

Space is the Place: Effects of Continuous Spatial Structure on Analysis of Population Genetic Data

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ABSTRACT Real geography is continuous, but standard models in population genetics are based on discrete, well-mixed populations. As a result many methods of analyzing genetic data assume that samples are a random draw from a well-mixed population, but are applied to clustered samples from populations that are structured clinally over space. Here we use simulations of populations living in continuous geography to study the impacts of dispersal and sampling strategy on population genetic summary statistics, demographic inference, and genome-wide association studies. We find that most common summary statistics have distributions that differ substantially from that seen in well-mixed populations, especially when Wright's neighborhood size is less than 100 and sampling is spatially clustered. Stepping-stone models reproduce some of these effects, but discretizing the landscape introduces artifacts which in some cases are exacerbated at higher resolutions. The combination of low dispersal and clustered sampling causes demographic inference from the site frequency spectrum to infer more turbulent demographic histories, but averaged results across multiple simulations revealed surprisingly little systematic bias. We also show that the combination of spatially autocorrelated environments and limited dispersal causes genome-wide association studies to identify spurious signals of genetic association with purely environmentally determined phenotypes, and that this bias is only partially corrected by regressing out principal components of ancestry. Last, we discuss the relevance of our simulation results for inference from genetic variation in real organisms.

KEYWORDS Space; Population Structure; Demography; Haplotype block sharing; GWAS

Introduction

The inescapable reality that biological organisms live, move, and reproduce in continuous geography is usually omitted from population genetic models. However, mates tend to live near to one another and to their offspring, leading to a positive correlation between genetic differentiation and geographic distance. This pattern of “isolation by distance” (Wright 1943) is one of the most widely replicated empirical findings in population genetics (Aguillon *et al.* 2017; Jay *et al.* 2012; Sharbel *et al.* 2000). Despite a long history of analytical work describing the genetics of populations distributed across continuous geography (e.g., Wright (1943); Rousset (1997); Barton *et al.* (2002, 2010); Ringbauer *et al.* (2017); Robledo-Arnuncio and Rousset (2010); Wilkins and Wakeley (2002); Wilkins (2004)), much modern work still describes geographic structure as a set of discrete populations connected by migration (e.g., Wright 1931; Epperson 2003; Rousset and Leblois 2011; Shirk and Cushman 2014; Lundgren and Ralph 2019)

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39 or as an average over such discrete models (Petkova *et al.* 2015; Al-Asadi *et al.* 2019). For this reason,
40 most population genetics statistics are interpreted with reference to discrete, well-mixed populations,
41 and most empirical papers analyze variation within clusters of genetic variation inferred by programs
42 like *STRUCTURE* (Pritchard *et al.* 2000) with methods that assume these are randomly mating units.

43 The assumption that populations are “well-mixed” has important implications for downstream
44 inference of selection and demography. Methods based on the coalescent (Kingman 1982; Wakeley
45 2009) assume that the sampled individuals are a random draw from a well-mixed population that is
46 much larger than the sample (Wakeley and Takahashi 2003). The key assumption is that the individuals
47 of each generation are *exchangeable*, so that there is no correlation between the fate or fecundity of a
48 parent and that of their offspring (Huillet and Möhle 2011). If dispersal or mate selection is limited by
49 geographic proximity, this assumption can be violated in many ways. For instance, if mean viability or
50 fecundity is spatially autocorrelated, then limited geographic dispersal will lead to parent–offspring
51 correlations. Furthermore, nearby individuals will be more closely related than an average random
52 pair, so drawing multiple samples from the same area of the landscape will represent a biased sample
53 of the genetic variation present in the whole population (Städler *et al.* 2009).

54 Two areas in which spatial structure may be particularly important are demographic inference and
55 genome-wide association studies (GWAS). Previous work has found that discrete population structure
56 can create false signatures of population bottlenecks when attempting to infer demographic histories
57 from microsatellite variation (Chikhi *et al.* 2010), statistics summarizing the site frequency spectrum
58 (SFS) (Ptak and Przeworski 2002; Städler *et al.* 2009; St. Onge *et al.* 2012), or runs of homozygosity in a
59 single individual (Mazet *et al.* 2015). The increasing availability of whole-genome data has led to the
60 development of many methods that attempt to infer detailed trajectories of population sizes through
61 time based on a variety of summaries of genetic data (Liu and Fu 2015; Schiffels and Durbin 2014;
62 Sheehan *et al.* 2013; Terhorst *et al.* 2016). Because all of these methods assume that the populations
63 being modeled are approximately randomly mating, they are likely affected by spatial biases in the
64 genealogy of sampled individuals (Wakeley 1999), which may lead to incorrect inference of population
65 changes over time (Mazet *et al.* 2015). However, previous investigations of these effects have focused on
66 discrete rather than continuous space models, and the level of isolation by distance at which inference
67 of population size trajectories become biased by structure is not well known. Here we test how two
68 methods suitable for use with large samples of individuals – stairwayplot (Liu and Fu 2015) and
69 SMC++ (Terhorst *et al.* 2016) – perform when applied to populations evolving in continuous space
70 with varying sampling strategies and levels of dispersal.

71 Spatial structure is also a major challenge for interpreting the results of genome-wide association
72 studies (GWAS). This is because many phenotypes of interest have strong geographic differences due
73 to the (nongenetic) influence of environmental or socioeconomic factors, which can therefore show
74 spurious correlations with spatially patterned allele frequencies (Bulik-Sullivan *et al.* 2015; Mathieson
75 and McVean 2012). Indeed, two recent studies found that previous evidence of polygenic selection on
76 human height in Europe was confounded by subtle population structure (Sohail *et al.* 2018; Berg *et al.*
77 2018), suggesting that existing methods to correct for population structure in GWAS are insufficient.
78 However we have little quantitative idea of the population and environmental parameters that can be
79 expected to lead to biases in GWAS.

80 Last, some of the most basic tools of population genetics are summary statistics like F_{IS} and
81 Tajima’s D , which are often interpreted as reflecting the influence of selection or demography on
82 sampled populations (Tajima 1989). Statistics like Tajima’s D are essentially summaries of the site
83 frequency spectrum, which itself reflects variation in branch lengths and tree structure of the underlying
84 genealogies of sampled individuals. Geographically limited mate choice distorts the distribution of
85 these genealogies (Maruyama 1972; Wakeley 1999), which can affect the value of Tajima’s D (Städler
86 *et al.* 2009). Similarly, the distribution of tract lengths of identity by state among individuals contains
87 information about not only historical demography (Harris and Nielsen 2013; Ralph and Coop 2013)
88 and selection (Garud *et al.* 2015), but also dispersal and mate choice (Ringbauer *et al.* 2017; Baharian
89 *et al.* 2016). We are particularly keen to examine how such summaries will be affected by models that
90 incorporate continuous space, both to evaluate the assumptions underlying existing methods and to
91 identify where the most promising signals of geography lie.

92 To study this, we have implemented an individual-based model in continuous geography that
93 incorporates overlapping generations, local dispersal of offspring, and density-dependent survival. We
94 simulate chromosome-scale genomic data in tens of thousands of individuals from parameter regimes
95 relevant to common subjects of population genetic investigation such as humans and *Drosophila*, and
96 output the full genealogy and recombination history of all final-generation individuals. We use these
97 simulations to test how sampling strategy interacts with geographic population structure to cause
98 systematic variation in population genetic summary statistics typically analyzed assuming discrete
99 population models. We then examine how the fine-scale spatial structures occurring under limited
100 dispersal impact demographic inference from the site frequency spectrum. Last, we examine the
101 impacts of continuous geography on genome-wide association studies (GWAS) and identify regions of
102 parameter space under which the results from GWAS may be misleading.

103 Materials and Methods

104 Modeling Evolution in Continuous Space

105 The degree to which genetic relationships are geographically correlated depends on the chance that
106 two geographically nearby individuals are close relatives – in modern terms, by the tension between
107 migration (the chance that one is descended from a distant location) and coalescence (the chance that
108 they share a parent). A key early observation by Wright (1946) is that this balance is often nicely
109 summarized by the “neighborhood size”, defined [in two dimensions](#) to be $N_W = 4\pi\rho\sigma^2$, where σ is
110 [the mean \$\sigma^2\$ is one half of the mean squared parent–offspring distance](#) and ρ is population density ([see](#)
111 [Rousset \(1997\) for further discussion of parameter definitions in one- and two-dimensional habitats](#)).
112 This can be thought of as proportional to the average number of potential mates for an individual (those
113 within distance 2σ), or the number of potential parents of a randomly chosen individual. Empirical
114 estimates of neighborhood size vary hugely across species – even in human populations, estimates
115 range from 40 to over 5,000 depending on the population and method of estimation (Table 1).

116 The first approach to modeling continuously distributed populations was to endow individuals in a
117 Wright-Fisher model with locations in continuous space. However, since the total size of the population
118 is constrained, this introduces interactions between arbitrarily distant individuals, which (aside from
119 being implausible) was shown by Felsenstein (1975) to eventually lead to unrealistic population
120 clumping if the range is sufficiently large. Another method for modeling spatial populations is to
121 assume the existence of a grid of discrete randomly mating populations connected by migration, thus
122 enforcing regular population density by edict. Among many other results drawn from this class of
123 “lattice” or “stepping stone” models (Epperson 2003), Rousset (1997) showed that the slope of the linear
124 regression of genetic differentiation (F_{ST}) against the logarithm of spatial distance is an estimate of
125 neighborhood size. Although these grid models may be good approximations of continuous geography
126 in many situations, they do not model demographic fluctuations, and limit investigation of spatial
127 structure below the level of the deme, assumptions whose impacts are unknown. An alternative
128 method for dealing with continuous geography is a new class of coalescent models, the Spatial Lambda
129 Fleming-Viot models (Barton *et al.* 2010; Kelleher *et al.* 2014).

130 To avoid hard-to-evaluate approximations, we here used forward-time, individual-based simulations
131 across continuous geographical space. The question of what regulates real populations has a
132 long history and many answers (e.g., Lloyd 1967; Antonovics and Levin 1980; Crawley 1990), but it is
133 clear that populations must at some point have density-dependent feedback on population size, or
134 else they would face eventual extinction or explosion. In the absence of unrealistic global population
135 regulation, this regulation must be local, and there are many ways to achieve this (Bolker *et al.* 2003). In
136 our simulations, each individual’s probability of survival is a decreasing function of local population
137 density, which shifts reproductive output towards low-density regions, and produces total census sizes
138 that fluctuate around an equilibrium. This also prevents the population clumping seen by Felsenstein
139 (1975) (Supplemental Figure S1)). Such models have been used extensively in ecological modeling
140 (Durrett and Levin 1994; Bolker and Pacala 1997; Law *et al.* 2003; Fournier and Méléard 2004; Champer
141 *et al.* 2019) but rarely in population genetics, where to our knowledge implementations of continuous
142 space models before their availability through SLiM (Haller and Messer 2019) have focused on a small

143 number of genetic loci (e.g., Slatkin and Barton 1989; Barton *et al.* 2002; Robledo-Arnuncio and Rousset
 144 2010; Rossine 2014; Jackson and Fahrig 2014), which limits the ability to investigate the impacts of
 145 continuous space on genome-wide genetic variation as is now routinely sampled from real organisms.
 146 By simulating chromosome-scale sequence alignments and complete population histories we are able
 147 to treat our simulations as real populations and replicate the sampling designs and analyses commonly
 148 conducted on real genomic data.

