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## 1 Introduction

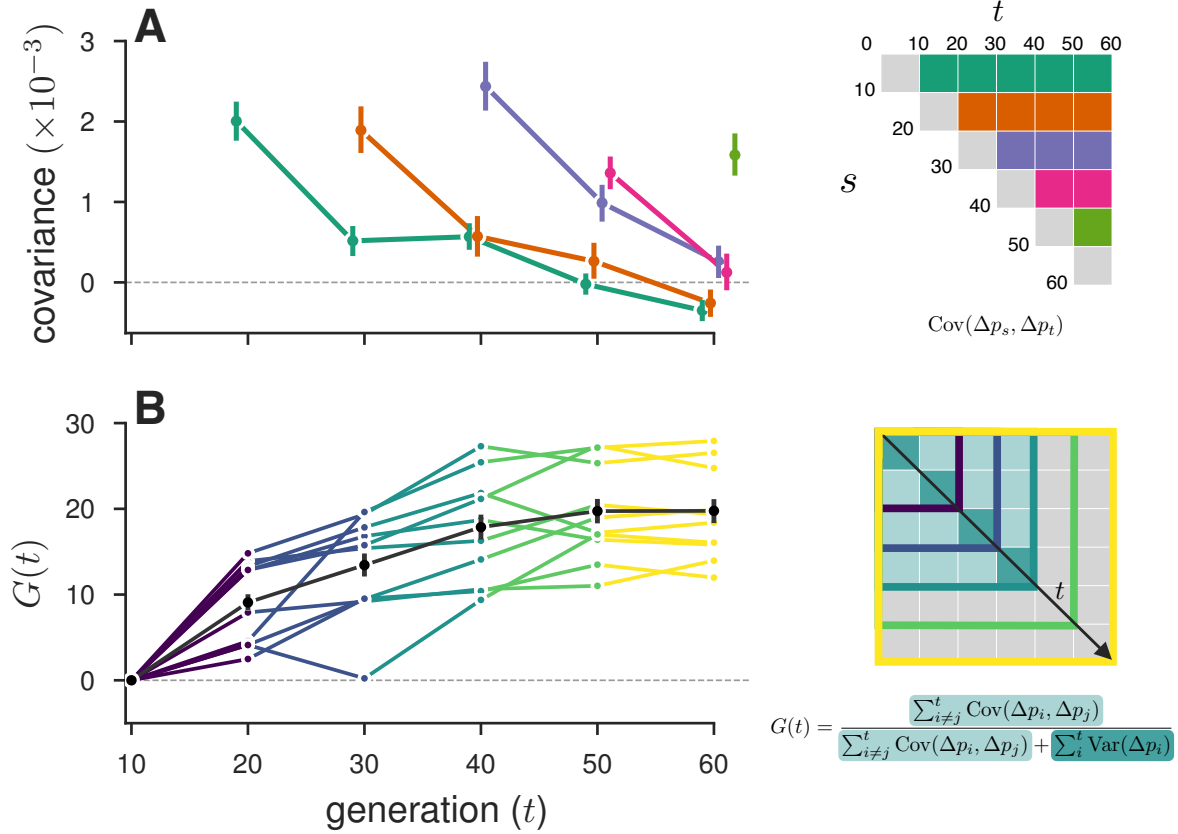
## 2 Results

Study	Species	Selection	Replicates	Pop. Size	Gens.	Timepoints
Kelly and Hughes (2019)	<i>D. simulans</i>	lab	3	~1100	14	2
Barghi et al. (2019)	<i>D. simulans</i>	lab	10	~1000	60	10
Castro et al. (2019)	<i>M. musculus</i>	tibiae length	2	32	20	2
		control	1	28		

We first analyzed Barghi et al. (2019), an evolve-and-resequence study with ten replicate populations exposed to a high temperature lab environment and evolved for 60 generations, and sequenced every ten generations. Using the seven timepoints and ten replicate populations, we estimated a bias-corrected genome-wide  $6 \times 6$  temporal covariance matrix (a timepoint is lost in calculating all adjacent allele frequency changes). This temporal covariance matrix contains

$60 \times 60$  temporal-replicate variance-covariance matrix (see XXX for details).

Since each replicate population was sequenced every ten generations, the timepoints  $t_0 = 0$  generations,  $t_1 = 10$  generations,  $t_2 = 20$  generations, etc., lead to observed allele frequency changes across ten generation blocks,  $\Delta p_{t_0}, \Delta p_{t_1}, \dots, \Delta p_{t_6}$ . Consequently, the ten temporal covariance matrices for each of the ten replicate populations have off-diagonal elements of the form  $\text{Cov}(\Delta p_{t_0}, \Delta p_{t_1}) = \text{Cov}(p_{t_1} - p_{t_0}, p_{t_2} - p_{t_1}) = \sum_{i=0}^{10} \sum_{j=10}^{20} \text{Cov}(\Delta p_i, \Delta p_j)$ . Each diagonal element has the form  $\text{Var}(\Delta p_{t_0}) = \sum_{i=0}^{t_0} \text{Var}(\Delta p_i) + \sum_{i \neq j}^{t_0} \text{Cov}(\Delta p_i, \Delta p_j)$ , and is thus a combination of the effects of drift and selection, as both the variance in allele frequency changes and cumulative temporal autocovariances terms increase the variance in allele frequency. With sampling each generation, one could more accurately partition the total variance in allele frequency change (Buffalo and Coop 2019); while we cannot directly estimate the contribution of linked selection to the variance in allele frequency change here, the presence of a positive observed covariance between allele frequency change can only be caused linked selection. Overall, we expect observed temporal covariances between timepoints to be weak, some fraction of the additive genetic variance and linkage disequilibrium that creates temporal autocovariance decays in the ten generations between sequencing.



**Figure 1:** A: Temporal covariance, averaged across all ten replicate populations, through time from the Barghi et al. (2019) study. Each line depicts the temporal covariance  $\text{Cov}(\Delta p_s, \Delta p_t)$  from some reference generation  $s$  to a later time  $t$  which varies along the x-axis; each line corresponds to a row of the upper-triangle of the temporal covariance matrix with the same color (upper right). The ranges around each point are 95% block-bootstrap confidence intervals. B: The proportion of the total variance in allele frequency change explained by linked selection,  $G(t)$ , as it varies through time  $t$  along the x-axis. The black line is the  $G(t)$  averaged across replicates, with the 95% block-bootstrap confidence interval. The other lines are the  $G(t)$  for each individual replicate, with colors indicating what subset of the temporal-covariance matrix to the right is being included in the calculation of  $G(t)$ .

