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asymptoticMK: A web-based tool for the asymptotic McDonald–Kreitman test

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ABSTRACT

The McDonald–Kreitman (MK) test is a widely used method for quantifying the role of positive selection in molecular evolution. One key shortcoming of this test lies in its sensitivity to the presence of slightly deleterious mutations, which can severely bias its estimates. An asymptotic version of the MK test was recently introduced that addresses this problem by evaluating polymorphism levels for different mutation frequencies separately, and then extrapolating a function fitted to that data. Here we present asymptoticMK, a web-based implementation of this asymptotic McDonald–Kreitman test. Our web service provides a simple R-based interface into which the user can upload the required data (polymorphism and divergence data for the genomic test region and a neutrally evolving reference region). The web service then analyzes the data and provides plots of the test results. This service is free to use, open-source, and available at http://benhaller.com/messerlab/asymptoticMK.html. We provide results from simulations to illustrate the performance and robustness of the asymptoticMK test under a wide range of model parameters.

INTRODUCTION

The extent to which molecular evolution is driven by positive selection, rather than neutral evolutionary processes such as random genetic drift, is one of the central questions of modern evolutionary biology. This question can be studied quantitatively by estimating the parameter α, which specifies the fraction of nucleotide substitutions in a given genomic region that were driven to fixation by positive selection (Eyre-Walker 2006). Values of α close to one indicate that most substitutions in the region were indeed the result of positive selection, whereas values close to zero indicate neutral evolution.

One of the most widely used approaches for inferring α from polymorphism and divergence data is the McDonald–Kreitman (MK) test (McDonald and Kreitman 1991; Eyre-Walker 2006), which compares levels of divergence between a genomic test region and a neutrally evolving reference region with the levels of polymorphism in the two regions. Early applications of the MK test typically focused on nonsynonymous sites in protein-coding regions as the test region, while synonymous sites were used as the neutral reference. However, the approach can also be applied to arbitrary genomic compartments or classes of mutations (Andolfatto 2005).

The original MK test makes several critical assumptions about the nature of the evolutionary process. First, it assumes that the positively selected mutations that ultimately contribute to divergence in the test region go to fixation quickly, such that they do not contribute noticeably to polymorphism levels. Second, it assumes that deleterious mutations in the test region are sufficiently deleterious to be lost quickly, such that they contribute to neither polymorphism nor divergence. Finally, neutral mutations in the test region are assumed to be subject to drift similar to the mutations in the neutral reference region and can therefore contribute to both polymorphism and divergence. Under these assumptions, it holds that

$$\alpha = 1 - \frac{d_0}{d}\frac{p}{p_0}, \quad (1)$$

where d and d_0 are substitution rates per site in the test region and neutral reference region, respectively, while p and p_0 specify the respective levels of polymorphism per site in the two regions (Eyre-Walker 2006). Note that if polymorphism and divergence levels are estimated over the

same region, the total number of sites cancels out in the ratios p/d and d_0/p_0 and one may then simply use the actual counts of observed substitutions (D and D_0) and polymorphic sites (P and P_0) instead of rates per site (Eyre-Walker 2006).

With the growing availability of genome-level polymorphism and divergence datasets, the MK test has become a popular method for inferring positive selection in various organisms (Fay 2011). Several software tools and web services with implementations of the test have also been developed (Egea *et al.* 2008; Librado and Rozas 2009; Eyre-Walker and Keightley 2009; Stoletzki and Eyre-Walker 2011; Vos *et al.* 2013). The estimates of α obtained in these studies range from as high as ~0.5 for nonsynonymous substitutions in *Drosophila* (Sella *et al.* 2009), to close to zero in organisms such as yeast (Elyashiv *et al.* 2010) or many plants (Gossmann *et al.* 2010). Indeed, estimates of α obtained from Equation (1) are often negative, indicating that at least some of the assumptions of the test were likely not met (since negative values of α have no biological meaning – estimates of α may be negative, but the true value cannot be).

One major problem with the original MK test lies in its assumption that deleterious mutations do not contribute to polymorphism in the test region. This stands in contrast to the frequent observation of weakly deleterious mutations in many organisms, and the fact that such mutations can substantially affect the site frequency spectrum (SFS) of polymorphisms in functional genomic regions (Bustamante *et al.* 2005; Eyre-Walker *et al.* 2006). In the presence of weakly deleterious mutations, the observed level of polymorphism in the test region (p) in Equation (1) will overestimate the rate at which neutral polymorphisms are expected to go to fixation in this region, which will bias estimates of α downwards (providing one possible explanation for the frequent observation of negative α estimates).

