

MOLECULAR ECOLOGY**Detecting natural selection across environmental gradients:
A performance evaluation of statistical genetic methods
using simulations**

Journal:	<i>Molecular Ecology</i>
Manuscript ID	MEC-16-0193
Manuscript Type:	Original Article
Date Submitted by the Author:	24-Feb-2016
Complete List of Authors:	Roffler, Gretchen; Alaska Department of Fish and Game, Division of Wildlife Conservation; United States Geological Survey, Alaska Science Center Landguth, Erin; University of Montana, Division of Biological Sciences Schwartz, Michael; US Forest Service Rocky Mountain Research Station, National Genomics Center for Wildlife and Fish Conservation Luikart, Gordon; University of Montana, Fish and Wildlife Genomics Group; Flathead Lake Biological Station of the University of Montana
Keywords:	adaptive differentiation, directional selection, environmental gradient, landscape genomics, outlier tests, simulations

Title: Detecting natural selection across environmental gradients: A performance evaluation of statistical genetic methods using simulations

Authors: Gretchen H. Roffler^{1, 2}, Erin L. Landguth³, Michael K. Schwartz⁴, Gordon Luikart^{5, 6}

¹Alaska Department of Fish and Game, Division of Wildlife Conservation, 802 3rd Street, Douglas, AK 99824, USA

²United States Geological Survey, Alaska Science Center, 4210 University Drive, Anchorage, AK 99508, USA

³Computational Ecology Laboratory, Division of Biological Sciences, University of Montana, Missoula, MT, 59812, USA

⁴US Forest Service Rocky Mountain Research Station, National Genomics Center for Wildlife and Fish Conservation, 800 E. Beckwith, Missoula, MT 59801, USA

⁵Fish and Wildlife Genomics Group, Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA

⁶Flathead Lake Biological Station, Fish and Wildlife Genomics Group, Division of Biological Sciences, University of Montana, Polson, MT 59860, USA

Corresponding author: Gretchen H. Roffler, Alaska Department of Fish and Game, Division of Wildlife Conservation, 802 3rd Street, Douglas, AK 99824, fax: (907) 465-4272, email: gretchen.roffler@alaska.gov

Key words: adaptive differentiation, directional selection, environmental gradient, landscape genomics, local adaptation, outlier tests, simulations

Running title: Methods to detect natural selection

Abstract

Identifying loci under selection is important for understanding how populations adapt to different environments. Most research on natural selection, gene flow, and effective population size interactions has focused on idealized theoretical populations without accounting for spatial complexities including selective gradients or discrete environments. Using spatially-explicit, individual-based simulations, we incorporated realistic demographic parameters and landscapes to address the following questions: (1) How strong must selection be for distributions of adaptive versus neutral alleles to diverge given different strengths of genetic drift (population size), gene flow (dispersal distance), and spatial patterns of selection, and (2) What is the relative power and error rate of population- and individual-based statistical tests for detecting loci under selection? The development of adaptive differentiation was most influenced by low dispersal ($N_m = 1-2$), and low drift ($N_e \leq 475$). Type I error rates (false positives) were highest with individual-based (e.g., Samβada and GLMs) and lowest with population-based methods (LOSITAN and BayeScan). Alternatively, population- and individual-based methods produced low type II error rates (false negatives) given strong regional differences in selection, but only the individual-based methods consistently performed well when selection was weak. Further, in a selection gradient, only individual-based methods consistently detected the locus under selection. Our results suggest that individual-based methods will most often correctly identify genotype-environment interactions, especially with large populations and low dispersal (type II error rate 0.0 – 0.3, in comparison to population-based methods 0.7 – 1.0). We suggest using



both population- and individual-based approaches for conservative detection of false positives and negatives.

Introduction

Understanding how populations adapt to spatial and temporal heterogeneity of their environments is a key topic in ecology, evolution, and conservation biology (Schoville *et al.* 2010; Hoffmann & Sgrò 201; Savolainen *et al.* 2013). Integral to this line of research is identification of genes underlying adaptation and signatures of natural selection (Storz 2005; Neale & Kremer 2011; Allendorf *et al.* 2010). A proven and increasingly common way to test for signatures of selection in natural populations involves detection of the geographical distribution of candidate adaptive alleles using statistical outlier tests and related methods (Luikart *et al.* 2003; Narum *et al.* 2011). Detection of adaptive alleles and correlating their spatial distributions with key landscape and environmental variables can help in understanding and verifying patterns of selection and local adaptation (Foll & Gaggiotti 2006; Joost *et al.* 2007). Moreover, quantifying the geographic distribution of adaptive alleles can help identify adaptively differentiated populations and predict population persistence and shifts in species distributions due to climate fluctuations, or anthropogenic changes of landcover (Hancock *et al.* 2008; Manel *et al.* 2010; Kovach *et al.* 2015).

Although much theory exists, and several model systems have been examined (Hohenlohe *et al.* 2010; Fournier-Level *et al.* 2011; Hancock *et al.* 2011), little is currently known concerning the pattern or rate of the spread of adaptive alleles across populations in

natural landscapes (Holderegger & Wagner 2008; Schoville *et al.* 2012). Advancement of research in detecting distinct signatures of adaptive allele distributions (e.g., outliers) across realistic landscapes is currently limited by poor understanding of the accuracy and precision of statistical tests and empirical approaches (Yang *et al.* 2012), and failure of methods to account for complications of spatial autocorrelation (Meirmans 2012; Neel *et al.* 2013). Genetic adaptive differentiation among populations often evolves if distinct environmental gradients are present or strong variation exists between regions, and if the selection is not countered by gene flow or genetic drift (Hohenlohe *et al.* 2010; Narum *et al.* 2010; Zardi *et al.* 2011). Given strong enough selection, frequencies of adaptive alleles (influenced by differences in individual fitness) will diverge between populations (Lewontin & Krakauer 1973; Beaumont 2005; Savolainen *et al.* 2013), and are predicted to have a different spatial distribution than neutral alleles (influenced by genetic drift, migration and mutation; Schwartz *et al.* 2010).

There is debate over whether neutral and adaptive markers will be spatially correlated (McKay & Latta 2002; Coop *et al.* 2009), or have different spatial distributions, as adaptive alleles can respond to environmental differences among populations (Hohenlohe *et al.* 2010; Limborg *et al.* 2012). Population genetics theory predicts large populations with limited gene flow will develop significant adaptive genetic differences among them (Crow & Kimura 1970; Waples 1998; Lenormand 2002; Hedrick 2005), due to the efficiency of natural selection differentiating adaptive allele frequencies, but not neutral alleles (Kohn *et al.* 2003; Franks *et al.* 2007, Lowe & Allendorf 2010). Conversely, gene flow may play a role in spreading advantageous mutations, whereas balancing selection could homogenize allele frequencies at certain adaptive loci (Rieseberg & Burke 2001; Charbonnel & Pemberton 2005; Holderegger &

Wagner 2008). However, population genetics theory has not been extensively tested or extended to complex landscapes or tested for using individual-based approaches. Advances have been made in identifying adaptive differentiation at broad geographic scales including globally (Prugnolle *et al.* 2005; Hancock *et al.* 2008; Coop *et al.* 2009), continent or ocean-wide (Joost *et al.* 2007, Limborg *et al.* 2012), and over multiple ecotypes (Matala *et al.* 2014; Cheviron *et al.* 2014), but less work has been accomplished at smaller, landscape level scales.

To understand the roles and interaction between gene flow, population size, and natural selection, it is necessary to answer these questions: **how does gene flow distribute adaptive genes across a heterogeneous landscape given different selection regimes and population sizes**, and **how does adaptive gene flow differ from neutral gene flow patterns**? Comparing loci that are outliers to what would be expected under neutral distributions is necessary to identify locally adapted populations, and prevent biases associated with using loci under selection when testing for neutral genetic structure (Luikart *et al.* 2003). Once they are identified, adaptive loci can be used to facilitate the study of interactions between environmental selective pressures and genes by examining patterns of adaptive divergence (Hand *et al.* 2015). In addition, adaptive loci can be used in combination with neutral loci to elucidate the effects of population genetic parameters such as gene flow and effective population size (N_e) on the spatial scale of adaptive differentiation.

Until recently, the majority of research on the role and interaction between natural selection, gene flow, and effective population size has focused on idealized theoretical populations without accounting for spatial complexities such as selective gradients or spatially distinct environments (Epperson *et al.* 2010). Using discrete, population-based models under

109 ideal Wright-Fisher assumptions limits their broad application, and makes interpretation of
110 results in complex environments equivocal. Incorporating biologically realistic demographic and
111 life history parameters into spatially explicit, individual-based models will increase our
112 understanding of how population genetic structure is influenced by environmental variability
113 (Landguth *et al.* 2010). This is now possible with the development of landscape genetics
114 approaches and modeling tools that specifically seek to reveal how spatial complexity of the
115 environment affects population genetic processes (Landguth & Cushman 2010).

116 Simulation studies are useful for understanding how complex systems function (Hoban *et*
117 *al.* 2011), how population genetic theory may be informed by approaches that incorporate
118 realistic landscape and environmental information (Hedrick 2005; Epperson *et al.* 2010;
119 Landguth *et al.* 2010), how sampling impacts results (Schwartz & McKelvey 2009), and
120 furthermore may be combined with empirical data to improve inference (Cushman & Landguth
121 2010). Recent simulation studies incorporating spatially-explicit data evaluated the performance
122 of **population-based** (Narum & Hess 2011; Villas *et al.* 2012; Lotterhos & Whitlock 2015) and
123 **correlation-based** (Jones *et al.* 2013; De Mitta *et al.* 2013; Forester *et al.* 2015; Lotterhos &
124 Whitlock 2015) methods to detect loci under selection. However, additional quantification of the
125 strengths and weaknesses of these methods is necessary, particularly in a spatially-explicit
126 modeling scenario. Whereas some studies have examined how environmental gradients affect
127 selection (Jones *et al.* 2013; Forester *et al.* 2015), very few have used scenarios with >1
128 population (De Mitta *et al.* 2013). **Finally, in addition to examining the effect of gene flow by**
129 **varying dispersal distance, it is also necessary to vary population sizes to assess the effects of**
130 **genetic drift on the development of adaptive differentiation.**

We used a spatially-explicit, individual-based simulation approach and incorporated biologically realistic demographic parameters and heterogeneous landscapes to evaluate the power and sensitivity of multiple widely-used methods and software programs to identify candidate loci under selection given varying strengths of natural selection, population sizes, and dispersal distances. Using this approach, we addressed the following questions: (1) How strong must selection be for spatial distributions of adaptive alleles to differ significantly from neutral patterns given different strengths of genetic drift (population size), gene flow (approximated through dispersal distance), and spatial pattern of selection, and (2) What is the relative power and error rate (type I and II) of population-based and individual-based statistical tests for detecting loci under selection? We predicted that adaptive and neutral alleles would become more differentiated with increasing population size and strength of selection, and decreasing dispersal distances, and these differences would become more distinct over time (generations). We further predicted that signatures of selection would be stronger in large populations with low dispersal, and therefore have lower type I and II error rates for the outlier loci detection methods.