149 **A Forward-Time Model of Evolution in Continuous Space**

150 We simulated populations using the program SLiM v3.1 (Haller and Messer 2019). Each time step
 151 consists of three stages: reproduction, dispersal, and mortality. To reduce the parameter space number
of parameters we use the same parameter, denoted σ , to modulate the spatial scale of interactions
 152 at all three stages by adjusting the standard deviation of the corresponding Gaussian functions. As
in previous work (Wright 1943; Ringbauer *et al.* 2017), Informally, we think of σ is equal to the mean
parent-offspring distanceas the “dispersal distance”, although only one of those stages is dispersal.
 153

154 At the beginning of the simulation individuals are distributed uniformly at random on a continuous,
 155 square landscape. Individuals are hermaphroditic, and each time step, each produces a Poisson number
 156 of offspring with mean $1/L$. Offspring disperse a Gaussian-distributed distance away from the parent
 157 with mean zero and standard deviation σ in both the x and y coordinates. Each offspring is produced
 158 with a mate selected randomly from those within distance 3σ , with probability of choosing a neighbor at
 159 distance d proportional to $\exp(-d^2/2\sigma^2)$ the Gaussian density with mean zero and standard deviation
 160 σ , which is $g(d) = \exp(-d^2/2\sigma^2)/(2\sigma^2)$.
 161

162 To maintain a stable population, mortality increases with local population density. To do this
 163 we say that individuals at distance d have a competitive interaction with strength $g(d)$, where g is
the Gaussian density with mean zero and standard deviation σ . Then, the sum of all competitive
 164 interactions with individual i is $n_i = \sum_j g(d_{ij})$, where d_{ij} is the distance between individuals i and j
 165 and the sum is over all neighbors within distance 3σ . Since g is a probability density, n_i is an estimate
 166 of the number of nearby individuals per unit area. Then, given a per-unit carrying capacity K , the
 167 probability of survival until the next time step for individual i is
 168

$$p_i = \min \left(0.95, \frac{1}{1 + n_i/(K(1+L))} \right). \quad (1)$$

169 We chose this functional form so that the equilibrium population density per unit area is close to K ,
 170 and the mean lifetime is around L ; for more description see the Appendix.

171 An important step in creating any spatial model is dealing with range edges. Because local popula-
 172 tion density is used to model competition, edge or corner populations can be assigned artificially high
 173 fitness values because they lack neighbors within their interaction radius but outside the bounds of the
 174 simulation. We approximate a decline in habitat suitability near edges by decreasing the probability
 175 of survival proportional to the square root of distance to edges in units of σ . The final probability of
 176 survival for individual i is then
 177

$$s_i = p_i \min(1, \sqrt{x_i/\sigma}) \min(1, \sqrt{y_i/\sigma}) \min(1, \sqrt{(W-x_i)/\sigma}) \min(1, \sqrt{(W-y_i)/\sigma}) \quad (2)$$

178 where x_i and y_i are the spatial coordinates of individual i , and W is the width (and height) of the
 179 square habitat. This buffer roughly counteracts the increase in fitness individuals close to the edge
 180 would otherwise have, though the effect is relatively subtle (Figure S2).

181 To isolate spatial effects from other components of the model such as overlapping generations,
 182 increased variance in reproductive success, and density-dependent fitness, we also implemented
 183 simulations identical to those above except that mates are selected uniformly at random from the
 184 population, and offspring disperse to a uniform random location on the landscape. We refer to this
 185 model as the “random mating” model, in contrast to the first, “spatial” model.

186 We stored the full genealogy and recombination history of final-generation individuals as tree
 187 sequences (Kelleher *et al.* 2018), as implemented in SLiM (Haller *et al.* 2019). Scripts for figures and

188 analyses are available at <https://github.com/kern-lab/spaceness>.

189 We ran 400 simulations for the spatial and random-mating models on a square landscape of width
190 $W = 50$ with per-unit carrying capacity $K = 5$ (census $N \approx 10,000$), average lifetime $L = 4$, genome
191 size 10^8 bp, recombination rate 10^{-9} per bp per generation, and drawing σ values from a uniform
192 distribution between 0.2 and 4. To speed up the simulations and limit memory overhead we used a
193 mutation rate of 0 in SLiM and later applied mutations to the tree sequence with msprime's `mutate`
194 function (Kelleher *et al.* 2016). Because msprime applies mutations proportionally to elapsed time,
195 we divided the mutation rate of 10^{-8} mutations per site per generation by the average generation
196 time estimated for each value of σ (see 'Demographic Parameters' below) to convert the rate to units
197 of mutations per site per unit time. We verify that this procedure produces the same site frequency
198 spectrum as applying mutations directly in SLiM in Figure S3, in agreement with theory (Ralph *et al.*
199 2019b). Simulations were run for 1.6 million timesteps (approximately $30N$ generations).

200 We also compared our model's output to a commonly-used approximation of continuous space,
201 the stepping-stone model, which we simulated with msprime (Kelleher *et al.* 2016). These results are
202 discussed in detail in the Appendix, but in general we find that the demographic structure of a stepping-
203 stone model can depend strongly on the chosen discretization, and some artifacts of discretization seem
204 to become stronger in the limit of a fine grid. For many summary statistics, finer discretizations (we
205 used a 50×50 grid) produced similar results to the continuous model, but this was not true for others
206 (e.g., F_{IS} and Tajima's D), which differed from the continuous model *more* at finer discretizations.

207 **Demographic Parameters**

208 Our demographic model includes parameters that control population density (K), mean life span (L),
209 and dispersal distance (σ). However, nonlinearity of local demographic stochasticity causes actual
210 realized averages of these demographic quantities to deviate from the specified values in a
211 way that depends on the neighborhood size. Therefore, to properly compare to theoretical expectations,
212 we empirically calculated these demographic quantities in simulations. We recorded the census
213 population size in all simulations, and used mean population density (ρ , census size divided by total
214 area) to compute neighborhood size as $N_W = 4\pi\rho\sigma^2$. To estimate generation times, we stored ages
215 of the parents of every new individual born across 200 timesteps, after a 100 generation burn-in, and
216 took the mean. To estimate variance in offspring number, we tracked the lifetime total number of
217 offspring for all individuals for 100 timesteps following a 100-timestep burn-in period, and calculated
218 the variance in number of offspring across all individuals in timesteps 50-100. All calculations were
219 performed with information recorded in the tree sequence, using pyslim (Ralph *et al.* 2019a).

220 Note that σ controls the dispersal of offspring away from only one parent (e.g. the seed parent
221 for plants), while population genetics usually defines "dispersal distance" to be the distance to a
222 randomly chosen one of the two parents (thus taking into account the distance from pollen parent
223 as well). This second component – the distance between mates – has in our simulations a distribution
224 that is of order σ but that depends on the population's patchiness. If both between-mate distance
225 and dispersal distance has variance σ^2 along each axis, then the mean squared distance to a randomly
226 chosen parent along that axis would be $(\sigma^2 + 2\sigma^2)/2 = 3\sigma^2/2$. To match theory, neighborhood size
227 should be defined in terms of effective dispersal distance, i.e., the mean squared displacement along an
228 axis between parent-child pairs found moving back along a lineage (Barton *et al.* 2002), and effective
229 population density (Rousset 1997). However, we use σ and ρ as defined here to compute N_W because
230 these quantities are more easily observable in practice than their "effective" versions.

231 **Sampling**

232 Our model records the genealogy and sequence variation of the complete population, but in real data,
233 genotypes are only observed from a relatively small number of sampled individuals. We modeled three
234 sampling strategies similar to common data collection methods in empirical genetic studies (Figure 1).
235 "Random" sampling selects individuals at random from across the full landscape, "point" sampling
236 selects individuals proportional to their distance from four equally spaced points on the landscape,
237 and "midpoint" sampling selects individuals in proportion to their distance from the middle of the

238 landscape. Downstream analyses were repeated across all sampling strategies.

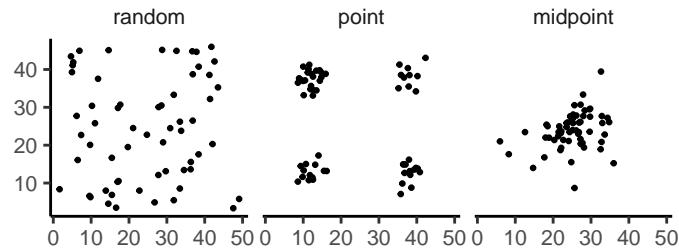


Figure 1 Example sampling maps for 60 individuals on a 50×50 landscape for midpoint, point, and random sampling strategies, respectively.

239 **Summary Statistics**

240 We calculated the site frequency spectrum and a set of 18 summary statistics (Table S1) from 60 diploid
241 individuals sampled from the final generation of each simulation using the python package scikit-
242 allel (Miles and Harding 2017). Statistics included common single-population summaries including
243 mean pairwise divergence (π), inbreeding coefficient (F_{IS}), and Tajima's D , as well as (motivated by
244 Rousset (1997)'s results) the correlation coefficient between the logarithm of the spatial distance and
245 the proportion of identical base pairs across pairs of individuals.

246 Following recent studies that showed strong signals for dispersal and demography in the distribution
247 of shared haplotype block lengths (e.g., Ringbauer *et al.* 2017; Baharian *et al.* 2016), we also
248 calculated various summaries of the distribution of pairwise identical-by-state (IBS) block lengths
249 among sampled chromosomes, defined to be the set of distances between adjacent sites that differ
250 between the two chromosomes. The full distribution of lengths of IBS tracts for each pair of chromo-
251 somes was first calculated with a custom python function. We then calculated the first three moments
252 of this distribution (mean, variance, and skew) and the number of blocks over 10^6 base pairs both
253 for each pair of individuals and for the full distribution across all pairwise comparisons. We then
254 calculated correlation coefficients between spatial distance and each moment of the pairwise IBS tract
255 distribution. Because more closely related individuals on average share longer haplotype blocks we
256 expect that spatial distance will be negatively correlated with mean haplotype block length, and that
257 this correlation will be strongest (i.e., most negative) when dispersal is low. The variance, skew, and
258 count of long haplotype block statistics are meant to reflect the relative length of the right (upper) tail
259 of the distribution, which represents the frequency of long haplotype blocks, and so should reflect
260 recent demographic events (Chapman and Thompson 2002). For a subset of simulations, we also
261 calculated cumulative distributions for IBS tract lengths across pairs of distant (more than 40 map
262 units) and nearby (less than 10 map units) individuals. Last, we examined the relationship between
263 allele frequency and the spatial dispersion of an allele by calculating the average distance between
264 individuals carrying each derived allele.

265 The effects of sampling on summary statistic estimates were summarized by testing for differences in
266 mean (ANOVA, [\(R Core Team 2018\)](#)) and variance (Levene's test, [\(Fox and Weisberg 2011\)](#)
267 [\(Fox and Weisberg 2011\)](#)) across sampling strategies for each summary statistic.

268 **Demographic Inference**

269 To assess the impacts of continuous spatial structure on demographic inference we inferred population
270 size histories for all simulations using two approaches: stairwayplot (Liu and Fu 2015) and SMC++
271 (Terhorst *et al.* 2016). Stairwayplot fits its model to a genome-wide estimate of the SFS, while SMC++
272 also incorporates linkage information. For both methods we sampled 20 individuals from all spatial
273 simulations using random, midpoint, and point sampling strategies.

274 As recommended by its documentation, we used stairwayplot to fit models with multiple bootstrap
275 replicates drawn from empirical genomic data, and took the median inferred N_e per unit time as
276 the best estimate. We calculated site frequency spectra with scikit-allel (Miles and Harding 2017),
277 generated 100 bootstrap replicates per simulation by resampling over sites, and fit models for all
278 bootstrap samples using default settings.

279 For SMC++, we first output genotypes as VCF with msprime and then used SMC++'s standard
280 pipeline for preparing input files assuming no polarization error in the SFS. We used the first individual
281 in the VCF as the "designated individual" when fitting models, and allowed the program to estimate
282 the recombination rate during optimization. We fit models using the 'estimate' command rather than
283 the now recommended cross-validation approach because our simulations had only a single contig.

284 To evaluate the performance of these methods we binned simulations by neighborhood size, took a
285 rolling median of inferred N_e trajectories across all model fits in a bin for each method and sampling
286 strategy. We also examined how varying levels of isolation by distance impacted the variance of N_e
287 estimates by calculating the standard deviation of N_e from each best-fit model.

288 **Association Studies**

289 To assess the degree to which spatial structure confounds GWAS we simulated four types of nongenetic
290 phenotype variation for 1000 randomly sampled individuals in each spatial SLiM simulation and
291 conducted a linear regression GWAS with principal components as covariates in PLINK (Purcell *et al.*
292 2007). SNPs with a minor allele frequency less than 0.5% were excluded from this analysis. Phenotype
293 values were set to vary by two standard deviations across the landscape in a rough approximation
294 of the variation seen in height across Europe (Turchin *et al.* 2012; Garcia and Quintana-Domeque
295 2006, 2007). Conceptually our approach is similar to that taken by Mathieson and McVean (2012),
296 though here we model fully continuous spatial variation and compare GWAS output across a range of
297 dispersal distances.