As one strategy to address this problem, it has been proposed to only consider polymorphisms for which the derived allele is above a certain threshold frequency when estimating p and p_0 (Charlesworth and Eyre-Walker 2008). This is because the fraction of weakly deleterious mutations among all polymorphisms should be lower for higher derived-allele frequencies. Ideally, one would wish to set this cutoff high, to minimize the bias due to weakly deleterious mutations; however, the higher this cutoff, the fewer polymorphisms will actually remain in the dataset, thus increasing statistical noise. To circumvent this problematic tradeoff, more sophisticated extensions of the original MK test first attempt to infer the actual distribution of fitness effects among new mutations in the test region from the SFS, and then

correct fixation probabilities accordingly (Boyko *et al.* 2008; Eyre-Walker and Keightley 2009). Yet these approaches can still suffer from unknown effects of demography or linked selection that are also expected to affect the shape of the SFS. The most sophisticated extensions of the test therefore additionally incorporate basic demographic models to improve their estimates (Keightley and Eyre-Walker 2007; Boyko *et al.* 2008; Eyre-Walker and Keightley 2009), which requires additional (and often uncertain) assumptions about the demographic history of the population of interest.

In contrast to such model-based approaches, a considerably simpler, heuristic approach was recently proposed by Messer and Petrov (2013). This approach generalizes the frequency-cutoff approach described above, without the need to discard polymorphism data. Instead of setting a specific frequency cutoff, it separately estimates α for each of a set of discrete mutational frequency classes:

$$\alpha(x) = 1 - \frac{d_0}{d}\frac{p(x)}{p_{0(x)}}. \quad (2)$$

Here $p(x)$ and $p_0(x)$ specify the levels of polymorphism per site in the test and reference regions, respectively, considering only those polymorphisms for which the derived allele is present at frequency x in the population (estimated from a population sample, for example). In the presence of deleterious mutations, $\alpha(x)$ will underestimate the true value of α for small x, yet should converge to the correct value as x approaches one (Messer and Petrov 2013). The asymptotic estimate of α is then obtained by fitting a function $\alpha_{\text{fit}}(x)$ to the empirical $\alpha(x)$ values and extrapolating this function to $x = 1$:

$$\alpha_{\text{asymptotic}} = \alpha_{\text{fit}}(x = 1). \quad (3)$$

One key advantage of this approach is that because $\alpha(x)$ does not depend on the individual functions $p(x)$ and $p_0(x)$ but only on their ratio, any biases due to demography or linked selection that affect the SFS in the test and reference regions in the same way will effectively cancel out (Messer and Petrov 2013). Another advantage over model-based approaches is that the asymptotic McDonald–Kreitman approach is much more computationally efficient, as it requires only fitting a simple curve to the data.

In this paper, we present asymptoticMK, a web-based tool for executing the asymptotic McDonald–Kreitman test quickly and easily in any web browser. After the necessary values are entered, asymptoticMK generates analyses and plots that are directly usable in publications. It is based internally on R, but no knowledge of R is needed to use it, nor does the user of asymptoticMK need to have R installed on their computer. For those who do wish to run the test themselves in R, the necessary code is freely available online. The asymptoticMK service can also be run in an automated fashion at the command line, for bulk analysis in script-based workflows. Finally, we present results from forward genetic simulations to illustrate the performance and robustness of the asymptotic MK test in various scenarios.

MATERIALS AND METHODS

Implementation

The asymptoticMK web service is implemented in R (R Development Core Team 2016). It uses the package `FastRWeb` (Urbanek 2008) to parse HTTP requests and generate responses, and uses the package `Rserve` (Urbanek 2003) as the lower-level interface that communicates with the web server through the standard CGI mechanism. A version of asymptoticMK that runs in R on the user's local machine is also provided. Source code and additional resources related to asymptoticMK are posted on GitHub at https://github.com/MesserLab/asymptoticMK.

Usage

The web service is free to use, without license restrictions of any kind, and is available at http://benhaller.com/messerlab/asymptoticMK.html. That URL displays an entry page (Figure 1) with an input form in which the user may enter the necessary data for the test: d (the substitution rate in the test region), d_0 (the substitution rate in the neutral reference region), and an uploaded file containing tab-delimited rows of data with values for x (the derived allele frequency), $p(x)$ (the polymorphism level in the test region at that frequency), and $p_0(x)$ (the polymorphism level in the neutral reference region at that frequency). A sample polymorphism file is provided on the website. In practice, it is often advisable to combine polymorphism levels into a smaller number of frequency bins, where x then specifies the central frequency of the bin. This is particularly relevant when the data includes frequencies at which no polymorphisms are actually present in

the neutral region, in which case $\alpha(x)$ would be undefined for those particular frequencies according to Equation (2). The frequency bins supplied to asymptoticMK do not need to be equally spaced, but to obtain the best possible α estimate it is preferable to have bins providing good coverage across the full frequency spectrum. The input form also allows entry of minimum and maximum values defining a cutoff interval for x, such that the test is run using only the polymorphisms whose frequencies fall within that cutoff interval; this is usually desirable as a means of excluding the lowest- and highest-frequency polymorphisms, where SNP quality issues and polarization errors are generally most pronounced. This frequency cutoff is set to [0.1, 0.9] by default, but should be adjusted as needed.

