COMICS: A pipeline for the composite identification of selection across multiple genomic scans using Invariant Coordinate Selection in R

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Running Head: composite identification of selection signals

Abstract

Identifying loci that are under selection versus those that are evolving neutrally is a common challenge in evolutionary genetics. Moreover, with the increase in sequence data, genomic studies have begun to incorporate the use of multiple methods to identify candidate loci under selection. Composite methods are usually implemented to transform the data into a multi-dimensional scatter where outliers are identified using a distance metric, the most common being Mahalanobis distance. However, studies have shown that the power of Mahalanobis distance reduces as the number of dimensions increases. Because the number of methods for detecting selection continue to grow, this is an undesirable feature of Mahalanobis distance. Other composite methods such as invariant coordinate selection (ICS) have proven to be a robust method for identifying outliers in multi-dimensional space; though, this method has not been implemented for genomic data. Here we use simulated genomic data to test the performance of ICS in identifying outlier loci from multiple selection scans and compare the results to the performance of Mahalanobis distances. We show that the ICS outperformed Mahalanobis distance in all aspects including false positives, false negatives, and recall. Furthermore, ICS also performed better when identifying loci with weaker selection coefficients. We also introduce a pipeline in a R-Shiny smart wrapper environment that implements the ICS on multiple scans of selection. Importantly, we show that the ICS is a robust method for identifying outliers in multi-dimensional space and recommend its use for studies aimed at identifying loci under selection in the genome.

Introduction

A main goal of evolutionary genetics and ecological genomics is to identify candidate regions of the genome that show patterns consistent with selection (Beaumont and Balding 2004; Campbell-Staton et al. 2016; Forester et al. 2016; Martins et al. 2016). Highly differentiated loci are often interpreted as regions of the genome under selection, potentially due to local adaptation of a population to a novel or changing environment (Beaumont and Balding 2004; Campbell-Staton et al. 2016; Bekkevold et al. 2016; Rellstab et al. 2016). With the improvement of sequencing methods, the quantity and quality of genetic data continues to increase, allowing a more rigorous analysis of highly differentiated loci and how they are distributed throughout the genome. Despite this increase in genetic data methods to identify putatively selected loci (Gunther and Coop 2013; Lotterhos and Whitlock 2015; Capblancq et al. 2018), identifying candidate loci under selection and differentiating between true outliers and false positives still proves to be a difficult task.

Many selection methods/scans include the analysis of single nucleotide polymorphisms (SNPs), where each SNP is treated as an independent hypothesis when tested against the rest of the genome (Nielsen et al. 2005; Lotterhos and Whitlock 2014; Lotterhos and Whitlock 2015). The increase in SNP data ultimately decreases our ability to differentiate between neutral and selective hypotheses, because of concomitant increase in false positives.. This problem is also present when referring to methods that use discrete windows containing multiple SNPs to detect overall deviations in the pattern of variation in local regions when compared to the rest of the genome (Sabeti et al. 2002; Nielsen et al. 2005; Sabeti et al. 2007; Chen et al. 2010; Zhong et al. 2011; Alachiotis et al. 2012; Pavlidis et al. 2013). To circumvent this issue, some studies have implemented the use of multiple selection methods to identify regions of interest and combine

them in a meaningful way (*Anopheles gambiae* 1000 Genomes Consortium 2017; Zueva et al. 2018; Hodel et al. 2018). For example, a common method is to only define genomic regions as under selection if they are identified as outliers by multiple selection methods. Because of the nuances across selection methods and the differences in the assumptions and types of signals, the number of intersections identified may underestimate the true number of regions under selection, increasing the false negative rate and making this approach very conservative. For instance, methods that detect selection using the site frequency spectrum (SFS) identify a selective sweep as an area with higher than expected levels of allele differentiation (Nielsen et al. 2005), while methods using linkage disequilibrium identify selective sweeps as regions with larger than expected associations among SNPs (Alachiotis et al. 2012). When looking at the intersection between these methods, regions under moderate selection near high levels of recombination would most likely be considered neutral.

In lieu of looking at the intersection across multiple selection methods to identify outlier loci, composite methods should be implemented. A composite method includes the use of multiple selection statistics to identify regions of the genome under selection (Verity et al. 2017; Capblancq et al. 2018). In this case, each method/statistic will result in a distribution that is described by its location and dispersion. A multi-dimensional transformation creates a cluster of points, derived from the distributions of the selection/population statistics, where the location is defined as the center of the cluster and the spread of the data are defined as the scatter (Verity et al. 2017; Capblancq et al. 2018; Archimbaud, Nordhausen, Ruiz-Gazen 2018a). Moreover, multidimensional analyses create ample opportunity to take different selection metrics and compare them linearly under one distribution (Verity et al. 2017). In the case of multi-dimensional data, most outliers are identified by a distance metric, where they are expected to

have a larger than average distances from the central location of the cluster (Archimbaud, Nordhausen, Ruiz-Gazen 2018a).

