442–455 (2015).
48. Bullaughney, K. L., Przeworski, M. & Coop, G. No effect of recombination on the efficacy of natural selection in primates. Genome Res. 18, 544–554 (2008).
49. Hussin, J. G. et al. Recombination affects accumulation of damaging and disease-associated mutations in human populations. Nature Genet. 47, 400 (2015).
50. Jensen, J. D., Thornton, K. R. & Andolfatto, P. An approximate Bayesian estimator suggests strong, recurrent selective sweeps in Drosophila. PLoS Genet. 4, e1000198 (2008).
51. Hernandez, R. D. et al. Classic selective sweeps were rare in recent human evolution. Science 331, 920–924 (2011).
52. Enard, D., Messer, P. W. & Petrov, D. A. Genome-wide signals of positive selection in human evolution. Genome Res. 24, 885–895 (2014).
53. Comeron, J. M. & Kreitman, M. Population, evolutionary and genomic consequences of interference selection. Genetics 161, 389–410 (2002).
54. Ursichio, L. H. & Hernandez, R. D. Robust forward simulations of recurrent hitchhiking. Genetics 197, 221–236 (2014).
55. Hernandez, R. D., Williamson, S. H. & Bustamante, C. D. Context dependence, ancestral misidentification, and spurious signatures of natural selection. Mol. Biol. Evol. 24, 1792–1800 (2007).
56. Ewing, G. B. & Jensen, J. D. The consequences of not accounting for background selection in demographic inference. Mol. Ecol. 25, 135–141 (2016).
57. Torres, R., Sipiec, Z. A. & Hernandez, R. D. Human demographic history has amplified the effects of background selection across the genome. PLoS Genet. 14, e1007387 (2018).
58. Huang, Y.-F. & Siepel, A. Estimation of allele-specific fitness effects across human protein-coding sequences and implications for disease. Preprint at bioRxiv https://doi.org/10.1101/441337 (2018).
59. Huber, C. D., Kim, B. Y., Marsden, C. D. & Lohmueller, K. E. Determining the factors driving selective effects of new non-synonymous mutations. Proc. Natl Acad. Sci. USA 114, 4465–4470 (2017).
60. Yates, A. et al. Ensembl 2016. Nucleic Acids Res. 44, D710–D716 (2015).
61. Kent, W. J. BLAT—the BLAST-like alignment tool. Genome Res. 12, 656–664 (2002).
62. Löytynoja, A. & Goldman, N. webPRANK: a phylogeny-aware multiple sequence aligner with interactive alignment browser. BMC Bioinformatics 11, 579 (2010).
63. Hernandez, R. D. & Uricchio, L. H. SFS_CODE: more efficient and flexible forward simulations. Preprint at bioRxiv https://doi.org/10.1101/025064 (2015).
64. Uricchio, L. H., Torres, R., Witte, J. S. & Hernandez, R. D. Population genetic simulations of complex phenotypes with implications for rare variant association tests. Genet. Epidemiol. 39, 35–44 (2015).
65. Beaumont, M. A., Zhang, W. & Balding, D. J. Approximate Bayesian computation in population genetics. Genetics 162, 2025–2035 (2002).
66. Tennessen, J. A. et al. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. Science 337, 64–69 (2012).
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Software and code

Policy information about availability of computer code

Data collection

We used open access human genetic data from the Thousand Genome Project (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/) and B-values (i.e., estimates of Background selection strength in humans) from previous research (http://www.phrap.org/othersoftware.html) as described in our methods section. Our final dataset, which summarizes data from these datasets, is available at https://github.com/uricchio/mktest in the data folder.

Data analysis

We used the Blat tool (https://genome.ucsc.edu/FAQ/FAQblat.html) to identify orthologs as described in the methods section. We used custom software for the remaining analyses. Our software is available at https://github.com/uricchio/mktest -- note that the software is freely available but is currently set to compile on the Stanford cluster and run on the Stanford Sherlock cluster.

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The supplemental table, along with the data that we used to parameterize our model, is available online at https://github.com/uricchio/mktest
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Study description We use models of weak adaptation to show that previous methods to estimate adaptation rate provide inaccurate estimates when adaptation is weak. We then extend existing methods to correct this issue, and show that variation in the strength of linked selection can be used to additionally estimate both adaptation rate and strength. We apply our method to human genomes to show that human polymorphism and divergence data is consistent with weak adaptation genome-wide, while virus interacting proteins support both faster and stronger adaptation than the genome background.

Research sample We used genetic data from the 661 samples of African origin from the Thousand Genomes Project in our study. We chose these samples because 1) African samples have been understudied in previous human genetics research and 2) the best-fitting demographic models for this continental group are simpler than European demographic models, simplifying our analysis.

Sampling strategy Since we used previously sampled data we were restricted to the samples available and did not make any sampling design choices herein.

Data collection We used publicly available data.

Timing and spatial scale The data were collected by the 1000 genomes consortium. Sample collection is described here: https://media.nature.com/original/nature-assets/nature/journal/v526/n7571/extref/nature15393-s1.pdf. According to this document, the samples were sequenced between October 2012 and March 2013.

Data exclusions We included genetic data from all human coding regions for which we could map an ortholog and a B-value, as described in the methods section. The total data set is described in Table 1.

Reproducibility We validated our estimation procedures by simulating a large number of datasets and applying our approach to the simulated data.

Randomization All samples were combined into a single group (i.e., we perform estimation on genetic data from individuals from a single continental group, and do not compare across groups).

Blinding Blinding was not necessary because we do not tune our analysis to the data in any way -- we prepared our estimation procedure by applying it to simulated data and simply report the estimates obtained from the real data.

Did the study involve field work? ☑ Yes  ☐ No

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