Upon submission of the web form, asymptoticMK conducts its analysis and then opens a results page in a new browser tab, presenting a summary of the input data and the results from the analysis. The first plot on this results page shows binned polymorphism counts, $p_0(x)$ and $p(x)$, for the submitted data; the second plot shows that same data normalized (i.e., the normalized SFS in the test and reference regions). A third plot shows the calculated empirical $\alpha(x)$ as a function of x, estimated from the input data according to Equation (2). The fourth plot shows the same $\alpha(x)$ data, with the best-fitting model and the asymptotic estimate of α from Equation (3) superimposed upon the data.

Below these plots, the results of the analysis are presented in two tables. The first table provides the coefficients a, b, and (for exponential fits) c of the model yielding the best fit to the data. The second table provides the estimated $\alpha_{\text{asymptotic}}$ according to Equation (3), and the upper and lower limits of the 95% confidence interval around that estimate, as well as the estimated α from the original non-asymptotic McDonald–Kreitman test (α_{original}) for comparison (also estimated from all polymorphisms falling within the frequency cutoff interval specified on the input page).

For purposes of automation, the asymptoticMK web service can also be run at the command line using the Linux/Unix `curl` command. For example, the command

```
curl -F"d=593" -F"d0=930" -F"xlow=0.1" -F"xhigh=0.9" -
F"datafile=@polymorphisms.txt" -F"reply=table" -o "MK_table.txt"
http://benhaller.com/cgi-bin/R/asymptoticMK_run.html
```

would run asymptoticMK with the given values of d and d_0, the given x cutoff interval, and polymorphism data uploaded from the local file `polymorphisms.txt`, and would output a simple table of results to the file `MK_table.txt`. Further documentation on the use of this feature is provided on the asymptoticMK web page.

Finally, it is also possible to run asymptoticMK in R on the user's local machine. The R code for doing so can be found on asymptoticMK's GitHub repository. In addition to allowing the user to modify asymptoticMK's analysis as desired, this option also allows PDF plots to be created, rather than the PNG plots provided by the web-based service.

Fitting and analysis procedure

The asymptotic McDonald–Kreitman test first involves calculating values of $\alpha(x)$ by applying Equation (2) to each frequency bin provided, as described by Messer and Petrov (2013). The next step involves fitting a function $\alpha_{\text{fit}}(x)$ to these empirical $\alpha(x)$ values. For greater robustness, asymptoticMK fits two functions to the data. The first function is exponential, of the form $\alpha_{\text{fit}}(x) = a + b \exp(-cx)$, and is fitted using the `nls2()` function, from the R package `nls2` (Grothendieck 2013). This fit is done in two steps. First, a brute-force scan for the closest fit is conducted across the likely portion of the three-dimensional parameter space defined by a, b, and c, by exhaustive search. This supplies reasonably good starting values for the second step, which refines those starting values using standard nonlinear least-squares regression. While this two-step procedure generally works well, it can fail to converge if the data is not exponential in form.

To address this possibility of nonconvergence of the exponential fit, asymptoticMK also fits a linear function of the form $\alpha_{\text{fit}}(x) = a + bx$, with the `lm()` function that is part of the `stats` package included in R. This fit always converges, and thus provides a backstop that allows the test to complete even when given irregular or extremely noisy data; however, it is always recommended that the results of the analysis be inspected visually to confirm that they are in fact meaningful.

Once these two models have been fitted, asymptoticMK chooses which model will be used for the remainder of the analysis. If the exponential fit failed to converge, then the linear model is chosen; if both fits succeeded, then the better model is chosen using the Akaike Information Criterion (AIC). Occasionally, in pathological cases, the exponential fit will have

the better AIC but will have extremely large coefficient standard error(s); in this case, the linear fit is chosen since predictions from the exponential model would be effectively worthless.

The chosen model is then used to provide an estimate of the value of $\alpha_{\text{asymptotic}}$ according to Equation (3), by evaluating the fitted function $\alpha_{\text{fit}}(x)$ at $x = 1$; this is the primary result of the test, and provides the test's estimate of the true value of α within the test region. A 95% confidence interval around this estimate is also calculated. For the exponential model, this is done using Monte Carlo simulation based upon the fitted model, using the `predictNLS()` function published online by Spiess (2013); for the linear model, it is done using the standard R function `predict()`.

Test datasets

To provide a test of asymptoticMK using empirical data, we used the same *Drosophila melanogaster* dataset that Messer and Petrov (2013) used in their Figure 3C. This data set consists of SNPs obtained from the genome sequences of 162 inbred fly lines generated by the *Drosophila* genetic reference panel (Mackay *et al.* 2012). Divergence data was obtained from genome alignments between *D. melanogaster* and *D. simulans*, extracted from the 12 *Drosophila* genomes data (Clark *et al.* 2007). The test data in the asymptoticMK analysis (d and p) are genome-wide nonsynonymous mutations, while synonymous sites were used as the neutral reference (d_0 and p_0). The polymorphism data is available online at on asymptoticMK's GitHub repository, with associated values $d = 59570$ and $d_0 = 159058$. The default frequency cutoff interval of [0.1, 0.9] was used in the analysis of this dataset with asymptoticMK.