A popular composite statistic recently proposed to identify outliers across multiple tests of selection is the Mahalanobis distance, a measure of the number of standard deviations that a given data point is from the multi-dimensional mean (Mahalanobis 1936; Gnanadesikan and Kettenring 1972; De Maesschalck et al. 2000; Verity et al. 2017; Archimbaud, Nordhausen, Ruiz-Gazen 2018a). Though the Mahalanobis distance has been implemented for identifying outlier loci in a multi-dimensional data set, recent studies have shown that an increase in the number of dimensions (the number of metrics estimated within a data set) results in an inflation of false negatives and false positives (Leys et al. 2018; Archimbaud, Nordhausen, Ruiz-Gazen 2018a). Specifically, results from Archimbaud et al. (2018) show that if outliers belong to a reduced dimension space, the probability that the Mahalanobis distance of a neutral locus exceeds the distance of a true outlier is low; however, this is not the case when dimension space is increased. In other words, as the number of selection methods used increases, it becomes more difficult to differentiate between true positives and true-negatives. This phenomenon is known as the "curse of dimensionality," (Trunk 1979; Bellman, Richard 2013; Bellman, Richard E. 2015).

We propose the use of invariant coordinate selection (ICS) as an alternative method to combine multiple selection scans and identify true outliers in growing multi-dimensional space. Here, we also present a pipeline to easily implement the method. Specifically, ICS is a method for identifying outlier data points in multi-dimensional space with coordinates derived from eigenvalues and eigenvectors (Tyler et al. 2007; Archimbaud, Nordhausen, Ruiz-Gazen 2018a). The ICS is analogous to a principal component analysis (PCA), however, it differs in two ways. First, instead of using one scatter matrix (the covariance matrix commonly used in PCA), ICS

adds further constraints by solving inequality conditions on a second matrix with either the third moment (skewness) or the fourth moment (kurtosis) (Tyler et al. 2007; Archimbaud, Aurore, Nordhausen, Ruiz-Gazen 2018a). Second, the invariant components are maintained in parallel while principal components are orthogonal (Tyler et al. 2007; Archimbaud, Aurore, Nordhausen, Ruiz-Gazen 2018a). Once the univariate data are transformed into a multi-dimensional data set, data points with the largest ICS distance (i.e., the Euclidean distance) in the transformed space are deemed as outliers. Importantly, because of the information drawn from the two different scatter matrices, ICS methods are less susceptible to the inflation of distance with an increase in the number of dimensions (Tyler et al. 2007; Archimbaud, Aurore, Nordhausen, Ruiz-Gazen 2018a).

ICS has previously been identified as a robust method for identifying outlier in multidimensional space and has been implemented to detect subgrouping within populations based on genotypic data, however, it has not been implemented for identifying outlier regions based on the input of selection scans. (Archimbaud, A. et al. 2016; Fischer et al. 2017; Archimbaud, Nordhausen, Ruiz-Gazen 2018b). We use simulated genetic data to compare the number of false positives, false negatives, and recall between Mahalanobis and ICS distances. We also introduce an easy to use R-Shiny wrapper specifically designed for performing ICS using genomic data. We call this app COMICS (**C**alling **O**utlier loci from **M**ulti-dimensional data using **I**nvariant **C**oordinate **S**election) and make it available at: https://github.com/JTNelsonWSU/COMICS.

Results

The COMICS R-package

COMICS was implemented with the R-Shiny environment, which is an open source R-package that provides a framework for building web applications using R (Chang et al. 2015; Team 2018). Specifically, the Shiny environment allows for the development of a graphical user interface (GUI) that permits the user to operate the ICS proficiently. Using the COMICS package, the user is able to analyze data in several different ways. First, COMICS has the option to observe the data before the multi-dimensional transformation; this feature allows the user to view the results from the different selection scans used to generate the multi-dimensional data in individual plots. COMICS allows for the customization of the end results of the ICS identification of outliers by modifying statistical cutoffs, and specific chromosome intervals. COMICS allows the user to download the multi-dimensional data frame and corresponding figures in csv and pdf formats. The pipeline is user friendly while maintaining flexibility in the implementation of the method. You can find the COMICS app and example files at: https://github.com/JTNelsonWSU/COMICS.