Methods

Simulation of spatially-explicit selection

We used the spatially-explicit, individual-based, landscape genetics program CDPOP v1.0 (Landguth *et al.* 2012) to simulate the effects of varying selection strength, population size, and dispersal distances across a hypothetical landscape of size 200 x 300 km. CDPOP is a simulation program that has been demonstrated to accurately reproduce natural selection

(Landguth *et al.* 2012), movement, and mating processes that affect spatial population genetic substructure (Landguth *et al.* 2010). Therefore, ecological processes that affect genetic patterns can be tested by varying model parameters and evaluating the results. We conducted a series of simulation modeling experiments with the following factors: (1) Population size (small = 1,000, large = 10,000), (2) Dispersal distance (5 % and 50 % of maximum dispersal distance in study area; 9 and 93 km respectively), (3) Strengths of selection (strong [selection coefficient (s) = 0.5] and weak [s = 0.01], absent [s = 0.00]; 50 %, 10 %, and 0 % reduced survival, respectively), and (4) Spatial patterns of selection: selection present in the northern half and absent in the southern half, a continuous selection gradient, and selection absent (Fig. 1). We subsequently ran 10 Monte Carlo replicates for each simulation scenario across 500 non-overlapping generations which resulted in 2,640 simulated datasets (Table 1), a computationally challenging and time-consuming endeavor.

The landscape was initially populated with random locations representing individuals in 2 subpopulations with an isolation-by-distance (IBD) genetic pattern (Fig. 1). We limited migration between the 2 subpopulations by maintaining a barrier to dispersal (Landguth *et al.* 2010, Landguth & Balkenhol 2012) for 50 generations to achieve desired levels of population differentiation (F_{ST} = 0.02). We then used the genotypes of these 2 populations as the starting point for subsequent simulations. We simulated genetic exchange as functions of individual-based movement, mating, and dispersal acting on offspring at a given location. The subpopulations had an equal ratio of males to females, a polygynous mating system, and an inverse square probability function for dispersal. Individual genotypes were composed of 100 bi-allelic loci (99 neutral and 1 under selection), with a k -allele mutation rate of 2.5×10^{-8} per

nucleotide per generation, based on previous estimates of SNP (single nucleotide polymorphism) mutation rate (Nachman & Crowell 2000; Kondrashov 2002, and references therein) included in the genetic model.

We then generated differences in selection by portraying individual fitness as a function of the number of surviving offspring, which was determined by the interaction of the offspring's genotype and spatial patterns of selection. We created 3 simulated landscape surfaces based on different spatial configuration of directional genetic selection: (a) two adjacent discrete regions with selection present in one and absent in the other to represent spatially distinct environments, (b) a gradient with the strength of selection continuously distributed from north to south to represent a clinal environment, and (c) a uniform surface with no selection (Fig. 1). For the first 2 surfaces, we simulated both strong and weak selection. In surface "a", we represented strong selection by assigning a relative fitness value of 1.0 to the genotype *AA* in the northern region (selection "absent"), and a value of 0.5 in the southern (selection "present") region. Weak selection was simulated by assigning a relative fitness value of 1.0 for the *AA* genotype in the north, and 0.9 in the south. The genotypes *Aa* and *aa* had a uniform fitness value of 1.0. In the selection surface "b" (selection gradient), the relative fitness value of the *AA* genotype was 1.0 (for both strong and weak selection) at the northern end, and either 0.5 (strong) or 0.9 (weak) at the southern end, with intermediate values in between. The *Aa* and *aa* genotypes had a uniform fitness value of 1.0 for both strong selection weak selection throughout the study area (Fig. 1). Pixel size on the selection surface was 100 m².

We estimated N_e using the bias-corrected version of the linkage disequilibrium method with 95 % confidence intervals generated with a jack-knife approach (Waples & Do 2008; Do *et*

al. 2014). We determined pairwise population F_{ST} values (Weir and Cockerham 1984) using GENEPOP, and calculated number of migrants per generation using the following equation: $N_m = (1 - F_{ST}) / 4 F_{ST}$.

Genetic differentiation of adaptive vs. neutral markers

To measure the degree of divergence of adaptive and neutral alleles, we estimated genetic differentiation at each simulated generation using both a population-based and an individual-based approach. For the **population-based approach**, we used the estimator D_{est} (Jost 2008) to quantify genetic differentiation over time using only the 99 neutral loci, the 1 loci under selection, and all 100 loci. Although several genetic differentiation metrics are available, we selected D_{est} because it is not constrained by measures of within-population diversity as much as F_{ST} , it scales linearly, and the low mutation rates common in SNPs would have a negligible effect (Meirmans & Hedrick 2011).

For the **landscape genetics approach**, we used Mantel tests (Mantel 1967) to compare pairwise genetic distance of all individuals to geographic distance using only neutral loci, only adaptive loci, and all loci combined. Genetic distance among individuals was calculated using the proportion of shared alleles (Dps; Bowcock *et al.* 1994). For each scenario, selection strength, and selection surface we evaluated the 10 independent Monte Carlo replicates to assess variability in results. To calculate changes in genetic differentiation across time, we plotted the average D_{est} and average Mantel correlation coefficient r across 500 generations in 4 scenarios portraying combinations of genetic drift (population size) and dispersal distance (Table 2). We

compared resulting test statistics and assessed differences using the neutral r values as the null distribution.

Identifying loci under selection

We computed the type I and II error rates of population- and individual-based methods to correctly detect loci under selection in the simulated datasets. We used the simulated allele frequencies at generations 50 and 500 under 4 different scenarios (Table 2), with both strong and weak selection and using 3 selection surfaces (present/absent, latitudinal gradient, selection absent), averaged across the 10 Monte Carlo replicates, resulting in 400 tests of each population- and individual-based method. We enumerated how often each method falsely identified loci under selection in each of the surfaces (type I error). We also summarized the number of times outlier programs failed to detect the 1 locus under selection in each run and under each scenario (type II error).

Population-based methods. – We tested the ability of the frequentist $D_{\text{FDIST}} F_{\text{ST}}$ outlier approach (Beaumont & Nichols 1996) implemented in to detect loci under selection in the simulated dataset. Using LOSITAN (Antão *et al.* 2008), distributions of F_{ST} values were simulated from thousands of loci under neutral expectations and candidate loci were identified as falling outside of confidence intervals from a plot of average locus heterozygosity versus F_{ST} (Beaumont & Nichols 1996; Beaumont & Balding 2004). We conducted pairwise population comparisons using 500,000 replicates for the 100 loci under an infinite alleles mutation model. We tested for

237 outliers at both the 95 % and 99 % confidence interval levels and used a false discovery rate
238 (FDR) of 0.1.

239 We compared results to those produced using **BayeScan v.2.1** (Foll & Gaggiotti 2008),
240 which incorporates a Bayesian approach and directly estimates the posterior probability of a
241 locus being under selection by comparing global and population-specific allele frequencies
242 derived from F_{ST} coefficients. The probabilities of models including and excluding selection are
243 estimated separately through a reversible-jump Markov chain Monte Carlo algorithm. BayeScan
244 is based on a multinomial-Dirichlet distribution, and incorporates estimates of gene flow based
245 on detection of linkage disequilibrium among loci pairs. **BayeScan is considered to be more**
246 **realistic in describing ecological scenarios such as migration in comparison to the Beaumont &**
247 **Nichols (1996) method, which applies more simplified demographic models** (Beaumont 2005;
248 Foll & Gaggiotti 2008).

249 To identify loci under selection using BayeScan, we first performed 20 pilot runs of
250 50,000 iterations, then used 100,000 iterations on a sample size of 5,000 and thinning interval of
251 10, setting the prior odds value to 10. We used posterior probabilities establishing Log10 values
252 of the posterior odds > 1 and 2 (“strong” and “decisive” support [Jefferys 1961]) that the model
253 included loci under selection. This probability is not directly comparable to P values (Foll &
254 Gaggiotti, 2008), but does allow for using threshold values obtained from the FDR to calculate q
255 values, which are analogous to P values. We identified outlier loci as those with FDR q values $<$
256 0.05 and 0.01, meaning outlier loci are identified by values lower than the threshold, and are
257 expected to have 5 % and 1 % false positive rate, respectively.

Individual-based methods. – We tested the ability of the individual-based spatial analysis method *Samβada* (Joost *et al.* 2007) to estimate the probability of the presence of an allele given the sampling site environmental characteristics. *Samβada* tests for associations between all possible pairs of each environmental variable and each allele frequency at spatially referenced sample locations using logistic regressions. In our simulations, the environmental variable used as the response was the latitude at each individual’s location, corresponding to the north-south selection gradient, and selection surfaces with selection present in the north and absent in the south (Fig. 1). Models including and excluding the latitude selection surface were compared through examination of the significance of regression coefficients. Models were considered significant at the Bonferroni-corrected alpha levels of 0.05 and 0.01 if both likelihood ratio (G) and Wald tests indicated latitude was more informative than a model with a constant only (Joost *et al.* 2007). We also used generalized linear models (GLMs) to detect loci under selection identified as alleles correlated with environmental characteristics (latitude). Alleles for each locus were defined as “present” or absent”, and used as the response variable in the models. We identified loci as under selection when they were significant at 95% and 99% confidence levels (CIs) after a Bonferonni correction.

Results

Genetic differentiation of adaptive vs. neutral markers

Linkage disequilibrium-based estimates of N_e in local subpopulation ranged from approximately 10–1,741, and were higher in the scenarios with longer dispersal distances (Table 3). F_{ST}

estimates were highest in the low N_m , high drift (small N_e) scenarios, and lowest in the high N_m , low drift (large N_e) scenarios, as expected. Values of N_m calculated from F_{ST} were accordingly lowest in the small populations with highest genetic differentiation between the two populations.

Results from the population-based genetic distance estimator D_{est} revealed development of substantial differences between adaptive and neutral alleles over time in the selection present/absent surface, but little difference in the selection gradient (Fig. 2 and Appendix S2, Supporting Information). Differences were especially clear with strong selection present/absent (Fig. 1a) in the scenarios with short dispersal distances (5 % of maximum dispersal distance in the study area [9 km]; Fig. 2ai and iii). Differences between adaptive and neutral genetic structure developed rapidly with the adaptive locus reaching the maximum D_{est} value (0.88 for adaptive loci versus only 0.06 and 0.01 for neutral loci in the populations with both low and high genetic drift, respectively) by 200 generations, and remaining at this level throughout the rest of the 500 simulated generations.

The greatest differentiation occurred in the low dispersal, low drift scenario, as neutral structure did not develop over time (Fig. 2aiii), whereas in the low dispersal, high drift scenario neutral D_{est} values gradually increased to 0.1 by 500 generations (Fig. 2ai). In the scenarios with strong selection and high dispersal, D_{est} values increased at a similar rate initially to the low dispersal scenarios, but then declined slightly and plateaued after approximately 100 generations (Fig. 2aiv). Adaptive and neutral alleles also showed substantial differentiation where present/absent selection was weak and dispersal low, but developed at a slower rate reaching D_{est} = 0.62 and 0.57 at 500 generations for populations with high and low drift, respectively (Fig. 2bi and iii).

