CLEAR: Composition of Likelihoods for Evolve And Resequence Experiments

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ABSTRACT The advent of next generation sequencing technologies has made whole-genome and whole-population sampling possible, even for eukaryotes with large genomes. With this development, experimental evolution studies can be designed to observe molecular evolution "in-action" via Evolve-and-Resequence (E&R) experiments. Among other applications, E&R studies can be used to locate the genes and variants responsible for genetic adaptation. Most of existing literature on time-series data analysis often assumes large population size, accurate allele frequency estimates, or wide time spans. These assumptions do not hold in many E&R studies.

In this article, we propose a method–Composition of Likelihoods for Evolve-And-Resequence experiments (CLEAR)–to identify signatures of selection in small population E&R experiments. CLEAR takes whole-genome sequence of pool of individuals (pool-seq) as input, and properly addresses heterogeneous ascertainment bias resulting from uneven coverage. CLEAR also provides unbiased estimates of model parameters, including population size, selection strength and dominance, while being computationally efficient. Extensive simulations show that CLEAR achieves higher power in detecting and localizing selection over a wide range of parameters, and is robust to variation of coverage. We applied CLEAR statistic to multiple E&R experiments, including, data from a study of *D. melanogaster* adaptation to alternating temperatures and a study of outcrossing yeast populations, and identified multiple regions under selection with genome-wide significance.

KEYWORDS Experimental Evolution; Selection; Genetic Drift; Time Series Data; Hidden Markov Model; Wright-Fisher Process

Introduction

Natural selection is a key force in evolution, and a mechanism by which populations can adapt to external 'selection' pressure. Examples of adaptation abound in the natural world Fan *et al.* (2016), including for example, classic examples like lactose tolerance in Northern Europeans Bersaglieri *et al.* (2004), human adaptation to high altitudes Yi *et al.* (2010); Simonson *et al.* (2010), but also drug resistance in pests Daborn *et al.* (2001), HIV Feder *et al.* (2016), cancer Gottesman (2002); Zahreddine and Borden (2013), malarial parasite Ariey *et al.* (2014); Nair *et al.* (2007), and others Spellberg *et al.* (2008). In these examples, understanding the genetic basis of adaptation can provide valuable information, underscoring the importance of the problem.

Experimental evolution refers to the study of the evolutionary processes of a model organism in a controlled Hegreness *et al.* (2006); Lang *et al.* (2013); Orozco-ter Wengel *et al.* (2012); Lang *et al.* (2011); Barrick *et al.* (2009); Bollback and Huelsenbeck (2007); Oz *et al.* (2014) or natural Maldarelli *et al.* (2013); Reid *et al.* (2011); Denef and Banfield (2012); Winters *et al.* (2012); Daniels *et al.* (2013); Barrett *et al.* (2008); Bergland *et al.* (2014) environment. Recent advances in whole genome sequencing have enabled us to sequence populations at a reasonable cost, even for large genomes. Perhaps more important for experimental evolution studies, we can now evolve and resequence (E&R) multiple replicates of a population to obtain *longitudinal time-series data*, in order to investigate the dynamics of evolution at molecular level. Although constraints such as small sizes, limited timescales, and oversimplified laboratory environments may limit the interpretation of E&R results, these studies are increasingly being used to test a wide range of hypotheses Kawecki *et al.* (2012)

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and have been shown to be more predictive than static data analysis Boyko *et al.* (2008); Desai and Plotkin (2008); Sawyer and Hartl (1992). In particular, longitudinal E&R data is being used to estimate model parameters including population size Williamson and Slatkin (1999); Wang (2001); Pollak (1983); Waples (1989); Terhorst *et al.* (2015); Jónás *et al.* (2016), strength of selection Mathieson and McVean (2013); Illingworth and Mustonen (2011); Terhorst *et al.* (2015); Bollback *et al.* (2008); Illingworth *et al.* (2012); Malaspinas *et al.* (2012); Steinrücken *et al.* (2014), allele age Malaspinas *et al.* (2012) recombination rate Terhorst *et al.* (2015), mutation rate Barrick and Lenski (2013); Terhorst *et al.* (2015), quantitative trait loci Baldwin-Brown *et al.* (2014) and for tests of neutrality hypotheses Feder *et al.* (2014); Terhorst *et al.* (2015); Burke *et al.* (2010); Bergland *et al.* (2014).

While many E&R study designs are being used Barrick and Lenski (2013); Schlötterer *et al.* (2015), we restrict our attention to the adaptive evolution due to standing variation in fixed size populations. This regime has been considered earlier, typically with *D. melanogaster* as the model organism of choice, to identify adaptive genes in longevity and aging Burke *et al.* (2010); Remolina *et al.* (2012) (600 generations), courtship song Turner *et al.* (2011) (100 generations), hypoxia tolerance Zhou *et al.* (2011) (200 generations), adaptation to new laboratory environments Orozco-ter Wengel *et al.* (2012); Franssen *et al.* (2015) (59 generations), egg size Jha *et al.* (2015) (40 generations), C virus resistance Martins *et al.* (2014) (20 generations), and dark-fly Izutsu *et al.* (2015) (49 generations).

The task of identifying selection signatures can be addressed at different levels of specificity. At the coarsest level, identification could simply refer to deciding whether some genomic region (or a gene) is under selection or not. In the following, we refer to this task as *detection*. In contrast, the task of *site-identification* corresponds to the process of finding the favored mutation/allele at nucleotide level. Finally, *estimation of model parameters*, such as strength of selection and dominance at the site, can provide a comprehensive description of the selection process.

In the effort to analyze E&R selection experiments, many authors chose to adapt existing tests that were originally used for static data, pairwise comparisons (two time-points) and single replicates to perform a null scan. For instance, Zhu *et al.* Zhou *et al.* (2011) used the ratio of the estimated population size of case and control populations to compute test statistic for each genomic region. Burke *et al.* Burke *et al.* (2010) applied Fisher exact test to the last observation of data on case and control populations. Orozco-terWengel *et al.* Orozco-ter Wengel *et al.* (2012) used the Cochran-Mantel-Haenszel (CMH) test Agresti and Kateri (2011) to detect SNPs whose read counts change consistently across all replicates of two time-point data. Turner *et al.* Turner *et al.* (2011) proposed the diffStat statistic to test whether the change in allele frequencies of two populations deviate from the distribution of change in allele frequencies of two drifting populations. Bergland *et al.* Bergland *et al.* (2014) calculated F_{st} to populations throughout time to signify their differentiation from ancestral (two time-point data) as well as geographically different populations. Jha *et al.* Jha *et al.* (2015) computed test statistic of generalized linear-mixed model directly from read counts.

Alternatively, *direct* methods have been developed to analyze time-series data by taking a likelihood approach, and estimating population genetics parameters. Bollback *et al.* Bollback *et al.* (2008) proposed a Hidden Markov Model (HMM) to estimate the selection coefficient s and population size by using a diffusion approximation to the Wright Fisher process. Steinrücken *et al.* Steinrücken *et al.* (2014) proposed a general diploid selection model which takes into account of dominance of the favored allele and approximates likelihood analytically. Recently, Schraiber *et al.* Schraiber *et al.* (2016) proposed a Bayesian framework to estimate parameters using Monte Carlo Markov chain sampling. Mathieson and McVean Mathieson and McVean (2013) adopted HMMs to structured populations and estimated parameters using an Expectation Maximization (EM) procedure on discretized allele frequency. Feder *et al.* Feder *et al.* (2014) modeled increments in allele frequency with a Brownian motion process, proposed the Frequency Increment Test (FIT). More recently, Topa *et al.* Topa *et al.* (2015) proposed a Gaussian Process (GP) for modeling single-locus time-series pool-seq data. Terhorst *et al.* Terhorst *et al.* (2015) extended GP to compute joint likelihood of multiple loci under null and alternative hypotheses. Finally, Levy *et al.* Levy *et al.* (2015) proposed a Bayesian model to handle sequencing, amplification and growth noise in a large population of barcoded lineages.

Among the methods specifically designed for time-series data, many make assumptions which may not hold in E&R studies. One common assumption is that the underlying population size is large, so it is reasonable to model dynamics of allele frequencies using continuous state models Bollback *et al.* (2008); Feder *et al.* (2014); Terhorst *et al.* (2015). Second, many existing methods were originally designed to process wider time spans seen in ancient DNA studies, an assumption that does not hold for E&R experiments Steinrücken *et al.* (2014); Schraiber *et al.* (2016). Finally, many E&R analysis tools assume that allele frequencies in the input data are unbiased (e.g. Bollback *et al.* (2008)), which may not be valid for shotgun sequencing experiments.