298 In all simulations, the phenotype of each individual is determined by drawing from a Gaussian
299 distribution with standard deviation 10 and a mean that may depend on spatial position. In spatially
300 varying models, the mean phenotype differs by two standard deviations across the landscape. We
301 then adjust the geographic pattern of mean phenotype to create four types of spatially autocorrelated
302 environmental influences on phenotype. In the first simulation of *nonspatial* environments, the mean
303 did not change, so that all individuals' phenotypes were drawn independently from a Gaussian
304 distribution with mean 110 and standard deviation 10. Next, to simulate *clinal* environmental influences
305 on phenotype, we increased the mean phenotype from 100 on the left edge of the range to 120 on the
306 right edge (two phenotypic standard deviations). Concretely, the mean phenotype p for an individual
307 at position (x, y) is $p = 100 + 2x/5$. Third, we simulated a more concentrated "*corner*" environmental
308 effect by setting the mean phenotype to 120 for individuals with both x and y coordinates below 20
309 (two standard deviations above the rest of the map). Finally, in "*patchy*" simulations we selected 10
310 random points on the map and set the mean phenotype of all individuals within three map units of
311 each of these points to 120.

312 We performed principal components analysis (PCA) using scikit-allel (Miles and Harding 2017) on
313 the matrix of derived allele counts by individual for each simulation. SNPs were first filtered to remove
314 strongly linked sites by calculating LD between all pairs of SNPs in a 200-SNP moving window and
315 dropping one of each pair of sites with an R^2 over 0.1. The LD-pruned allele count matrix was then
316 centered and all sites scaled to unit variance when conducting the PCA, following recommendations
317 in Patterson *et al.* (2006).

318 We ran linear-model GWAS both with and without the first 10 principal components as covariates
319 in PLINK and summarized results across simulations by counting the number of SNPs with p -value
320 below 0.05 after adjusting for an expected false positive rate of less than 5% (Benjamini and Yekutieli
321 2001). We also examined p values for systematic inflation by comparing to the values expected from
322 a uniform distribution (because no SNPs were used when generating phenotypes, well-calibrated
323 p -values should be uniform).

324 Results from all analyses were summarized and plotted with the "ggplot2" (Wickham 2016) and
325 "cowplot" (Wilke 2019) packages in R (R Core Team 2018).

326 **Results**

327 **Demographic Parameters and Run Times**

328 Adjusting the spatial dispersal and interaction distance, σ , has a surprisingly large effect on de-
 329 mographic quantities that are usually fixed in Wright-Fisher models – the generation time, census
 330 population size, and variance in offspring number, shown in Figure 2. Because our simulation is
 331 parameterized on an individual level, these population parameters emerge as a property of the inter-
 332 actions among individuals rather than being directly set. Variation across runs occurs because, even
 333 though the parameters K and L that control population density and mean lifetime respectively were
 334 the same in all simulations, the strength of stochastic effects depends strongly on the spatial interaction
 335 distance σ . For instance, the population density near to individual i (denoted n_i above) is computed
 336 by averaging over roughly $N_W = 4\pi K\sigma^2$ individuals, and so has standard deviation proportional to
 337 $1/\sqrt{N_W}$ – it is more variable at lower densities. (Recall that N_W is Wright’s neighborhood size.) Since
 338 the probability of survival is a nonlinear function of n_i , actual equilibrium densities and lifetimes differ
 339 from K and L . This is the reason that we included *random mating* simulations – where mate choice and
 340 offspring dispersal are both nonspatial – since this should preserve the random fluctuations in local
 341 population density while destroying any spatial genetic structure. We verified that random mating
 342 models retained no geographic signal by showing that summary statistics did not differ significantly
 343 between sampling regimes (Table S2), unlike in spatial models (discussed below).

344 There are a few additional things to note about Figure 2. First, all three quantities are non-monotone
 345 with neighborhood size. Census size largely declines as neighborhood size increases for both the spatial
 346 and random mating models. However, for spatial models this decline only begins for neighborhood
 347 size ≥ 10 . Spatial and random mating models are indistinguishable from one another for neighborhood
 348 sizes larger than 100. Census sizes range from around 14,000 at low σ in the random mating model
 349 to 10,000 for both models when neighborhood sizes approach 1,000. The scaling of census sizes in
 350 both random-mating and spatial models appears to be related to two consequences of the spatial
 351 competition function: the decline of fitness at range edges, which effectively reduces the habitable area
 352 by one σ around the edge of the map and so results in a smaller habitable area at high σ values; and
 353 variation in the equilibrium population density given varying competition radii. Furthermore, census
 354 size increases in spatial models as neighborhood size increases from 2 to 10. This may reflect an Allee
 355 effect (Allee *et al.* 1949) in which some individuals are unable to find mates when the mate selection
 356 radius is very small.

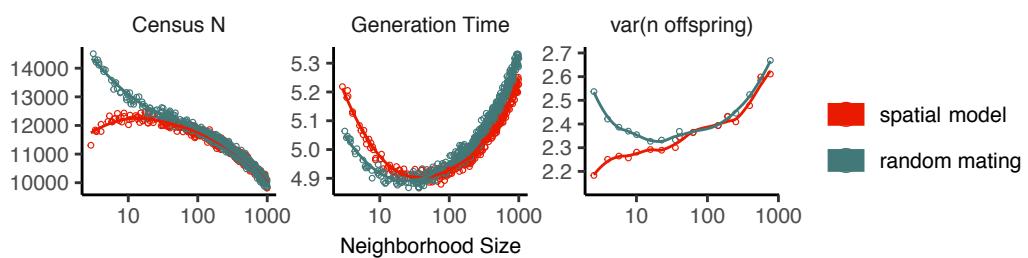


Figure 2 Genealogical parameters from spatial and random mating SLiM simulations, by neighborhood size.

357 Generation time similarly shows complex behavior with respect to neighborhood sizes, and varies
 358 between 5.2 and 4.9 timesteps per generation across the parameter range explored. Under both the
 359 spatial and random mating models, generation time reaches a minimum at a neighborhood size of
 360 around 50. Interestingly, under the range of neighborhood sizes that we examined, generation times
 361 between the random mating and spatial models are never quite equivalent – presumably this would
 362 cease to be the case at neighborhood sizes higher than we simulated here.

363 Last, we looked at the variance in number of offspring – a key parameter determining the effective

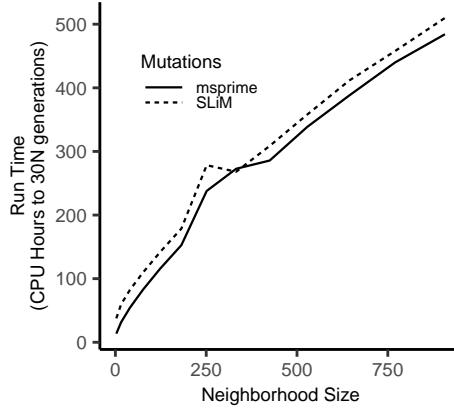


Figure 3 Run times of continuous space simulations with landscape width 50 and expected density 5 under varying neighborhood size. Times are shown for simulations run with mutations applied directly in SLiM (dashed lines) or later applied to tree sequences with msprime (solid lines). Times for simulations run with tree sequence recording disabled are shown in grey.

population size. Surprisingly, the spatial and random mating models behave quite differently: while the variance in offspring number increases nearly monotonically under the spatial model, the random mating model actually shows a decline in the variance in offspring number until a neighborhood size of around 10 before it increases and eventually equals what we observe in the spatial case.

Run times for our model scale approximately linearly with neighborhood size (Figure 3), with the lowest neighborhood sizes reaching 30N generations in around a day and those with neighborhood size approaching 1,000 requiring up to three weeks of computation. As currently implemented, running simulations at neighborhood sizes more than 1,000 to coalescence is likely impractical, though running these models for more limited timescales and then “recapitulating” the simulation using reverse-time simulation from the resulting tree sequence in msprime is possible (Haller *et al.* 2019).

Impacts of Continuous Space on Population Genetic Summary Statistics

Even though certain aspects of population demography depend on the scale of spatial interactions, it still could be that population genetic variation is well-described by a well-mixed population model. Indeed, mathematical results suggest that genetic variation in some spatial models should be well-approximated by a Wright-Fisher population if neighborhood size is large and all samples are geographically widely separated (Wilkins 2004; Zähle *et al.* 2005). However, the behavior of most common population genetic summary statistics other than Tajima’s D (Städler *et al.* 2009) has not yet been described in realistic geographic models. Moreover, as we will show, spatial sampling strategies can affect summaries of genetic variation at least as strongly as the underlying population dynamics.

Site Frequency Spectra and Summaries of Diversity Figure 4 shows the effect of varying neighborhood size and sampling strategy on the site frequency spectrum (Figure 4, Figure S5) and several standard population genetic summary statistics (Figure 4B; additional statistics are shown in Figure S4). Consistent with findings in island and stepping stone simulations (Städler *et al.* 2009), the SFS shows a significant enrichment of intermediate frequency variants in comparison to the nonspatial expectation. This bias is most pronounced below a neighborhood size of 100 and is exacerbated by midpoint and point sampling of individuals (depicted in Figure 1). Reflecting this, Tajima’s D is quite positive in the same situations (Figure 4B). Notably, the point at which Tajima’s D approaches 0 differs strongly across sampling strategies – varying from a neighborhood size of roughly 50 for random sampling to at least 1000 for midpoint sampling.

One of the most commonly used summaries of variation is Tajima’s summary of nucleotide diversity, θ_π , calculated as the mean density of nucleotide differences averaged across pairs of samples. As can



Figure 4 Site frequency spectrum (A; note axes are log-scaled) and summary statistic distributions (B) by sampling strategy and neighborhood size.

395 be seen in Figure 4B, θ_π in the spatial model is inflated by up to three-fold relative to the random
396 mating model. This pattern is opposite the expectation from census population size (Figure 2), because
397 the spatial model has *lower* census size than the random mating model at neighborhood sizes less than
398 100. Differences between these models likely occur because θ_π is a measure of mean time to most recent
399 common ancestor between two samples, and at small values of σ , the time for dispersal to mix ancestry
400 across the range exceeds the mean coalescent time under random mating. (For instance, at the smallest
401 value of $\sigma = 0.2$, the range is 250 dispersal distances wide, and since the location of a diffusively
402 moving lineage after k generations has variance $k\sigma^2$, it takes around $250^2 = 62500$ generations to
403 mix across the range, which is roughly ten times larger than the random mating effective population
404 size). θ_π using each sampling strategy approaches the random mating expectation at its own rate, but
405 by a neighborhood size of around 100 all models are roughly equivalent. Interestingly, the effect of
406 sampling strategy is reversed relative to that observed in Tajima's D – midpoint sampling reaches
407 random mating expectations around neighborhood size 50, while random sampling is inflated until
408 around neighborhood size 100.

409 Values of observed heterozygosity and its derivative F_{IS} also depend heavily on neighborhood size
410 under spatial models as well as the sampling scheme. F_{IS} is inflated above the expectation across
411 most of the parameter space examined and across all sampling strategies. This effect is caused by
412 a deficit of heterozygous individuals in low-dispersal simulations – a continuous-space version of
413 the Wahlund effect (Wahlund 1928). Indeed, for random sampling under the spatial model, F_{IS} does
414 not approach the random mating equivalent until neighborhood sizes of nearly 1000. On the other
415 hand, the dependency of raw observed heterozygosity on neighborhood size is not monotone. Under
416 midpoint sampling observed heterozygosity is inflated even over the random mating expectation, as a
417 result of the a higher proportion of heterozygotes occurring in the middle of the landscape (Figure S6).
418 This echoes a report from Shirk and Cushman (2014) who observed a similar excess of heterozygosity
419 in the middle of the landscape when simulating under a lattice model.