Data upload and input file format

COMICS requires two input data files. The first file is a multivariate data file containing a list of each genomic position with the corresponding likelihood estimate, or effect size estimate, from each scan of selection used. The first two columns contain the chromosome and physical position along the chromosome (or equivalent). COMICS can handle SNP or window analyses, as long as the windows are consistent across scans of selection; in the latter case, the position will correspond to the midpoint of the window. The physical positions need to be numerically organized relative to only the corresponding chromosome; COMICS is designed to convert all physical positions to the length of the genome.. The second data file is known as the

genome configuration file. Specifically, the configuration file is used to define the number of chromosomes (or scaffolds) used, including the total length of each chromosomes in base pairs. The main role of this file is to tell COMICS how large the genome (or assembly) is and the order in which the positions align along the genome; this is taken into account when COMICS generates genome-wide selection scan figures.

COMICS features and figure generation

In addition to customizing statistical thresholds (e.g., quantiles of interest), COMICS will generate several different figures for both single statistics and ICS distances. For example, two different figures are generated from the multivariate data prior to the analysis. The first is a scatter plot generated by the ggplot and reshape R-packages (Wickham 2007; Wickham 2016), consolidating all genome scans into one panel and color codes them by chromosome (Figure 1). The second figure is a user-defined representation of one particular scan of selection at a time. After analyses with ICS, COMICS will produce two histograms of the log ICS distance for 1) the entire genome and 2) a user defined chromosome. Each of these has a vertical line that defines a cutoff based on the quantile of interest. The main figure that COMICS produces is a genome scan of the logged ICS Euclidean distance. Like the univariate genome scans, the ICS genome scan is color coded by chromosome and has a horizontal line representing the statistical cutoff (Figure 2). Another feature of the COMICS package is the ability to download the data frame generated from the ICS. Specifically, the output file contains six different columns, including the chromosome, the SNP position (or midpoint position in a window), outlier status (Boolean form), ICS Distance, logged ICS, and the position with respect to the chromosome. Importantly, the downloadable content in COMICS allows for the flexibility of both downstream analyses of multi-dimensional data and novel figure generation.

171 *Testing the ICS with genomic data*

172 Since the development of the ICS, studies have shown that it is a robust method for
173 detecting outliers in a multidimensional space (Tyler et al. 2007; Archimbaud, Aurore,
174 Nordhausen, Ruiz-Gazen 2018a). However, none of these analyses have been geared toward
175 genomic data. To test the robustness of the ICS, we used simulated genomic data from
176 previously published analyses (Lotterhos and Whitlock 2014; Lotterhos and Whitlock 2015;
177 Verity et al. 2017). Specifically,, genetic data were simulated under several demographic models
178 and include populations that experience either an equilibrium or non-equilibrium state. For
179 example, we used data simulated under four different demographic scenarios: a non-equilibrium
180 isolation by distance due to a one refugium and two refugia demographic expansion, an
181 equilibrium isolation by distance model where the landscape was started at carrying capacity and
182 was run until equilibrium, and a island model at equilibrium with a migration rate of 0.01 among
183 demes. All simulated scenarios contained 30 populations that consisted of 20 individuals. The
184 simulated data also consisted of 10,000 independent SNPs where 9,900 SNPs were evolving
185 neutrally and the remaining 100 SNPs were under the influence of natural selection with
186 different selection coefficients: 12 SNPs where s = 0.1, 38 SNPs where s = 0.01, and 50 SNPs
187 where s = 0.005. Specifically, the strength of selection within a simulated population was
188 determined by the standardized environment for that specific population; if observed
189 environmental values were greater than the mean environment then selection was positive,
190 however neutrality was concluded if there were differences between observed environmental
191 values and the average environment (cite). For each SNP, five different univariant statistics were
192 used that were calculated from previously published studies (cite sim studies). These statistics
193 include 1) log-Bayes factor (a measure of the association between allele frequency and the

194 environment calculated in BAYENV2, 2) Spearman's rho (additional measure of association
195 between alleles and the environment from BAYENV2, 3) X^TX (measure of genetic
196 differentiation among populations from BAYENV2, 4) Z-score (a measure of association
197 between genotypes and the environment from the LFMM R-package),(Gunther and Coop 2013)
198 (cite LFMM). For a more comprehensive description of the previously simulated data see
199 (Lotterhos and Whitlock 2014; Lotterhos and Whitlock 2015).