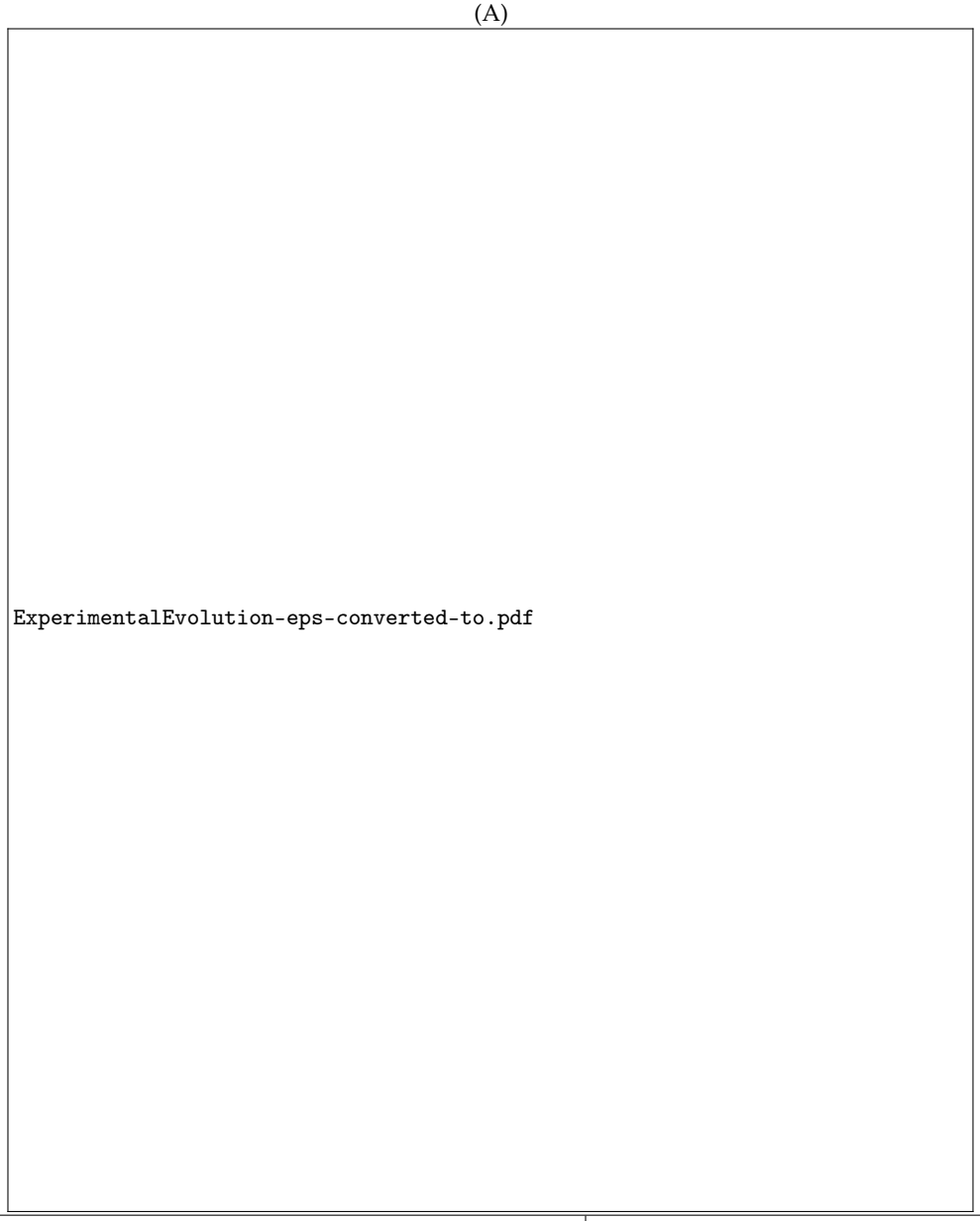
Here, we consider a Hidden Markov Model (HMM), similar to Williamson *et al.* Williamson and Slatkin (1999) and Bollback *et al.*'s Bollback *et al.* (2008) but under a "small-population-size" regime. Specifically, we use a discrete state (frequency) model. We show that for small population sizes, discrete models can compute likelihood exactly, which improves statistical performance, especially for short time-span experiments. Additionally, we add another level of sampling-noise to the traditional HMM model, allowing for heterogeneous ascertainment bias due to uneven coverage among variants. We show that for a wide range of parameters, CLEAR provides higher power for detecting selection, estimates model parameters consistently, and localizes favored allele more accurately compared to the state-of-the-art methods, while being computationally efficient.

Materials and Methods

Consider a panmictic diploid population with fixed size of N individuals. Let $\boldsymbol{\nu} = \{\nu_t\}_{t\in\mathcal{T}}$ be frequencies of the derived allele at generations $t \in \mathcal{T}$ for a given variant, where at generations $\mathcal{T} = \{\tau_i : 0 \leq \tau_0 < \tau_1 \ldots < \tau_T\}$ samples of n individuals are chosen for pooled sequencing. The experiment is replicated R times. We denote allele frequencies of the R replicates by the set $\{\boldsymbol{\nu}\}_R$. To identify the genes and variants that are responding to selection pressure, we use the following procedure:

1. **Estimating population size.** The procedure starts by estimating the effective population size, $\widehat{N}$, under the assumption that much of the genome is evolving neutrally.

(A)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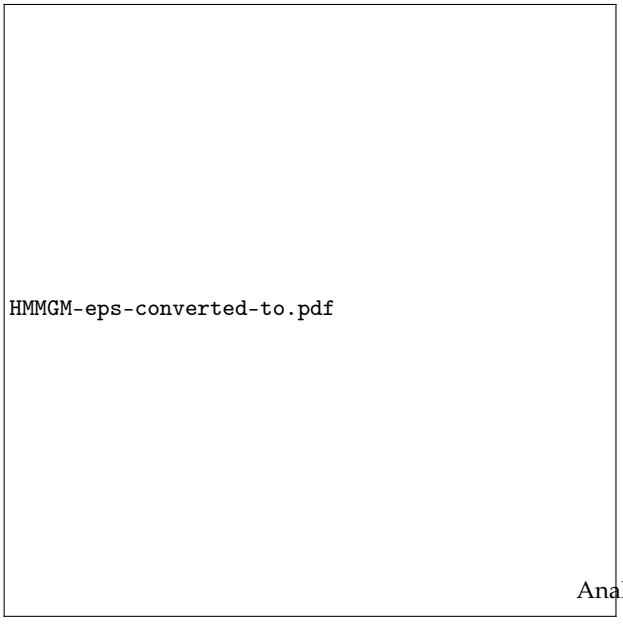
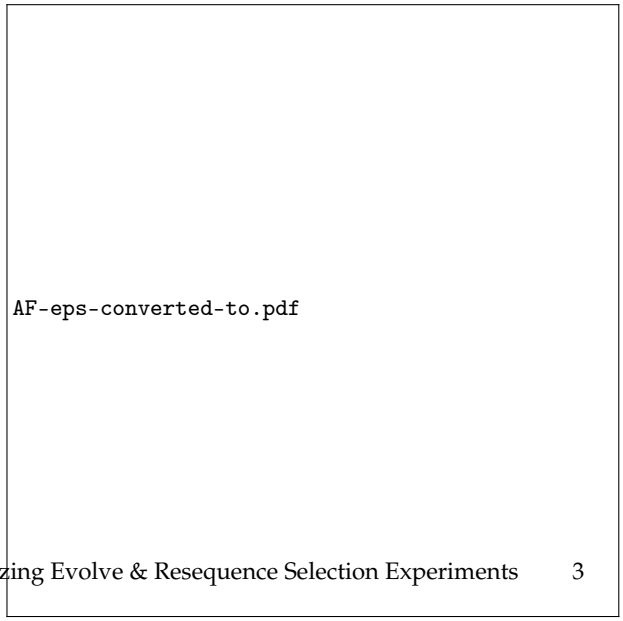
(B)


HMMGM-eps-converted-to.pdf

(C)


AF-eps-converted-to.pdf

2. **Estimating selection parameters.** For each polymorphic site, selection and dominance parameters s, h are estimated so as to maximize the likelihood of the time series data, given $\widehat{N}$.

3. **Computing likelihood statistics.** For each variant, a log-odds ratio of the likelihood of selection model ($s > 0$) to the likelihood of neutral evolution/drift model is computed. Likelihood ratios in a genomic region are combined to compute the CLEAR statistic for the region.

4. **Hypothesis testing.** An empirical null distribution of the CLEAR statistic is calculated using genome-wide drift simulations, and used to compute p-values and thresholds for a specified FDR. We perform single locus hypothesis testing within selected regions to identify significant variants and report genes that intersect with the selected variants.

These steps are described in detail below.

Estimating Population Size

Methods for estimating population sizes from temporal neutral evolution data have been developed Williamson and Slatkin (1999); Anderson *et al.* (2000); Bollback *et al.* (2008); Terhorst *et al.* (2015); Jónás *et al.* (2016). Here, we aim to extend these models to explicitly model the sampling noise that arise in pool-seq data. Specifically, we model the variation in sequence coverage over different locations, and the noise due to sequencing only a subset of the individuals in the population. In addition, many existing methods Bollback *et al.* (2008); Feder *et al.* (2014); Topa *et al.* (2015); Terhorst *et al.* (2015) are designed for large populations, and model frequency as a continuous quantity. We observed that using Brownian motion to model frequency drift may be inadequate for small populations, low starting frequencies and sparse sampling (in time), factors that are common in experimental evolution (see Results, Figure 3A-C, and Figure 2). To this end, we model the Wright-Fisher Markov process for generating pool-seq data (**??**) via a discrete HMM (Figure 1-B). We start by computing a likelihood function for the population size given neutral pool-seq data.