The adaptive locus and neutral loci both showed minimal differentiation in selection gradients as measured by the D_{est} estimator ($D_{est} \leq 0.25$). With weak selection, high drift, and low dispersal, D_{est} based on the adaptive locus rose more rapidly than D_{est} based on neutral loci and reached its highest genetic differentiation between marker types at approximately 300 generations (Appendix S2, Supporting Information). In scenarios with relatively low drift, slightly more genetic structure developed for both marker types within 100 generations, but both followed the same pattern. D_{est} values of the combined neutral and adaptive loci followed the same trend as the neutral loci in all selection surfaces and scenarios. In contrast, when selection was absent genetic structure did not develop except for in the low dispersal, high drift scenario reaching $D_{est} = 0.12$ after 500 generations (Appendix S3, Supporting Information).

Results from the landscape genetics approach measured by Mantel r indicated substantial development of neutral and adaptive genetic structure over time in both the selection present/absent and selection gradient surfaces, whereas the population-based approach only detected this pattern in the selection present/absent surface. Genetic structure was strongest and developed the quickest in scenarios with low dispersal in both selection surfaces. Adaptive differentiation between neutral and adaptive loci was most pronounced in the selection gradient surfaces with low dispersal, for both weak and strong selection. In this case, the genetic structure based on neutral and combined loci increased to $r = 0.7$ with both strong and weak selection after 500 generations, whereas the genetic structure based on the adaptive locus remained $r < 0.2$ for the same duration of time (Fig. 3 bi and biii).

322 *Identifying loci under selection*

323 Our results revealed that high dispersal ($N_m > 19$) and genetic drift ($N_e < 121$) can reduce the
324 ability of both population- and individual-based methods to detect loci under selection. All 4
325 methods had low type II error rates when selection was strong in present/absent scenarios, but
326 individual-based methods performed best in selection gradients, and in the present/absent surface
327 when selection was weak. Overall population-based methods had the lowest type I error rates,
328 and GLMs had the highest.

329 *Population-based methods.* – With strong selection in the present/absent surface, both
330 population-based methods (LOSITAN and BayeScan) rarely failed to reject the false null
331 hypothesis (failed to detect the adaptive locus, type II error; Table 4). With weak selection
332 however, LOSITAN failed to detect the adaptive locus in $\geq 90\%$ of the Monte Carlo replicates at
333 both confidence levels in all scenarios after 50 generations (Appendix S4, Supporting
334 Information). After 500 generations Type II error rates declined to 0% in the scenarios with
335 large populations, but remained high for the small populations (high drift) with both high and
336 low dispersal (Table 4). Bayescan failed to detect the adaptive locus in 60–100 % of the Monte
337 Carlo replicates in all scenarios after 50 generations. BayeScan also performed better after 500
338 generations in the large populations, but still failed to reject the false null hypothesis in $\geq 70\%$ of
339 the replicates in the scenarios with high drift (Table 4). Type I error rates were low (≤ 0.1) for
340 both methods at the 99 % CI levels, but high at the 95 % CI for the low dispersal, high drift
341 scenario with LOSITAN (Table 5).

Both population-based methods had high type II error rates in both the weak and strong selection gradient after 50 and 500 generations (Table 4, Appendix S4, Supporting Information). The exception was BayeScan, which detected the locus under selection in 100 % of the replicates after 50 generations when selection was strong, the population large, and dispersal low. Neutral markers were incorrectly identified as outliers with both population-based methods in all scenarios (Table 5). In particular LOSITAN identified between 1–5 false outliers in 10–60 % of the replicates at 95 % CI with selection both weak and strong and both the present/absent and environmental gradient selection surfaces. Type II error rate decrease when using 99 % CIs. The low dispersal, high drift scenario had the highest frequency of neutral loci identified as outliers by LOSITAN. When selection was absent, population-based methods rarely falsely detected loci under selection, except for LOSITAN when dispersal was low and drift high (error rate = 0.8; Appendix S4, Supporting Information).

Individual-based methods. – Both methods (GLMs and Samβada) consistently detected the adaptive locus in the present/absent surface with selection both weak and strong and at 50 and 500 generations (Table 4, Appendix S4, Supporting Information), with one exception. In the high dispersal, high drift scenario, both methods failed to reject the false null hypothesis (that an adaptive locus was present), but still type II error rates were low (0.1–0.2). When a selection gradient was present, error rate increased, especially in small populations with high dispersal, after 500 generations. Type II error rate also increased over time when the selection gradient was strong. Whereas both Samβada and GLMs had low type II error after 50 generations, both methods failed to detect the adaptive locus in any of the replicates with small populations after 500 generations (adaptive locus was not significantly correlated with latitude; Table 4). When

the selection gradient was weak, Samβada detected the adaptive locus more often than GLMs. Type I error rates (neutral markers were significantly correlated with latitude = false positive) were quite high for both classes of methods (Table 6, Appendix S1, Supporting Information). Type I error rates were high for both individual-based methods in all scenarios, but the highest when dispersal levels were low, and the lowest in the selection gradient when dispersal was high. When selection was absent, individual-based methods also performed poorly, especially GLMs which falsely identified neutral loci as under selection in 100 % of replicates in all scenarios (Appendix S1, Supporting Information). Overall, type I error rate increased over time, demonstrated by an escalation of false positives between 50 and 500 generations (Table 6, Appendix S1, Supporting Information). This was especially the case in scenarios with low dispersal distances regardless of selection surface or strength.

Discussion

We used simulations to gain understanding of the relationship between complex environments, demographic parameters, including population size and dispersal distances, and the development of adaptive genetic differentiation over time. Specifically, we wished to refine understanding of our capacity to quantify and avoid large type I and II error in outlier loci detection methods and correlative approaches between genetic and environmental variables.

We found that individual-based methods performed best in selection gradients and more often correctly identified gene-environment interactions, but that type I error rates were quite high, especially when dispersal levels were low and drift high. Our results suggest caution is

required in applying both population- and individual- based methods to detect molecular signals of local adaptation, and careful consideration of N_m , genetic drift, and patterns of IBD in the study population is recommended when interpreting results. A combination of methods and using corroborating results is the most conservative approach to identifying loci under selection; an even better approach is to additionally conduct simulations and combine with empirical data to improve inference.

Influence of dispersal, genetic drift and selection on adaptive differentiation

Our research expanded upon recent simulation studies by introducing a spatial component (Narum & Hess 2011; Villas *et al.* 2012; Lotterhos & Whitlock 2014; Forester *et al.* 2015; Lotterhos & Whitlock 2015) and by examining temporal trends in adaptive differentiation by simulating genetic exchange over 500 generations (DeMita *et al.* 2013; Jones *et al.* 2013). Our study is unique in varying population size to assess the effect of genetic drift on adaptive differentiation.

Our results revealed that the strength and rate of development of different genetic structure between neutral and adaptive loci was most highly influenced by low dispersal (e.g., 5 % of maximum dispersal distance in the study area [9 km]; $N_m = 1-2$), and low drift ($N_e \leq 475$). As predicted, we found different geographic distributions of adaptive versus neutral alleles in landscapes where natural selection results in reduced survival for particular genotypes, and these differences became more distinct over time. Furthermore, the differences in adaptive and neutral allele distribution was more distinct with strong selection, suggesting adaptive differentiation can

both occur over fine spatial scales and be detected given strong enough differences in individual survival.

Our simulation results confirm the importance of distinguishing between adaptive and neutral loci, and measuring genetic differentiation separately in studies of population structure. When measured together, the combined neutral and adaptive loci generally followed the same trend of genetic structure over time as the neutral loci assessed independently (Figs. 2, 3). Further, when neutral and adaptive loci were combined, there was little difference in the rate or strength of IBD development among the same scenarios comparing strong and weak selection, or the selection present/absent or gradient surfaces. Without testing for loci under selection and assessing genetic structure of adaptive and neutral loci separately, these patterns of genetic variation resulting from adaptation to different environmental conditions would not be detected. Significantly, identifying and removing adaptive loci improves inference of widely applied population genetics parameters such as F_{ST} and N_m by removing bias of loci that do not behave according to neutral expectations.

We also found differences in the ability of the population-based (D_{est}) and individual-based (Mantel r) tests to measure development in genetic structure given different selection surfaces. D_{est} was effective at detecting development of adaptive differentiation only in the selection present/absent surface, whereas Mantel r detected this pattern in both the selection present/absent and selection gradient surfaces. These results suggest that the individual-based methods for detecting structure commonly used in landscape genetic studies are most appropriate when a selection gradient is present, or when populations have a clinal genetic pattern. As

demonstrated with simulation modeling (Landguth & Schwartz 2014), matching the metric used to detect differentiation given continuous or clustered genetic patterns is a key consideration.

Power and limitations of methods

We predicted that selection would be stronger in large populations with shorter simulated dispersal distances, and therefore adaptive loci would be more readily detectable. We further expected that outlier loci detection methods would have corresponding lower type I and II error rates under these scenarios. We found that the performance of both population- and individual-based methods to detect loci under selection (type II error) was in fact improved with low dispersal and low genetic drift (large populations), but that type I error (falsely identifying neutral loci as under selection) was highest using individual-based methods under all scenarios, in particular low dispersal and high drift.

The efficacy of natural selection is predicted as the product of the effective population size and the strength of selection ($N_e * s$), and selection is expected to be less effective than genetic drift in changing alleles frequencies when the value of this equation is < 1 (Li 1978). The low dispersal, high drift scenario with weak selection ($s = 0.01$) produced a value < 1 (0.15 in the present/absent selection surface, and 0.25 in the selection gradient surface); all the other scenarios were > 1 . We would therefore expect that the high type I error rates produced by the individual-based methods in this scenario are a result of drift overpowering selection in small populations. These results suggest that caution is required when interpreting results of outlier and correlative tests for adaptive differentiation, especially in small and isolated populations where genetic drift exerts a greater influence than natural selection.

We were able to directly compare population- and individual-based methods in our analyses by examining 2 simulated populations separated by a partial barrier. In addition to dispersal and drift, differences in rates of type II error (false negatives) between the methods were influenced by the strength of selection and the selection surfaces. When environments were distinct (present versus absent selection), and selection was strong, both classes of methods performed well, whereas with weak selection, only the individual-based methods consistently detected the locus under selection. In contrast, type II error was very high (0.7–1.0) in the selection gradient surface when using population-based methods but generally low for individual-based methods (with the exception of in populations with high dispersal and high drift). DeMita *et al.* (2013) also compared individual- and population-based methods and found that the former was more powerful in detecting adaptive loci in a selection gradient. These results suggest that in selection gradients, individual-based methods most often correctly identify gene-environment interactions, especially with large populations and low dispersal (type II error rate 0–0.3) in comparison to population-based methods (type II error rate 0.7–1.0; Table 4).