We also tested asymptoticMK on simulated data, using the forward genetic simulation framework SLiM 2 (Haller and Messer 2017). A population of 1000 diploid individuals was simulated to evolve in a total of 13 different scenarios, with 20 replicates for each scenario. Simulation runs depended upon six free parameters (T, L, μ, r_b, s_d, s_b) as described hereafter. After an initial burn-in period of 10,000 generations to equilibrate the model, runs executed for T additional generations. The simulated chromosome was L base pairs long. Nucleotide mutations occurred uniformly at a rate of μ per base per generation, and recombination occurred uniformly at a rate of 10^{-7} per base per generation. Each new mutation was either of neutral type "m1" (relative proportion of 0.5 of all new mutations), of functional non-beneficial type "m2" (relative proportion of 0.5 of all new mutations), or of functional beneficial type "m3" (relative proportion

of r_b of all new mutations); these relative proportions were automatically rescaled by SLiM to be absolute proportions. The neutral m1 mutations always had a selection coefficient of $s = 0.0$; the selection coefficients of m2 mutations were drawn from a gamma distribution with a mean of s_d and a shape parameter of 0.2; and m3 mutations always had a selection coefficient of s_b. Fitness effects were assumed to be codominant. Every 500 generations after the burn-in period, all polymorphisms were recorded in the population by dividing them according to their frequency into 50 equal-width frequency bins, and then adding them to an ongoing binned tabulation. The SLiM configuration script used for these simulations is provided on asymptoticMK's GitHub repository.

The "baseline" parameterization of this model utilized parameter values of $L = 10^7$, $\mu = 10^{-9}$, $r_b = 0.0005$, $s_b = 0.1$, $s_d = -0.02$, and $T = 2\times10^5$. The other twelve parameterizations involved either a "high" or a "low" value of one of the six parameters, replacing the "central" value used in the baseline scenario: $L = 10^8$ or 10^6, $\mu = 10^{-8}$ or 10^{-10}, $r_b = 0.001$ or 0.0001, $s_b = 0.2$ or 0.02, $s_d = -0.2$ or -0.002, and $T = 2\times10^6$ or 2×10^4. At the end of each model run, we obtained binned values for $p(x)$ and $p_0(x)$, where p_0 was estimated from all polymorphisms involving mutations of type m1, while p was estimated from the combined mutations of types m2 and m3. Values for d and d_0 were obtained from the set of mutations fixed during the simulation; as with p_0 and p, d_0 was estimated from all mutations of type m1, while d was estimated from the combined mutations of types m2 and m3. These values, output by the model, were used in asymptoticMK with the default x cutoff interval of [0.1, 0.9] to calculate an α estimate. The α estimate from the original MK test was also calculated using the data within the same interval. Finally, the true value of α was estimated from the simulation run as the fraction $d_3 / (d_2 + d_3)$, where d_2 is the number of m2 mutations fixed and d_3 is the number of m3 mutations fixed. This value provides a metric for the accuracy of the α estimates – a benefit of using simulated data, where the true α can be calculated.

From this raw data provided by each set of 20 replicates for a given parameterization, summary statistics for that parameterization were computed. In particular, we calculated (i) the mean and standard deviation of the true α values, (ii) the mean and standard deviation of the asymptoticMK α estimates, (iii) the mean of the absolute differences between the true α and the asymptoticMK estimate (i.e., the mean estimation error for asymptoticMK), (iv) the mean and standard deviation of the estimates of α using the original non-asymptotic MK test, (v) the mean

of the absolute differences between the true α and the original MK test estimate (i.e., the mean estimation error for the original MK test), and (vi) the fraction of the 20 replicates for which asymptoticMK chose an exponential (as opposed to linear) fit (Table 1).

RESULTS AND DISCUSSION

Results from our test of asymptoticMK with the empirical *D. melanogaster* dataset are shown in Figures 2A and 2B. The fitted exponential function is: $\alpha_{\text{fit}}(x) = 0.585 - 0.622 \exp(-3.80x)$. The asymptotic McDonald–Kreitman estimate provided by this model is $\alpha_{\text{asymptotic}} = 0.571$. These results match those obtained by Messer and Petrov (2013) using the same dataset (their Figure 3C), as expected. The estimate provided by the original McDonald–Kreitman test is $\alpha_{\text{original}} = 0.407$, by comparison (shown in Figure 2B).