Likelihood for Neutral Model. We model the allele frequency counts $2N\nu_t$ as being sampled from a Binomial distribution. Specifically,

$$\begin{aligned} \nu_0 &\sim \pi, \\ 2N\nu_t|\nu_{t-1} &\sim \text{Binomial}(2N, \nu_{t-1}) \end{aligned}$$

where π is the global distribution of allele frequencies in the base population. Note that π depends on the demographic history of the founder lines and can be estimated from site frequency spectrum(see **??**) of the initial population. For notational convenience, henceforth we omit the dependence of likelihoods to the parameter π.

To estimate frequency after τ transitions, it is enough to specify the $2N \times 2N$ transition matrix $P^{(\tau)}$, where $P^{(\tau)}[i,j]$ denotes probability of change in allele frequency from $i/2N$ to $j/2N$ in τ generations:

$$\begin{aligned} P^{(1)}[i,j] &= \Pr\left(\nu_{t+1} = \frac{j}{2N} \mid \nu_t = \frac{i}{2N}\right) \\ &= \binom{2N}{j} s\nu_t^j (1-\nu_t)^{2N-j}, \end{aligned} \tag{1}$$

$$P^{(\tau)} = P^{(\tau-1)} P^{(1)} \tag{2}$$

Furthermore, in an E&R experiment, $n \leq N$ individuals are randomly selected for sequencing. The sampled allele frequencies, $\{y_t\}_{t\in\mathcal{T}}$, are also Binomially distributed

$$2ny_t \sim \text{Binomial}(2n, \nu_t) \tag{3}$$

We introduce the $2N \times 2n$ sampling matrix Y, where $Y[i,j]$ stores the probability that the sample allele frequency is $j/2n$ given that the true allele frequency is $i/2N$.

We denote the pool-seq data for that variant as $\{x_t = \langle c_t, d_t\rangle\}_{t\in\mathcal{T}}$ where d_t, c_t represent the coverage, and the read count of the derived allele, respectively. Let $\{\lambda_t\}_{t\in\mathcal{T}}$ be the sequencing coverage at different generations. Then, the observed data are sampled according to

$$d_t \sim \text{Poisson}(\lambda_t), \qquad c_t \sim \text{Binomial}(d_t, y_t) \tag{4}$$

The emission probability for a observed tuple $x_t = \langle d_t, c_t\rangle$ is

$$\mathbf{e}_i(x_t) = \binom{d_t}{c_t} \left(\frac{i}{2n}\right)^{c_t} \left(1 - \frac{i}{2n}\right)^{d_t - c_t}. \tag{5}$$

For $1 \leq t \leq T, 1 \leq j \leq 2N$, let $\alpha_{t,j}$ denote the probability of emitting $x_1, x_2, \ldots, x_t$ and reaching state j at τ_t. Then, α_t can be computed using the forward-procedure Durbin *et al.* (1998):

$$\alpha_t^T = \alpha_{t-1}^T P^{(\delta_t)} \text{diag}(Y\mathbf{e}(x_t)) \tag{6}$$

where $\delta_t = \tau_t - \tau_{t-1}$. The joint likelihood of the observed data from R independent observations is given by

$$\begin{aligned} \mathcal{L}(N|\{\boldsymbol{x}\}_R, n) &= \prod_{r=1}^{R} \mathcal{L}(N|\boldsymbol{x}^{(r)}, n) = \Pr(\{\boldsymbol{x}\}_R|N, n) \\ &= \prod_{r=1}^{R} \sum_i \alpha_{T,i}^{(r)} \end{aligned} \tag{7}$$

where $\boldsymbol{x} = \{x_t\}_{t\in\mathcal{T}}$. The graphical model and the generative process for which data is being generated is depicted in Figure 1-B and **??**, respectively.

Finally, the last step is to compute an estimate $\widehat{N}$ that maximizes the likelihood of all M variants in whole genome. Let $\boldsymbol{x}_i^{(r)}$ denote the time-series data of the i-th variant in replicate r. Then,

$$\widehat{N} = \arg\max_N \prod_{i=1}^{M} \prod_{r=1}^{R} \mathcal{L}(N|\boldsymbol{x}_i^{(r)}) \tag{8}$$

Estimating Selection Parameters

Likelihood for Selection Model. Assume that the site is evolving under selection constraints $s \in \mathbb{R}, h \in \mathbb{R}_+$, where s and h denote selection strength and dominance parameters , respectively. By definition, the relative fitness values of genotypes $0|0$, $0|1$ and $1|1$ are given by $w_{00} = 1$, $w_{01} = 1 + hs$ and $w_{11} = 1 + s$. Then, ν_{t^+}, the frequency at time $\tau_t + 1$ (one generation ahead), can be estimated using:

$$\begin{aligned} \hat{\nu}_{t^+} = \mathbb{E}[\nu_{t^+}|s,h,\nu_t] &= \frac{w_{11}\nu_t^2 + w_{01}\nu_t(1-\nu_t)}{w_{11}\nu_t^2 + 2w_{01}\nu_t(1-\nu_t) + w_{00}(1-\nu_t)^2} \\ &= \nu_t + \frac{s(h + (1-2h)\nu_t)\nu_t(1-\nu_t)}{1 + s\nu_t(2h + (1-2h)\nu_t))}. \end{aligned} \tag{9}$$

The machinery for computing likelihood of the selection parameters is identical to that of population size, except for transition matrices. Hence, here we only describe the definition transition

matrix $Q_{s,h}$ of the selection model. Let $Q^{(\tau)}_{s,h}[i,j]$ denote the probability of transition from $i/2N$ to $j/2N$ in τ generations, then (See Ewens (2012), Pg. 24, Eqn. 1.58-1.59):

$$\begin{aligned} Q^{(1)}_{s,h}[i,j] &= \Pr\left(\nu_{t^+} = \frac{j}{2N} \,\middle|\, \nu_t = \frac{i}{2N}; s, h, N\right) \\ &= \binom{2N}{j} \hat{\nu}^j_{t^+} (1-\hat{\nu}_{t^+})^{2N-j} \end{aligned} \tag{10}$$

$$Q^{(\tau)}_{s,h} = Q^{(\tau-1)}_{s,h} Q^{(1)}_{s,h} \tag{11}$$

The maximum likelihood estimates are given by

$$\widehat{s}, \widehat{h} = \arg\max_{s,h} \prod_{r=1}^{R} \mathcal{L}(s, h | \boldsymbol{x}^{(r)}, \widehat{N}) \tag{12}$$

Using grid search, we first estimate N (Eq. 8), and subsequently, we estimate parameters s, h (Eq. 12, **??**). By broadcasting and vectorizing the grid search operations across all variants, the genome scan on millions of polymorphisms can be done in significantly smaller time than iterating a numerical optimization routine for each variant(see Results and Figure 6).

Empirical Likelihood Ratio Statistics

The likelihood ratio statistic for testing directional selection, to be computed for each variant, is given by

$$H = -2 \log \left(\frac{\mathcal{L}(\bar{s}, 0.5 | \{\boldsymbol{x}\}_R, \widehat{N})}{\mathcal{L}(0, 0.5 | \{\boldsymbol{x}\}_R, \widehat{N})} \right), \tag{13}$$

where $\bar{s} = \arg\max_s \prod_{r=1}^{R} \mathcal{L}(s, 0.5 | \boldsymbol{x}^{(r)}, \widehat{N})$. Similarly we can define a test statistic for testing if selection is dominant by

$$D = -2 \log \left(\frac{\mathcal{L}(\widehat{s}, \widehat{h} | \{\boldsymbol{x}\}_R, \widehat{N})}{\mathcal{L}(\bar{s}, 0.5 | \{\boldsymbol{x}\}_R, \widehat{N})} \right). \tag{14}$$

While extending the single-locus WF model to a multiple linked-loci can improve the power of the model Terhorst *et al.* (2015), it is computationally and statistically expensive to compute exact likelihood. In addition, computing linked-loci joint likelihood requires haplotype resolved data, which pool-seq does not provide. Here, similar to Nielsen *et al* Nielsen *et al.* (2005), we calculate *composite likelihood ratio* score for a genomic region.

$$\mathcal{H} = \frac{1}{|L|} \sum_{\ell \in L} H_\ell. \tag{15}$$

where L is a collection of segregating sites and H_ℓ is the likelihood ratio score based for each variant ℓ in L. The optimal value of the hyper-parameter L depends upon a number of factors, including initial frequency of the favored allele, recombination rates, linkage of the favored allele to neighboring variants, population size, coverage, and time since the onset of selection (duration of the experiment). In **??**, we provide a heuristic to compute a reasonable value of L, based on experimental data.

We work with a normalized value of $\mathcal{H}$, given by

$$\mathcal{H}^*_i = \frac{\mathcal{H}_i - \mu_{\mathcal{C}}}{\sigma_{\mathcal{C}}}, \qquad \forall i \in \mathcal{C}, \tag{16}$$

where $\mu_{\mathcal{C}}$ and $\sigma_{\mathcal{C}}$ are the mean and standard deviation of $\mathcal{H}$ values in a large region $\mathcal{C}$. We found different chromosomes to have different distribution of $\mathcal{H}_i$ values, and therefore decided to use single chromosomes as $\mathcal{C}$.