Averaging across replicate populations, we find positive temporal covariances across time that are statistically significant ( $p < XXX$ ) consistent with linked selection acting to affect allele frequency changes over very short time periods. We visualize these covariances in Figure (A), which depicts the temporal covariances through time, for each of the five rows covariance matrix. Each row represents the temporal covariance  $\text{Cov}(\Delta p_s, \Delta p_t)$ , between some initial reference generation  $s$  (the row of the matrix), and some later timepoint  $t$  (the column of the matrix). For each row, the covariances at first are positive, and then decay towards zero as expected when directional selection affects linked variants' frequency trajectories until ultimately linkage disequilibrium and additive genetic variance for decay (Buffalo and Coop 2019). Note that per replicate, the signal is a bit noisier; see Supplementary Figures XXX.

While the presence of positive temporal covariances is consistent with linked selection affecting

allele frequencies over time, this measure not easily interpretable. Additionally, we can quantify the impact of linked selection on allele frequency change as the ratio of total covariance in allele frequency change to the total variance in allele frequency change. Since the total variation in allele frequency change can be decomposed into variance and covariance components,  $\text{Var}(p_t - p_0) = \sum_{i \neq j} \text{Cov}(\Delta p_i, \Delta p_j) / \text{Var}(p_t - p_0)$ , and the covariances are zero when drift acts alone, this is a lower bound on how much of the variance in allele frequency change is caused by linked selection (Buffalo and Coop 2019). We call this measure  $G(t)$ , which is the total effect of linked selection between the initial generation 0 and some later generation  $t$ , which can be varied to see how this quantity grows through time. As with the temporal covariances, the study design of **Barghi2019** leads our measure  $G(t)$  to be even more conservative, since the temporal covariances with each ten-generation block between sequenced timepoints are not directly observable, and are not included in the numerator of  $G(t)$ . Still, we find a remarkably strong signal that greater than 20% of total variation in allele frequency change over 60 generations is directly the result of linked selection.

The replicate design of Barghi et al. (2019) also allows us to quantify another covariance: the covariance in allele frequency change between replicate populations experiencing convergent selection pressure. These between-replicate covariances are created in the same way as temporal covariances are: neutral alleles linked to a particular fitness background experience are expected to have allele frequency changes in the same direction if the selection pressures are similar. We measure this through a statistic similar to a correlation, which we call the convergent correlation; this is the ratio of average between-replicate covariance across all pairs to the average standard deviation across all pairs of replicates,

$$\text{cor}(\Delta p_s, \Delta p_t) = \frac{\mathbb{E}_{A \neq B} (\text{Cov}(\Delta p_{s,A}, \Delta p_{t,B}))}{\mathbb{E}_{A \neq B} (\sqrt{\text{Var}(\Delta p_{s,A}) \text{Var}(\Delta p_{t,B})})} \quad (1)$$

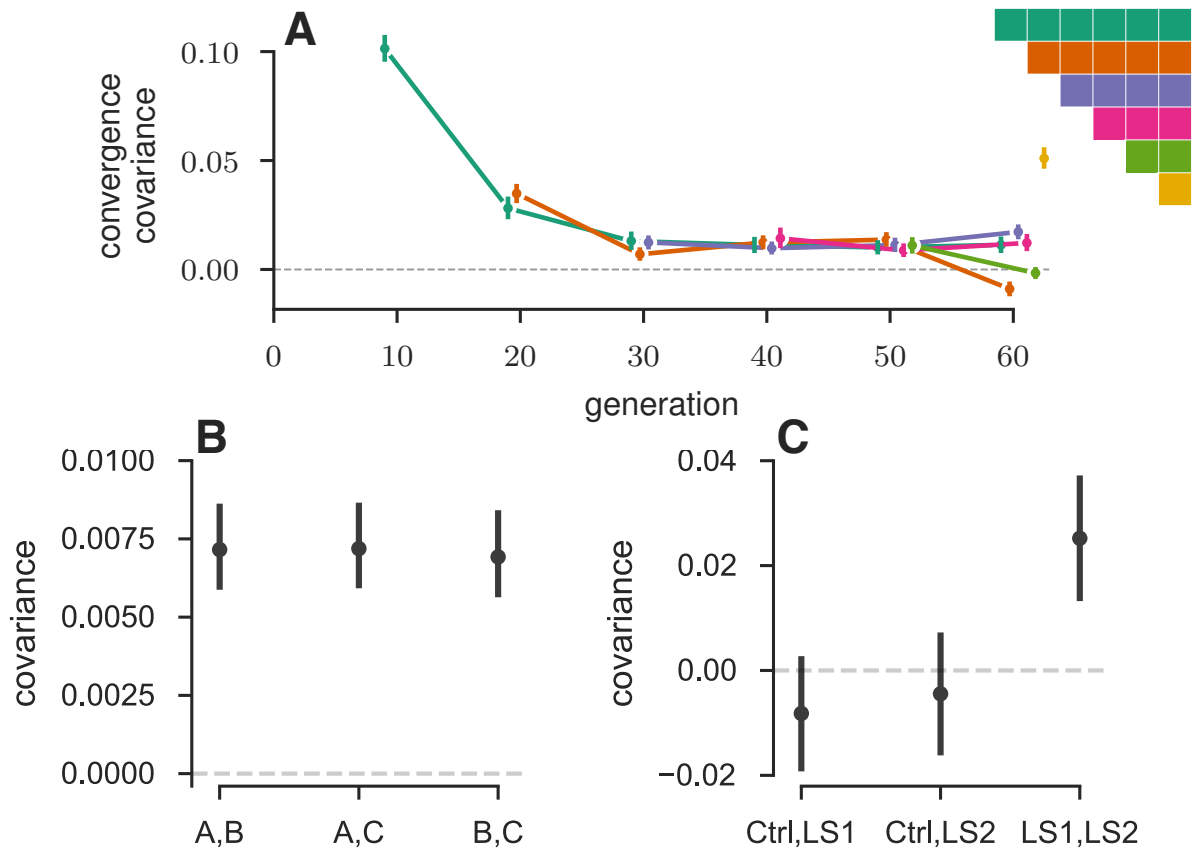
where  $A$  and  $B$  here are two replicate labels.

We’ve calculated the convergent correlation for all rows of the replicate covariance matrices, which unlike temporal covariance matrices have diagonal elements that are also covariances (e.g.  $\text{Cov}(\Delta p_{t,A}, \Delta p_{t,B})$ ). Like temporal covariances, we visualize these through time (Figure 2 A), with each line representing the convergent correlation from a particular reference generation  $s$  as it varies with  $t$  (shown on the x-axis). In other words, each of the colored lines corresponds to the like colored row of the convergence correlation matrix (upper left in Figure 2 A). We find these decay very quickly, from an initial convergence correlation coefficient of about 0.1 (block bootstrap confidence intervals XXX; see the note in Supplementary Material XXX why these are so narrow), to around 0.01 within 20 generations.