420 **IBS tracts and correlations with geographic distance** We next turn our attention to the effect of
421 geographic distance on haplotype block length sharing, summarized for sets of nearby and distant
422 individuals in Figure 5. There are two main patterns to note. First, nearby individuals share more
423 long IBS tracts than distant individuals (as expected because they are on average more closely related).
424 Second, the difference in the number of long IBS tracts between nearby and distant individuals
425 decreases as neighborhood size increases. This reflects the faster spatial mixing of populations with
426 higher dispersal, which breaks down the correlation between the IBS tract length distribution and
427 geographic distance. This can also be seen in the bottom row of Figure 4B, where the correlation
428 coefficients between the summaries of the IBS tract length distribution (the mean, skew, and count of
429 tracts over 10^6 bp) and geographic distance approaches 0 as neighborhood size increases.

430 The patterns observed for correlations of IBS tract lengths with geographic distance are similar
431 to those observed in the more familiar correlation of allele frequency measures such as D_{xy} (i.e.,
432 "genetic distance") or F_{ST} against geographic distance (Rousset 1997). D_{xy} is positively correlated
433 with the geographic distance between the individuals, and the strength of this correlation declines
434 as dispersal increases (Figure 4B), as expected (Wright 1943; Rousset 1997). This relationship is very
435 similar across random and point sampling strategies, but is weaker for midpoint sampling, perhaps
436 due to a dearth of long-distance comparisons. In much of empirical population genetics a regression
437 of genetic differentiation against spatial distance is a de-facto metric of the significance of isolation
438 by distance. The similar behavior of moments of the pairwise distribution of IBS tract lengths shows
439 why haplotype block sharing has recently emerged as a promising source of information on spatial
440 demography through methods described in Ringbauer *et al.* (2017) and Baharian *et al.* (2016).

441 **Spatial distribution of allele copies** Mutations occur in individuals and spread geographically over
442 time. Because low frequency alleles generally represent recent mutations (Sawyer 1977; Griffiths *et al.*
443 1999), the geographic spread of an allele may covary along with its frequency in the population. To
444 visualize this relationship we calculated the average distance among individuals carrying a focal
445 derived allele across simulations with varying neighborhood sizes, shown in Figure 6. On average

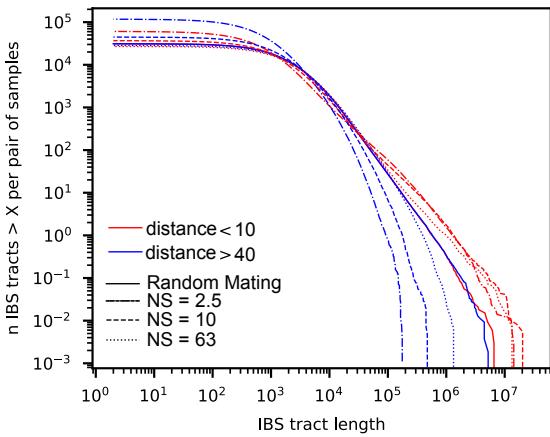


Figure 5 Cumulative distributions for IBS tract lengths per pair of individuals at different geographic distances, across three neighborhood sizes (NS). Nearby pairs (red curves) share many more long IBS tracts than do distant pairs (blue curves), except in the random mating model. The distribution of long IBS tracts between nearby individuals are very similar across neighborhood sizes, but distant individuals are much more likely to share long IBS tracts at high neighborhood size than at low neighborhood size.

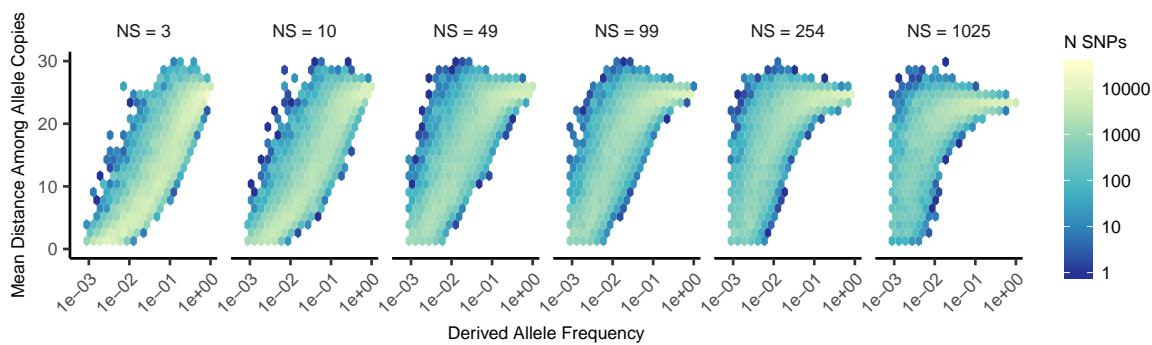


Figure 6 Spatial spread of rare alleles by neighborhood size (NS): Each plot shows the distribution (across derived alleles and simulations) of average pairwise distance between individuals carrying a focal derived allele and derived allele frequency.

446 we find that low frequency alleles are the most geographically restricted, and that the extent to which
447 geography and allele frequency are related depends on the amount of dispersal in the population.
448 For populations with large neighborhood sizes we found that even very low frequency alleles can be
449 found across the full landscape, whereas in populations with low neighborhood sizes the relationship
450 between distance among allele copies and their frequency is quite strong. This is the basic process
451 underlying Novembre and Slatkin's (2009) method for estimating dispersal distances based on the
452 distribution of low frequency alleles, and also generates the greater degree of bias in GWAS effect sizes
453 for low frequency alleles identified in Mathieson and McVean (2012).

454 **Effects of Space on Demographic Inference**

455 One of the most important uses for population genetic data is inferring demographic history of popu-
456 lations. As demonstrated above, the site frequency spectrum and the distribution of IBS tracts varies
457 across neighborhood sizes and sampling strategies. Does this variation lead to different inferences of
458 past population sizes? To ask this we inferred population size histories from samples drawn from our
459 simulated populations with two approaches: stairwayplot (Liu and Fu 2015), which uses a genome-
460 wide estimate of the SFS, and SMC++ (Terhorst *et al.* 2016), which incorporates information on both the
461 SFS and linkage disequilibrium across the genome.

462 Figure 7A shows rolling medians of inferred population size histories from each method across all
463 simulations, grouped by neighborhood size and sampling strategy. In general these methods tend to
464 slightly overestimate ancient population sizes and infer recent population declines when neighborhood
465 sizes are below 20 and sampling is spatially clustered. The overestimation of ancient population sizes
466 however is relatively minor, averaging around a two-fold inflation at 10,000 generations before present
467 in the worst-affected bins. For stairwayplot we found that many runs infer dramatic population
468 bottlenecks in the last 1,000 generations when sampling is spatially concentrated, resulting in ten-fold
469 or greater underestimates of recent population sizes. However SMC++ appeared more robust to
470 this error, with runs on point- and midpoint-sampled simulations at the lowest neighborhood sizes
471 underestimating recent population sizes by roughly half and those on randomly sampled simulations
472 showing little error. Above neighborhood sizes of around 100, both methods performed relatively well
473 when averaging across results from multiple simulations.

474 However, individual simulations were often inferred to have turbulent demographic histories, as
475 shown by the individually inferred histories (shown in Figure S7). Indeed, the standard deviation of
476 inferred N_e across time points (shown in Figure 7B) often exceeds the expected N_e for both methods.
477 That is, despite the nearly constant population sizes in our simulations, both methods tended to infer
478 large fluctuations in population size over time, which could potentially result in incorrect biological
479 interpretations. On average the variance of inferred population sizes was elevated at the lowest
480 neighborhood sizes and declines as dispersal increases, with the strongest effects seen in stairwayplot
481 results with clustered sampling and neighborhood sizes less than 20 (Figure 7B).

482 **GWAS**

483 To ask what confounding effects spatial genetic variation might have on genome-wide association
484 studies we performed GWAS on our simulations using phenotypes that were determined solely by
485 the environment – so, any SNP showing statistically significant correlation with phenotype is a false
486 positive. As expected, spatial autocorrelation in the environment causes spurious associations across
487 much of the genome if no correction for genetic relatedness among samples is performed (Figures 8 and
488 S8). This effect is particularly strong for clinal and corner environments, for which the lowest dispersal
489 levels cause over 60% of SNPs in the sample to return significant associations. Patchy environmental
490 distributions, which are less strongly spatially correlated (Figure 8A), cause fewer false positives
491 overall but still produce spurious associations at roughly 10% of sites at the lowest neighborhood
492 sizes. Interestingly we also observed a small number of false positives in roughly 3% of analyses
493 on simulations with nonspatial environments, both with and without PC covariates included in the
494 regression.

495 The confounding effects of geographic structure are well known, and it is common practice to
496 control for this by including principal components (PCs) as covariates to control for these effects. This

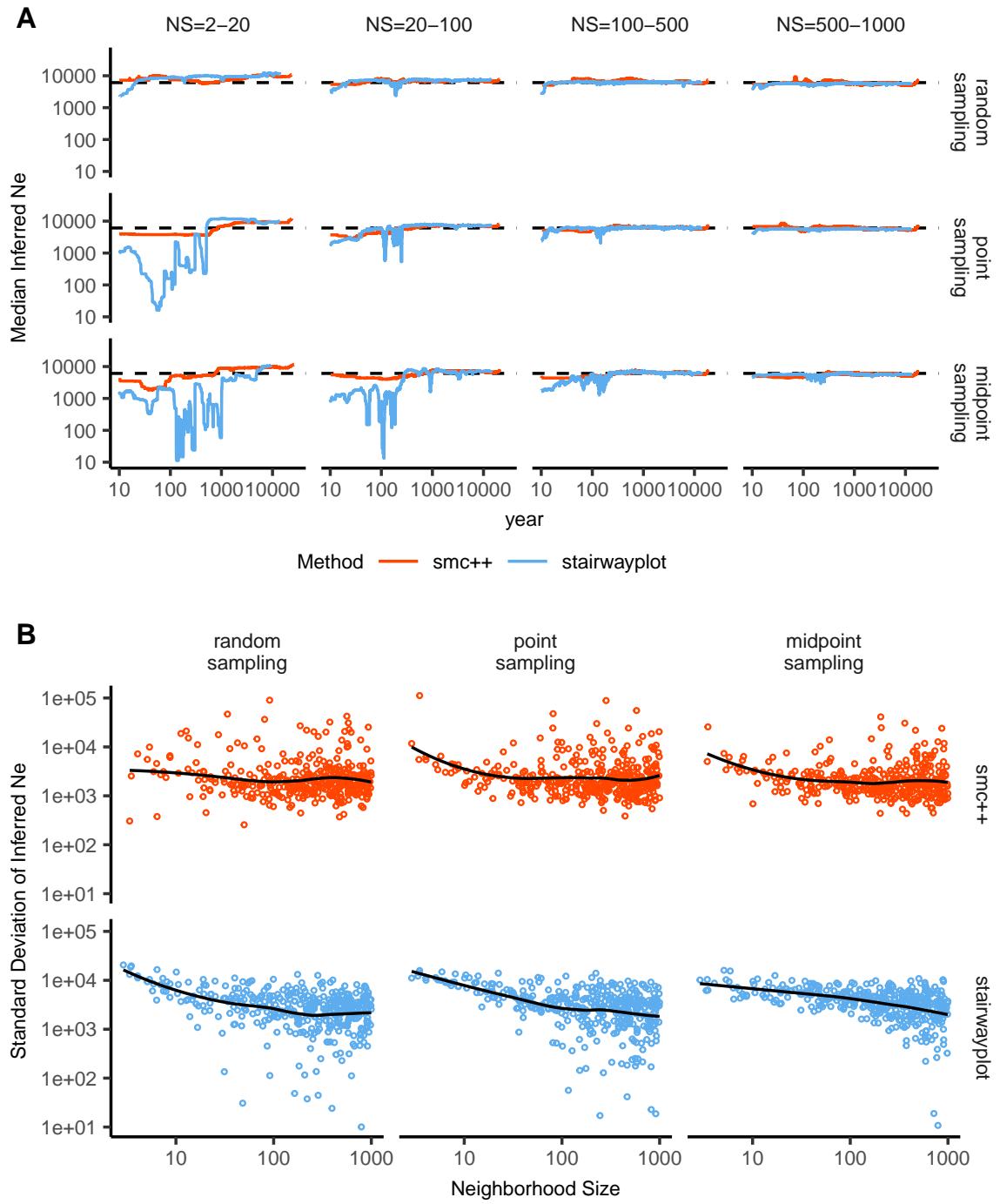


Figure 7 A: Rolling median inferred N_e trajectories for stairwayplot and smc++ across sampling strategies and neighborhood size bins. The dotted line shows the mean N_e of random-mating simulations. B: Standard deviation of individual inferred N_e trajectories, by neighborhood size and sampling strategy. Black lines are loess curves. Plots including individual model fits are shown in Figure S7.

mostly works in our simulations – after incorporating the first ten PC axes as covariates, the vast majority of SNPs no longer surpass a significance threshold chosen to have a 5% false discovery rate (FDR). However, a substantial number of SNPs – up to 1.5% at the lowest dispersal distances – still surpass this threshold (and thus would be false positives in a GWAS), especially under “corner” and “patchy” environmental distributions (Figure 8C). At neighborhood sizes larger than 500, up to 0.31% of SNPs were significant for corner and clinal environments. Given an average of 132,000 SNPs across simulations after MAF filtering, this translates to up to 382 false-positive associations; for human-sized genomes, this number would be much larger. In most cases the p values for these associations were significant after FDR correction but would not pass the threshold for significance under the more conservative Bonferroni correction (see example Manhattan plots in figure S8).