Hypothesis Testing

Single-Locus tests. Under neutrality, Log-likelihood ratios can be approximated by $\mathcal{X}^2$ distribution Williams and Williams (2001), and p-values can be computed directly. However, Feder *et al.* Feder *et al.* (2014) showed that when the number of independent samples (replicates) is small, $\mathcal{X}^2$ is a crude approximation to the true null distribution and results in more false positive. Following their suggestion, we first compute the empirical null distribution using simulations with the estimated population size (See **??**). The empirical null distribution of statistic H is used to compute p-values as the fraction of null values that exceed the test score. Finally, we use Storey and Tibshirani's method Storey and Tibshirani (2003) to control for False Discovery Rate in multiple testing.

Composite likelihood tests. Similar to single-locus tests, we compute the null distribution of the $\mathcal{H}^*$ statistic using whole-genome simulations with the estimated population size, and subsequently compute FDR. The simulations for generating the null distribution of $\mathcal{H}^*$ are described next.

Simulations

We use the same simulation procedure for two purposes. First, we use them to test the power of CLEAR against other methods in small genomic windows. Second, we use the simulations to generate the distribution of null values for the statistic to compute empirical p-values. We mainly chose parameters that are relevant to *D. melanogaster* experimental evolution Kofler and Schlötterer (2013). See also Figure 1-A for illustration.

1. **Creating initial founder line haplotypes.** Using `msms` Ewing and Hermisson (2010), we created neutral populations for F founding haplotypes with command `$./msms <F> 1 -t <2μWN_o> -r <2rWN_o> <W>`, where $F = 200$ is number of founder lines, $N_o = 10^6$ is effective founder population size, $r = 2 \times 10^{-8}$ is recombination rate, $\mu = 2 \times 10^{-9}$ is mutation rate. The window size W is used to compute $\theta = 2\mu N_o W$ and $\rho = 2N_o r W$. We chose $W = 50$Kbp for simulating individual windows for performance evaluations, and $W = 20$Mbp for simulating *D. melanogaster* chromosomes for p-value computations.

2. **Creating initial diploid population.** An initial set of $F = 200$ haplotypes was created from step I, and duplicated to create F homozygous diploid individuals to simulate generation of inbred lines. N diploid individuals were generated by sampling with replacement from the F individuals.

3. **Forward Simulation.** We used forward simulations for evolving populations under selection. We also consider selection regimes which the favored allele is chosen from standing variation (not *de novo* mutations). Given initial diploid population, position of the site under selection, selection strength s, number of replicates $R = 3$, recombination rate $r = 2 \times 10^{-8}$ and sampling times $\mathcal{T} = \{0, 10, 20, 30, 40, 50\}$, `simuPop` Peng and Kimmel (2005) was used to perform forward simulation and compute allele frequencies for all of the R replicates. For hard sweep (respectively, soft sweep) simulations we randomly chose a site with initial frequency of $\nu_0 = 0.005$ (respectively, $\nu_0 = 0.1$) to be the favored allele. For generating the null distribution with drift for p-value computations, we used this procedure with $s = 0$.

4. **Sequencing Simulation.** Given allele frequency trajectories we sampled depth of each site in each replicate identically and independently from Poisson(λ), where $\lambda \in \{30, 100, 300\}$ is the coverage for the experiment. Once depth d is drawn for the site with frequency ν, the number of reads c carrying the derived allele are sampled according to Binomial(d, ν). For experiments with finite depth the tuple $\langle c, d \rangle$ is the input data for each site.

Results

Modeling Allele Frequency Trajectories in Small Populations. We first tested the goodness of fit of the discrete versus Brownian motion (a continuous-state model) in modeling allele frequency trajectories, under general E&R parameters. For this purpose, we conducted 100K simulations with two time samples $\mathcal{T} = \{0, \tau\}$ where $\tau \in \{1, 10, 100\}$ is the parameter controlling the density of sampling in time. In addition, we repeated simulations for different values of starting frequency $\nu_0 \in \{0.005, 0.1\}$ (i.e., hard and soft sweep) and selection strength $s \in \{0, 0.1\}$ (i.e., neutral and selection). Then, given initial frequency ν_0, we computed the expected distribution of the frequency of the next sample ν_τ under two models to make a comparison. Figure 2A-F shows that Brownian motion (continuous model) is inadequate when ν_0 is far from 0.5, or when sampling times are sparse ($\tau > 1$). If the favored allele arises from standing variation in a neutral population, it is unlikely to have frequency close to 0.5, and the starting frequencies are usually much smaller (see **??**). Moreover, in typical *D. melanogaster* experiments for example, sampling is sparse. Often, the experiment is designed so that $10 \leq \tau \leq 100$ Kofler and Schlötterer (2013); Orozco-ter Wengel *et al.* (2012); Zhou *et al.* (2011); Franssen *et al.* (2015).

In contrast to the Brownian motion approximation, discrete Markov chain predictions (Eq. 11) are highly consistent with empirical data for a wide range of simulation parameters (Figure 2A-M). Moreover, the discrete markov chain can be modified to model the case when the the allele is under selection.

Detection Power. We compared the performance of CLEAR against other methods for detecting selection. For each method we calculated detection power as the percentage of true-positives identified with false-positive rate ≤ 0.05. For each configuration (specified with values for selection coefficient s, starting allele frequency ν_0 and coverage λ), power of each method is evaluated over 2000 distinct simulations, half of which modeled neutral evolution and the rest modeled positive selection.

We compared the power of CLEAR with Gaussian process (GP) Terhorst *et al.* (2015), FIT Feder *et al.* (2014), and CMH Agresti and Kateri (2011) statistics. FIT and GP convert read counts to allele frequencies prior to computing the test statistic. CLEAR shows the highest power in all cases and the power stays relatively high even for low coverage (Figure 3 and **??**). In particular, the difference in performance of CLEAR with other methods is pronounced when starting frequency is low. The advantage of CLEAR stems from the fact that favored allele with low starting frequency might be missed by low coverage sequencing. In this case, incorporating the signal from linked sites becomes increasingly important. We note that methods using only two time points, such as CMH, do relatively well for high selection values and high coverage. However, the use of time-series data can increase detection power in low coverage experiments or when starting frequency is low. Moreover, time-series data provide means for estimating selection parameters s, h (see below). Finally, as CLEAR is robust to change of coverage, our results (Figure 3B,C) suggest that taking many samples with lower coverage is preferable to sparse sampling with higher coverage. For comparison purposes, we also tested CLEAR using the single locus statistic ($L = 1$). For the most part, CLEAR showed an improvement over other methods even with $L = 1$, or showed similar performance. The performance improved with higher L.

Site-identification. In general, localizing the favored variant, using pool-seq data is a nontrivial task due to extensive linkage disequilibrium Tobler *et al.* (2014). To measure performance, we sorted variants by their H scores and computed rank of the favored allele for each method. For each setting of ν_0 and s, we conducted 1000 simulations and computed the rank of the favored mutation in each simulation. The cumulative distribution of the rank of the favored allele in 1000 simulation for each setting (Figure 4) shows that CLEAR outperforms other statistics.

An interesting observation is revisiting the contrast between site-identification and detection Long *et al.* (2013); Tobler *et al.* (2014). When selection strength is high, detection is easier (Figure 3A-F), but site-identification is harder, due to the high LD between flanking variants and the favored allele (Figure 4A-F). Moreover, site-identification becomes more difficult whenever the initial frequency of the favored allele is low, i.e., at the onset of selection, LD between favored allele and its nearby variants is high. For example, when coverage $\lambda = 100$ and selection coefficient $s = 0.1$, the detection power is 75% for hard sweep, but 100% for soft sweep (Figure 3B-E). In contrast, the favored site was ranked as the top in 14% of hard sweep cases, compared to and 95% of soft sweep simulations.

Estimating Parameters. CLEAR estimates effective population size $\widehat{N}$ and selection parameters, $\hat{s}$ and $\hat{h}$, as a byproduct of the hypothesis testing. We computed bias of selection fitness $(s - \hat{s})$ and dominance $(h - \hat{h})$ for of CLEAR and GP for 1000 simulations in each setting. The distribution of the error (bias) for $100\times$ coverage is presented in Figure 5 for different configurations. **??** and **??** provide the distribution of estimation errors for $30\times$, and $300\times$ coverage, respectively. For hard sweep, CLEAR provides estimates of s with lower variance of bias (Figure 5A and **??**). In soft sweep, GP and CLEAR both provide unbiased estimates of s with low variance (Figure 5B). Figure 5 C-D shows that CLEAR provides unbiased estimates of h as well when $h \in \{0, 0.5, 1, 2\}$ and $s = 0.1$. We also tested if CLEAR provide unbiased estimates of N, by estimating population size on 1000 simulations when $N \in \{200, 600, 1000\}$. As shown in Figure 7-A and **??**A-C, maximum likelihood is attained at true value of the parameter.

Running Time. As CLEAR does not compute exact likelihood of a region (i.e., does not explicitly model linkage between sites), the complexity of scanning a genome is linear in number of polymorphisms. Calculating score of each variant requires and $\mathcal{O}(TRN^3)$ computation for $\mathcal{H}$. However, most of the operations are can be vectorized for all replicates to make the effective running time for each variant. We conducted 1000 simulations and measured running times for computing site statistics H, FIT, CMH and GP with different number of linked-loci. Our analysis reveals (Figure 6) that CLEAR is orders of magnitude faster than GP, and comparable to FIT. While slower than CMH on the time per variant, the actual running times are comparable after vectorization and broadcasting over variants (see below).