A benefit of between-replicate covariances is unlike temporal covariances, these can be calculated with only two sequenced timepoints and a replicated study design. This allowed us to assess the impact of linked selection in driving convergent patterns of allele frequency change across replicate populations in two other studies. First, we reanalyzed the selection experiment of Kelly and Hughes (2019), which evolved three replicate populations of *Drosophila simulans* for 14 generations in a novel laboratory environment. Since each replicate was exposed to the same selection pressure and share linkage disequilibria common to the original natural founding population, we expected each of the three replicate populations to have positive between-replicate covariances. We find all three pairwise between-replicate covariances are positive and statistically significant ( $p < \text{XXX}$ , estimated via block bootstrap). We estimate the convergent correlation coefficient across these replicates as 0.36 (95% block-bootstrap confidence interval [0.31, 0.40]).

Second, we reanalyzed the Longshanks selection experiment, which selected for longer tibiae length relative to body size in mice, leading to a response of selection of about 5 standard deviations over the course of twenty generations (Castro et al. 2019). This study includes two independent selection lines, Longshanks 1 and 2 (LS1 and LS2), and an unselected control line (Ctrl). Consequently, this selection experiment offers a useful control to test our between-replicate covariances: we expect to see positive between-replicate covariance in the comparison between the two Longshanks selection lines, but not between the two pairwise comparisons between the control line and the two Longshanks lines. We find that this is the case (Figure 2 C), with the two Longshanks comparisons to the control line not being significantly different from zero, while the comparison between the two Longshanks line is statistically significantly different from zero (CIs XXX).

Paragraph about large effect loci.



**Figure 2:** A: The convergence correlation, averaged across replicate pairs, through time. Each line represents the convergence correlation  $\text{cor}(\Delta p_s, \Delta p_t)$  from a starting reference generation  $s$  to a later time  $t$ , which varies along the x-axis; each line corresponds to a row of the temporal convergence correlation matrix depicted to the right.

Finally, we observed that in the longest run evolve-and-resequence study we analyzed (Barghi et al. 2019), some temporal covariances appeared to become negative at future timepoints (see the first two rows in Figure 1 A). This could be caused by advantageous fitness backgrounds later becoming disadvantageous, either due to frequency-dependent selection, recombination breaking up coupled beneficial and deleterious alleles, or an environmental change in the direction of selection.

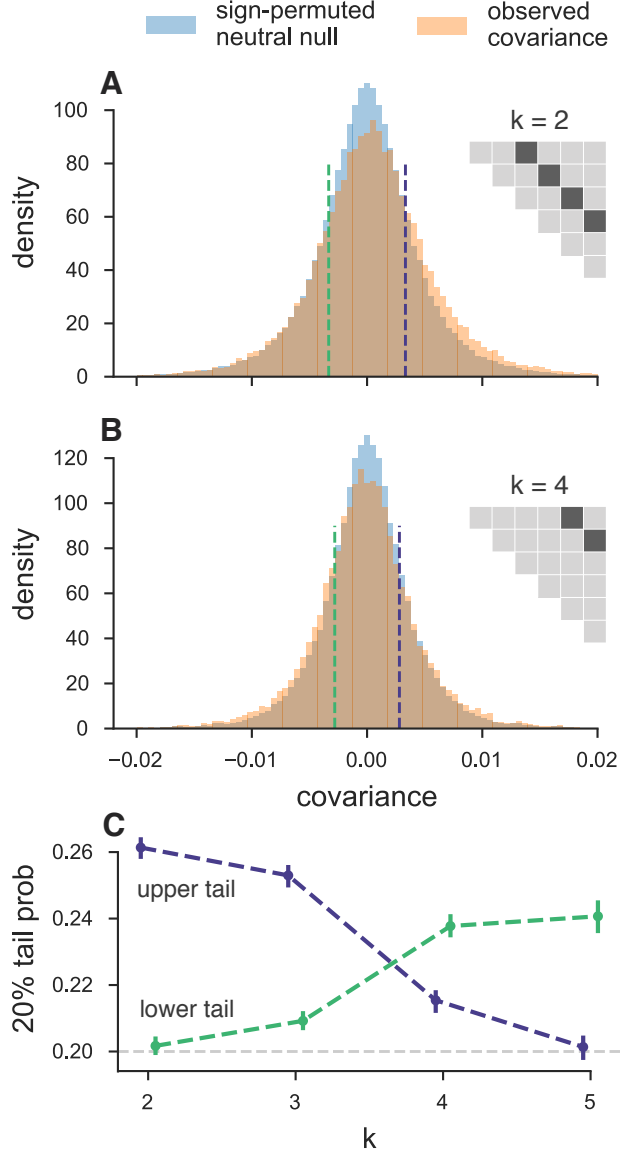
However, the signal of a change in the direction of selection localized to a particular region of the genome may be washed out when we calculate genome-wide temporal covariances. Averaging over such large numbers of loci is necessary to detect a signal of covariance in allele frequency changes when both drift (inflated in evolve-and-sequence studies which use small population sizes) and sampling variance can create spurious covariances. To address this limitation, we calculated temporal covariances over 100kb genomic windows, with on average XXX loci per window. While the covariance of each tile is noisy, we can analyze the genome-wide distribution of tile covariances for changes through time. We would like to compare the distribution of these tile covariances to a neutral expectation, but using a theoretic neutral null distribution would be inappropriate given complex dependencies across the genome due to linkage disequilibria (which can occur over long physical distances in E&R and selection studies, Baldwin-Brown et al. 2014; Nuzhdin and Turner 2013).

Instead, we have developed a permutation-based procedure that constructs a null distribution of the genome-wide covariances as they would look if only neutral genetic drift was acting. We do this by randomly flipping the signs of the allele frequency changes per-genomic window 1,000 times, which acts to destroy the systematic covariances created by linked selection but creates a sampling distribution of the covariances spuriously created by neutral genetic drift. This empirical neutral null distribution is conservative in the sense that the variances of the covariances are wider than expected under drift alone, since drift also acts to inflate the magnitude of allele frequency change. By flipping the sign at the block-level, we preserve the complex dependencies between adjacent loci created by linkage disequilibrium; see Supplementary Material Section 5.6 for more details on this approach.