Clinal environments cause an interesting pattern in false positives after PC correction: at low neighborhood sizes the correction removes nearly all significant associations, but at neighborhood sizes above roughly 250 the proportion of significant SNPs increases to up to 0.4% (Figure 8). This may be due to a loss of descriptive power of the PCs – as neighborhood size increases, the total proportion of variance explained by the first 10 PC axes declines from roughly 10% to 4% (Figure 8B). Essentially, PCA seems unable to effectively summarize the weak population structure present in large-neighborhood simulations ~~, given the sample sizes we tested,~~ but these populations continue to have enough spatial structure to create significant correlations between genotypes and the environment. A similar process can also be seen in the corner phenotype distribution, in which the count of significant SNPs initially declines as neighborhood size increases and then increases at approximately the point at which the proportion of variance explained by PCA approaches its minimum.

Figure 8D shows quantile-quantile plots for a subset of simulations that show the degree of genome-wide inflation of test statistics in PC-corrected GWAS across all simulations and environmental distributions. An alternate visualization is also included in figure S9. For clinal environments, $-\log_{10}(p)$ values are most inflated when neighborhood sizes are large, consistent with the pattern observed in the count of significant associations after PC regression. In contrast corner and patchy environments cause the greatest inflation in $-\log_{10}(p)$ at neighborhood sizes less than 100, which likely reflects the inability of PCA to account for fine-scale structure caused by very limited dispersal. Finally, we observed that PC regression appears to overfit to some degree for all phenotype distributions, visible in Figure 8D as points falling below the 1:1 line.

Discussion

In this study, we have used efficient forward time population genetic simulations to describe the myriad influence of continuous geography on genetic variation. In particular, we examine how three main types of downstream empirical inference are affected by unmodeled spatial population structure – population genetic summary statistics, inference of population size history, and genome-wide association studies (GWAS). As discussed above, space often matters (and sometimes dramatically), both because of how samples are arranged in space, and because of the inherent patterns of relatedness established by geography.

Effects of Dispersal

Limited dispersal inflates effective population size, creates correlations between genetic and spatial distances, and introduces strong distortions in the site frequency spectrum that are reflected in a positive Tajima’s D (Figure 4). At the lowest dispersal distances, this can increase genetic diversity threefold relative to random-mating expectations. These effects are strongest when neighborhood sizes are below 100, but in combination with the effects of nonrandom sampling they can persist up to neighborhood sizes of at least 1000 (e.g., inflation in Tajima’s D and observed heterozygosity under midpoint sampling). If samples are chosen uniformly from across space, the general pattern is similar to expectations of the original analytic model of Wright (1943), which predicts that populations with neighborhood sizes under 100 will differ substantially from random mating, while those above 10,000 will be nearly indistinguishable from panmixia.

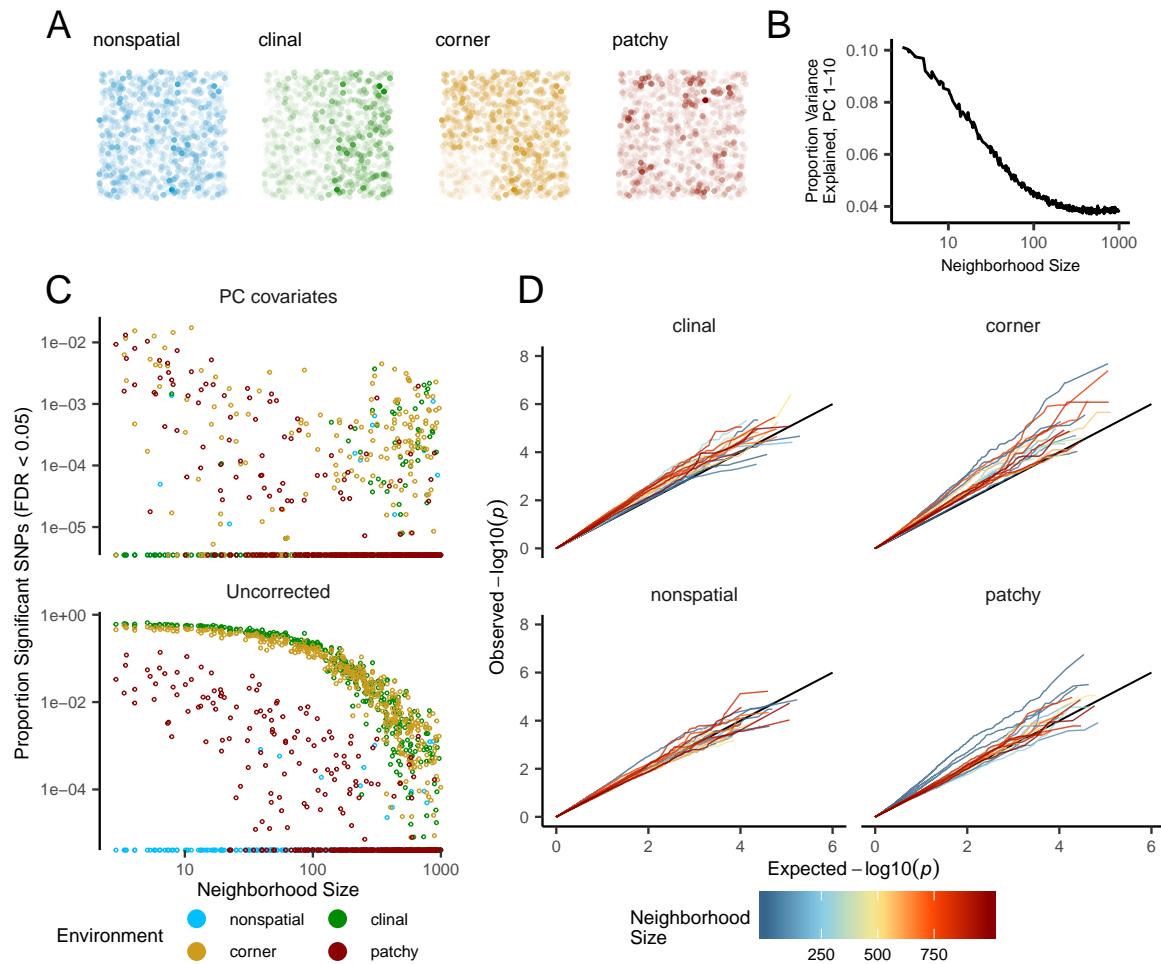


Figure 8 Impacts of spatially varying environments and isolation by distance on linear regression GWAS. Simulated quantitative phenotypes are determined only by an individual's location and the spatial distribution of environmental factors. In **A** we show the phenotypes and locations of sampled individuals under four environmental distributions, with transparency scaled to phenotype. As neighborhood size increases a PCA explains less of the total variation in the data (**B**). Spatially correlated environmental factors cause false positives at a large proportion of SNPs, which is partially but not entirely corrected by adding the first 10 PC coordinates as covariates (**C**). Quantile-quantile plots in (**D**) show inflation of $-\log_{10}(p)$ after PC correction for simulations with spatially structured environments, with line colors showing the neighborhood size of each simulation.

546 The patterns observed in sequence data reflect the effects of space on the underlying genealogy.
547 Nearby individuals coalesce rapidly under limited dispersal and so are connected by short branch
548 lengths, while distant individuals take much longer to coalesce than they would under random
549 mating. Mutation and recombination events in our simulation both occur at a constant rate along
550 branches of the genealogy, so the genetic distance and number of recombination events separating
551 sampled individuals simply gives a noisy picture of the genealogies connecting them. Tip branches
552 (i.e., branches subtending only one individual) are then relatively short, and branches in the middle of
553 the genealogy connecting local groups of individuals relatively long, leading to the biases in the site
554 frequency spectrum shown in Figure 4.

555 The genealogical patterns introduced by limited dispersal are particularly apparent in the distribution
556 of haplotype block lengths (Figure 4). This is because identical-by-state tract lengths reflect the
557 impacts of two processes acting along the branches of the underlying genealogy – both mutation and
558 recombination – rather than just mutation as is the case when looking at the site frequency spectrum or
559 related summaries. This means that the pairwise distribution of haplotype block lengths carries with
560 it important information about genealogical variation in the population, and correlation coefficients
561 between moments of the this distribution and geographic location contain signal similar to the correla-
562 tions between F_{ST} or D_{xy} and geographic distance (Rousset 1997). Indeed this basic logic underlies
563 two recent studies explicitly estimating dispersal from the distribution of shared haplotype block
564 lengths (Ringbauer *et al.* 2017; Baharian *et al.* 2016). Conversely, because haplotype-based measures of
565 demography are particularly sensitive to variation in the underlying genealogy, inference approaches
566 that assume random mating when analyzing the distribution of shared haplotype block lengths are
567 likely to be strongly affected by spatial processes.

568 **Effects of Sampling**

569 One of the most important differences between random mating and spatial models is the effect of
570 sampling: in a randomly mating population the spatial distribution of sampling effort has no effect on
571 estimates of genetic variation (Table S1), but when dispersal is limited sampling strategy can compound
572 spatial patterns in the underlying genealogy and create pervasive impacts on all downstream genetic
573 analyses (see also Städler *et al.* (2009)). In most species, the difficulty of traveling through all parts
574 of a species range and the inefficiency of collecting single individuals at each sampling site means
575 that most studies follow something closest to the “point” sampling strategy we simulated, in which
576 multiple individuals are sampled from nearby points on the landscape. For example, in ornithology a
577 sample of 10 individuals per species per locality is a common target when collecting for natural history
578 museums. In classical studies of *Drosophila* variation the situation is considerably worse, in which a
579 single orchard might be extensively sampled.

580 When sampling is clustered at points on a landscape and dispersal is limited, the sampled indi-
581 viduals will be more closely related than a random set of individuals. Average coalescence times of
582 individuals collected at a locality will then be more recent and branch lengths shorter than expected by
583 analyses assuming random mating. This leads to fewer mutations and recombination events occurring
584 since their last common ancestor, causing a random set of individuals to share longer average IBS tracts
585 and have fewer nucleotide differences. For some data summaries, such as Tajima’s D , Watterson’s
586 θ , or the correlation coefficient between spatial distance and the count of long haplotype blocks, this
587 can result in large differences in estimates between random and point sampling (Figure 4). Inferring
588 underlying demographic parameters from these summary statistics – unless the spatial locations of the
589 sampled individuals are somehow taken into account – will likely be subject to bias.