These times can have a practical consequence. For instance, to run GP in the single locus mode on the entire pool-seq data

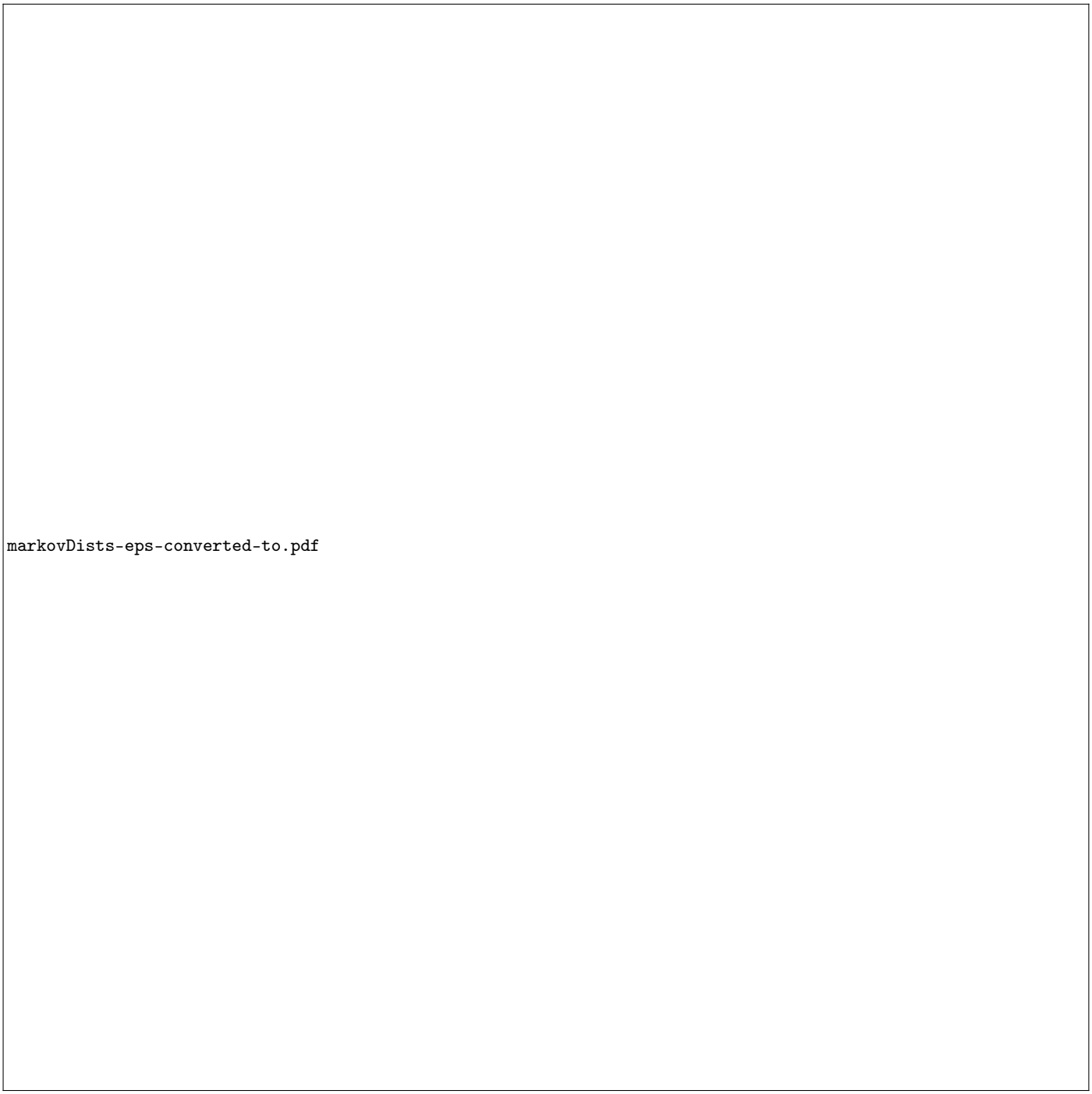


Figure 2 Comparison of empirical distributions of allele frequencies (red) versus predictions from Brownian Motion (green), and Markov chain (blue).
Comparison of empirical and theoretical distributions under neutral evolution (panels A-F) and selection (panels G-M) with different starting frequencies $\nu_0 \in \{0.005, 0.1\}$ and sampling times of $\mathcal{T} = \{0, \tau\}$, where $\tau \in \{1, 10, 100\}$ and $N = 1000$. For each panel, the empirical distribution was computed over 100,000 simulations. Brownian motion (Gaussian approximation) provides poor approximations when initial frequency is far from 0.5 (A) or sampling is sparse (B,C,E,F). In addition, Brownian motion can only provide approximations under neutral evolution. In contrast, Markov chain consistently provides a good approximation in all cases.

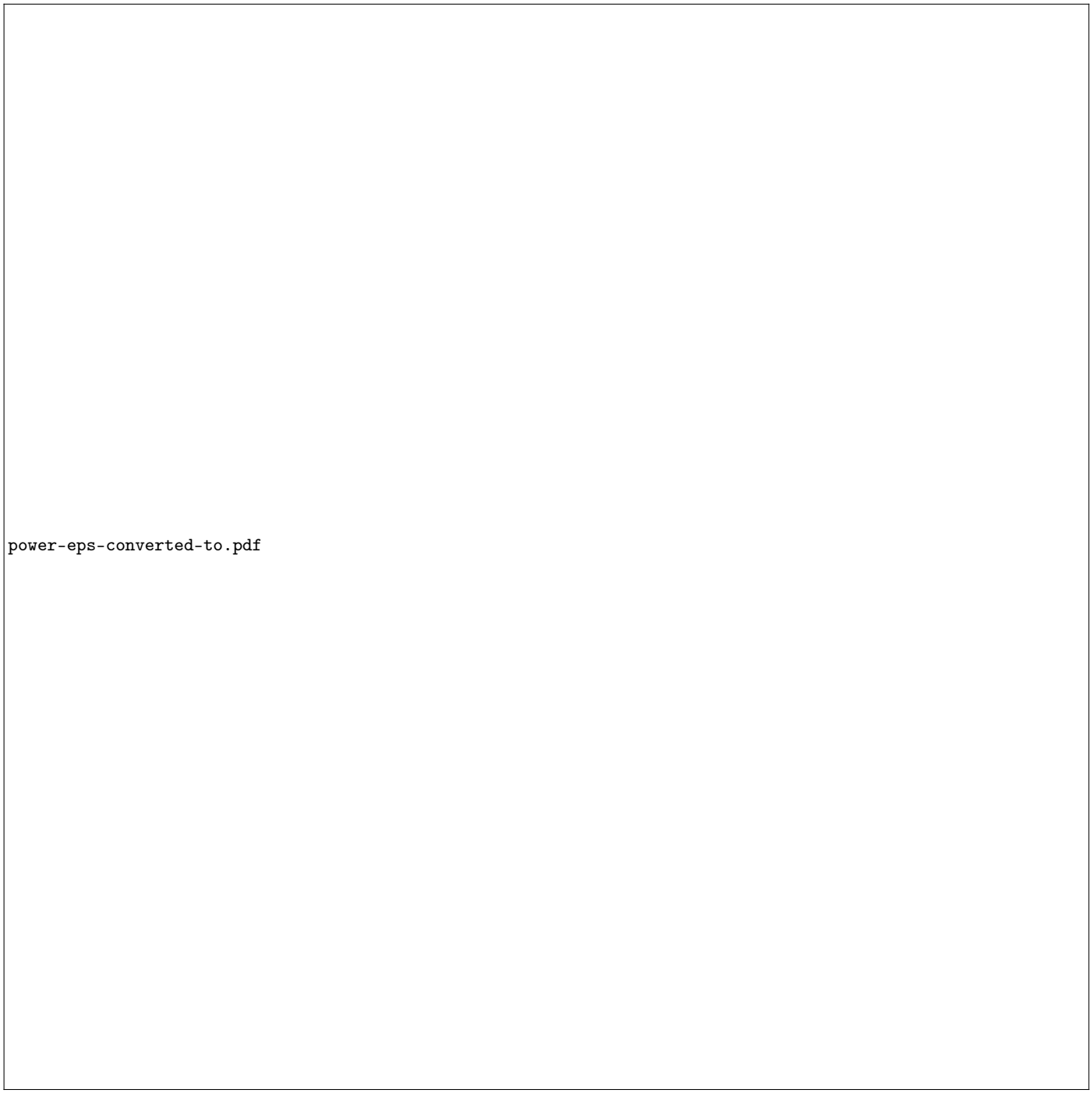
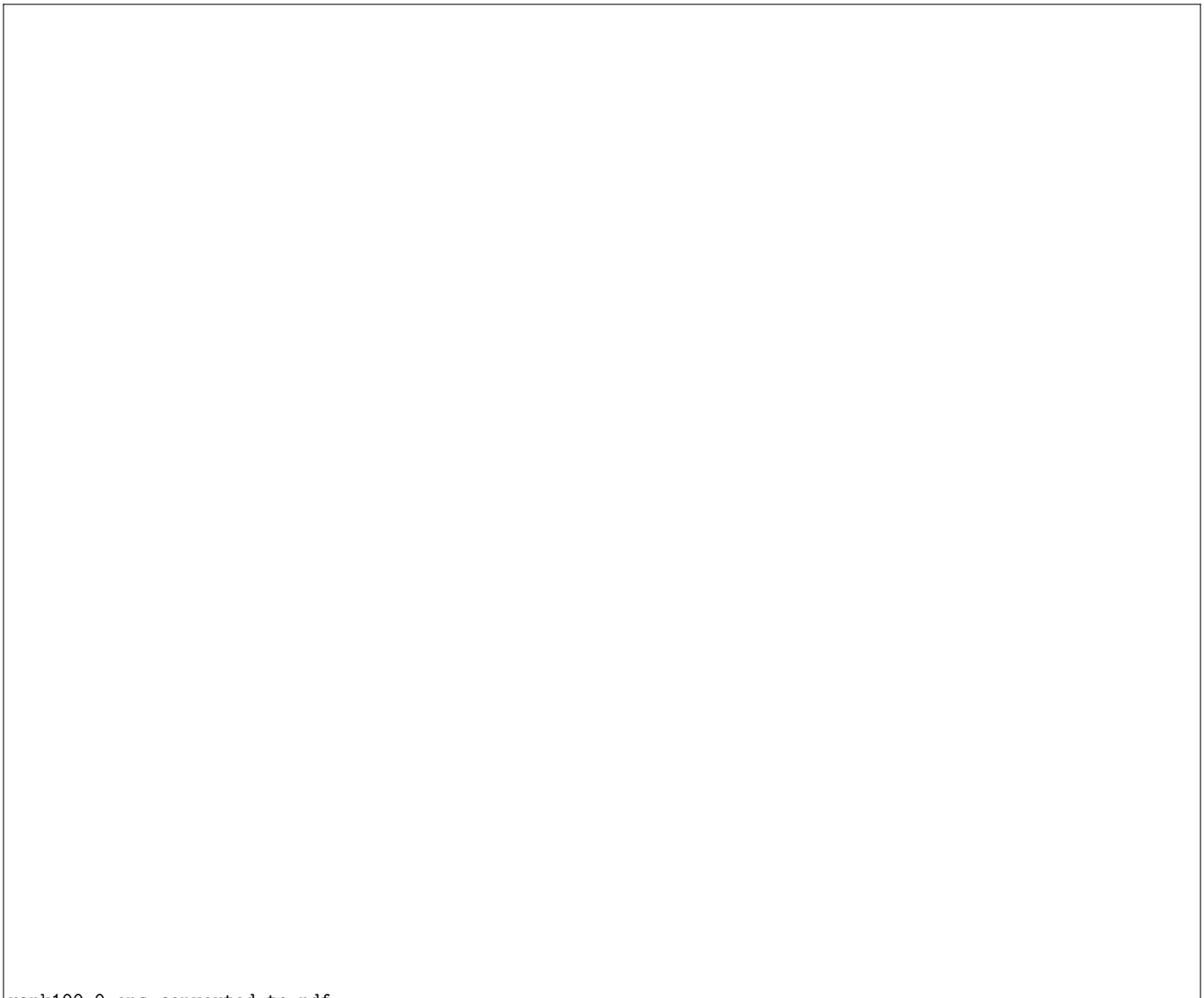