Using the Barghi et al. (2019) study’s data, we used the empirical neutral null to investigate whether there was a general trend for the temporal covariance  $\text{Cov}(\Delta p_t, \Delta p_{t+k})$  to be positive for small  $k$  (as expected under selection), but then become systematically negative for larger  $k$  (as would occur under evolutionary processes that reverse the fitness of regions across the genome). Such a change in the fitness direction of haplotypes would not be discernible from genome-wide covariances like those in Figure 1. We find (Figure 3 A and B), pooling across all replicates (see Supplementary Figure 6 for individuals replicates), that windowed temporal covariances between close timepoints are skewed positive (a heavy right tail), while between more distant timepoints these windowed temporal covariances tend to shift to become more negative (a heavy left tail). We additionally quantified the degree to which the left and right tails change as we look at covariances between timepoints further apart (Figure 3 C). We find that for covariances 2 timepoints apart ( $k = 2 \times 10$  generations), the 20% right tail is inflated by a of 1.3 (95% CI [1.29, 1.32]), consistent with linked selection creating positive covariance in allele frequency change. Under constant directional selection, we expect these positive covariances between increasingly distant timepoints to decay to zero as additive genetic variance is exhausted by selection and linkage disequilibrium decays. Instead we find that for covariances 4 timepoints apart ( $k = 4 \times 10$  generations), the left tail of negative covariances is inflated by a factor of 1.19 (95% CI [1.17, 1.21]). Overall, we see a striking pattern that covariances not only decay towards zero, but in fact become negative through time, consistent with some regions in the genome having had a reversed fitness effect at later timepoints.

### 3 Acknowledgments

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**Figure 3:** A, B: The distribution of temporal covariances calculated in 100kb genomic windows from the Barghi et al. (2019) study, plotted alongside an empirical neutral null distribution created by recalculating the windowed covariances on sign permuting the allele frequency changes. In subfigure A, windowed covariances  $\text{Cov}(\Delta p_t, \Delta p_{t+k})$  are separated by  $k = 2 \times 10$  generations and in subfigure B the covariances are separated by  $k = 4 \times 10$  generations; each  $k$  is an off-diagonal from the variance diagonal of the temporal covariance matrix (see cartoon of upper-triangle of covariance matrix in subfigures A and B, where the first diagonal is the variance, and the dark gray indicates which off-diagonal of the covariance matrix is plotted in the histograms). C: The lower and upper tail probabilities of the observed windowed covariances, at 20% and 80% quintiles of the empirical neutral null distribution, for varying time between allele frequency changes (i.e. which off-diagonal  $k$ ). The confidence intervals are 95% block-bootstrap confidence interval, and the light gray dashed line indicates the 20% tail probability expected under the neutral null.

## 4 Appendix

### 5 Estimator Bias Correction

#### 5.1 Correcting variance bias with a single depth sampling process

Following Waples (1989), we have that the variance in the initial generation, which is entirely due to the binomial sampling process, is  $\text{Var}(p_0) = p_0(1-p_0)/d_0$  where  $d_0$  is the number of binomial draws (e.g. read depth). At a later timepoint, the variance in allele frequency is a result of both the binomial sampling process at time  $t$  and the evolutionary process.

Using the law of total variation,

$$\text{Var}(\tilde{p}_t) = \mathbb{E}(\text{Var}(\tilde{p}_t|p_t)) + \text{Var}(\mathbb{E}(\tilde{p}_t|p_t)) \quad (2)$$

$$= \underbrace{\frac{p_t(1-p_t)}{d_t}}_{\text{generation } t \text{ sampling noise}} + \underbrace{\text{Var}(p_t)}_{\text{variance due to evolutionary process}} \quad (3)$$

Under a drift-only process,  $\text{Var}(p_t) = p_0(1-p_0) \left[1 - \left(1 - \frac{1}{2N}\right)^t\right]$ . However, with heritable variation in fitness, we need to consider the covariance in allele frequency changes across generations (Buffalo and Coop 2019). We can write

$$V(p_t) = V(p_0 + (p_1 - p_0) + (p_2 - p_1) + \dots + (p_t - p_{t-1})) \quad (4)$$

$$= V(p_0 + \Delta p_0 + \Delta p_1 + \dots + \Delta p_{t-1}) \quad (5)$$

$$= V(p_0) + \sum_{i=0}^{t-1} \text{Cov}(p_0, \Delta p_i) + \sum_{i=0}^{t-1} \text{Var}(\Delta p_i) + \sum_{0 \leq i < j}^{t-1} \text{Cov}(\Delta p_i, \Delta p_j). \quad (6)$$

Each allele frequency change is equally like to be positive as it is to be negative; thus by symmetry this second term is zero. Additionally  $V(p_0) = 0$ , as we treat  $p_0$  as a fixed initial frequency. We can write,

$$V(p_t) = \sum_{i=0}^{t-1} \text{Var}(\Delta p_i) + \sum_{0 \leq i < j}^{t-1} \text{Cov}(\Delta p_i, \Delta p_j). \quad (7)$$

The second term, the cumulative impact of variance in allele frequency change can be partitioned into heritable fitness and drift components (Buffalo and Coop 2019; Santiago and Caballero 1995)

$$V(p_t) = \sum_{i=0}^{t-1} \text{Var}(\Delta_D p_i) + \sum_{i=0}^{t-1} \text{Var}(\Delta_H p_i) + \sum_{0 \leq i < j}^{t-1} \text{Cov}(\Delta p_i, \Delta p_j). \quad (8)$$

where  $\Delta_H p_t$  and  $\Delta_D p_t$  indicate the allele frequency change due to heritable fitness variation and drift respectively. Then, sum of drift variances in allele frequency change is

$$\sum_{i=0}^{t-1} \text{Var}(\Delta_D p_i) = \sum_{i=0}^{t-1} \frac{p_i(1-p_i)}{2N} \quad (9)$$

replacing the heterozygosity in generation  $i$  with its expectation, we have

$$\sum_{i=0}^{t-1} \text{Var}(\Delta_D p_i) = p_0(1-p_0) \sum_{i=0}^{t-1} \frac{1}{2N} \left(1 - \frac{1}{2N}\right)^i \quad (10)$$

$$= p_0(1-p_0) \left[1 - \left(1 - \frac{1}{2N}\right)^t\right] \quad (11)$$

which is the usual variance in allele frequency change due to drift. Then, the total allele frequency change from generations 0 to  $t$  is  $\text{Var}(\tilde{p}_t - \tilde{p}_0) = \text{Var}(\tilde{p}_t) + \text{Var}(\tilde{p}_0) - 2 \text{Cov}(\tilde{p}_t, \tilde{p}_0)$ , where the covariance depends on the nature of the sampling plan (see Nei and Tajima 1981; Waples 1989). In the case where there is heritable variation for fitness, and using the fact that  $\text{Cov}(\tilde{p}_t, \tilde{p}_0) = p_0(1-p_0)/2N$  for Plan I sampling procedures (Waples 1989), we write,

