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

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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 (Lewotin & 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; Hanncock *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

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

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

The main focus of the study

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 m^2.

Continue here.

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*

196 *al*. 2014). We determined pairwise population F_{ST} values (Weir and Cockerham 1984) using
197 GENEPOP, and calculated number of migrants per generation using the flowing equation: N_m =
198 (1- F_{ST}) / 4 F_{ST}.

199

200 *Genetic differentiation of adaptive vs. neutral markers*

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

209 For the landscape genetics approach, we used Mantel tests (Mantel 1967) to compare
210 pairwise genetic distance of all individuals to geographic distance using only neutral loci, only
211 adaptive loci, and all loci combined. Genetic distance among individuals was calculated using
212 the proportion of shared alleles (Dps; Bowcock *et al*. 1994). For each scenario, selection
213 strength, and selection surface we evaluated the10 independent Monte Carlo replicates to assess
214 variability in results. To calculate changes in genetic differentiation across time, we plotted the
215 average D_{est} and average Mantel correlation coefficient *r* across 500 generations in 4 scenarios
216 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_{FDIST} F_{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 to10. We used posterior probabilities establishing Log10 values
252 of the posterior odds > 1and 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}

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

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

292 The greatest differentiation occurred in the low dispersal, low drift scenario, as neutral
293 structure did not develop over time (Fig. 2aiii), whereas in the low dispersal, high drift scenario
294 neutral D_{est} values gradually increased to 0.1 by 500 generations (Fig. 2ai). In the scenarios with
295 strong selection and high dispersal, D_{est} values increased at a similar rate initially to the low
296 dispersal scenarios, but then declined slightly and plateaued after approximately 100 generations
297 (Fig. 2aii and iv). Adaptive and neutral alleles also showed substantial differentiation where
298 present/absent selection was weak and dispersal low, but developed at a slower rate reaching D_{est}
299 = 0.62 and 0.57 at 500 generations for populations with high and low drift, respectively (Fig. 2bi
300 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).

Identifying loci under selection

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

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

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

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

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

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

428 *Power and limitations of methods*

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

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

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

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

The use of adaptive genetic markers is a promising approach for defining management 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.

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721

722 **Author Contributions**

723

724 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.
725 G.H.R. performed the analyses, interpreted the data, and wrote the manuscript with input from
726 all authors.

727

728 **Data accessibility**

729

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

732

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 (LOSITAN) 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.

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.).