Figure 3 Power calculations for detection of selection.
Detection power for CLEAR($\mathcal{H}$), Frequency Increment Test (FIT), Gaussian Process (GP), and CMH under hard (A-C) and soft sweep (D-F) scenarios. λ, s denote the mean coverage and selection coefficient, respectively. Orange hexagons represent the performance of CLEAR when the maximum of the single-locus statistic is used to make a decision for the genomic region, while the red circle corresponds to the performance of CLEAR when single locus statistics are averaged over the region. The y-axis measures power – sensitivity with false positive rate FPR $\leq$ 0.05 – for 2,000 simulations with $N = 1{,}000$, $L = 50$Kbp. The horizontal line reflects the power of a random classifier. In all simulations, 3 replicates are evolved and sampled at generations $\mathcal{T} = \{0, 10, 20, 30, 40, 50\}$.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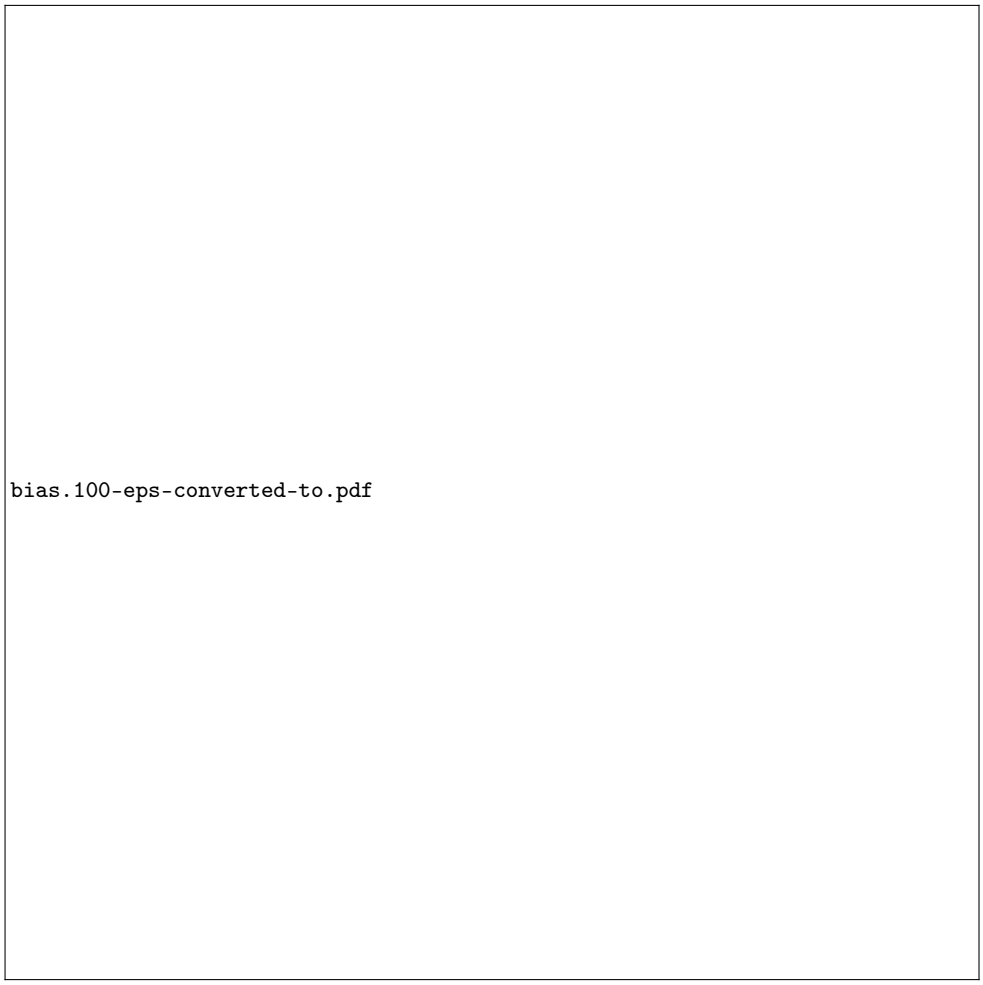


Figure 5 Distribution of bias for 100× coverage.
The distribution of bias ($s - \hat{s}$) in estimating selection coefficient over 1000 simulations using Gaussian Process (GP) and CLEAR (H) is shown for a range of choices for the selection coefficient s and starting carrier frequency ν_0, when coverage $\lambda = 100$ (Panels A,B). GP and CLEAR have similar variance in estimates of s for soft sweep, while CLEAR provides lower variance in hard sweep. Also see **??**. Panels C,D show the variance in the estimation of h. In all simulations, 3 replicates are evolved and sampled at generations $\mathcal{T} = \{0, 10, 20, 30, 40, 50\}$.

of the *D. melanogaster* genome from a small sample (≈1.6M variant sites), it would take 1444 CPU-hours (≈ 1 CPU-month). In contrast, after vectorizing and broadcasting operations for all variants operations using `numba` package, CLEAR took 75 minutes to perform an scan, including precomputation, while the fastest method, CMH, took 17 minutes.

Analysis of a D. melanogaster *Adaptation to Alternating Temperatures*

We applied CLEAR to the data from a study of *D. melanogaster* adaptation to alternating temperatures Orozco-ter Wengel *et al.* (2012); Franssen *et al.* (2015), where 3 replicate samples were chosen from a population of *D. melanogaster* for 59 generations under alternating 12-hour cycles of hot stressful (28°C) and non-stressful (18°C) temperatures and sequenced. In this dataset, sequencing coverage is different across replicates and generations (see S2 Fig of Terhorst *et al.* (2015)) which makes variant depths highly heterogeneous (**??**).

We first filtered out heterochromatic, centromeric and telomeric regions Fiston-Lavier *et al.* (2010), and those variants that have collective coverage of more that 1500 in all 13 populations: three replicates at the base population, two replicates at generation 15, one replicate at generation 23, one replicate at generation 27, three replicates at generation 37 and three replicates at generation 59. After filtering, we ended up with 1,605,714 variants.