The results from the analysis of the SLiM simulations are shown in Table 1. In 12 of the 13 parameterizations, the mean estimation error of asymptoticMK was markedly lower than that of the original MK test; in the other parameterization ($T = 2\times10^4$) the tests performed similarly (mean estimation errors of 0.126 and 0.120). For 3 of the 13 parameterizations ($L = 10^6$, $\mu = 10^{-10}$, and $T = 2\times10^4$), however, the mean estimation error of asymptoticMK was greater than 0.1, indicating that α estimates were relatively inaccurate for those simulations. These three parameterizations involved a shorter chromosome, a lower mutation rate, or a shorter duration, and thus all provided approximately ten times less polymorphism data upon which to base estimations than our baseline scenario. Accordingly, parameterizations that provided more polymorphism data ($L = 10^8$, $\mu = 10^{-8}$, and $T = 2\times10^6$) provided more accurate α estimates (mean estimation errors below 0.03). This pattern was weak or absent for the original MK test; even for the high-data parameterizations the original MK test always showed a mean estimation error greater than 0.1, and its mean estimation error for the high-data $L = 10^8$ case was actually substantially higher than for the low-data $L = 10^6$ case (0.151 versus 0.120). This is consistent with the fact that the original MK test systematically underestimates α in the presence of deleterious mutations (as discussed in the Introduction). The asymptotic MK test may still have a tendency toward underestimation as well, but errors are much smaller.

Another noteworthy observation is that in the high-data parameterizations the exponential fit was chosen by asymptoticMK in 100% of cases, whereas in the low-data parameterizations

the linear fit was chosen a majority of the time (Table 1). It is not the case that the linear model always produces a poor α estimate; we observed many runs where the linear fit performed well. However, it may indicate that a poor cutoff interval was chosen, that the binning of the polymorphism data ought to be done differently, or that the data is simply too noisy. We suggest that the result of the asymptotic MK test should always be inspected visually to verify that the fit is reasonable and that appropriate cutoff intervals and bin sizes were used.

To illustrate how such manual inspection can help improve estimates, we examine two of the simulation runs from Table 1 in more detail. Figure 2C shows the automated fit for one of the simulations in the low-data $L = 10^6$ scenario. A linear fit function produced an asymptotic α estimate of 0.0313, which is quite distant from the true value of 0.3462. The binned polymorphism data within the cutoff interval of [0.1, 0.9] is rather flat, but the data appears reasonable across the whole frequency spectrum in this case, and the upward trend of the data is much more visible outside of the cutoff interval used. Changing the cutoff interval to [0.0, 1.0] produces the fit shown in Figure 2D, with an asymptotic α estimate of 0.2829 – much closer to the true value. Figure 2E shows the automated fit for another $L = 10^6$ scenario run. This fit also used a linear fit function, producing an asymptotic α estimate of 0.0103 compared to the true value of 0.2462. Here, the data are very noisy, which could be an indication that more bins have been used than can be robustly supported by the data. Re-binning the polymorphism data into half as many bins provides a less noisy dataset that results in a much better fit (Figure 2F), with an α estimate of 0.1813 – again, a substantial improvement. These examples illustrate that automated fits can be particularly problematic in low-data situations such as the $L = 10^6$ scenario, but that hand inspection and tailoring of the fitting process can sometimes improve the result noticeably.

CONCLUSIONS

In this paper, we presented asymptoticMK, a new web-based tool for executing the asymptotic McDonald–Kreitman test. To demonstrate its functionality, we analyzed both empirical and simulation-generated datasets. Our results illustrate the greater power of the asymptotic McDonald–Kreitman test to estimate the true value of α, compared to the original non-asymptotic test. However, our results also underline the need for a large dataset to obtain

reasonably accurate results from the asymptotic test; estimates of α from a single gene, or from a system with a very short divergence time, are unlikely to be meaningful. In addition, visual inspection of the quality of the fit used to estimate α is necessary for accuracy. With attention to these caveats, the asymptoticMK service presented here allows the user to obtain α estimates quickly and easily through any web browser, or using R on any machine.

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LITERATURE CITED

Andolfatto, P., 2005 Adaptive evolution of non-coding DNA in *Drosophila*. Nature 437: 1149–1152.

Boyko, A. R., S. H. Williamson, A. R. Indap, J. D. Degenhardt, R. D. Hernandez *et al.*, 2008 Assessing the evolutionary impact of amino acid mutations in the human genome. PLoS Genet. 4: e1000083.

Bustamante, C. D., A. Fledel-Alon, S. Williamson, R. Nielsen, M. T. Hubisz *et al.*, 2005 Natural selection on protein-coding genes in the human genome. Nature 437: 1153–1157.

Charlesworth, J., and A. Eyre-Walker, 2008 The McDonald-Kreitman test and slightly deleterious mutations. Mol. Biol. Evol. 25: 1007–15.

Clark, A. G., M. B. Eisen, D. R. Smith, C. M. Bergman, B. Oliver *et al.*, 2007 Evolution of genes and genomes on the *Drosophila* phylogeny. Nature 450: 203–218.

Egea, R., S. Casillas, and A. Barbadilla, 2008 Standard and generalized McDonald-Kreitman

test: a website to detect selection by comparing different classes of DNA sites. Nucleic Acids Res. 36: W157-62.