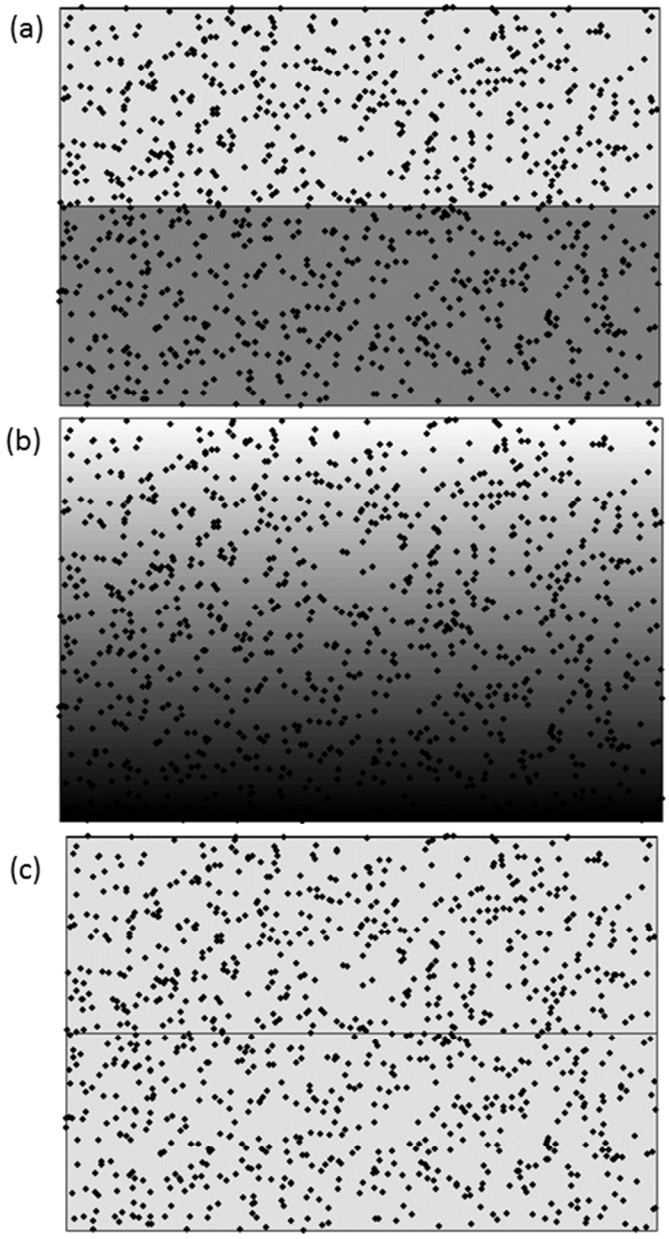


Figure 2. D_{est} results depicting genetic differentiation for adaptive (selection-driven), neutral and combined loci. The present/absent selection surface is shown with selection strong (a) and weak (b), for the i) low N_m, high drift, ii) high N_m, high drift, iii) low N_m, low drift, iv) high N_m,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