Next, we estimated genome-wide population size $\widehat{N} = 250$ (Figure 7-B and **??**-E) which is consistent with previous studies Orozco-ter Wengel *et al.* (2012); Jónás *et al.* (2016). The likelihood curves of CLEAR are sharper around the optimum compared to that of Bollback et. al Bollback *et al.* (2008)'s method (see Supplementary Fig. 1 in Orozco-ter Wengel *et al.* (2012)). Also, chromosomes 3L and 3R appear to have smaller population size, $\widehat{N} = 200, 150$, respectively. Others have made similar observations on this data. In particular, Jónás *et al.* Jónás *et al.* (2016) shown that the chromosome-wise population size varies even more when it is computed for each replicate separately (see Table 1 in Jónás *et al.* (2016)). For instance, $\widehat{N}$ is 131 for chromosome 3R replicate 1, while it is 328 for chromosome X replicate 2.

While it would be ideal to compute CLEAR statistic for each replicate and chromosome separately, computing empirical p-values and significant regions become computationally intensive as empirical null distribution of each replicate and each chromosome needs to be computed. Hence, we use a single genome-wide estimate $\widehat{N} = 250$ in all analyses, but we normalize statistic $\mathcal{H}^*$ separately for each chromosome.

We use a heuristic calculation (See **??**) to choose the sliding window size L as the distance where the LD between the favored mutation and a site $L/2$bp away remains strong. For *D. melanogaster* parameters, we obtained $L = 30$Kbp. We computed the normalized test statistic $\mathcal{H}^*$ on sliding windows of size of 30Kbp and step size of 5Kbp over the genome (See Figure 8-A).

Empirical null distribution of $\mathcal{H}^*$ was estimated by creating 100 whole genome simulations (400K statistic values) as described in Section . Then, p-value of the test statistic in each region in the experimental data was calculated as the fraction of the null statistic values that are greater than or equal to the test statistic(see **??**). After correcting for multiple testing, we identified 5 contiguous intervals (Figure 8) satisfying FDR$\leq$ 0.05, and covering 2,829 polymorphic sites. We further performed single-locus hypothesis testing on the 2,829 sites to identify 174 individual variants with FDR $\leq$ 0.01 (Figure 8-B).

The final set of 174 variants fall within 32 genes(**??**) including many Serine inhibitory proteases (serpins), and other genes involved in endocytosis. Recycling of synaptic vesicles is seen to be blocked at high temperature in temperature sensitive Drosophila mutants Kosaka and Ikeda (1983). This is also supported by GO enrichment analysis, where a single GO term 'inhibition of proteolysis' is found to enriched (corrected p-value:0.0041). To test for dominant selection, we computed D statistic on simulated neutral and experimental data, and computed p-values accordingly. After correcting for multiple testing, 96 variants were discovered with FDR$\leq$ 0.01 (**??**).

Analysis of Outcrossing Yeast Populations

We also applied CLEAR to 12 replicate samples of outcrossing yeast populations Burke *et al.* (2014), where samples are taken at generations $\mathcal{T} = \{0, 180, 360, 540\}$. We observed a significant variation in the genome-wide site frequency spectrum of certain populations over different time points for some replicates (**??**). The variation does not have an easily identifiable cause. Therefore, we focused analysis on seven replicates $r \in \{3, 7, 8, 9, 10, 11, 12\}$ with genome-wide site-frequency spectrum over the time range (**??**).

We estimated population size to be $\widehat{N} = 2000$ haplotypes (Figure 7-C and **??**-F), and computed $\widehat{s}$, $\widehat{h}$ and H statistic accordingly. To compute p-values, we created 1M single-locus neutral simulations according to experimental data's initial frequency and coverage. By setting FDR cutoff to 0.05, only 18 and 16 variants show significant signal for directional and dominant selection, respectively (**??**). Selected variants for directional selection are clustered in two regions, which match 2 of the 5 regions (regions C and E in Fig. 2-a in Burke *et al.* (2014)) identified by Burke *et al.* in their preliminary analysis.

Discussion

We developed a computational tool, CLEAR, that can detect regions and variants under selection E&R experiments. Using extensive simulations, we show that CLEAR outperforms existing methods in detecting selection, locating the favored allele, and estimating model parameters. Also, while being computationally efficient, CLEAR provide means for estimating populations size and hypothesis testing.

Many factors such as small population size, finite coverage, linkage disequilibrium, finite sampling for sequencing, duration of the experiment and the small number of replicates can limit the power of tools for analyzing E&R. Here, by an discrete modeling, CLEAR estimates population size, and provides unbiased estimates of s, h. It adjusts for heterogeneous coverage of pool-seq data, and exploits presence of linkage within a region to compute composite likelihood ratio statistic.

It should be noted that, even though we described CLEAR for small fixed-size populations, the statistic can be adjusted for other scenarios, including changing population sizes when the demography is known. For large populations, transitions can be computed on sparse data structures, as for large N the transition matrices become increasingly sparse. Alternatively, frequencies can be binned to reduce dimensionality.

The comparison of hard and soft sweep scenarios showed that initial frequency of the favored allele can have an nontrivial effect on the statistical power for identifying selection. Interestingly, while it is easier to detect a region undergoing strong selection, it is harder to locate the favored allele in that region.

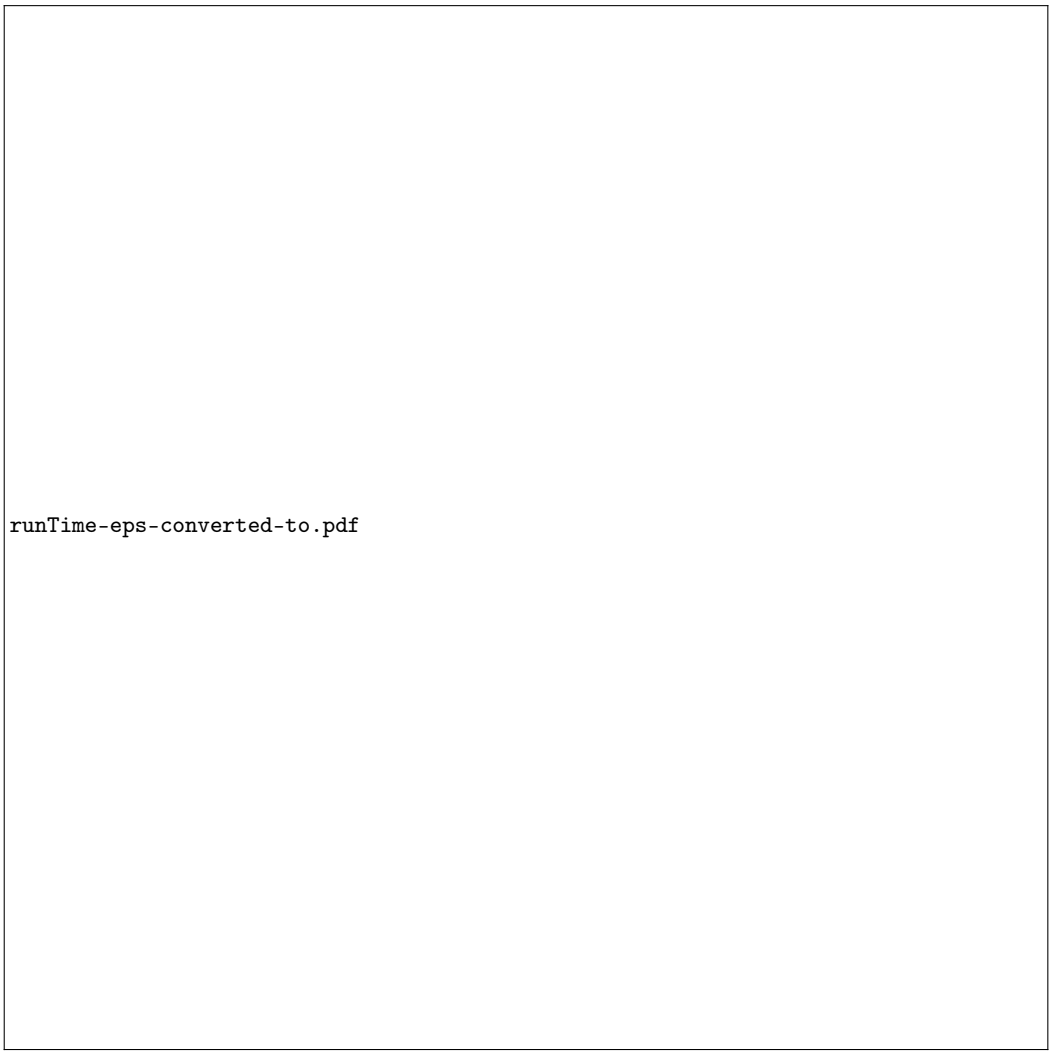


Figure 6 Running time.
Box plots of running time per variant (CPU-secs.) of CLEAR($\mathcal{H}$), CMH, FIT, and GP with single, 3, 5, 7, and 10 loci over 1000 simulations conducted on a workstation with Intel Core i7 processor. The average running time for each method is shown on the x-axis. In all simulations, 3 replicates are evolved and sampled at generations $\mathcal{T} = \{0, 10, 20, 30, 40, 50\}$.