590 We observed the largest sampling effects using “midpoint” sampling. This model is meant to
591 reflect a bias in sampling effort towards the middle of a species’ range. In empirical studies this
592 sampling strategy could arise if, for example, researchers choose to sample the center of the range
593 and avoid range edges to maximize probability of locating individuals during a short field season.
594 Because midpoint sampling provides limited spatial resolution it dramatically reduces the magnitude
595 of observed correlations between spatial and genetic distances. More surprisingly, midpoint sampling
596 also leads to strongly positive Tajima’s D and an inflation in the proportion of heterozygous individuals
597 in the sample – similar to the effect of sampling a single deme in an island model as reported in Städler

598 *et al.* (2009). This increase in observed heterozygosity appears to reflect the effects of range edges,
599 which are a fundamental facet of spatial genetic variation. If individuals move randomly in a finite
600 two-dimensional landscape then regions in the middle of the landscape receive migrants from all
601 directions while those on the edge receive no migrants from at least one direction. The average number
602 of new mutations moving into the middle of the landscape is then higher than the number moving
603 into regions near the range edge, leading to higher heterozygosity and lower inbreeding coefficients
604 (F_{IS}) away from range edges. Though here we used only a single parameterization of fitness decline at
605 range edges we believe this is a general property of non-infinite landscapes as it has also been observed
606 in previous studies simulating under lattice models (Neel *et al.* 2013; Shirk and Cushman 2014).

607 In summary, we recommend that empirical researchers collect individuals from across as much
608 of the species' range as practical, choosing samples separated by a range of spatial scales. Many
609 summary statistics are designed for well-mixed populations, and so provide different insights into
610 genetic variation when applied to different subsets of the population. Applied to a cluster of samples,
611 summary statistics based on segregating sites (e.g., Watterson's θ and Tajima's D), heterozygosity, or
612 the distribution of long haplotype blocks, can be expected to depart significantly from what would be
613 obtained from a wider distribution of samples. Comparing the results of analyses conducted on all
614 individuals versus those limited to single individuals per locality can provide an informative contrast.
615 Finally we wish to point out that the bias towards intermediate allele frequencies that we observe may
616 mean that the importance of linked selection, at least as is gleaned from the site frequency spectrum,
617 may be systematically underestimated currently.

618 Demography

619 Previous studies have found that population structure and nonrandom sampling can create spuri-
620 ous signals of population bottlenecks when attempting to infer demographic history with microsatellite
621 variation, summary statistics, or runs of homozygosity ([Chikhi *et al.* 2010](#); [Städler *et al.* 2009](#); [Ptak and Przeworski 2002](#); [Ma](#)
622 ([Chikhi *et al.* 2010](#); [Städler *et al.* 2009](#); [Ptak and Przeworski 2002](#); [Mazet *et al.* 2015](#); [Leblois *et al.* 2006](#)). Here
623 we found that methods that infer detailed population trajectories through time based on the SFS and
624 patterns of LD across the genome are also subject to this bias, with some combinations of dispersal
625 and sampling strategy systematically inferring deep recent population bottlenecks and overestimating
626 ancient N_e by around a factor of 2. We were surprised to see that both stairwayplot and SMC++ can tol-
627 erate relatively strong isolation by distance – i.e., neighborhood sizes of 20 – and still perform well when
628 averaging results across multiple simulations. ([However, note the high amount of between-simulation](#)
629 [variance seen in Figure S7](#).[\)](#) Inference in populations with neighborhood sizes over 20 was relatively
630 unbiased unless samples were concentrated in the middle of the range (Figure 7). Although median
631 demography estimates across many independent simulations were fairly accurate, empirical work
632 has only a single estimate to work with, and individual model fits (Figure S7) suggest that spuriously
633 inferred population size changes and bottlenecks are common, especially at small neighborhood sizes.
634 As we will discuss below, most empirical estimates of neighborhood size, including all estimates for
635 human populations, are large enough that population size trajectories inferred by these approaches
636 should not be strongly affected by spatial biases created by dispersal in continuous landscapes. In
637 contrast, Mazet *et al.* (2015) found that varying migration rates through time could create strong biases
638 in inferred population trajectories from an n -island model with parameters relevant for human history,
639 suggesting that changes in migration rates through time are more likely to drive variation in inferred
640 N_e than isolation by distance.

641 We found that SMC++ was more robust to the effects of space than stairwayplot, underestimating
642 recent populations by roughly half in the worst time periods rather than nearly 10-fold as with
643 stairwayplot. Though this degree of variation in population size is certainly meaningful in an ecological
644 context, it is relatively minor in population genetic terms. Methods directly assessing haplotype
645 structure in phased data example, (e.g., Browning and Browning 2015) are thought to provide increased
646 resolution for recent demographic events, but in this case the error we observed was essentially an
647 accurate reflection of underlying genealogies in which terminal branches are anomalously short.
648 Combined with our analysis of IBS tract length variation (Figure 5) this suggests that haplotype-based
649 methods are likely to be affected by similar biases.

650 A more worrying pattern was the high level of variance in inferred N_e trajectories for individual
651 model fits using these methods, which was highest in simulations with the smallest neighborhood
652 size (Figure 7, Figure S7). This suggests that, at a minimum, researchers working with empirical data
653 should replicate analyses multiple times and take a rolling average if model fits are inconsistent across
654 runs. Splitting samples and running replicates on separate subsets – the closest an empirical study can
655 come to our design of averaging the results from multiple simulations – may also alleviate this issue.

656 Our analysis suggests that many empirical analyses of population size history using methods like
657 SMC++ are robust to error caused by spatial structure within continuous landscapes. Inferences drawn
658 from static SFS-based methods like stairwayplot should be treated with caution when there are signs
659 of isolation by distance in the underlying data (for example, if a regression of F_{ST} against the logarithm
660 of geographic distance has a significantly positive slope), and in particular an inference of population
661 bottlenecks in the last 1000 years should be discounted if sampling is clustered, but estimates of deeper
662 time patterns are likely to be fairly accurate. The biases in the SFS and haplotype structure identified
663 above (see also Wakeley 1999; Chikhi *et al.* 2010; Städler *et al.* 2009) are apparently small enough that
664 they fall within the range of variability regularly inferred by these approaches, at least on datasets of
665 the size we simulated.

666 GWAS

667 Spatial structure is particularly challenging for genome-wide association studies, because the effects of
668 dispersal on genetic variation are compounded by spatial variation in the environment (Mathieson
669 and McVean 2012). Spatially restricted mate choice and dispersal causes variation in allele frequencies
670 across the range of a species. If environmental factors affecting the phenotype of interest also vary over
671 space, then allele frequencies and environmental exposures will covary over space. In this scenario an uncorrected GWAS will infer genetic associations with a purely environmental phenotype at any site
672 in the genome that is differentiated over space, and the relative degree of bias will be a function of the
673 degree of covariation in allele frequencies and the environment (i.e., Figure 8C, bottom panel). This
674 pattern has been demonstrated in a variety of simulation and empirical contexts (Price *et al.* 2006; Yu
675 *et al.* 2005; Young *et al.* 2018; Mathieson and McVean 2012; Kang *et al.* 2008, 2010; Bulik-Sullivan *et al.*
676 2015; Berg *et al.* 2018; Sohail *et al.* 2018).

677 Incorporating PC positions as covariates in a linear-regression GWAS (Price *et al.* 2006) is designed
678 to address this challenge by regressing out a baseline level of “average” differentiation. In essence, a
679 PC-corrected GWAS asks “what regions of the genome are more associated with this phenotype than
680 the average genome-wide association observed across populations?” In our simulations, we observed
681 that this procedure can fail under a variety of circumstances. If dispersal is limited and environmental
682 variation is clustered in space (i.e., corner or patchy distributions in our simulations), PC positions fail
683 to capture the fine-scale spatial structure required to remove all signals of association. Conversely, as
684 dispersal increases, PCA loses power to describe population structure before spatial mixing breaks
685 down the relationship between genotype and the environment. These effects were observed with all
686 spatially correlated environmental patterns, but were particularly pronounced if environmental effects
687 are concentrated in one region, as was also found by Mathieson and McVean (2012). Though increasing
688 the number of PC axes used in the analysis may reduce the false-positive rate, this may also decrease
689 the power of the test to detect truly causal alleles (Lawson *et al.* 2019).

690 In this work we simulated a single chromosome with size roughly comparable to one human
691 chromosome. If we scale the number of false-positive associations identified in our analyses to a
692 GWAS conducted on whole-genome data from humans, we would expect to see several thousand
693 weak false-positive associations after PC corrections in a population with neighborhood sizes up to at
694 least 1000 (which should include values appropriate for many human populations). Notably, very few
695 of the spurious associations we identified would be significant at a conservative Bonferroni-adjusted
696 p -value cutoff (see Figure S8). This suggests that GWAS focused on finding strongly associated
697 alleles for traits controlled by a limited number of variants in the genome are likely robust to the
698 impacts of continuous spatial structure. However, methods that analyze the combined effects of
699 thousands or millions of weakly associated variants such as polygenic risk scores (Khera *et al.* 2018)
700 (Wray *et al.* 2007; International Schizophrenia Consortium *et al.* 2009) are likely to be affected by subtle

702 population structure. Indeed as recently identified in studies of genotype associations for human height
703 in Europe (Berg *et al.* 2018; Sohail *et al.* 2018), PC regression GWAS in modern human populations
704 do include residual signal of population structure in large-scale analyses of polygenic traits. ~~When~~
705 ~~attempting to make predictions across populations with different environmental exposures, polygenic~~
706 ~~risk scores affected~~
~~In addition to error associated with varying patterns of linkage disequilibrium~~
707 ~~and allele frequency among populations, the confounding of environmental and genetic effects on~~
708 ~~phenotypes introduced~~
~~by population structure can be expected to offer is expected to lead to low~~
709 ~~predictive power ,when polygenic scores are generated for populations outside the original GWAS~~
710 ~~cohort,~~
711 as was shown in a recent study finding lower ~~performance~~ polygenic score predictive power
outside European populations (Martin *et al.* 2019).

712 In summary, spatial covariation in population structure and the environment confounds the interpretation
713 of GWAS *p*-values, and correction using principal components is insufficient to fully separate these signals for polygenic traits under a variety of environmental and population parameter
714 regimes. Other GWAS methods such as mixed models (Kang *et al.* 2008) may be less sensitive to this confounding, but there is no obvious reason that this should be so. One approach to estimating the degree of bias in GWAS caused by population structure is LD score regression (Bulik-Sullivan
715 *et al.* 2015). Though this approach appears to work well in practice, its interpretation is not always straightforward and it is likely biased by the presence of linked selection (Berg *et al.* 2018). In addition, we observed that in many cases the false-positive SNPs we identified appeared to be concentrated in LD peaks similar to those expected from truly causal sites (Figure S8), which may confound LD score regression.

723 We suggest a straightforward alternative for species in which the primary axes of population differentiation ~~is space~~
724 ~~are associated with geography~~ (note this is likely not the case for some modern human populations): run a GWAS with spatial coordinates as phenotypes and check for *p*-value inflation or significant associations. If significant associations with sample locality are observed after correcting for population structure, the method is sensitive to false positives induced by spatial structure. This is essentially the approach taken in our “clinal” model (though we add normally distributed noise to our phenotypes). This approach has recently been taken with polygenic scores for UK Biobank samples in Haworth *et al.* (2019), finding that scores are correlated with birth location even in this relatively homogenous sample. Of course, it is possible that genotypes indirectly affect individual locations by adjusting organismal fitness and thus habitat selection across spatially varying environments, but we believe that this hypothesis should be tested against a null of stratification bias inflation rather than accepted as true based on GWAS results.

735 **Where are natural populations on this spectrum?**

736 For how much of the tree of life do spatial patterns circumscribe genomic variation? In Table 1 we
737 gathered estimates of neighborhood size from a range of organisms to get an idea of how strongly
738 local geographic dispersal affects patterns of variation. This is an imperfect measure: some aspects
739 of genetic variation are most strongly determined by neighborhood size (Wright 1946), others (e.g.,
740 number of segregating sites) ~~are more strongly determined~~ by global N_e ~~or by~~, or the ratio of the two. In
741 addition, ~~definitions of "population density" in genetic versus ecological studies may lead to varying~~
742 ~~estimates of neighborhood size for a given species, and~~ these empirical examples ~~are likely~~ may be
743 biased towards small-neighborhood species ~~(because few studies have quantified neighborhood size~~
744 ~~in species with very high dispersal or population density).~~

745 However, from the available data we find that neighborhood sizes in the range we simulated are
746 fairly common across a range of taxa. At the extreme low end of empirical neighborhood size estimates
747 we see some flowering plants, large mammals, and colonial insects like ants ~~-with neighborhood sizes~~
748 ~~less than roughly 100~~. Species such as this have neighborhood size estimates small enough that spatial
749 processes are likely to strongly influence inference. These include some human populations such as
750 the Gainj- and Kalam-speaking people of Papua New Guinea, in which the estimated neighborhood
751 sizes in Rousset (1997) range from 40 to 410 depending on the method of estimation. Many more
752 species occur in a middle range of neighborhood sizes between 100 and 1000 – a range in which spatial
753 processes play a minor role in our analyses under random spatial sampling but are important when

Table 1 Neighborhood size estimates from empirical studies.