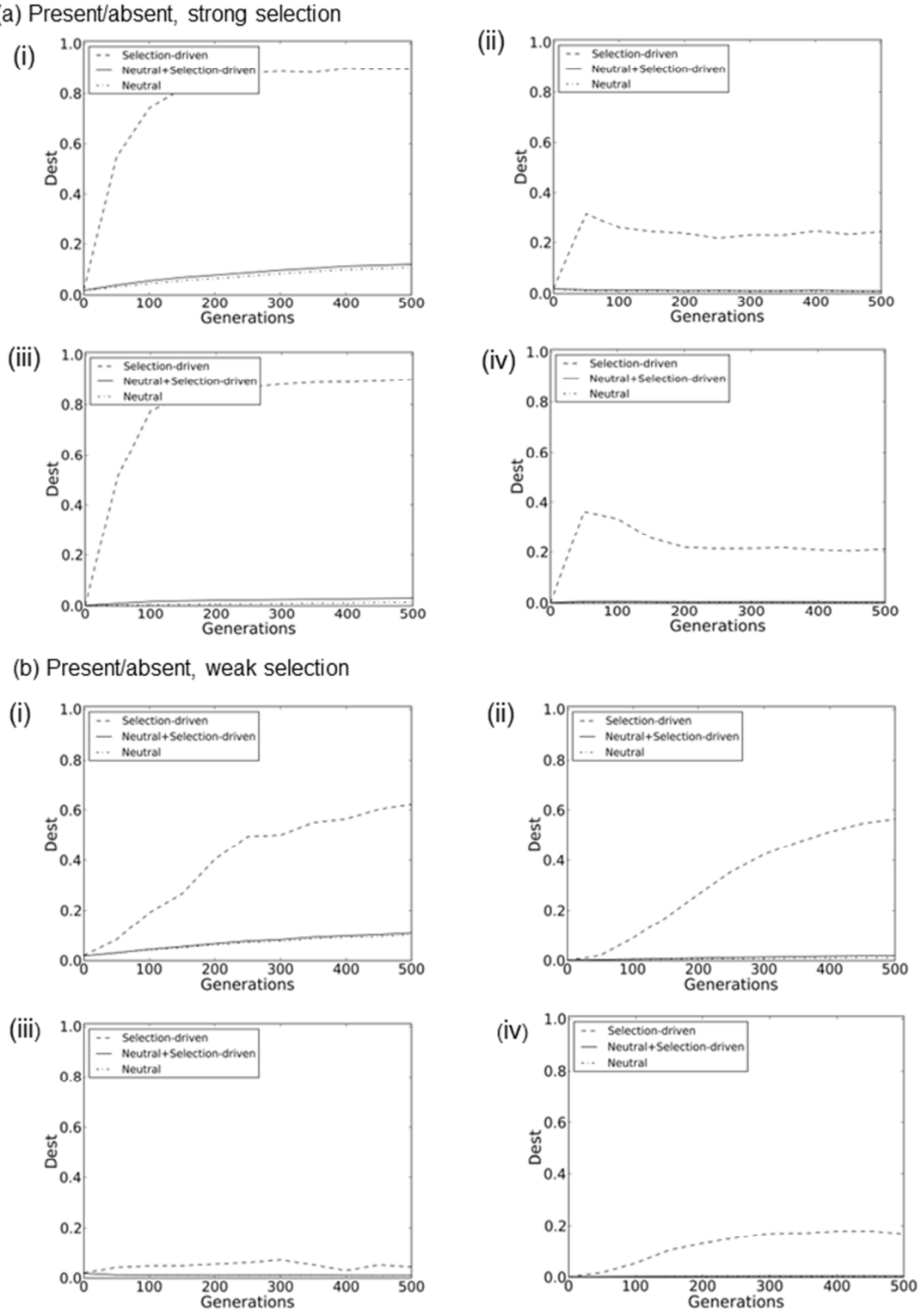
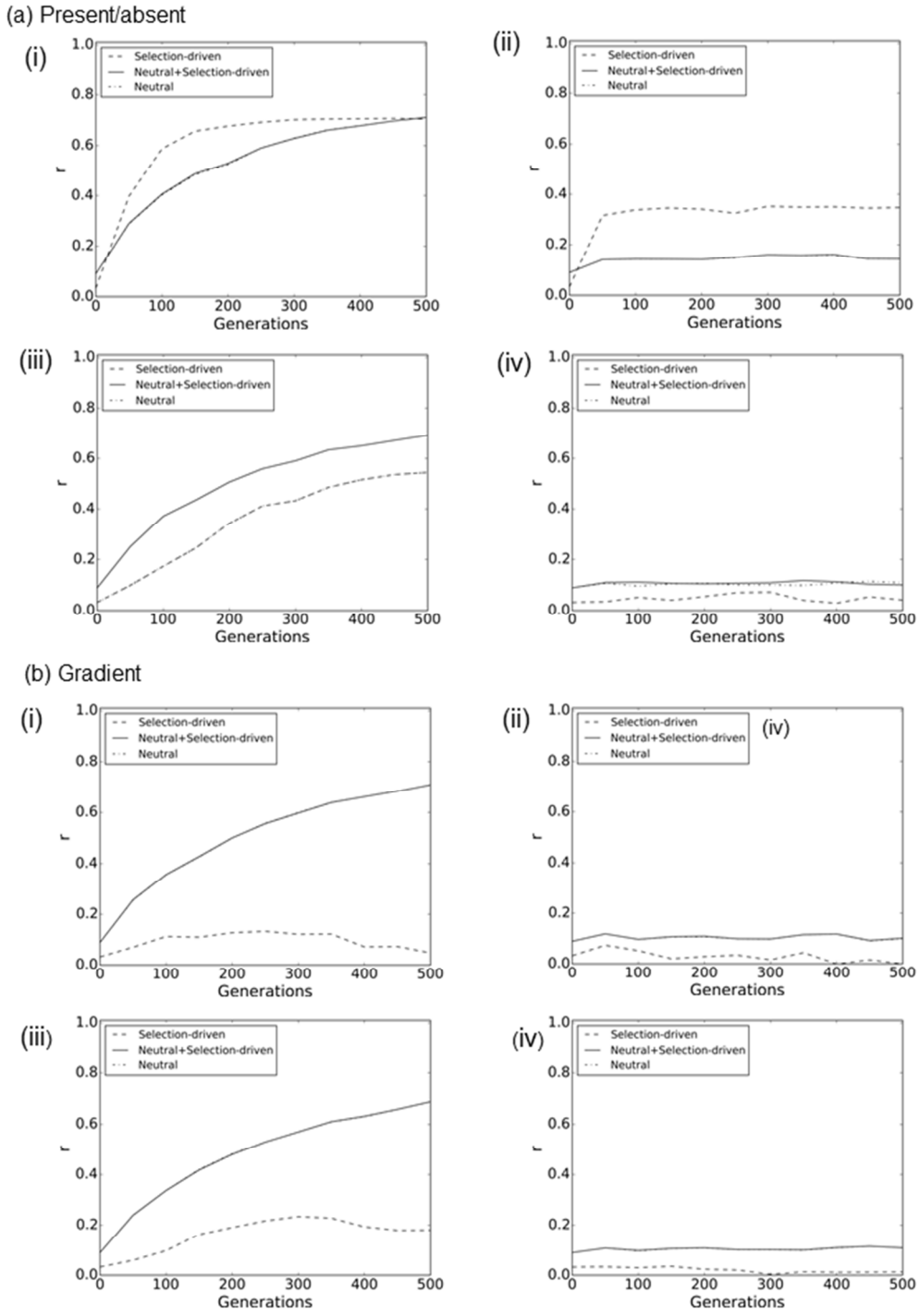