Elyashiv, E., K. Bullaughey, S. Sattath, Y. Rinott, M. Przeworski *et al.*, 2010 Shifts in the intensity of purifying selection: an analysis of genome-wide polymorphism data from two closely related yeast species. Genome Res. 20: 1558–73.

Eyre-Walker, A., 2006 The genomic rate of adaptive evolution. Trends Ecol. Evol. 21: 569–575.

Eyre-Walker, A., and P. D. Keightley, 2009 Estimating the rate of adaptive molecular evolution in the presence of slightly deleterious mutations and population size change. Mol. Biol. Evol. 26: 2097–108.

Eyre-Walker, A., M. Woolfit, and T. Phelps, 2006 The distribution of fitness effects of new deleterious amino acid mutations in humans. Genetics 173: 891–900.

Fay, J. C., 2011 Weighing the evidence for adaptation at the molecular level. Trends Genet. 27: 343–9.

Grothendieck, G., 2013 nls2: Non-linear regression with brute force. Available at: https://CRAN.R-project.org/package=nls2. Accessed: December 14, 2016.

Gossmann, T. I., B.-H. Song, A. J. Windsor, T. Mitchell-Olds, C. J. Dixon *et al.*, 2010 Genome wide analyses reveal little evidence for adaptive evolution in many plant species. Mol. Biol. Evol. 27: 1822–1832.

Haller, B. C., and P. W. Messer, 2017 SLiM 2: Flexible, interactive forward genetic simulations. Mol. Biol. Evol. 34: 230–240.

Keightley, P. D., and A. Eyre-Walker, 2007 Joint inference of the distribution of fitness effects of deleterious mutations and population demography based on nucleotide polymorphism frequencies. Genetics 177: 2251–2261.

Librado, P., and J. Rozas, 2009 DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452.

Mackay, T. F., S. Richards, E. A. Stone, A. Barbadilla, J. F. Ayroles *et al.*, 2012 The *Drosophila melanogaster* genetic reference panel. Nature 482: 173–178.

McDonald, J. H., and M. Kreitman, 1991 Adaptive protein evolution at the *Adh* locus in *Drosophila*. Nature 351: 652–4.

Messer, P. W., and D. A. Petrov, 2013 Frequent adaptation and the McDonald-Kreitman test. Proc. Natl. Acad. Sci. U. S. A. 110: 8615–20.

R Development Core Team, 2016 *R: A language and for statistical computing*. Vienna, Austria.

Sella, G., D. A. Petrov, M. Przeworski, and P. Andolfatto, 2009 Pervasive natural selection in the *Drosophila* genome? PLoS Genet. 5: e1000495.

Spiess, A.-N. predictNLS (Part 1, Monte Carlo simulation): confidence intervals for "nls" models. R-bloggers. Available at: https://www.r-bloggers.com/predictnls-part-1-monte-carlo-simulation-confidence-intervals-for-nls-models/. Accessed: December 14, 2016.

Stoletzki, N., and A. Eyre-Walker, 2011 Estimation of the neutrality index. Mol. Biol. Evol. 28: 63–70.

Urbanek, S., 2008 FastRWeb: Fast interactive web framework for data mining using R, in *IASC 2008 World Congress*. Available at: https://rforge.net/FastRWeb/. Accessed: December 14, 2016.

Urbanek, S., 2003 Rserve - A fast way to provide R functionality to applications. In *Proceedings of the 3rd International Workshop on Distributed Statistical Computing*. Available at: https://rforge.net/Rserve/. Accessed: December 14, 2016.

Vos, M., T. A. H. te Beek, M. A. van Driel, M. A. Huynen, A. Eyre-Walker *et al.*, 2013 ODoSE: a webserver for genome-wide calculation of adaptive divergence in prokaryotes. PLoS One 8: e62447.