	Species	Description	Neighborhood Size
<i>Ipomopsis aggregata</i>	flowering plant	12.60 - 37.80	
<i>Borrichia frutescens</i>	salt marsh plant	20 - 30	
<i>Oreamnos americanus</i>	mountain goat	36 - 100	
<i>Homo sapiens</i>	Gainj- and Kalam-speaking people, Papua New Guinea	40 - 213	
<i>Formica sp.</i>	colonial ants	50 - 100	
<i>Astrocaryum mexicanum</i>	palm tree	102 - 895	
<i>Spermophilus mollis</i>	ground squirrel	204 - 480	
<i>Sceloporus olivaceus</i>	lizard	225 - 270	
<i>Dieffenbachia longispatha</i>	beetle-pollinated colonial herb	227 - 611	
<i>Aedes aegypti</i>	Yellow-fever mosquito	268	
<i>Homo sapiens</i>	Gainj- and Kalam-speaking people, Papua New Guinea	410	
<i>Quercus laevis</i>	Oak tree	> 440	
<i>Drosophila pseudoobscura</i>	fruit fly	500 - 1,000	
<i>Homo sapiens</i>	POPRES data NE Europe	1,342 - 5,425	

intertidal snail 240,000 Survey (Rousset 1997) intertidal snail 360,000 Genetic (Rousset 1997) height

754 sampling of individuals in space is clustered. Surprisingly, even some flying insects with huge census
755 population sizes fall in this group, including fruit flies (*D. melanogaster*) and mosquitoes (*A. aegypti*).
756 Last, many species likely have neighborhood sizes much larger than we simulated, including the
757 recent ancestors of modern humans in northeastern Europe (Ringbauer *et al.* 2017). For these species
758 demographic inference and summary statistics are likely to reflect minimal bias from spatial effects as
759 long as dispersal is truly continuous across the landscape. While that is so we caution that association
760 studies in which the effects of population structure are confounded with spatial variation in the
761 environment are still sensitive to dispersal even at these large neighborhood sizes.

762 **Other demographic models**

763 Any simulation of a population of reproducing organisms requires some kind of control on population
764 sizes, or else the population will either die out or grow very large after a sufficiently long period of time.
765 The usual choice of population regulation for population genetics – a constant size, as in the Wright–
766 Fisher model – implies biologically unrealistic interactions between geographically distant parts of the
767 species range. Our choice to regulate population size by including a local density-dependent control
768 on mortality is only one of many possible ways to do this. We could have instead regulated fecundity,
769 or recruitment, or both; this general class of models is sometimes referred to as the “Bolker–Pacala
770 model” (Bolker and Pacala 1997). It is not currently clear how much different choices of demographic
771 parameters, or of functional forms for the regulation, might quantitatively affect our results, although
772 the general predictions should be robust to similar forms of regulation. ~~Since populations are still~~
773 ~~As is usual in population genetics, the populations are~~ entirely *intrinsically* regulated, ~~our model still~~
774 ~~has a very strong “population genetics” flavor.~~ Alternatively, population size could be regulated
775 by interactions with other species (e.g., a Lotka–Volterra model), or extrinsically specified by local
776 resource availability (e.g., by food or nest site availability). Indeed, our model could be interpreted
777 as a caricature of such a model: as local density increases, good habitat is increasingly occupied,
778 pushing individuals into more marginal habitat and increasing their mortality. Many such models
779 should behave similarly to ours, but others (especially those with local population cycling), may differ
780 dramatically.

781 Population genetic simulations often use grids of discrete demes, which are assumed to approximate
782 continuous space. However, there are theoretical reasons to expect that increasingly fine grids of
783 discrete demes do not approach the continuous model (Barton *et al.* 2002). If continuous space can
784 be approximated by a limit of discrete models, this should be true regardless of the precise details
785 of the discrete model. Although we carefully chose parameters to match our continuous models, we
786 found that some aspects of genetic variation diverged from the continuous case as the discretization
787 got finer. This suggests that these models do not converge in the limit. However, many populations
788 may indeed be well-modeled as a series of discrete, randomly-mating demes if, for example, suitable
789 habitats are patchily distributed across the landscape. There is a clear need for greater exploration of
790 the consequences for population genetics of ecologically realistic population models.

791 **Future Directions and Limitations**

792 As we have shown, a large number of population genetic summary statistics contain information about
793 spatial population processes. We imagine that combinations of such summaries might be sufficient
794 for the construction of supervised machine learning regressors (e.g., Schrider and Kern 2018) for the
795 accurate estimation of dispersal from genetic data. Indeed, Ashander *et al.* (2018) found that inverse
796 interpolation on a vector of summary statistics provided a powerful method of estimating dispersal
797 distances. Expanding this approach to include the haplotype-based summary statistics studied here
798 and applying machine learning regressors built for general inference of nonlinear relationships from
799 high-dimensional data may allow precise estimation of spatial parameters under a range of complex
800 models.

801 One facet of spatial variation that we did not address in this study is the confounding of dispersal
802 and population density implicit in the definition of Wright’s neighborhood size. Our simulations
803 were run under constant densities, but Guindon *et al.* (2016) and Ringbauer *et al.* (2017) have shown

that these parameters are identifiable under some continuous models. Similarly, though the scaling effects of dispersal we show in Figure 4 should occur in populations of any total size, other aspects such as the number of segregating sites are also likely affected by the total landscape size (and so total census size). Indeed, our finding that stepping-stone and continuous-space models match in only certain aspects of genetic variation (Figure A1) shows that qualitatively similar models can produce different results dependent on the specific parameterizations used. While we believe our continuous model is a more appropriate depiction of many species' demographies than lattice models, it is likely that some populations and breeding systems do more closely resemble a series of interconnected random-mating populations. As with all population models, the best approximation for any empirical system will depend on the natural history of the species in question. Much additional work remains to be done to better understand how these parameters life history, range size, and habitat geometry interact to shape genetic variation in continuous space, which we leave to future studies.

Though our simulation allows incorporation of realistic demographic and spatial processes, it is inevitably limited by the computational burden of tracking tens or hundreds of thousands of individuals in every generation. In particular, computations required for mate selection and spatial competition scale approximately with the product of the total census size and the neighborhood size and so increase rapidly for large populations and dispersal distances. The reverse-time spatial Lambda–Fleming–Viot model described by Barton *et al.* (2010) and implemented by Kelleher *et al.* (2014) allows exploration of larger population and landscape sizes, but the precise connection of these models to forward-time demography is not yet clear. Alternatively, implementation of parallelized calculations may allow progress with forward-time simulations.

Finally, we believe that the difficulties in correcting for population structure in continuous populations using principal components analysis or similar decompositions is a difficult issue, well worth considering on its own. How can we best avoid spurious correlations while correlating genetic and phenotypic variation without underpowering the methods? Perhaps optimistically, we posit that process-driven descriptions of ancestry and/or more generalized unsupervised methods may be able to better account for carry out this task.

832 Data Availability

833 Scripts used for all analyses and figures are available at <https://github.com/kern-lab/spaceness>.

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1063 **Comparisons with Stepping-Stone Models**

1064

1065 We also compared our model results to a regular grid of discrete populations, which is commonly
 1066 used as an approximation of continuous geography. An important reason that this approximation
 1067 is often made is that it allows more efficient, coalescent simulations; we implemented these using
 1068 `msprime` (Kelleher *et al.* 2016). In this class of models we imagine an $n \times n$ grid of populations
 1069 exchanging migrants with neighboring populations at rate m . If these models are good approximations
 1070 of the continuous case we expect that results will converge as $n \rightarrow \infty$ (while scaling m appropriately
 1071 and keeping total population size fixed), so we ran simulations while varying n from 5 to 50 (Table
 1072 A1). To compare with continuous models we first distributed the same “effective” number of
 1073 individuals across the landscape as in our continuous-space simulations (≈ 6100 , estimated from θ_π
 1074 of random-mating continuous-space simulations). We then approximate the mean per-generation
 1075 dispersal distance σ given a total landscape width W as the product of the probability of an individual
 1076 being a migrant and the distance traveled by migrants: $\sigma = 4m(W/n)$. This means that m in different
 1077 simulations with the same σ scales with \sqrt{n} . We ran 500 simulations for each value of n while
 1078 sampling σ from $U(0.2, 4)$. We then randomly selected 60 diploid individuals from each simulation
 1079 (approximating diploidy by combining pairs of chromosomes with contiguous indices within demes)
 1080 and calculated a set of six summary statistics using the scripts described in the summary statistics
 1081 portion of the main text.

demes per side (n)	N_e per deme	samples per deme
5	244	20
10	61	10
20	15.25	2
50	2.44	1

Table A1 stepping-stone simulation parameters

1082 In general we find many of the qualitative trends are similar among continuous and stepping-stone
 1083 models and that, at low neighborhood sizes, many (but not all) statistics from stepping-stone models
 1084 approach the continuous model as the resolution of the grid increases. For example, θ_π is **inflated**
 1085 **lower in stepping-stone models** at low neighborhood sizes (i.e., low m), **and the extent of the inflation**
 1086 **but** increases to approach the continuous case as the resolution of the landscape increases. Similar
 1087 patterns are observed for F_{IS} **and** observed heterozygosity. However, θ_W behaves differently, showing
 1088 a non-monotonic relationship with grid resolution. This results in an increasingly positive Tajima’s D
 1089 in grid simulations at small neighborhood sizes, to a much greater extent than seen in a continuous
 1090 model. In contrast to θ_π , increasing the resolution of the grid causes Tajima’s D to deviate *more* from
 1091 what is seen in the continuous model. **Similarly, although F_{IS} approaches the continuous case as the**
 1092 **resolution of the grid increases at very small neighborhood sizes, at intermediate neighborhood sizes**
 1093 **the continuous case best matches intermediate grid resolutions.**

1094 These differences relative to our continuous model mainly reflect two shortcomings of the reverse-
 1095 time stepping stone model. If we simulate a coarse grid with relatively large populations in each
 1096 deme, we cannot accurately capture the dynamics of small neighborhood sizes because mating within
 1097 each deme remains random regardless of the migration rate connecting demes. This likely explains
 1098 the trends in θ_π , observed heterozygosity, and F_{IS} . However increasing the number of demes while
 1099 holding the total number of individuals constant results in small within-deme populations for which
 1100 even the minimum sample size of 1 approaches the local N_e (Table A1). This results in an excess
 1101 of short terminal branches in the coalescent tree, which decreases the total branch length and leads
 1102 to fewer segregating sites, deflated θ_W , and inflated Tajima’s D . Overall, the stepping-stone model
 1103 reproduces important features of spatial structure in our continuous space model, such as a decline in

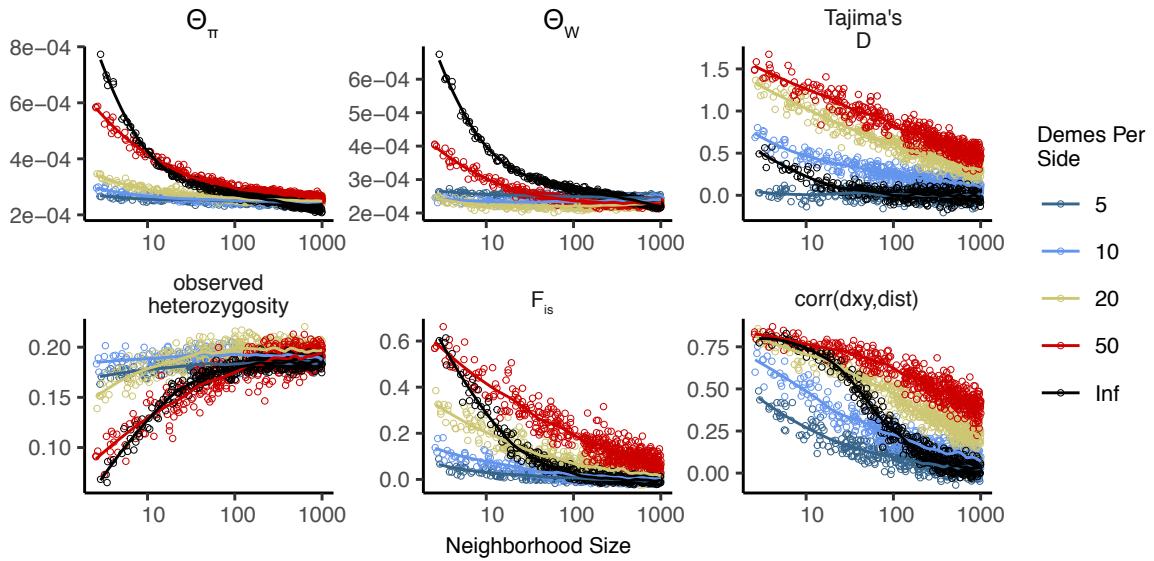


Figure A1 Summary statistics for 2-dimensional coalescent stepping-stone models with fixed total N_e and varying numbers of demes per side. The black “infinite” points are from our forward-time continuous space model. Inter-deme migration rates are related to σ as described above.