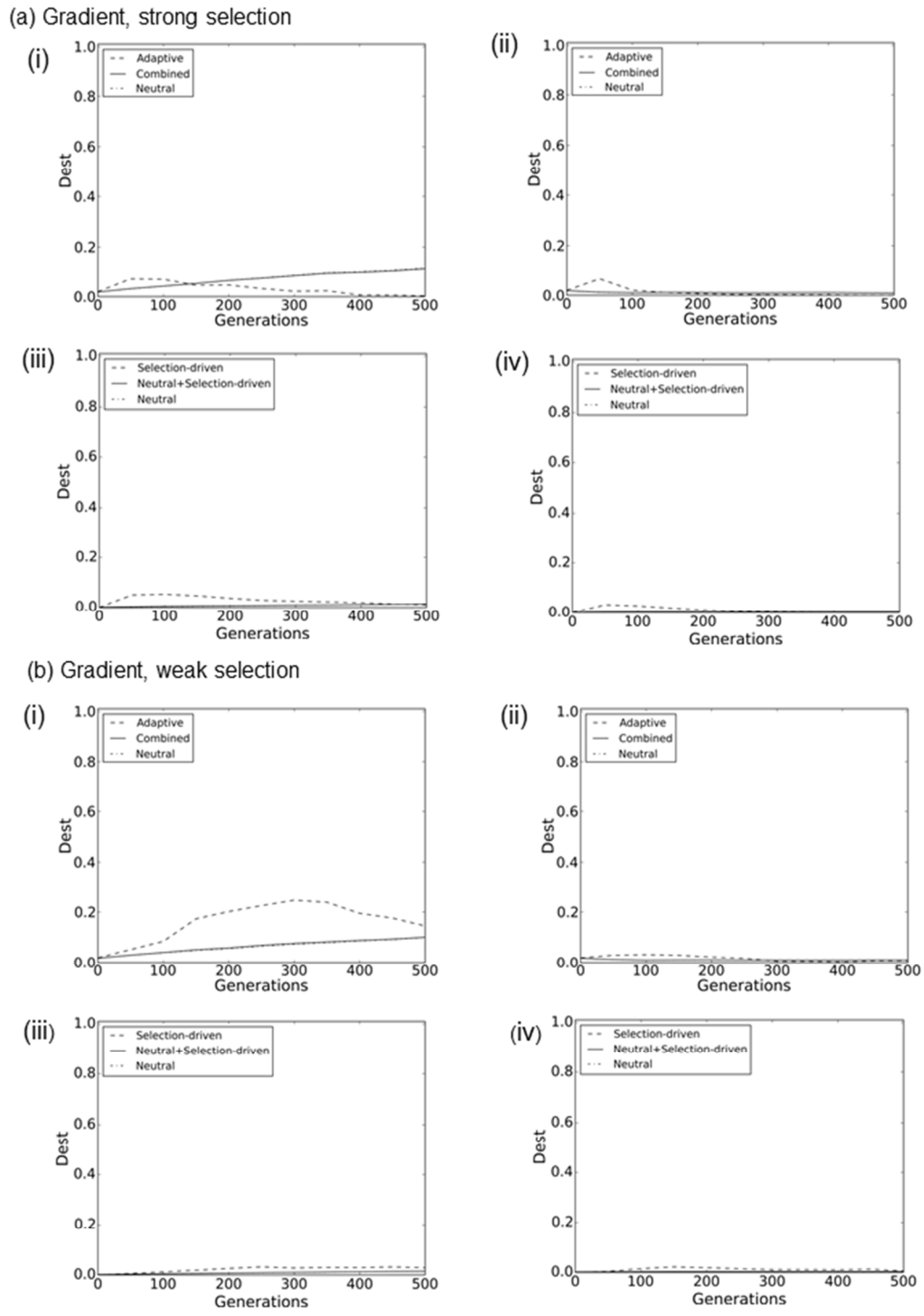
Figure 3. Mantel *r* results depicting genetic differentiation for adaptive (selection-driven), neutral and combined loci. The present/absent selection (a) and selection gradient (b) surfaces are shown with selection strong for the i) low N_m, high drift, ii) high N_m, high drift scenarios, and selection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