200 To directly compare between Mahalanobis and ICS, we identified the number of true-
201 positives/negatives, false-positives/negatives, and recall (the number of true-positives over the
202 number of putative positives identified) as a measure of performance. We display this
203 information in the form of a confusion matrix (Figure 3B & 3D). A confusion matrix is a table
204 that is used to describe the performance of a defined model (ICS and Mahalanobis distances) on
205 a test data set of known values (simulated data). In our confusion matrices, there are four
206 different categories that are defined by a Boolean format: Actual Neutral, Identified Neural,
207 Actual Selection, and Identified Selection; where "Actual" is defined by the known values from
208 the simulated data and "Identified" is defined by the outputs of the ICS and Mahalanobis. For
209 example, the number of loci that were "Actual Neutral" and "Identified Neutral" are true-
210 negatives and those that were "Actual Selection" and "Identified Selection" are true-positives.

211 In the COMICS application, outliers are determined by a statistical cutoff. Statistical
212 cutoffs in COMICS are defined by the user and are picked out of the distribution of ICS
213 distances, usually in the form of quantiles (i.e., top 1% of ICS distances). Importantly, the
214 detection of outliers only satisfies a statistical pattern which may or may not be indicative of the
215 biological process of selection. Because we are using simulated data we are able to make a clear
216 distinction between outliers that are the result of a statistical pattern (i.e., arbitrary statistical

217 cutoffs) versus those that are identified as an outlier from a biological process (i.e., natural
218 selection). For our comparative analyses we used a 1% cutoff. Of the 10,000 simulated loci
219 (9,900 neutral and 100 selected). Moreover, the ICS performed marginally better than
220 Mahalanobis distance when separating selected loci from the neutral background across loci
221 under varying selective coefficients. (Figure 3A). Out of the top 1% (the first 100 loci with the
222 largest ICS distance), there was a total of 32 false-positives, with the remaining 68 loci being
223 true-positives. In terms of neutral loci, the ICS identified a total of 9,868 loci as true-negatives
224 with 32 false-negatives (Figure 3B). Across different regimes of selective coefficients, all loci
225 with a large selective coefficient (s = 0.1) were identified as an outlier, 94.7% (35 of 38) of loci
226 with intermediate selection coefficient (s = 0.01) were correctly identified as an outlier, while
227 42% of weakly selected loci (s = 0.005) were identified as outliers. The fraction of relevant
228 outliers versusthe total of relevant outliers (recall) for ICS was 0.68; meaning that out of the top
229 100 ICS distances, 68% of loci were under selection (Figure 3A). Analysis with Mahalanobis
230 distances allowed us to correctly identify loci with strong selection coefficients (s = 0.1) as
231 outliers. 94.7% of loci with intermediate coefficients (s = 0.01) were correctly identified as
232 outliers, and only 38% of weakly selected (s = 0.005) loci were correctly identified as outliers
233 (Figure 3C). The total recall for selected loci under the Mahalanobis distance was 0.66 (Figure
234 3D). When ICS results are compared with those obtained with Mahalanobis distances, we found
235 nearly a threefold decrease in false-positives (total false-positives in Mahalanobis = 34, as
236 opposed to 32 in ICS) as well as an increase in the true-positives (true-positives in Mahalanobis
237 = 66 as opposed to 68 in ICS, Figure 3B & 3D).

238 **Discussion:**

239 As the amount of genetic data increase, so does the number of SNPs (or windows) that
240 can be tested for neutral or selective evolution. Because each SNP (or window) is considered an
241 individual hypothesis, confounding effects on genetic analyses including the inflation of false-
242 positives and false-negatives will become more prominent. The inflation of type I and type II
243 errors brings further issues pertaining to the interpretation of putatively selected genetic markers
244 and their implication on downstream analyses; for example, the misidentification of adaptive loci
245 or the inability to capture adaptive loci. There is a growing need in developing methods to
246 combine methods of selection, as the number of methods keeps growing in numbers. Here we
247 developed, tested, and introduced an R-package (COMICS) using the Shiny smart wrapper that
248 incorporates the use ICS specifically for genomic data.