Jones *et al.* (2013) found that individual-based methods produced few type I errors (0.0–0.2) within a selection gradient surface when selection varied from weak to strong (e.g., $s = 0.01$ –0.5). Low type I error rates were attributed to high dispersal in all of the simulated populations (equivalent to 25% maximum dispersal distance of their simulated study area) which resulted in minimal development of IBD patterns. Our analyses varied dispersal distances and thus expanded upon quantification of error rates using individual-based methods, and demonstrated generally high type I error rates (0.3–1, Appendix S1, Supporting Information, Table 6). Samβada performed better than GLMs for detecting selection in all scenarios, but still

produced high type I error rates between 0.7–1.0 except for the scenario with high dispersal (50% of landscape) and high drift (Appendix S1, Supporting Information). The high dispersal, high drift error rate (0.3) fell within the range of values of error rates reported by Jones *et al.* (0.1–0.4) reflecting similar dispersal distances relative to the scale of the simulated landscape.

These results corroborate that individual-based approaches may have a relatively low type I error rate (falsely identifying neutral loci as significantly correlated with environment) given comparatively high dispersal. However, our results support the conclusion of DeMita *et al.* (2013) that population-based methods have lower type I error rates in comparison to individual-based approaches and are particularly effective in detecting adaptive variation when selection is strong. Furthermore, our investigation into the effects of varying dispersal distances revealed limitations to the application of individual-based methods given low dispersal.

Our results indicate that dispersal influenced development of an IBD pattern in neutral and adaptive loci over time. Over the 500 simulated generations, IBD developed most quickly in the scenarios with the shortest dispersal distances, and had a diminishing rate of increase and lower resulting D_{est} and Mantel r values at generation 500 with longer dispersal distances. Spatial autocorrelation of genetic variation can arise in continuously distributed populations from limited dispersal, mating patterns, and other demographic processes. These spatially structured processes can be difficult to distinguish from spatial patterns of natural selection, and demonstrates a drawback of population genetics statistical methods that do not account for spatial autocorrelation (Neel *et al.* 2013).

Dispersal patterns leading to IBD can generate spurious correlations of selection gradients and allele frequencies, resulting in high type I error (De Mita *et al.* 2013). Our work supports this conclusion, as well as evidence from other research that a strong pattern of IBD can reduce the effectiveness of landscape genetic methods to detect loci affected by selection (Meirmans 2012; Jones *et al.* 2013; Lotterhos & Whitlock 2014; Forester *et al.* 2015) and produce high levels of false positives. We concur that these methods may be most applicable to situations where strong population structure is not present, such as in populations with clinal genetic patterns. However, in our analysis overall type I error rates were lower using population-based methods than with individual-based methods particularly when populations were large and dispersal distances short. Thus, we suggest choice of methods based on assessment of N_m , genetic drift, and patterns of IBD in the study population. In most situations it would be most appropriate to use both approaches for conservative detection of both false positives and false negatives (De Mita *et al.* 2013; Jones *et al.* 2013; Forester *et al.* 2015).

Conclusions

Our research provides insights into molecular adaptation and detection of natural selection. We performed our analyses in a spatially-explicit modelling scenario using realistic population structure, which provided richer insights into the influences of spatial genetic structure on adaptive divergence than simplistic ideal models (e.g., Wright-Fisher). Thus, this work advances knowledge about evolutionary mechanisms influencing adaptive differentiation. In this context we found individual-based methods performed well in selection gradients

510 suggesting these methods are more appropriate for continuously distributed populations.
511 However, considering the effects of spatial autocorrelation of genetic variation, our results
512 suggest some limitations in the capacity of these increasingly applied statistical methods to
513 detect molecular signatures of local adaptation, and therefore should be used with a great deal of
514 caution and in combination with other methods. Identification of correlations between candidate
515 loci and environmental variables does not necessarily indicate causation, as these patterns may
516 be spurious and moreover do not elucidate the actual mechanisms of selection. However,
517 identification of gene-environment correlations can inform further research into genes associated
518 with specific traits that may influence fitness (Hansen *et al.* 2012).

519 By improving our ability to detect and model the distribution and presence of adaptive
520 alleles across complex landscapes, this work should help transform population genetics by
521 facilitating discovery and use of adaptive genomic information (Allendorf *et al.* 2010), or
522 accurately exclude adaptive loci when only neutral loci should be used, e.g. for estimation of
523 parameters like N_e , N_m , and F_{ST} (Luikart *et al.* 2003). Our results and modeling provide guidance
524 for designing and conducting both genomic research and species monitoring or conservation
525 programs. For example, identification of patterns of adaptation is critical in designing predictive
526 models of a species' ability to persist, shift its geographic distribution (Allendorf *et al.* 2010;
527 Manel *et al.* 2010; Hoffmann & Sgrò 2011), and to maintain connectivity among populations
528 (Fortin & Dale 2005; Foll & Gaggiotti 2006) despite changes in climate patterns (Kovach *et al.*
529 2015).

530 The use of adaptive genetic markers is a promising approach for defining management
531 units and evolutionary significant units (Allendorf *et al.* 1997; Fraser & Bernatchez 2001; Funk

et al. 2012), and would provide useful insight to the appropriate scale for current and long-term population management and monitoring strategies. Further, discovery of locally-adapted populations may inform management actions and prioritize policy decisions so that the evolutionary processes themselves may be targeted for conservation (Funk *et al.* 2012; Limborg *et al.* 2012). However, we caution that verification that a genetic marker is adaptive (or linked to adaptive loci) can require detection of the same outlier behavior in multiple independent populations and/or experiments (Allendorf *et al.* 2013). We hope this work sparks additional research and understanding of natural selection in spatially heterogeneous environments by providing insights into factors that influence adaptive vs. neutral population genetic structure and appropriate methods to study them.

Acknowledgements

We thank B. Forester and B. Hand for analytical assistance and D. Whited and Allen Warren for computational support. L. Adams, S. Dobrowski, and M. Hebblewhite provided useful comments on early drafts of the manuscript. This work was supported by the U.S. National Science Foundation grant 1407300. G.R. was supported by the U.S. Geological Survey and 3 University of Montana scholarships (Bertha Morton, Les Pengelly, and George and Mildred Cirica). G.L. was supported by NSF grants DEB 1258203 and 1067613.

References

Allendorf FW, Bayles D, Bottom DL, Currens KP, Frissell CA, Hankin D, Lichatowich JA, Nehlsen W, Trotter PC, Williams TH (1997) Prioritizing Pacific salmon stocks for conservation. *Conservation Biology*, **11**, 140–152.

- 554 Allendorf FW, Hohenlohe PA., Luikart G (2010) Genomics and the future of conservation
555 genetics. *Nature Reviews Genetics*, **11**, 697–709.
- 556 Allendorf FW, Luikart G, Aitken SN (2013) Conservation and the genetics of populations. 2nd
557 ed. Oxford (UK): Wiley-Blackwell.
- 558 Antão T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: a workbench to
559 detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics*, **9**, 323.
- 560 Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population
561 structure. *Proceedings of the Royal Society B: Biological Sciences*, **263**, 1619–1626.
- 562 Beaumont MA., Balding DJ (2004) Identifying adaptive genetic divergence among populations
563 from genome scans. *Molecular Ecology*, **13**, 969–980.
- 564 Beaumont MA (2005) Adaptation and speciation: what can F(st) tell us? *Trends in Ecology &*
565 *Evolution*, **20**, 435–40.
- 566 Bowcock AM, Ruiz-Linares A, Tomfohrde J *et al.* (1994) High resolution of human evolutionary
567 trees with polymorphic microsatellites. *Letters to Nature*, **368**, 455–457.
- 568 Charbonnel N, Pemberton J (2005) A long-term genetic survey of an ungulate population reveals
569 balancing selection acting on MHC through spatial and temporal fluctuations in selection.
570 *Heredity*, **95**, 377–88.
- 571 Cheviron ZA, Natarajan C, Projecto-Garcia J *et al.* (2014) Integrating Evolutionary and
572 Functional Tests of Adaptive Hypotheses : A Case Study of Altitudinal Differentiation in
573 Hemoglobin Function in an Andean Sparrow, *Zonotrichia capensis*. *Molecular Biology*
574 *and Evolution*, **31**, 2948–2962.
- 575 Coop G, Pickrell JK, Novembre J *et al.* (2009) The role of geography in human adaptation. *PLoS*
576 *Genetics*, **5**, e1000500.
- 577 Crow JF, Kimura M (1970) An introduction to population genetics theory. New York: Harper
578 and Row.
- 579 De Mita S, Thuillet A-C, Gay L *et al.* (2013) Detecting selection along environmental gradients:
580 analysis of eight methods and their effectiveness for outbreeding and selfing populations.
581 *Molecular Ecology*, **22**, 1383–1399.
- 582 Do C, Waples RS, Peel D *et al.* (2014) NeEstimator v2: Re-implementation of software for the
583 estimation of contemporary effective population size (N_e) from genetic data. *Molecular*
584 *Ecology Resources*, **14**, 209–214.
- 585 Epperson BK, McRae BH, Scribner K *et al.* (2010) Utility of computer simulations in landscape
586 genetics. *Molecular Ecology*, **19**, 3549–3564.

- 587 Foll M, Gaggiotti O (2006) Identifying the environmental factors that determine the genetic
588 structure of populations. *Genetics*, **174**, 875–91.
- 589 Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both
590 dominant and codominant markers: a Bayesian perspective. *Genetics*, **180**, 977–93.
- 591 Forester BR, Jones MR, Joost S, Landguth EL, Lasky JR (2015) Detecting spatial genetic
592 signatures of local adaptation in heterogeneous landscapes. *Molecular Ecology*.
- 593 Fortin, M-J, Dale, MRT (2005) Spatial Analysis. Cambridge University Press, Cambridge.
- 594 Franks SJ, Sim S, Weis AE (2007) Rapid evolution of flowering time by an annual plant in
595 response to a climate fluctuation. *Proceedings of the National Academy of Sciences*, **104**,
596 1278–1282.
- 597 Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept
598 for defining conservation units. *Molecular Ecology*, **10**, 2741–52.
- 599 Fournier-Level A, Korte A, Cooper MD *et al.* (2011) A Map of local adaptation in *Arabidopsis*
600 *thaliana*. *Science*, 86–89.
- 601 Funk WC, McKay JK, Hohenlohe PA, Allendorf FW (2012) Harnessing genomics for
602 delineating conservation units. *Trends in Ecology & Evolution*, **27**, 489–496.
- 603 Hancock AM, Witonsky DB, Gordon AS *et al.* (2008) Adaptations to climate in candidate genes
604 for common metabolic disorders. *PLoS Genetics*, **4**, e32.
- 605 Hancock AM, Jarymowycz LB, Sperone FG *et al.* (2011) Adaptation to climate across the
606 *Arabidopsis thaliana* genome. *Science*, **334**, 83–86.
- 607 Hand BK, Lowe WH, Kovach RP, Muhlfeld CC, Luikart G (2015) Landscape community
608 genomics : understanding eco-evolutionary processes in complex environments. *Trends*
609 *in Ecology & Evolution*, **30**, 161–168.
- 610 Hansen MM, Olivieri I, Waller DM, Nielsen EE (2012) Monitoring adaptive genetic responses to
611 environmental change. *Molecular Ecology*, **21**, 1311–1329.
- 612 Hedrick PW (2005) A standardized genetic differentiation measure. *Evolution*, **59**, 1633–8.
- 613 Hoban S, Bertorelle G, Gaggiotti OE (2011) Computer simulations: tools for population and
614 evolutionary genetics. *Nature reviews. Genetics*, **13**, 110–22.
- 615 Hohenlohe PA, Bassham S, Etter PD *et al.* (2010) Population genomics of parallel adaptation in
616 threespine stickleback using sequenced RAD tags. *PLoS genetics*, **6**, e1000862.
- 617 Hoffmann AA, Sgrò CM (2011) Climate change and evolutionary adaptation. *Nature*, **470**, 479–
618 85.
- 619 Holderegger R, Wagner HH (2008) Landscape Genetics. *BioScience*, **58**, 199–207.