$$\text{Var}(\tilde{p}_t - \tilde{p}_0) = \text{Var}(\tilde{p}_t) + \text{Var}(\tilde{p}_0) - 2C \text{Cov}(\tilde{p}_t, \tilde{p}_0) \quad (12)$$

$$= \frac{p_t(1-p_t)}{d_t} + \frac{p_0(1-p_0)}{d_0} + p_0(1-p_0) \left[1 - \left(1 - \frac{1}{2N}\right)^t\right] + \quad (13)$$

$$\sum_{i=0}^{t-1} \text{Var}(\Delta_H p_i) + \sum_{0 \leq i < j}^{t-1} \text{Cov}(\Delta p_i, \Delta p_j) - \frac{C p_0(1-p_0)}{2N} \quad (14)$$

$$\frac{\text{Var}(\tilde{p}_t - \tilde{p}_0)}{p_0(1-p_0)} = 1 + \frac{p_t(1-p_t)}{p_0(1-p_0)d_t} + \frac{1}{d_0} - \left(1 - \frac{1}{2N}\right)^t + \quad (15)$$

$$\sum_{i=0}^{t-1} \frac{\text{Var}(\Delta_H p_i)}{p_0(1-p_0)} + \sum_{0 \leq i < j}^{t-1} \frac{\text{Cov}(\Delta p_i, \Delta p_j)}{p_0(1-p_0)} - \frac{C}{N} \quad (16)$$

where  $C = 1$  if Plan I is used, and  $C = 0$  if Plan II is used (see Waples 1989, p. 380 and Figure 1 for a description of these sampling procedures). We move terms creating a corrected estimator for the population variance in allele frequency change, and replace all population heterozygosity terms with the unbiased sample estimators, e.g.  $\frac{d_t}{d_t-1} \tilde{p}_t(1-\tilde{p}_t)$ ,

$$\frac{d_0-1}{d_0} \frac{\text{Var}(\tilde{p}_1 - \tilde{p}_0)}{\tilde{p}_0(1-\tilde{p}_0)} - \frac{(d_0-1)}{d_0(d_1-1)} \frac{\tilde{p}_1(1-\tilde{p}_1)}{\tilde{p}_0(1-\tilde{p}_0)} - \frac{1}{d_0} + \frac{C}{N} = \frac{\text{Var}(\Delta_H p_0)}{p_0(1-p_0)} + \frac{1}{2N} \quad (17)$$

## 5.2 Correcting variance bias with individual and depth sampling processes

Here, we extend the sampling bias correction described above to handle two binomial sampling processes: one as individuals are binomially sampled from the population, and another as reads are binomially sampled during sequencing. (see also Jónás et al. 2016). Let  $X_t \sim \text{Binom}(n_t, p_t)$



where  $X_t$  is the count of alleles and  $n_t$  is the number of diploids sampled at time  $t$ . Then, these individuals are sequenced at a depth of  $d_t$ , and  $Y_t \sim \text{Binom}(d_t, X_t/n_t)$  reads have the tracked allele. We let  $\tilde{p}_t = Y_t/d_t$  be the observed sample allele frequency. Then, the sampling noise is

$$\text{Var}(\tilde{p}_t|p_t) = \mathbb{E}(\text{Var}(\tilde{p}_t|X_t)) + \text{Var}(\mathbb{E}(\tilde{p}_t|X_t)) \quad (18)$$

$$= p_t(1-p_t) \left( \frac{1}{n_t} + \frac{1}{d_t} - \frac{1}{n_t d_t} \right) \quad (19)$$

$$\text{Var}(\tilde{p}_t - \tilde{p}_0) = p_t(1-p_t) \left( \frac{1}{n_t} + \frac{1}{d_t} - \frac{1}{n_t d_t} \right) + p_0(1-p_0) \left( \frac{1}{n_0} + \frac{1}{d_0} - \frac{1}{n_0 d_0} \right) \quad (20)$$

$$- \frac{C p_0(1-p_0)}{N} + p_0(1-p_0) \left[ 1 - \left( 1 - \frac{1}{2N} \right)^t \right] + \sum_{i=0}^{t-1} \text{Var}(\Delta_H p_i) \quad (21)$$

$$+ \sum_{0 \leq i < j}^{t-1} \text{Cov}(\Delta p_i, \Delta p_j) \quad (22)$$

Through the law of total expectation, one can find that an unbiased estimator of the heterozygosity is

$$\frac{n_t d_t}{(n_t - 1)(d_t - 1)} \tilde{p}_t(1 - \tilde{p}_t). \quad (23)$$

Replacing this unbiased heterozygosity into our expression above, the total sample variance is

$$\text{Var}(\tilde{p}_t - \tilde{p}_0) = \frac{n_t d_t \tilde{p}_t(1 - \tilde{p}_t)}{(n_t - 1)(d_t - 1)} \left( \frac{1}{n_t} + \frac{1}{d_t} - \frac{1}{n_t d_t} \right) + \frac{n_0 d_0 \tilde{p}_0(1 - \tilde{p}_0)}{(n_0 - 1)(d_0 - 1)} \left( \frac{1}{n_0} + \frac{1}{d_0} - \frac{1}{n_0 d_0} \right) + \quad (24)$$

$$\frac{n_0 d_0 \tilde{p}_0(1 - \tilde{p}_0)}{(n_0 - 1)(d_0 - 1)} \left[ 1 - \left( 1 - \frac{1}{2N} \right)^t \right] - \frac{C}{N} \frac{n_0 d_0 \tilde{p}_0(1 - \tilde{p}_0)}{(n_0 - 1)(d_0 - 1)} + \sum_{i=0}^{t-1} \text{Var}(\Delta_H p_i) + \sum_{0 \leq i < j}^{t-1} \text{Cov}(\Delta p_i, \Delta p_j). \quad (25)$$

As with equation (17), we can rearrange this to get a biased-correct estimate of the variance in allele frequency change between adjacent generations,  $\text{Var}(\Delta p_t)$ .

### 5.3 Covariance Correction

We also need to apply a bias correction to the temporal covariances (and possibly the replicate covariances if the initial sample frequencies are all shared).

The basic issue is that  $\text{Cov}(\Delta \tilde{p}_t, \Delta \tilde{p}_{t+1}) = \text{Cov}(\tilde{p}_{t+1} - \tilde{p}_t, \tilde{p}_{t+2} - \tilde{p}_{t+1})$ , and thus shares the sampling noise of timepoint  $t+1$ . Thus acts to bias the covariance by subtracting off the noise variance term of  $\text{Var}(\tilde{p}_{t+1})$ , so we add that back in.