249 We show that ICS is a robust method for identifying outlier loci in multi-dimensional
250 space and propose its use over other similar existing methods like Mahalanobis distances.
251 Specifically, the ICS identified fewer false-positives/negatives, more true-positives/negatives and
252 had a higher recall for true-positives. One of the more interesting aspects of the ICS was its
253 ability to detect a larger proportion of weakly selected loci. Across the genome and across a
254 geographic space, loci experience selective pressures at varying strengths. Across methods,
255 signatures of loci under strong selection are often identified as true-positives. As the strength of
256 selection acting on a locus is reduced, the chances of identifying it as a true outlier are also
257 reduced, even after combining multiple selection detection methods that vary in their ability to
258 detect signatures of selection. Compared to contemporary methods, the ICS offers an increase in
259 the recall for outlier loci even for weakly selected loci. We therefore recommend that future
260 studies using univariate statistics to identify loci under selection consider the ICS as means to
261 identify outliers in multi-dimensional space.

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Figure 1. A screenshot of the suite of simulated selection scans that can be generated with COMICS. COMICS also allows the user to create individual selection scans of each univariate statistic that contributes the multidimensional scatter. In this example, there are three different selection methods that contribute to the ICS distance, Scan1, Scan2, and Scan3. Note that each color change represents a different chromosome. For simplicity, this example figure contains only ten 2kb chromosomes.

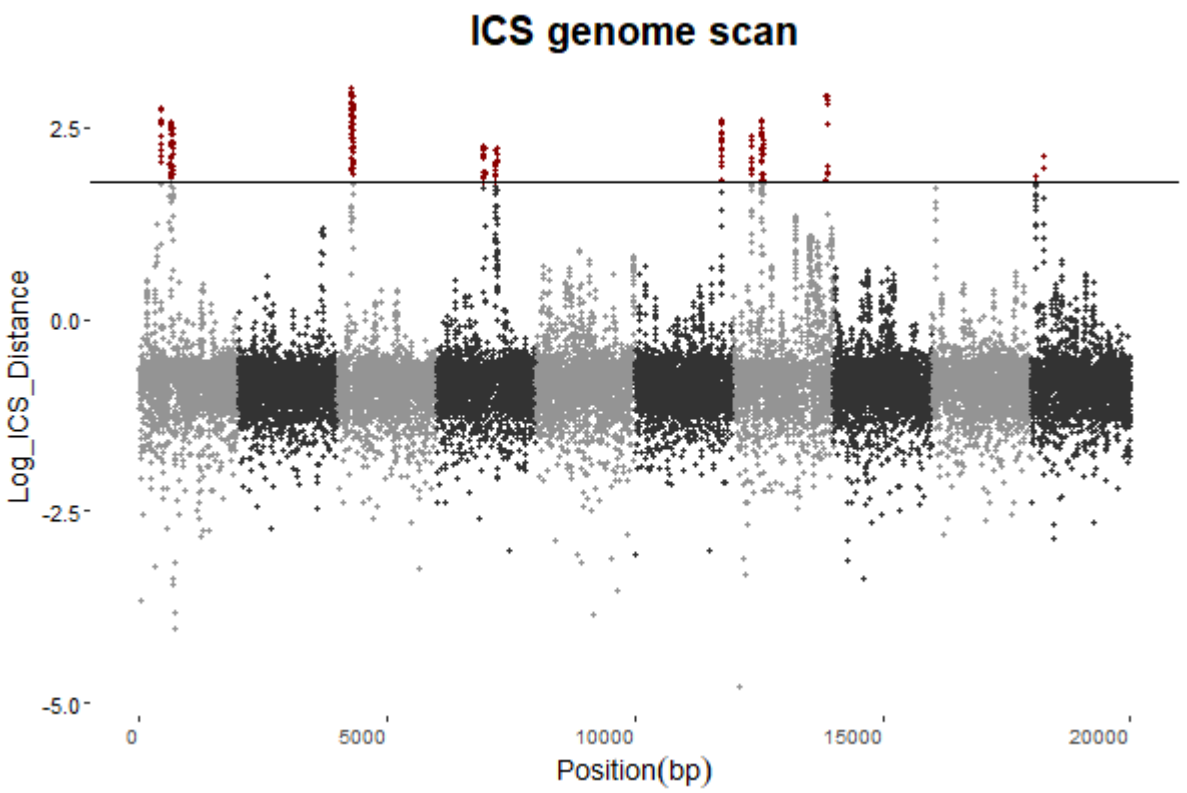
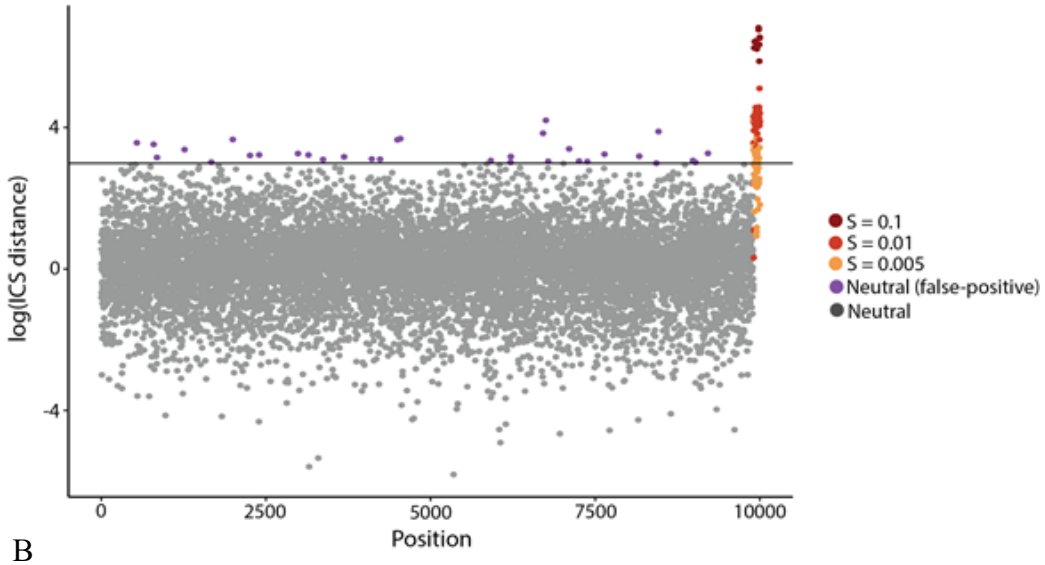