- 620 Jeffreys H (1961) Theory of Probability, 3rd edn. Oxford University Press, London, p. 432.
- 621 Jones MR, Forester BR, Teufel AI *et al.* (2013) Integrating landscape genomics and spatially
622 explicit approaches to detect loci under selection in clinal populations. *Evolution*, **67**,
623 3455–68.
- 624 Joost S, Bonin A, Bruford MW *et al.* (2007) A spatial analysis method (SAM) to detect
625 candidate loci for selection: towards a landscape genomics approach to adaptation.
626 *Molecular Ecology*, **16**, 3955–69.
- 627 Jost L (2008) GST and its relatives do not measure differentiation. *Molecular Ecology*, **17**,
628 4015–4026.
- 629 Kohn MH, Pelz H-J, Wayne RK (2003) Locus-specific genetic differentiation at Rw among
630 warfarin-resistant rat (*Rattus norvegicus*) populations. *Genetics*, **164**, 1055–70.
- 631 Kovach RP, Muhlfeld CC, Wade AA, Hand BK, Whited DC, DeHaan PW, Al-Chokhachy R,
632 Luikart G (2015) Genetic diversity is related to climatic variation and vulnerability in
633 threatened bull trout. *Global Change Biology*, **21**, 2510–2524.
- 634 Landguth EL, Cushman SA (2010) cdpop: A spatially explicit cost distance population genetics
635 program. *Molecular Ecology Resources*, **10**, 156–61.
- 636 Landguth EL, Cushman SA, Murphy MA, Luikart G (2010) Relationships between migration
637 rates and landscape resistance assessed using individual-based simulations. *Molecular*
638 *Ecology Resources*, **10**, 854–62.
- 639 Landguth EL, Cushman SA, Johnson NA (2012) Simulating natural selection in landscape
640 genetics. *Molecular Ecology Resources*, **12**, 363–368.
- 641 Landguth EL, Balkenhol N (2012) Relative sensitivity of neutral versus adaptive genetic data for
642 assessing population differentiation. *Conservation Genetics*, **13**, 1421–1426.
- 643 Landguth EL, Schwartz MK (2014) Evaluating sample allocation and effort in detecting
644 population differentiation for discrete and continuously distributed individuals.
645 *Conservation Genetics*, **15**, 981–992.
- 646 Lenormand T (2002) Gene flow and the limits to natural selection. *Trends in Ecology &*
647 *Evolution*, **17**, 183–189.
- 648 Lewontin RC, Krakauer J (1973) Distribution of gene frequency as a test of the theory of the
649 selective neutrality of polymorphisms. *Genetics*, **74**, 175–195.
- 650 Li, W.H. (1978) Maintenance of genetic variability under joint effect of mutation, selection and
651 random drift. *Genetics*, **90**, 349–382.

- 652 Limborg MT, Helyar SJ, De Bruyn M *et al.* (2012) Environmental selection on transcriptome-
653 derived SNPs in a high gene flow marine fish, the Atlantic herring (*Clupea harengus*).
654 *Molecular Ecology*, **21**, 3686–703.
- 655 Lotterhos KE, Whitlock MC (2014) Evaluation of demographic history and neutral
656 parameterization on the performance of FST outlier tests. *Molecular Ecology*, **23**, 2178–
657 2192.
- 658 Lotterhos KE, Whitlock MC (2015) The relative power of genome scans to detect local
659 adaptation depends on sampling design and statistical method. *Molecular Ecology*, **24**,
660 1031–1046.
- 661 Lowe WH, Allendorf FW (2010) What can genetics tell us about population connectivity?
662 *Molecular Ecology*, **19**, 3038–3051.
- 663 Luikart G, England PR, Tallmon D, Jordan S, Taberlet P (2003) The power and promise of
664 population genomics: from genotyping to genome typing. *Nature Reviews Genetics*, **4**,
665 981–994.
- 666 Kondrashov AS (2003) Direct estimates of human per nucleotide mutation rates at 20 loci
667 causing Mendelian diseases. *Human Mutation*, **21**, 12–27.
- 668 Kovach RP, Muhlfeld CC, Wade AA, Hand BK, Whited DC, DeHaan PW, Al-Chokhachy R,
669 Luikart G (2015) Genetic diversity is related to climatic variation and vulnerability in
670 threatened bull trout. *Global Change Biology*. doi: 10.1111/gcb.12850
- 671 McKay JK, Latta RG (2002) Adaptive population divergence: markers, QTL and traits. *Trends in*
672 *Ecology and Evolution*, **17**, 285–291.
- 673 Manel S, Joost S, Epperson BK *et al.* (2010) Perspectives on the use of landscape genetics to
674 detect genetic adaptive variation in the field. *Molecular Ecology*, **19**, 3760–72.
- 675 Mantel N (1967) The detection of disease clustering and a generalized regression approach.
676 *Cancer Research*, **27**, 209– 220.
- 677 Matala AP, Ackerman MW, Campbell MR, Narum SR (2014) Relative contributions of neutral
678 and non-neutral genetic differentiation to inform conservation of steelhead trout across
679 highly variable landscapes. *Evolutionary Applications*, **7**, 682–701.
- 680 Meirmans PG, Hedrick PW (2011) Assessing population structure: F_{ST} and related measures.
681 *Molecular Ecology Resources*, **11**, 5–18.
- 682 Meirmans PG (2012) The trouble with isolation by distance. *Molecular Ecology*, **21**, 2839–46.
- 683 Nachman MW, Crowell SL (2000) Estimate of the mutation rate per nucleotide in humans.
684 *Genetics*, **156**, 297–304.

- 685 Narum SR, Campbell NR, Kozfkay CC, Meyer K a (2010) Adaptation of redband trout in desert
686 and montane environments. *Molecular Ecology*, **19**, 4622–37.
- 687 Narum SR, Hess JE (2011) Comparison of F_{ST} outlier tests for SNP loci under selection.
688 *Molecular Ecology Resources*, **11**(s.1), 184–194.
- 689 Neale DB, Kremer A (2011) Forest tree genomics: growing resources and applications. *Nature*
690 *reviews. Genetics*, **12**, 111–122.
- 691 Neel MC, McKelvey K, Ryman N *et al.* (2013) Estimation of effective population size in
692 continuously distributed populations: there goes the neighborhood. *Heredity*, **111**, 189–
693 99.
- 694 Prugnolle F, Manica A, Balloux F (2005) Geography predicts neutral genetic diversity of human
695 populations. *Current Biology: CB*, **15**, R159–60.
- 696 Rieseberg, L.H., Burke, J.M. 2001. The biological reality of species: gene flow, selection, and
697 collective evolution. *Taxon*, **50**, 47–67.
- 698 Savolainen O, Lascoux M, Merilä J (2013) Ecological genomics of local adaptation. *Nature*
699 *Reviews Genetics*, **14**, 807–820.
- 700 Schoville SD, Bonin A, François O *et al.* (2012) Adaptive genetic variation on the landscape:
701 methods and cases. *Annual Review of Ecology, Evolution, and Systematics*, **43**, 23–43.
- 702 Schwartz MK, McKelvey KS (2009) Why sampling scheme matters: the effect of sampling
703 scheme on landscape genetic results. *Conservation Genetics*, **10**, 441–452.
- 704 Storz JF (2005) Using genome scans of DNA polymorphism to infer adaptive population
705 divergence. *Molecular Ecology*, **14**, 671–688.
- 706 Thibert-Plante X, Hendry AP (2010) When can ecological speciation be detected with neutral
707 loci? *Molecular Ecology*, **19**, 2301–14.
- 708 Vilas A, Pérez-Figueroa A, Caballero A (2012) A simulation study on the performance of
709 differentiation-based methods to detect selected loci using linked neutral markers.
710 *Journal of Evolutionary Biology*, **25**, 1364–76.
- 711 Waples RS (1998) Separating the wheat from the chaff: Patterns of genetic differentiation in high
712 gene flow species. *Journal of Heredity*, **89**, 438–450.
- 713 Waples RS, Do C (2008) Ldne: a Program for Estimating Effective Population Size From Data
714 on Linkage Disequilibrium. *Molecular Ecology Resources*, **8**, 753–756.
- 715 Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure.
716 *Evolution* **38**:1358–1370.
- 717 Yang W-Y, Novembre J, Eskin E, Halperin E (2012) A model-based approach for analysis of
718 spatial structure in genetic data. *Nature Genetics*, **44**, 725–31.

Zardi GI, Nicastro KR, Canovas F *et al.* (2011) Adaptive traits are maintained on steep selective gradients despite gene flow and hybridization in the intertidal zone. *PloS One*, **6**, e19402.

Author Contributions

G.H.R., E.L.L., M.K.S. and G.L. designed the study. G.H.R. and E.L.L. ran the simulations. G.H.R. performed the analyses, interpreted the data, and wrote the manuscript with input from all authors.

Data accessibility

Simulation data, landscape surfaces, and output files are available at Dryad doi:XXX. CDPOP software and user manual are available at <http://cel.dbs.umt.edu/cms/CDPOP>.

Table 1. Summary of factors we varied in simulated populations. For each population size (used to approximate genetic drift), different combinations of dispersal distance, and strength of selection, and selection surface were simulated.

Population size	Dispersal distance [*]	Strength of selection	Selection surface
1,000	5 %	Strong ($s = 0.5$) [†]	Present/absent [§]
10,000	50 %	Weak ($s = 0.1$) [‡]	Latitudinal gradient
		Absent	Uniform [¶]

^{*} Percentage of maximum possible dispersal distance (i.e., length the entire study area).
[†] 0.5 selection coefficient ($s = 1$ - relative fitness genotype AA). Aa and aa = uniform fitness value of 1.0.
[‡] 0.1 selection coefficient ($s = 1$ - relative fitness genotype AA). Aa and aa genotypes = uniform fitness value of 1.0.
[§] Two adjacent discrete regions with selection present in one and absent in the other.
[¶] A uniform surface with no selection to test for type I statistical errors.

Table 2. Scenarios used to quantify type I and II error rates for population- and individual-based tests to detect loci under selection. Scenarios were tested with both strong and weak selection (Table 1) and using 3 selection surfaces (present/absent, latitudinal gradient, selection absent uniformly).

	low N_m *, high drift	low N_m , low drift	high N_m , high drift	high N_m , low drift
population size	1,000	10,000	1,000	10,000
maximum % dispersal distance [†]	5 %	5 %	50 %	50 %

* N_m = effective population size (N_e) * rate of dispersal among populations; estimated through dispersal distance parameter (Table 1).