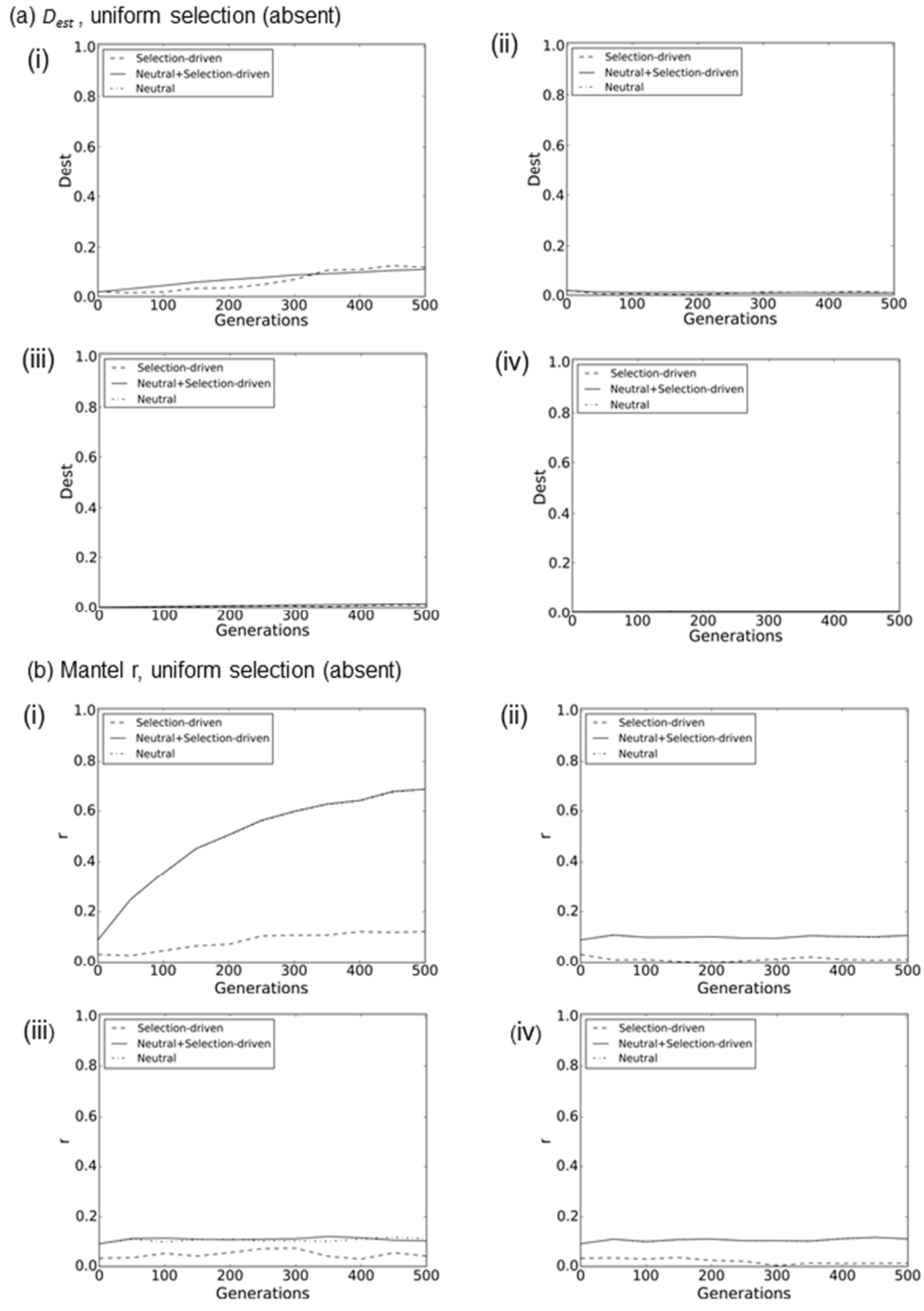
APPENDIX S1. Type I error rates of population- and individual-based methods when using the uniform selection surface (selection absent). Type I error rates are reported at 50 and 500 generations, and refer to the proportion of replicates where neutral loci were identified as under selection at the 95% 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	
Absent	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.



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.



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	
	CIs	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 N_m, high drift		high N_m, low drift	
	CIs	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