Figure 2. A screen shot from the COMICS GUI showing the final genome scan once transformed to the multi-dimensional scatter. The Y-axis is the ICS distance while the X-axis is the position along the genome. The color difference between grey and black represents transitions from one chromosome to another. The black horizontal line is the statistical cutoff that is defined by the user. Depending on the statistical cutoff, COMICS will render all data points above the threshold red where all other loci will conform to the default chromosome color. Note that the chromosomes numbers and sizes are the same as Figure 1.

A

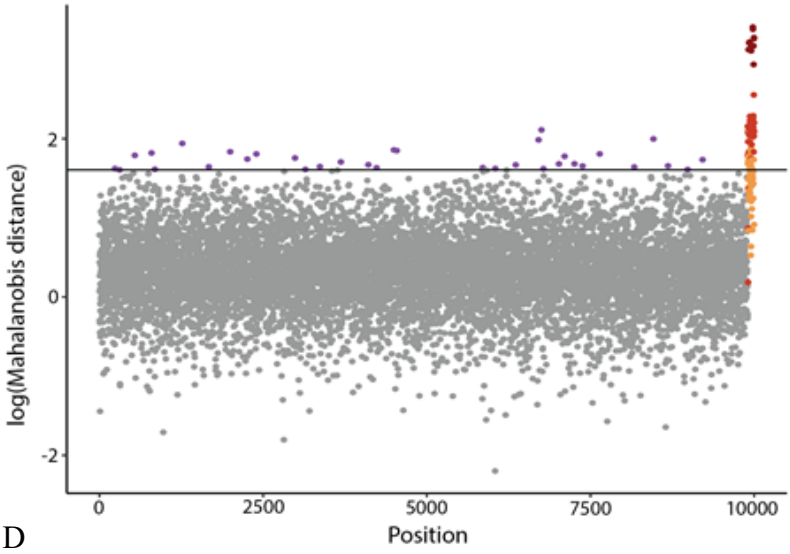


B

ICS	Identified: Neutral	Identified: Selection
Actual: Neutral	9,868	32
Actual: Selection	32	68

Recall: 0.68

C



D

Mahalanobis	Identified: Neutral	Identified: Selection
Actual: Neutral	9,866	34
Actual: Selection	34	66

Recall: 0.66

Figure 3. Selection scans and confusion matrices for the ICS (A & B) and Mahalanobis (C &D) distances calculated using simulated data. The horizontal line represents our statistical cutoff for outlier loci where all true-negatives are marked as grey and false-positives are purple. Loci that were simulated with a selection coefficient are in shades of red where the darkest represent loci with the largest selection coefficient. Importantly, loci with a selection coefficient that were below the statistical cutoff were deemed false-negatives and those above the line were true-positives. Within confusion matrices, the sum of the columns and the sum of the rows equals the total number of loci used in the simulated data.