FIGURES

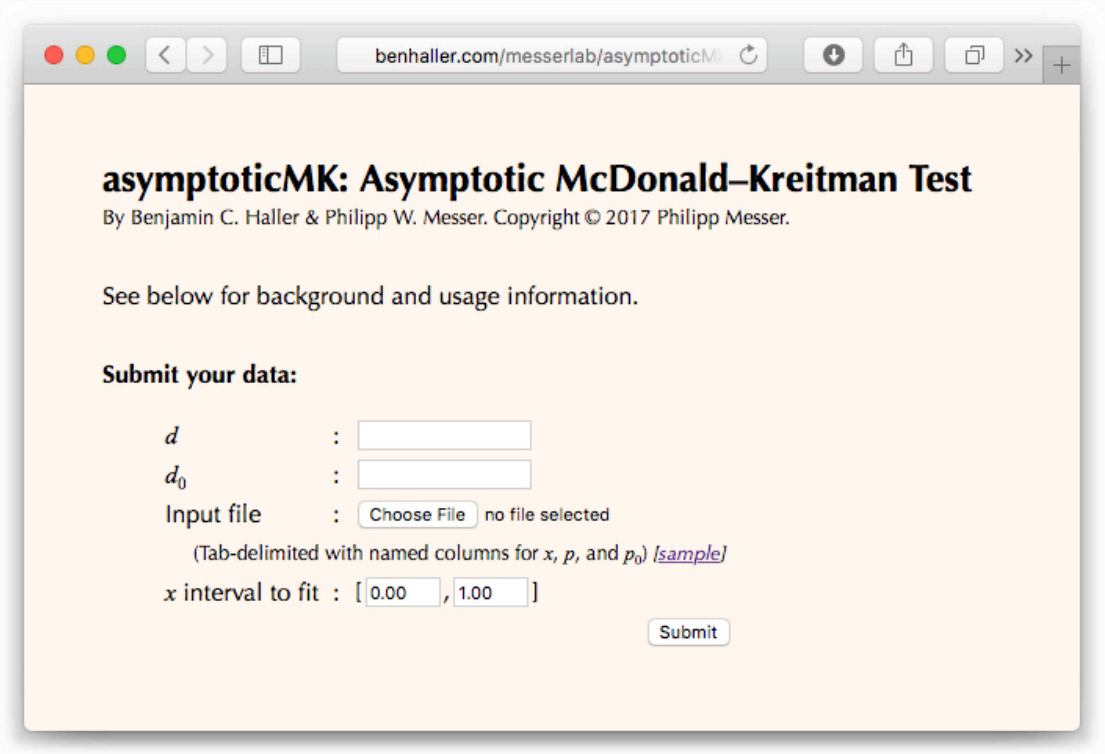

Figure 1. A screenshot of the web page for asymptoticMK. After entering values for d and d_0, choosing an input file with binned values for x, p, and p_0, and choosing the x interval to fit, the user can click the Submit button and asymptoticMK will provide its results in a new browser window or tab.