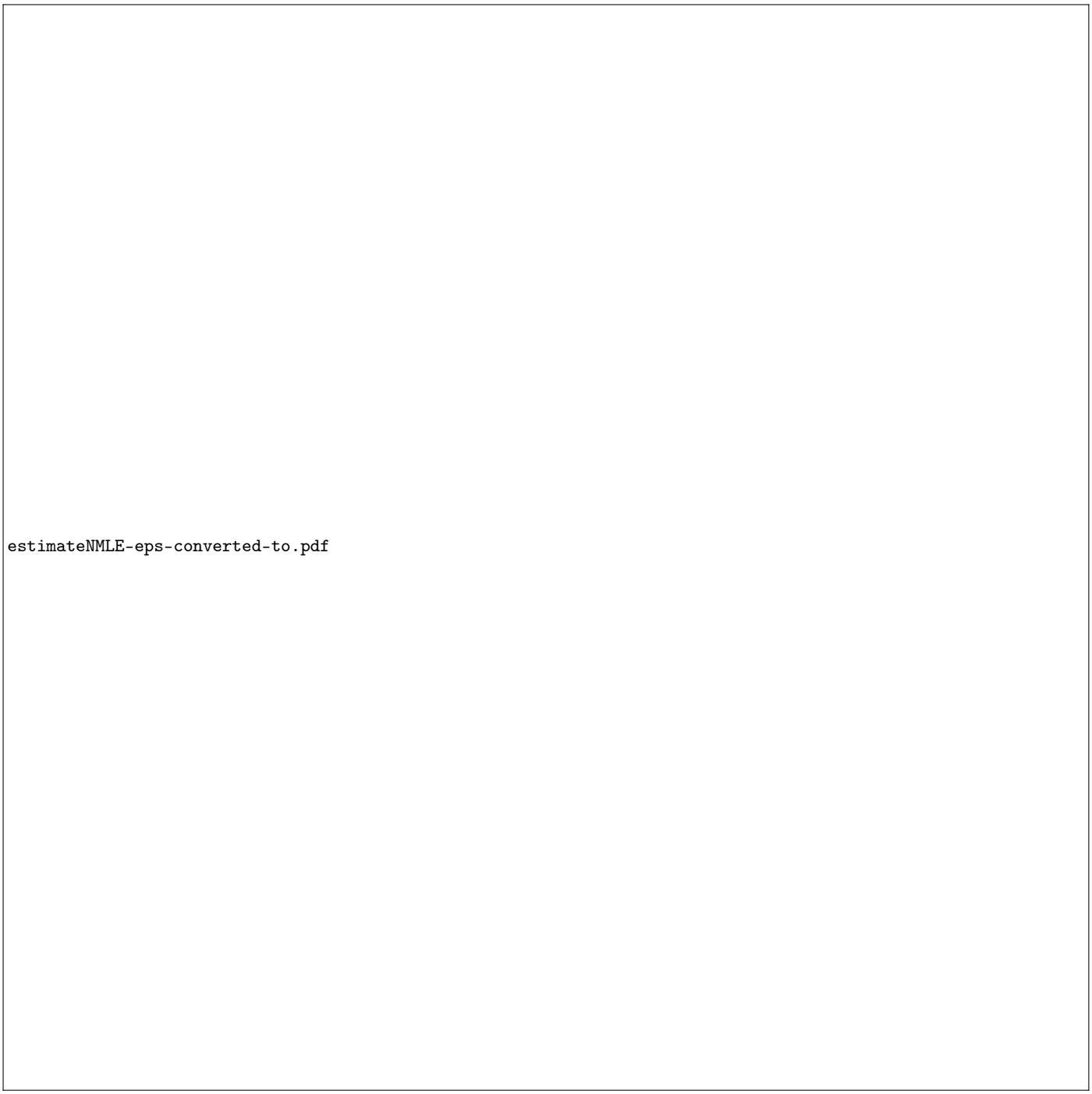
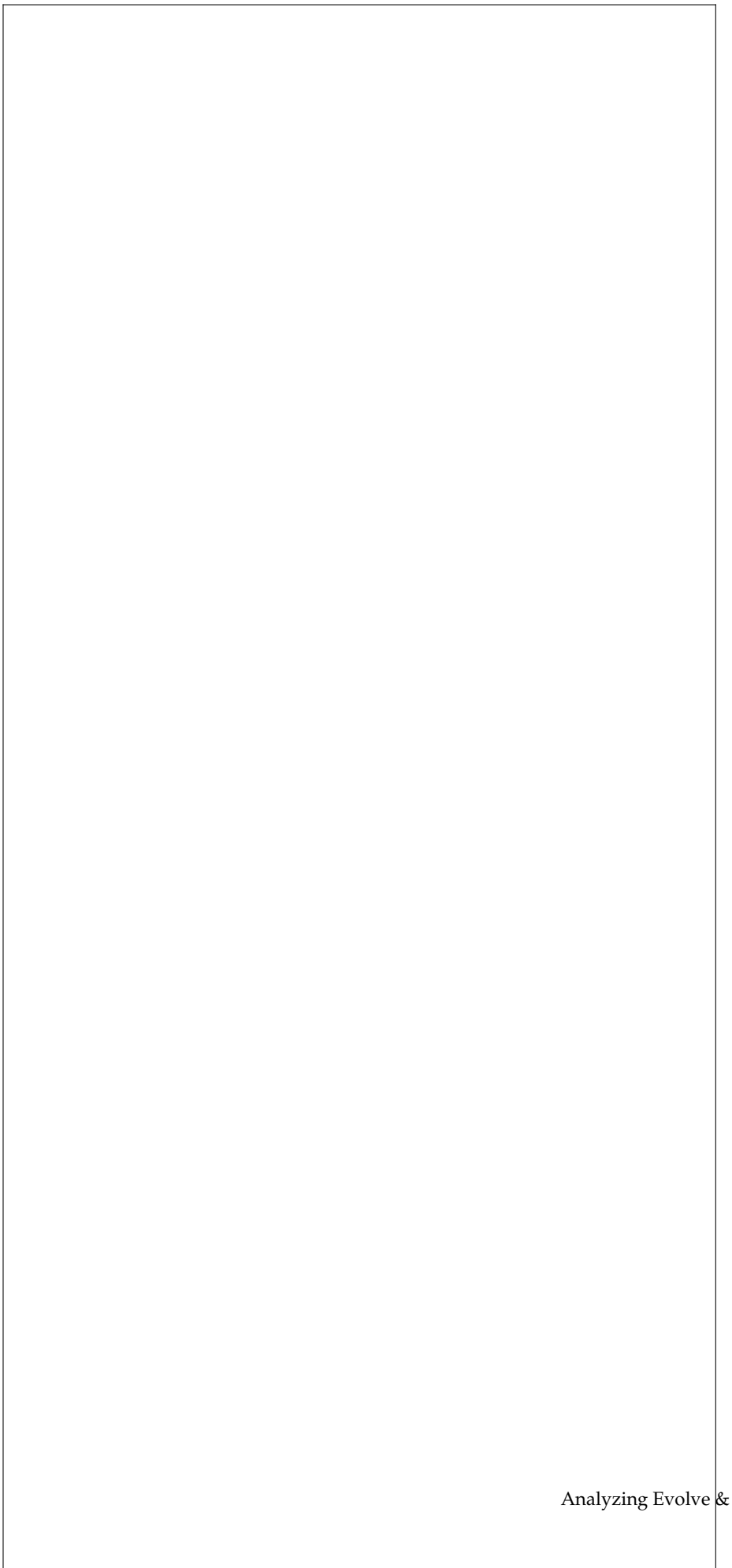


Figure 7 Estimating population size. (A) Distribution of bias in estimating N, computed on 1000 neutral simulations for each $N \in \{200, 600, 1000\}$ when $W = 10$Mbp and $r = 2 \times 10^{-8}$. (B) Estimates of population size for data from a study of *D. melanogaster* adaptation to alternating temperatures. For each case, the distribution of estimator is computed by 100 bootstrap computations using 1000 variants each. The multiple modes are an artifact of grid search used to speed up computation. (C) Distribution of the population size estimates on the yeast dataset. Despite large census population size ($10^6 - 10^7$ Burke *et al.* (2014)), this dataset exhibits much smaller effective population size ($\widehat{N} = 2000$).

(A)



There are many directions to improve the analyses presented here. In particular, we plan to focus our attention on other organisms with more complex life cycles, experiments with variable population size and longer sampling-time-spans. As evolve and resequencing experiments continue to grow, deeper insights into adaptation will go hand in hand with improved computational analysis.

Software and Data Availability. The source code and running scripts for CLEAR are publicly available at https://github.com/airanmehr/clear.

D. melanogaster data originally published Orozco-ter Wengel *et al.* (2012); Franssen *et al.* (2015). The dataset of the *D. melanogaster* study, until generation 37, is obtained from Dryad digital repository (http://datadryad.org) under accession DOI: 10.5061/dryad.60k68. Generation 59 of the *D. melanogaster* study is accessed from European Sequence Read Archive (http://www.ebi.ac.uk/ena/) under the project accession number: PRJEB6340. The dataset containing experimental evolution of Yeast populations Burke *et al.* (2014) is downloaded from http://wfitch.bio.uci.edu/~tdlong/PapersRawData/BurkeYeast.gz (last accessed 01/24/2017). UCSC browser tracks for *D. melanogaster* and Yeast data analysis are found in Suppl. Data 1 and 2, respectively.

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Conflict of interest

VB is a co-founder, has an equity interest, and receives income from Digital Proteomics, LLC (DP). The terms of this arrangement have been reviewed and approved by the University of California, San Diego in accordance with its conflict of interest policies. DP was not involved in the research presented here.

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