## 5.4 Variance-Covariance Matrix Correction

Now, we extend the bias corrections for single locus variance and covariance described in Supplementary Material Sections 5.1, 5.2, and 5.3 to multiple sampled loci. With frequency collected at  $T + 1$  timepoints across  $R$  replicate populations at  $L$  loci, we have  $\mathbf{F}$  of allele frequencies,  $\mathbf{D}$  multidimensional array of sequencing depths, and a  $\mathbf{N}$  multidimensional array of the number of individuals sequenced, each of dimension  $R \times (T + 1) \times L$ . We calculate the array  $\Delta\mathbf{F}$  which contains the allele frequency changes between adjacent generations, and has dimension  $R \times T \times L$ . The operation  $\text{flat}(\Delta\mathbf{F})$  flattens this array to a  $(R \cdot T) \times L$  matrix, such that rows are grouped by replicate, e.g. for timepoint  $t$ , replicate  $r$ , and locus  $l$  and allele frequencies  $p_{t,r}$ , for a single locus the entries are

$$\text{flat}(\Delta\mathbf{F}) = \begin{bmatrix} \Delta p_{1,0,0} & \Delta p_{2,0,0} & \dots & \Delta p_{1,1,0} & \Delta p_{2,1,0} & \dots & \Delta p_{T,R,0} \\ \Delta p_{1,0,1} & \Delta p_{2,0,1} & \dots & \Delta p_{1,1,1} & \Delta p_{2,1,1} & \dots & \Delta p_{T,R,1} \\ \vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\ \Delta p_{1,0,L} & \Delta p_{2,0,L} & \dots & \Delta p_{1,1,L} & \Delta p_{2,1,L} & \dots & \Delta p_{T,R,L} \end{bmatrix} \quad (26)$$

where each  $\Delta p_{t,r,l} = p_{t+1,r,l} - p_{t,r,l}$ . Then, the sample temporal-replicate covariance matrix  $\mathbf{Q}'$  calculated on  $\text{flat}(\Delta\mathbf{F})$  is a  $(R \cdot T) \times (R \cdot T)$  matrix, with the  $R$  temporal-covariance block submatrices along the diagonal, and the  $R(R - 1)$  replicate-covariance submatrices matrices in the upper and lower triangles of the matrix,

$$\mathbf{Q}' = \begin{bmatrix} \mathbf{Q}'_{1,1} & \mathbf{Q}'_{1,2} & \dots & \mathbf{Q}'_{1,R} \\ \mathbf{Q}'_{2,1} & \mathbf{Q}'_{2,2} & \dots & \mathbf{Q}'_{2,R} \\ \vdots & \vdots & \ddots & \vdots \\ \mathbf{Q}'_{R,1} & \mathbf{Q}'_{R,2} & \dots & \mathbf{Q}'_{R,R} \end{bmatrix} \quad (27)$$

where each submatrix  $\mathbf{Q}'_{i,j}$  is the  $T \times T$  sample covariance matrix for replicates  $i$  and  $j$ .

Given the bias of the sample covariance of allele frequency changes, we calculated an expected bias matrix  $\mathbf{B}$ , averaging over loci,

$$\mathbf{B} = \frac{1}{L} \sum_{l=1}^L \frac{\mathbf{h}_l}{2} \circ \left( \frac{1}{\mathbf{d}_l} + \frac{1}{2\mathbf{n}_l} + \frac{1}{2\mathbf{d}_l \circ \mathbf{n}_l} \right) \quad (28)$$

where  $\circ$  denotes elementwise product, and  $\mathbf{h}_l$ ,  $\mathbf{d}_l$ , and  $\mathbf{n}_l$ , are rows corresponding to locus  $l$  of the unbiased heterozygosity arrays  $\mathbf{H}$ , depth matrix  $\mathbf{D}$ , and number of diploids matrix  $\mathbf{N}$ . The unbiased  $R \times (T + 1) \times L$  heterozygosity array can be calculated as

$$\mathbf{H} = \frac{2\mathbf{D} \circ \mathbf{N}}{(\mathbf{D} - 1) \circ (\mathbf{N} - 1)} \circ \mathbf{F} \circ (1 - \mathbf{F}) \quad (29)$$

where division here is elementwise. Thus,  $\mathbf{B}$  is a  $R \times (T + 1)$  matrix. As explained in Supplementary Material Section 5.2 and 5.3, the temporal variances and covariances require bias corrections, meaning each temporal covariance submatrix  $\mathbf{Q}_{r,t}$  requires two corrections. For an element  $Q_{r,t,s} =$

$\text{Cov}(\Delta p_t, \Delta p_s)$  of the temporal covariance submatrix for replicate  $r$ ,  $\mathbf{Q}_{r,r}$ , we apply the following correction

$$Q_{r,t,s} = \begin{cases} Q'_{r,t,s} - b_{r,t} - b_{r,t+1}, & \text{if } t = s \\ Q'_{r,t,s} + b_{r,\max(t,s)}, & \text{if } |t - s| = 1 \end{cases} \quad (30)$$

where  $b_{r,t}$  is element in row  $r$  and column  $t$  of  $\mathbf{B}$ . Additionally, in some study designs, a single timepoint is shared for the initial generation across replicates. In this case, the sampling noise is shared between

## 5.5 Block Bootstrap Procedure

To infer the uncertainty of covariance, convergence correlation, and  $G(t)$  estimates, we used a block bootstrap procedure. This is a version of the bootstrap that resamples blocks of data points, rather than individual data points, to infer the uncertainty of an statistic in the presence of unknown correlation structure between data. With genome-wide data, linkage disequilibria between sites creates complex and unknown dependencies between variants. The estimators used in this paper are predominantly ratios, e.g. temporal-replicate covariance standardized by half the heterozygosity (XXX),  $G(t)$  which is the ratio of covariance to total variance (XXX), and the convergence correlation (equation (1)). In these cases, we can exploit the linearity of the expectation to make the bootstrap procedure more computationally efficient, by pre-calculating the statistics of the ratio's numerator and denominator,  $N(\mathbf{x}_i)$  and  $D(\mathbf{x}_i)$ , on the data  $\mathbf{x}_i$  for all blocks  $i \in \{1, 2, \dots, W\}$  in the genome. Then we draw  $W$  bootstrap samples with replacement, and compute the estimate for bootstrap sample  $b$  with an average weighted by the number of loci in all sampled blocks,

$$\tilde{\theta}_b = \sum_{i=1}^W w_i \frac{N(\mathbf{x}_i)}{D(\mathbf{x}_i)} \quad (31)$$

Note that computing the ratio of averages rather than the average of a ratio is a practice common for population genetic statistics like  $F_{ST}$  (**Bhatia2013-zy**). With these  $B$  bootstrap estimates, we calculate the  $\alpha/2$  and  $1 - \alpha/2$  quantiles, which we use to estimate the  $1 - \alpha = 95\%$  pivot confidence intervals (p. 33 **Wasserman2006-jl**, p. 194 **Davison2013-oy**) throughout the paper,

$$C_\alpha = \left( 2\hat{\theta} - q_{1-\alpha/2}, 2\hat{\theta} - q_{\alpha/2} \right). \quad (32)$$

where  $\hat{\theta}$  is the estimate, and  $q_x$  is bootstrap quantile for probability  $x$ .