[†]Percent of the length of the entire study area.

Table 3. Estimates of effective population size (N_e) using the linkage disequilibrium method, population differentiation (F_{ST}), and number of migrants per generation (N_m) for each of the 4 simulation scenarios, for strong and weak directional selection ($s = 0.5$ and 0.05 , respectively), in the selection present/absent and gradient surfaces.

a.) Present/Absent					
Selection strength	Estimated Metric	low N_m , high drift	low N_m , low drift	high N_m , high drift	high N_m , low drift
weak	N_e	15.25	475.20	126.90	1572.40
	F_{ST}	0.108	0.027	0.013	0.001
	N_m	2.06	8.26	19.45	249.00
strong	N_e	10.25	407.15	127.50	1549.60
	F_{ST}	0.162	0.012	0.014	0.001
	N_m	1.29	20.58	17.61	249.70
b.) Gradient					
Selection strength	Metric	low N_m , high drift	low N_m , low drift	high N_m , high drift	high N_m , low drift
weak	N_e	25.20	456.00	136.85	1741.85
	F_{ST}	0.143	0.015	0.110	0.002
	N_m	1.50	15.67	22.42	124.00
strong	N_e	13.00	394.20	121.20	1490.20
	F_{ST}	0.131	0.014	0.013	0.001
	N_m	1.66	17.61	18.88	249.75

Table 4. Type II error rates of population- and individual-based methods to detect loci under selection after 500 generations, and the proportion of replicates where this occurred at the 95% and 99% confidence interval levels (LOSITAN, GLM, Samβada) and with FDR q value thresholds < 0.05 and 0.01 (BayeScan), which are analogues of *P*-values (displayed under 95% and 99% columns). Type II error rates (false negatives) are shown for a.) selection present/absent surfaces, and b.) gradient surfaces, for both weak and strong selection scenarios.

a.)		Present/Absent							
Selection strength	Method	low N_m , high drift		low N_m , low drift		high N_m , high drift		high N_m , low drift	
	CIs	95%	99%	95%	99%	95%	99%	95%	99%
weak	LOSITAN	0.2	0.4	0	0	1	1	0	0
	BayeScan	0.7	0.6	0.1	0.1	1	1	0.3	0.3
	Samβada	0	0	0	0	0.2	0.2	0	0
	GLM	0	0	0	0	0.1	0.1	0	0
strong	LOSITAN	0	0	0	0	0	0	0	0
	BayeScan	0	0	0.2	0.2	0	0	0	0
	Samβada	0	0	0	0	0.2	0.2	0	0
	GLM	0	0	0	0	0	0	0	0
b.)		Gradient							
Selection strength	Method	low N_m , high drift		low N_m , low drift		high N_m , high drift		high N_m , low drift	
	CIs	95%	99%	95%	99%	95%	99%	95%	99%
weak	LOSITAN	0.8	0.9	1	1	0.8	0.8	1	1
	BayeScan	1	1	1	1	1	1	1	1
	Samβada	0	0	0	0	0.5	0.5	0	0
	GLM	0.2	0.2	0	0	0.7	0.7	0	0
strong	LOSITAN	0.8	0.7	1	1	0.9	0.9	1	1
	BayeScan	1	1	1	1	1	1	1	1
	Samβada	0.3	0.3	0.3	0.3	1	1	0	0
	GLM	1	1	0	0	1	1	0.4	0.4

Table 5. Type I error rates of population-based methods defined as the number of neutral loci (1 - 6) identified as outliers using LOSTIAN and BayeScan, and the proportion of replicates where this occurred at the 95% and 99% confidence interval levels (LOSTIAN) and with FDR q value thresholds < 0.05 and 0.01 (BayeScan), which are analogues of *P*-values (displayed under 95% and 99% columns). The expected number of outlier loci for 95% and 99% is 5 and 1, respectively (with 100 simulated loci).

		Present/absent								Gradient								
	Number of loci	low N_m , high drift		low N_m , low drift		high N_m , high drift		high N_m , low drift		Number of loci	low N_m , high drift		low N_m , low drift		high N_m , high drift		high N_m , low drift	
		95 %	99 %	95 %	99 %	95 %	99 %	95 %	99 %		95 %	99 %	95 %	99 %	95 %	99 %	95 %	99 %
weak selection	LOSITAN										LOSITAN							
	1	0.6	0.0	0.3	0.0	0.0	0.0	0.0	0.0	1	0.3	0.3	0.3	0.0	0.2	0.0	0.0	0.0
	2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	2	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	>4	0.1*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	>4	0.3 [§]	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	BayeScan										BayeScan							
	1	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
strong selection	LOSITAN										LOSITAN							
	1	0.3	0.3	0.1	0.0	0.2	0.1	0.0	0.0	1	0.2	0.1	0.2	0.0	0.1	0.1	0.0	0.0
	2	0.2	0.2	0.0	0.0	0.1	0.0	0.0	0.0	2	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3	0.2	0.0	0.0	0.0	0.1	0.0	0.0	0.0
	>4	0.3 [†]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	BayeScan										BayeScan							
	1	0.2	0.0	0.0	0.0	0.1	0.0	0.0	0.0	1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*One Monte Carlo replicate detected 5 false positive loci.
† Two Monte Carlo replicates detected 4, and one detected 6 false positive loci.

[§] Two Monte Carlo replicates detected 4, and one detected 5 false positive loci.

For Review Only

Table 6. Type I error rates (false positives) of individual-based methods (Samβada and GLMs) defined as the mean number of neutral loci significantly correlated with the selection gradient (latitude) at the 95% and 99% confidence interval levels after 500 generations. The expected number of significantly correlated loci for 95% and 99% is 5 and 1, respectively (with 100 simulated loci). Type I error rates are shown for a.) selection present/absent surfaces, and b.) gradient surfaces, for both weak and strong selection scenarios (where one locus was under selection).

a.) Present/absent									
Selection strength	Method	low N_m , high drift		low N_m , low drift		high N_m , high drift		high N_m , low drift	
	CIs	95%	99%	95%	99%	95%	99%	95%	99%
weak	Samβada	56	52	48	45	19	14	28	22
	GLM	57	54	49	47	18	14	30	25
strong	Samβada	57	54	50	47	16	13	21	17
	GLM	59	56	52	49	18	15	22	19
b.) Gradient									
Selection strength	Method	low N_m , high drift		low N_m , low drift		high N_m , high drift		high N_m , low drift	
	CIs	95%	99%	95%	99%	95%	99%	95%	99%
weak	Samβada	43	40	49	47	16	13	28	24
	GLM	58	54	50	48	18	15	30	26
strong	Samβada	56	53	50	47	14	10	26	23
	GLM	58	54	51	49	15	12	29	25

Figure 1. Simulated 200 x 300 km landscape with 1,000 individuals (black filled dots). In the selection present/absent scenario (a), individuals with the AA genotype have a relative fitness value of 1.0 in the light grey area, and 0.5 (strong selection), or 0.1 (weaker selection) in the dark grey area, and all other genotypes have a relative fitness value of 1.0. In the gradient scenario (b) individuals with the AA genotype have a relative fitness value of 1.0 on the northern (upper) end of the landscape (white), and linearly decreasing values to 0.5 (strong) or 0.1 (weak) at the lower end (dark grey). Scenario c.) represents a uniform surface with no selection. The Aa and aa genotypes have a uniform relative fitness value of 1.0 for both strong selection weak selection in scenarios a.), b.), and c.).

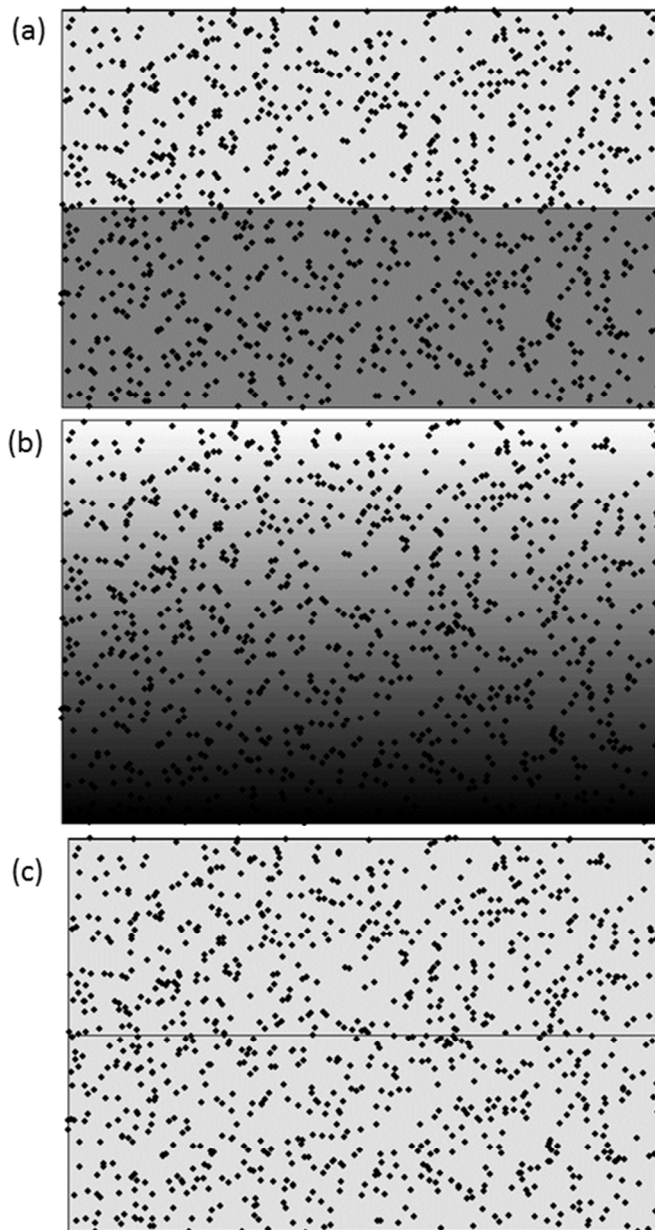
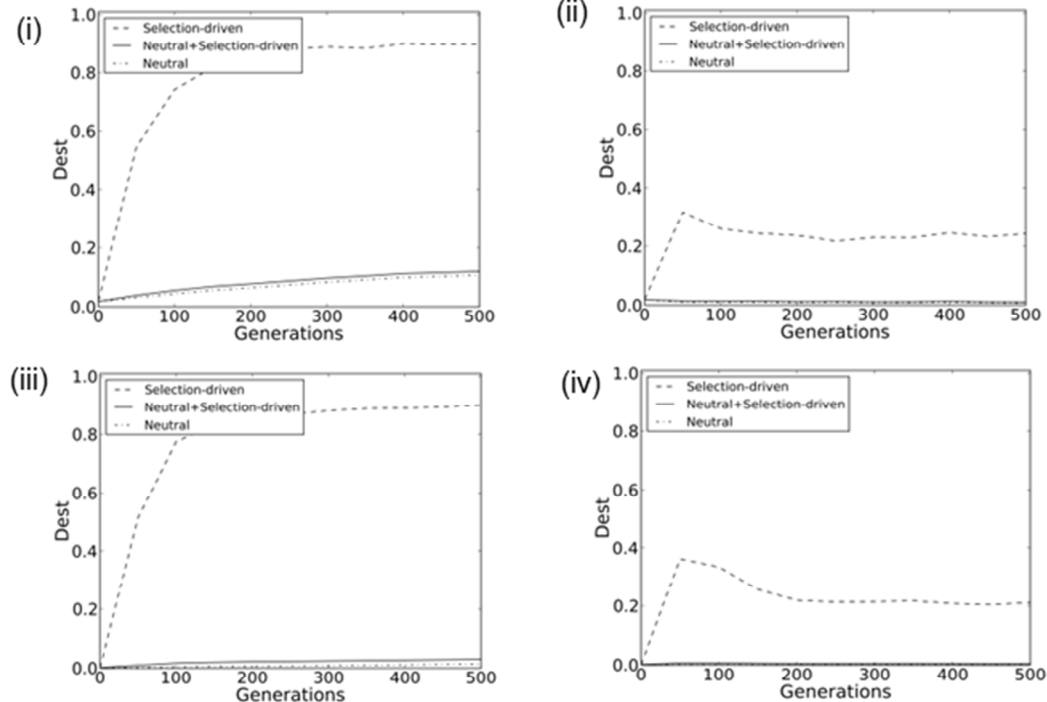


Figure 2. D_{est} results depicting genetic differentiation for adaptive (selection-driven), neutral and combined loci. The present/absent selection surface is shown with selection strong (a) and weak (b), for the i) low N_m , high drift, ii) high N_m , high drift, iii) low N_m , low drift, iv) high N_m , low drift scenarios. See Tables 5.1 and 5.2 for descriptions.