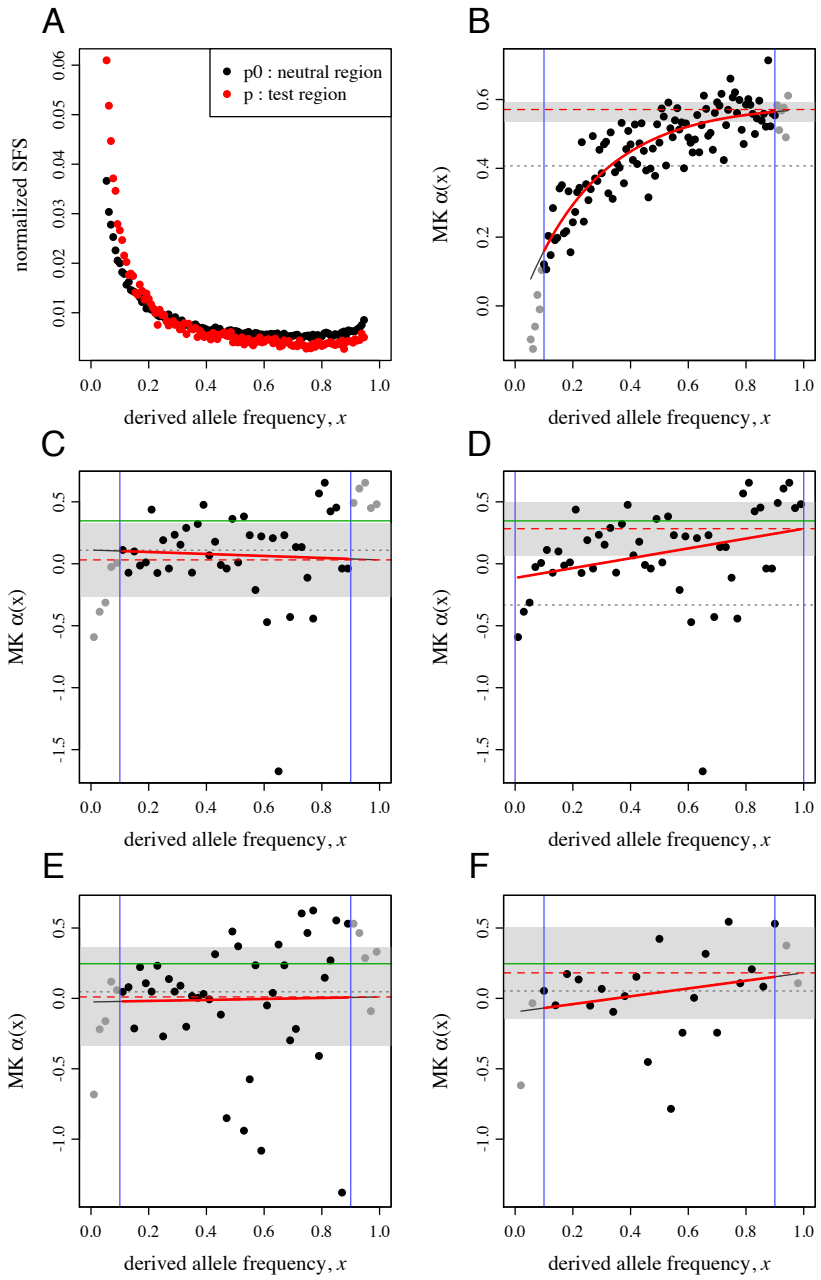

Figure 2. Results from asymptoticMK for three test datasets. (A) Normalized site frequency spectrum (SFS) for the *Drosophila* dataset used in Messer and Petrov (2013). Points show normalized binned polymorphism frequencies for the neutral region (black) and the test region (red). (B) Result of asymptoticMK's analysis of that dataset. The two vertical blue lines show the limits of the frequency cutoff interval used for fitting. Points indicate binned values of $\alpha(x)$, estimated according to Equation 2; points are gray if they are outside the cutoff interval (and thus not used in fitting). The solid red curve shows the fitted $\alpha_{\text{fit}}(x)$ (here, exponential). The dashed

red line shows the estimate of $\alpha_{asymptotic}$, obtained from the fitted function according to Equation 3. The gray band indicates the 95% confidence intervals around this $\alpha_{asymptotic}$ estimate. The dotted gray line shows the estimate of $\alpha_{original}$, obtained from the original (non-asymptotic) MK test, for comparison (also calculated using only the data within the cutoff interval). (C) and (D) show corresponding results from one SLiM simulation run, and (E) and (F) show results from another SLiM simulation run; in each case, the first panel shows the result of an automated fit using asymptoticMK, whereas the second shows the improvement after hand tailoring of the fit (see Results and Discussion). Note that in all four cases the linear fit was deemed more appropriate by asymptoticMK. The solid green horizontal lines, finally, show the true value of α in the simulation runs for comparison.

TABLES

Table 1. Results from asymptoticMK for simulation runs conducted with SLiM 2. The first row shows the averaged results from 20 replicate runs of the baseline SLiM model supplied on GitHub (see text). These runs used parameter values of mutation rate $\mu = 10^{-9}$ per base position per generation, chromosome length $L = 10^7$ base positions, beneficial mutation rate $r_b = 0.0005$, beneficial mutation selection coefficient $s_b = 0.1$, deleterious mutation selection coefficient $s_d = -0.02$, and time after burn-in $T = 2\times10^5$ generations. Each subsequent row shows the results from 20 replicate runs using the non-baseline parameter value shown. α_{true} specifies the true value of α averaged across the 20 replicates in each row; $\alpha_{asymptotic}$ and $\alpha_{original}$ specify the asymptoticMK estimate and the estimate from the original test, respectively. Standard deviations across the 20 replicates of each row are shown as ± values. $\Delta_{asymptotic} = |\alpha_{asymptotic} - \alpha_{true}|$ and $\Delta_{original} = |\alpha_{original} - \alpha_{true}|$ specify the mean absolute errors between true α values and the estimates from asymptoticMK and the original test, respectively, in each run, averaged over the 20 replicates. ρ_{exp} specifies the fraction of runs in which the exponential fit was chosen.

Model	α_{true}	$\alpha_{asymptotic}$	$\alpha_{original}$	$\Delta_{asymptotic}$	$\Delta_{original}$	ρ_{exp}
baseline	0.329 ± 0.015	0.307 ± 0.058	0.164 ± 0.035	0.045	0.165	0.75
$L = 10^8$	0.327 ± 0.008	0.301 ± 0.013	0.174 ± 0.012	0.025	0.152	1.00
$L = 10^6$	0.321 ± 0.067	0.246 ± 0.134	0.142 ± 0.141	0.120	0.191	0.15
$\mu = 10^{-8}$	0.306 ± 0.005	0.287 ± 0.016	0.173 ± 0.009	0.019	0.132	1.00
$\mu = 10^{-10}$	0.317 ± 0.057	0.288 ± 0.169	0.145 ± 0.074	0.134	0.173	0.05
$r_b = 0.0010$	0.493 ± 0.018	0.481 ± 0.045	0.378 ± 0.025	0.041	0.114	0.70
$r_b = 0.0001$	0.091 ± 0.014	0.115 ± 0.080	−0.103 ± 0.053	0.071	0.194	0.55
$s_b = 0.20$	0.477 ± 0.016	0.451 ± 0.032	0.366 ± 0.025	0.029	0.111	0.70
$s_b = 0.02$	0.096 ± 0.011	0.090 ± 0.068	−0.119 ± 0.047	0.057	0.215	0.50
$s_d = -0.200$	0.424 ± 0.024	0.422 ± 0.042	0.289 ± 0.036	0.032	0.135	0.60
$s_d = -0.002$	0.233 ± 0.011	0.234 ± 0.057	0.104 ± 0.039	0.045	0.129	0.50
$T = 2\times10^6$	0.324 ± 0.006	0.302 ± 0.014	0.173 ± 0.012	0.022	0.151	1.00
$T = 2\times10^4$	0.345 ± 0.063	0.369 ± 0.183	0.225 ± 0.113	0.126	0.120	0.05