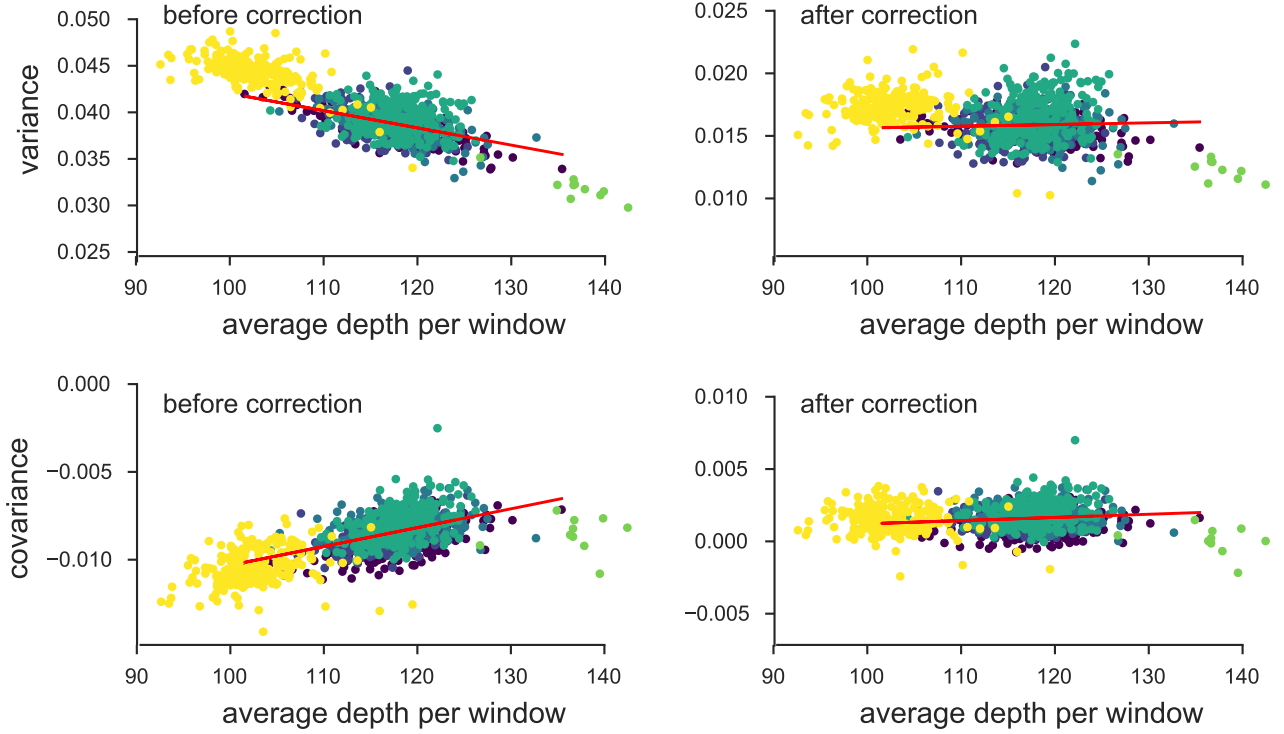
## 5.6 The Empirical Neutral Null Windowed Covariance Distribution

# 6 Supplementary Figures

## 6.1 Bias Correction for Barghi et al. (2019)

We have investigated the effectiveness of our correction on real data by exploiting the relationship between sampling depth and the magnitude of the variance and covariance biases, and comparing

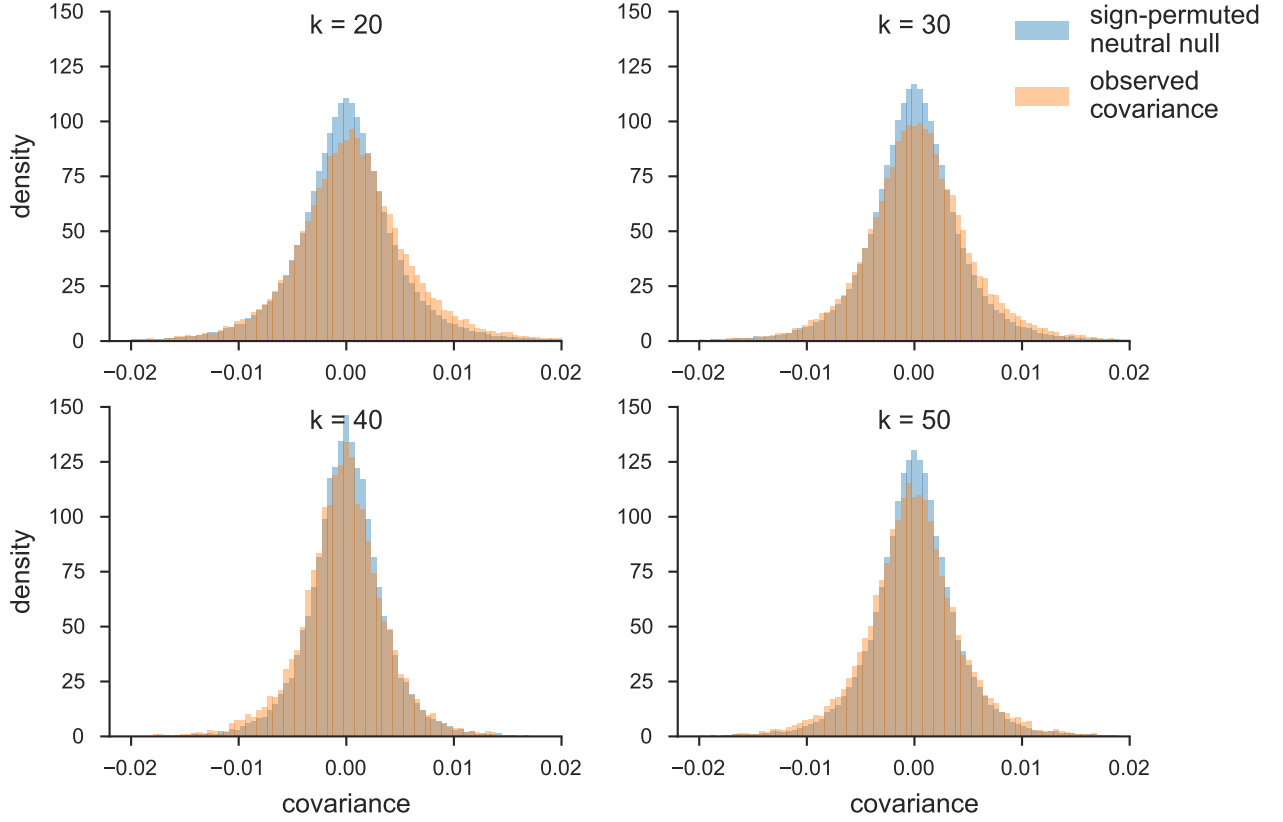
the observed variances and covariances before and after correction. We plot the variance and covariance (between adjacent timepoints) before and after the bias correction against the average sample depth in 100kb genomic windows in Figure 4. Overall, we find the correction strongly