(a) Present/absent, strong selection



(b) Present/absent, weak selection

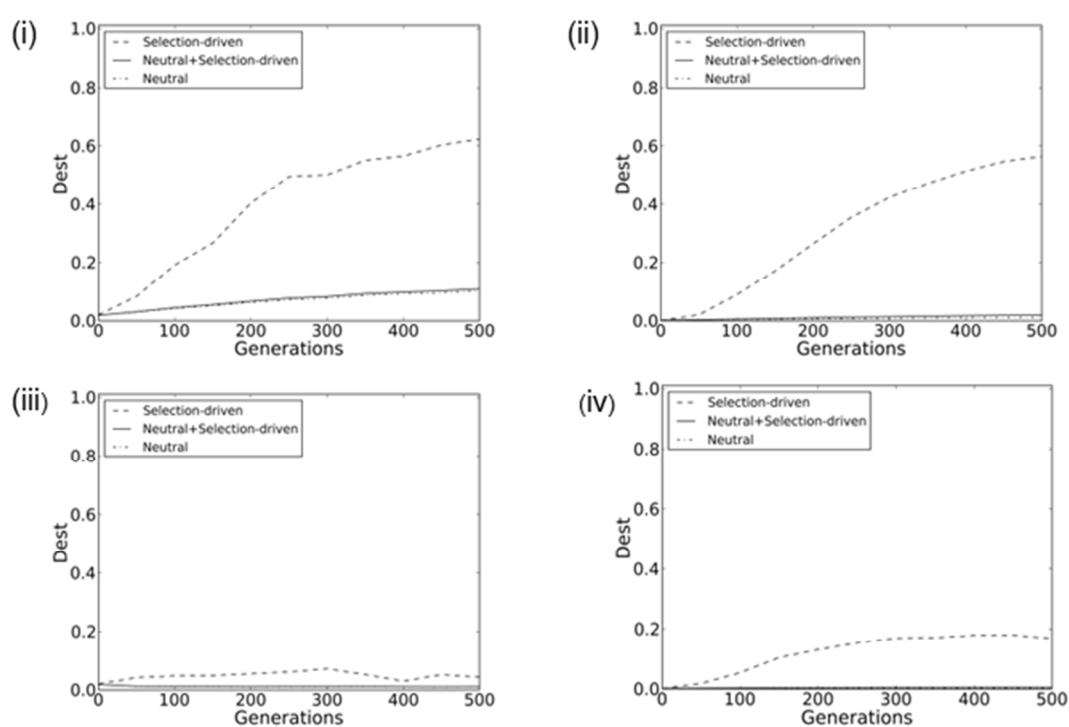
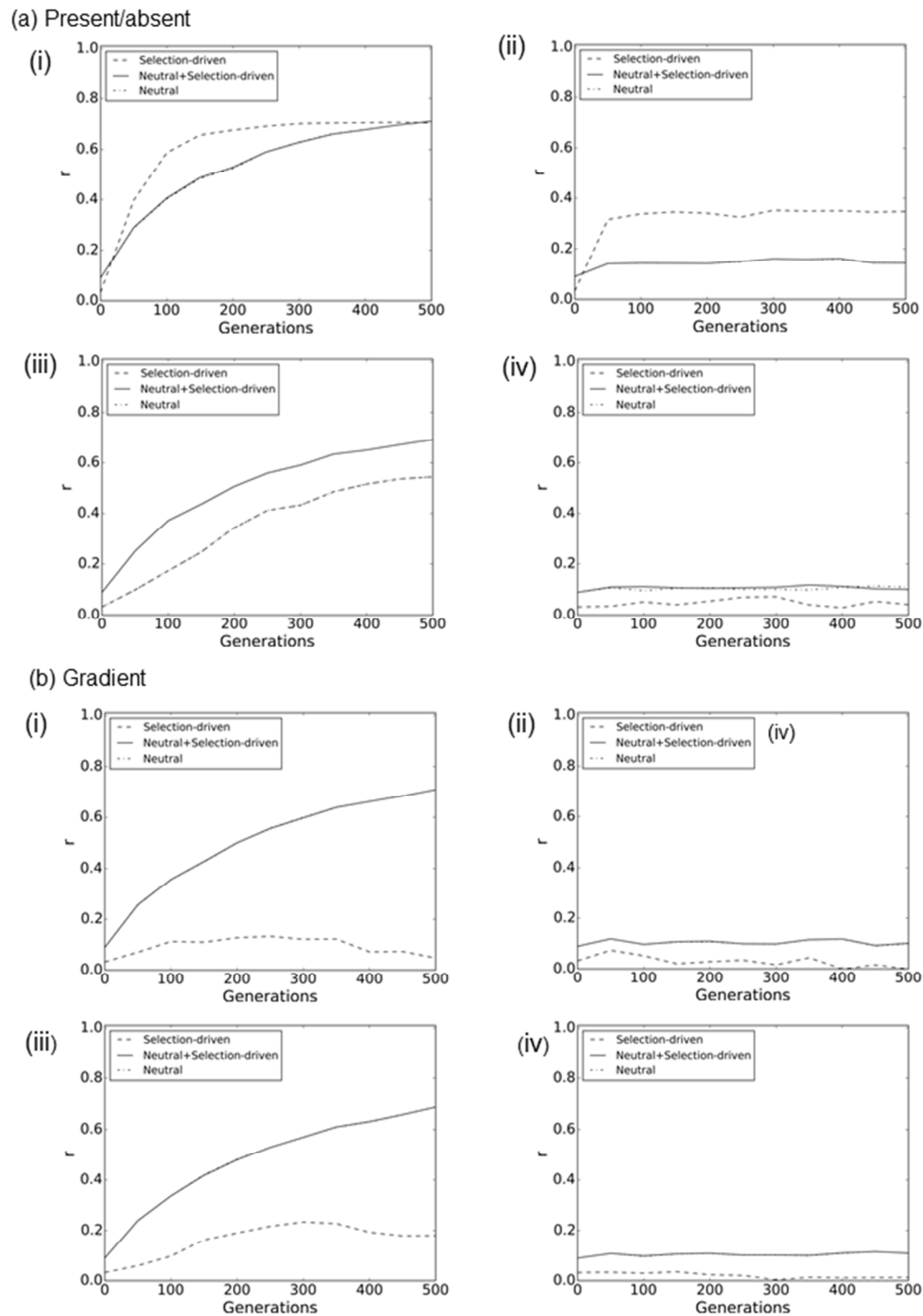


Figure 3. Mantel r results depicting genetic differentiation for adaptive (selection-driven), neutral and combined loci. The present/absent selection (a) and selection gradient (b) surfaces are shown with selection strong for the i) low N_m , high drift, ii) high N_m , high drift scenarios, and selection weak for the iii) low N_m , low drift, iv) high N_m , low drift scenarios. See Tables 5.1 and 5.2 for descriptions.

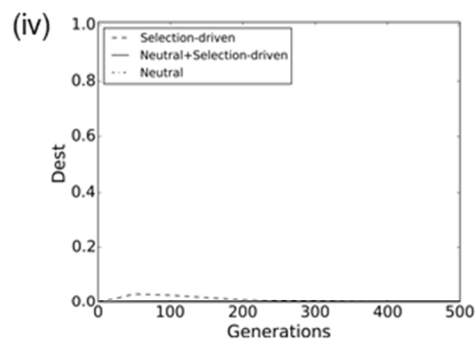
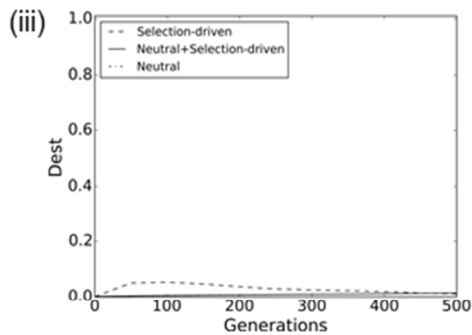
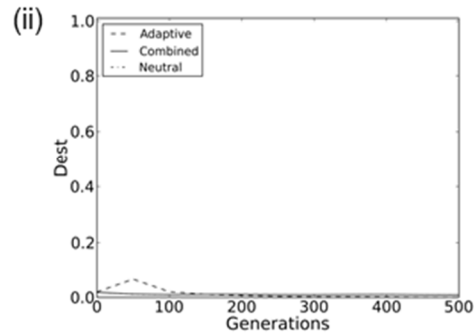
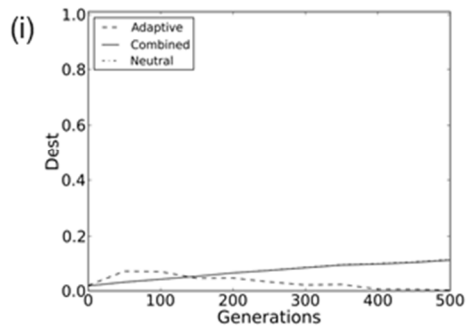


APPENDIX S1. Type I error rates of population- and individual-based methods when using the uniform selection surface (selection absent). Type I error rates are reported at 50 and 500 generations, and refer to the proportion of replicates where neutral loci were identified as under selection at the 95% and 99% confidence interval levels (LOSITAN, GLM, Samβada) and with FDR q value thresholds < 0.05 and 0.01 (BayeScan), which are analogues of *P*-values (displayed under 95% and 99% columns).