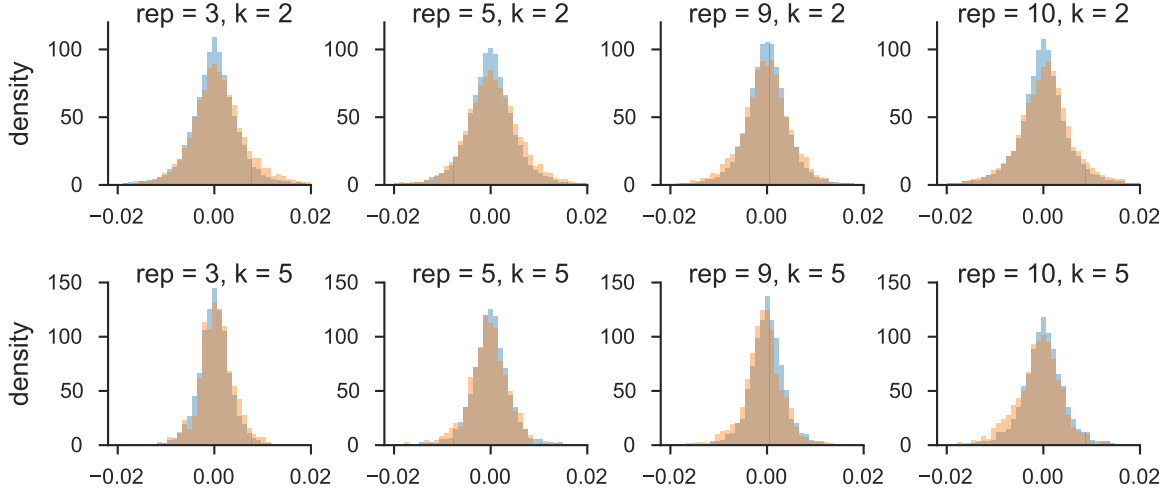
**Figure 4:** The variance and covariances calculated in 100kb genomic windows plotted against average depth in a window before and after bias correction. Each panel has a least-squares estimate between the variance and covariance, and the average depth. Overall, the bias correction corrects sampling bias in both the variance and covariance such that the relationship with depth is constant. Colors indicate the different chromosomes of *D. simulans*; we have excluded the X chromosome (yellow points) and chromosome 4 points (green points to far right) from the regression due to large differences in average coverage.

## 6.2 Barghi et al. (2019) Empirical Null and Windowed Covariance Distributions

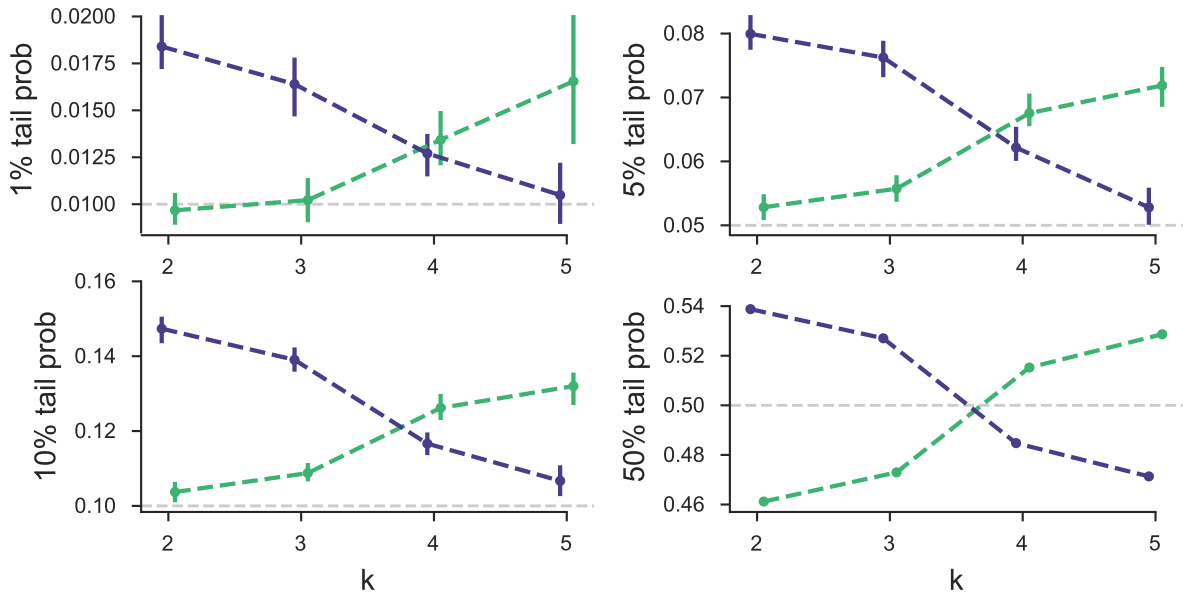
## 6.3 Barghi et al. (2019) Tail Probabilities for Windowed Covariances Distributions



**Figure 5:** The distribution of temporal covariances calculated across 100kb genomic windows from Barghi et al. (2019)’s study (orange) and the block sign permuted empirical neutral null distribution of the windowed covariances (blue). Each panel shows these windowed covariances and the empirical null distribution for covariances  $\text{Cov}(\Delta p_t, \Delta p_{t+k})$ ,  $k$  is the number of generations between allele frequency changes.



**Figure 6:** The distribution of windowed temporal covariances alongside the empirical neutral null for five randomly sampled replicates (columns), for  $k = 2$  (first row) and  $k = 5$  (second row). The main figure of the paper pools all replicate window and empirical neutral null covariances; we show here the windowed temporal covariances tend to shift from being positive (a heavier right tail) to become more negative (a heavier left tail) through time within particular replicates.



**Figure 7**