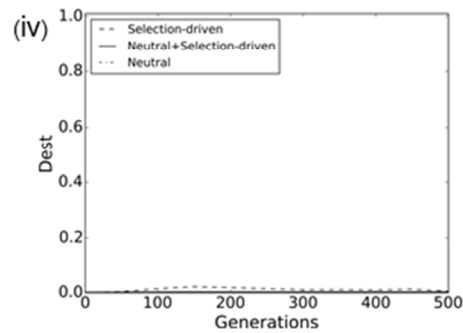
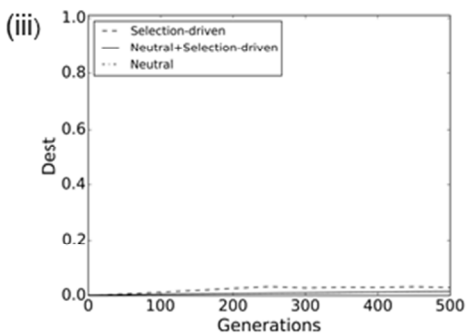
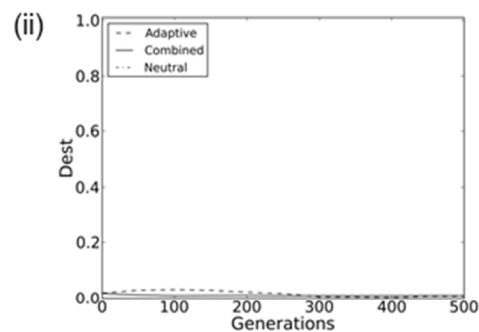
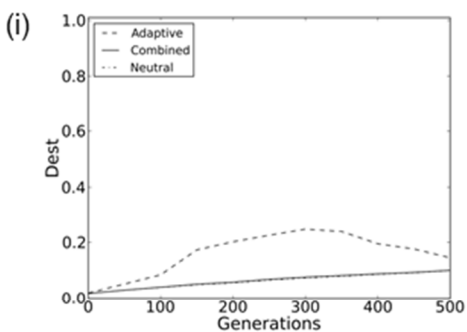
Selection surface	low N_m , high drift		low N_m , low drift		high N_m , high drift		high N_m , low drift	
	95%	99%	95%	99%	95%	99%	95%	99%
LOSITAN								
50	0.3	0.1	0	0	0.1	0	0	0
500	0.8	0.1	0.1	0	0.2	0.1	0	0
BayeScan								
50	0.1	0.1	0.1	0.1	0	0	0	0
500	0.1	0.1	0.1	0.1	0.1	0.1	0	0
Samβada								
50	1	1	0.7	0.7	0.8	0.8	0.8	0.8
500	1	1	0.7	0.7	0.3	0.3	0.7	0.7
GLM								
50	1	1	1	1	1	1	1	1
500	1	1	1	1	1	1	1	1

APPENDIX S2. D_{est} results depicting genetic differentiation for adaptive (selection-driven), neutral and combined loci. The selection gradient surface is shown with (a) selection strong ($s = 0.5$) and (b) weak ($s = 0.1$), for the i) low N_m , high drift, ii) high N_m , high drift, iii) low N_m , low drift, iv) high N_m , low drift scenarios. See Tables 1 and 2 for descriptions.

(a) Gradient, strong selection

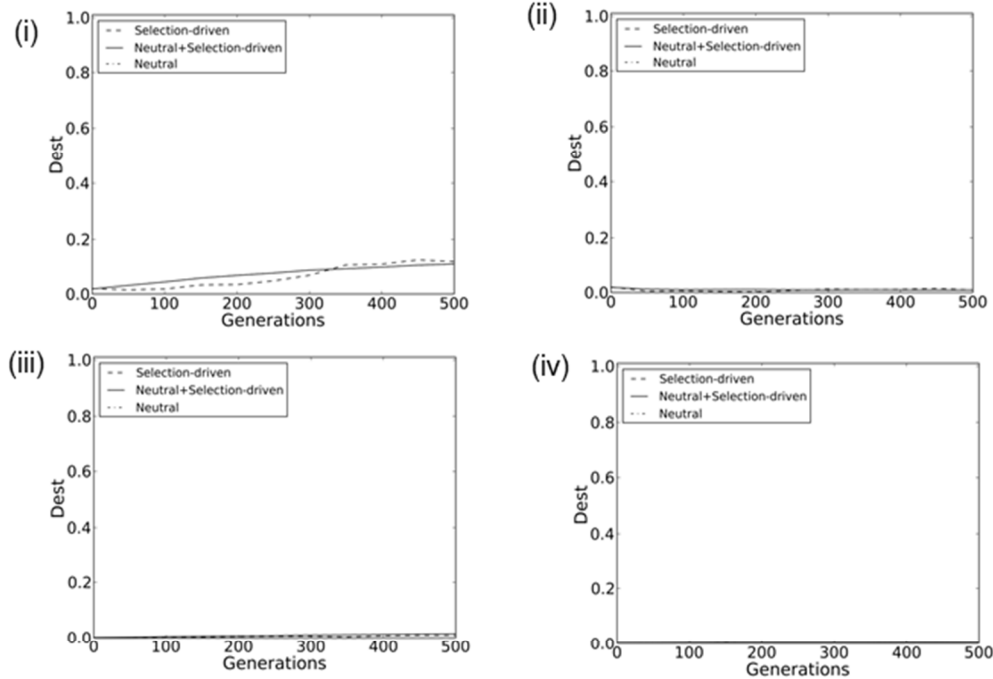


(b) Gradient, weak selection

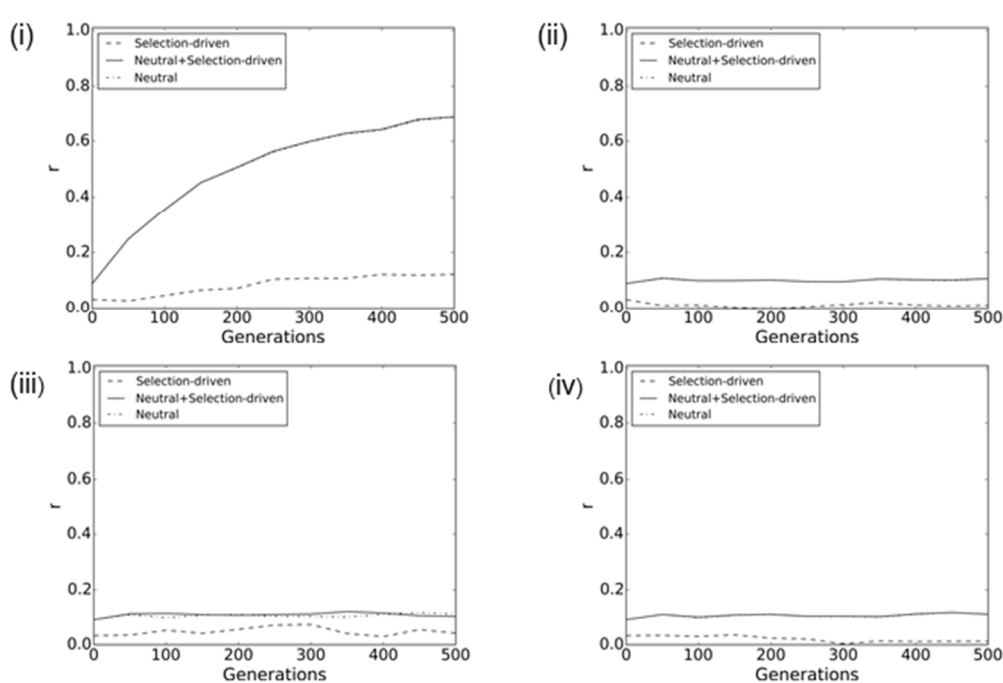


APPENDIX S3. D_{est} (a) and Mantel r (b) results depicting genetic differentiation for adaptive (selection-driven), neutral and combined loci in the uniform selection surface (selection absent) for the i) low Nm , high drift, ii) high Nm , high drift, iii) low Nm , low drift, iv) high Nm , low scenarios. See Tables 1 and 2 for descriptions.

(a) D_{est} , uniform selection (absent)



(b) Mantel r , uniform selection (absent)



APPENDIX S4. Type II error rates of population- and individual-based methods to detect loci under selection after 50 generations, and the proportion of replicates where this occurred at the 95% and 99% confidence interval levels (LOSITAN, GLM, Samβada) and with FDR q value thresholds < 0.05 and 0.01 (BayeScan), which are analogues of *P*-values (displayed under 95% and 99% columns). Type II error rates are shown for a.) selection present/absent surfaces, and b.) gradient surfaces, for both weak and strong selection scenarios.

a.)		Present/Absent							
Selection strength	Method	low N_m , high drift		low N_m , low drift		high N_m , high drift		high N_m , low drift	
	CI	95%	99%	95%	99%	95%	99%	95%	99%
weak	LOSITAN	1	1	1	1	0.9	1	1	1
	BayeScan	1	1	0.6	0.6	1	1	0.7	0.7
	Samβada	0	0	0	0	0.2	0.2	0	0
	GLM	0	0	0	0	0.2	0.2	0	0
strong	LOSITAN	0	0	0	0	0	0	0	0
	BayeScan	0	0	0	0	0	0	0	0
	Samβada	0	0	0	0	0.2	0.2	0	0
	GLM	0	0	0	0	0	0	0	0
b.)		Gradient							
Selection strength	Method	low N_m , high drift		low N_m , low drift		high N_m , high drift		high N_m , low drift	
	CI	95%	99%	95%	99%	95%	99%	95%	99%
weak	LOSITAN	1	1	1	1	0.9	1	1	1
	BayeScan	1	1	1	1	1	1	1	1
	Samβada	0.2	0.2	0.2	0.2	0.3	0.3	0	0
	GLM	0.3	0.3	0.1	0.1	0.3	0.3	0	0
strong	LOSITAN	0.9	1	0.7	1	0.7	0.9	1	1
	BayeScan	1	1	0	0	0.9	1	0.1	0.1
	Samβada	0.2	0.2	0	0	0	0	0	0
	GLM	0	0	0	0	0.1	0.1	0	0

APPENDIX S5. Type I error rates of individual-based methods (Samβada and GLMs) defined as the mean number of neutral loci significantly correlated with the selection gradient (latitude) at the 95% and 99% confidence interval levels after 50 generations. The expected number of significantly correlated loci for 95% and 99% is 5 and 1, respectively (with 100 simulated loci).

a.)		Present/absent							
Selection strength	Method	low N_m , high drift		low N_m , low drift		high N_m , high drift		high N_m , low drift	
	CIs	95%	99%	95%	99%	95%	99%	95%	99%
weak	Samβada	27	24	30	21	16	13	21	17
	GLM	28	26	25	22	18	14	22	19
strong	Samβada	29	25	25	21	16	13	20	17
	GLM	31	27	26	23	16	14	22	19
b.)		Gradient							
Selection strength	Method	low N_m , high drift		low N_m , low drift		high N_m , high drift		high N_m , low drift	
	CIs	95%	99%	95%	99%	95%	99%	95%	99%
weak	Samβada	26	22	27	22	15	12	22	18
	GLM	27	24	29	25	17	14	23	20
strong	Samβada	27	23	26	22	15	12	23	19
	GLM	35	29	27	25	17	14	25	22