1104 θ_π and correlations between spatial and genetic distance with increasing migration, but introduces
 1105 artifacts caused by binning the landscape into discrete demes.

1106 Demographic model

1107 Local population regulation is controlled by two parameters, L , and K . Here, we show that these
 1108 should be close to the average lifespan of an individual and the average number of individuals per
 1109 unit area, respectively. We chose our demographic model so that every individual has on average $1/L$
 1110 offspring each time step, and if the local population density of an individual is n , then their probability
 1111 of survival until the next time step is (equation (1)):

$$p = \min \left(0.95, \frac{1}{1 + n/(K(1 + L))} \right). \quad (3)$$

1112 We capped survival at 0.95 so that we would not have exceptionally long-lived individuals in sparsely
 1113 populated areas – otherwise, an isolated individual might live for a very long time. Since $1 - p \approx$
 1114 $n/(K(1 + L))$, mortality goes up roughly linearly with the number of neighbors (on a scale given by
 1115 K), as would be obtained if, for instance, mortality is due to agonistic interactions. Ignoring migration,
 1116 a region is at demographic equilibrium if the per-capita probability of death is equal to the birth rate,
 1117 i.e., if $1 - p = 1/L$. (Note that there is no effect of age in the model, which would make the analysis
 1118 more complicated.) Solving this for n , we get that in a well-mixed population, the equilibrium density
 1119 should be around

$$n = K \frac{L + 1}{L - 1} \quad (4)$$

1120 individuals per unit area. At this density, the per-capita death rate is $1/L$, so the mean lifetime is L .
 1121 This equilibrium density is *not* K , but (since $L = 4$) is two-thirds larger. However, in practice this model
 1122 leads to a total population size which is around K multiplied by total geographic area (but which
 1123 depends on σ , as discussed above). The main reason for this is that since offspring tend to be near
 1124 their parents, individuals tend to be “clumped”, and so experience a higher average density than the

¹¹²⁵ “density” one would compute by dividing census size by geographic area (Lloyd 1967). To maintain a
¹¹²⁶ constant expected total population size would require making (say) K depend on σ ; however, typical
¹¹²⁷ local population densities might then be more dissimilar.

¹¹²⁸ **Supplementary Figures and Tables**

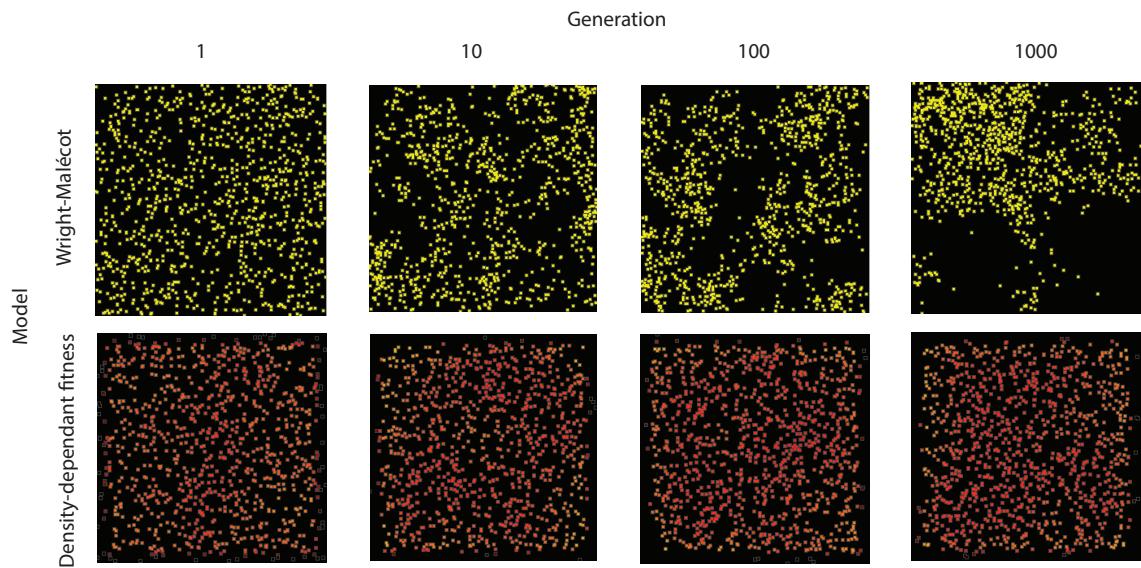


Figure S1 Maps of individual locations in a continuous-space Wright-Malécot model with independent dispersal of all individuals (top) and under our continuous space model incorporating density-dependant fitness (bottom). The clustering seen in the top row is the “Pain in the Torus” described by Felsenstein (1975).

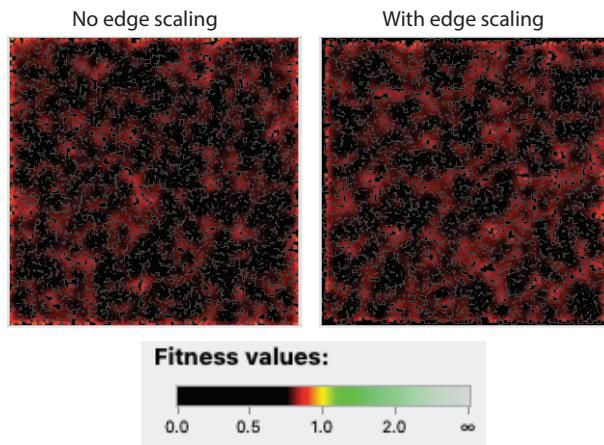


Figure S2 Comparison of individual fitness across the landscape in simulations with (right) and without (left) a decline in fitness approaching range edges. Note the slight excess of high-fitness individuals at edges on the left, which is (partially) counteracted by the scaling procedure.

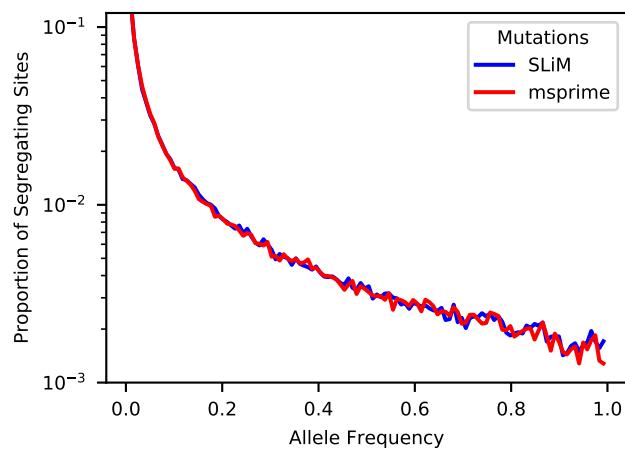


Figure S3 Site frequency spectra from a simulation with neighborhood size = 12.5 when mutations are recorded directly in SLiM (blue line) or applied later in msprime (red line).

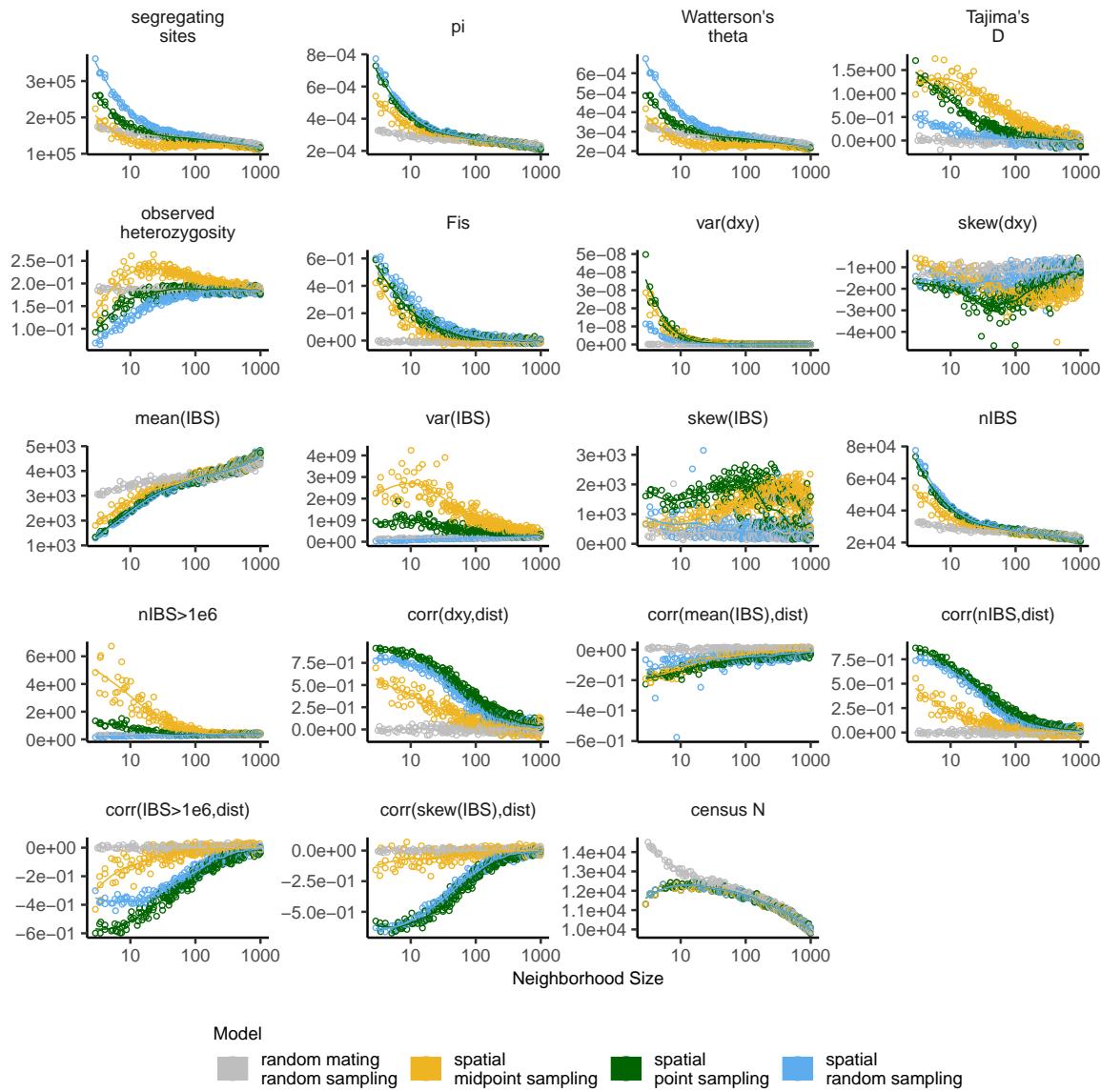


Figure S4 Change in summary statistics by neighborhood size and sampling scheme calculated from simulated sequence data of 60 individuals.

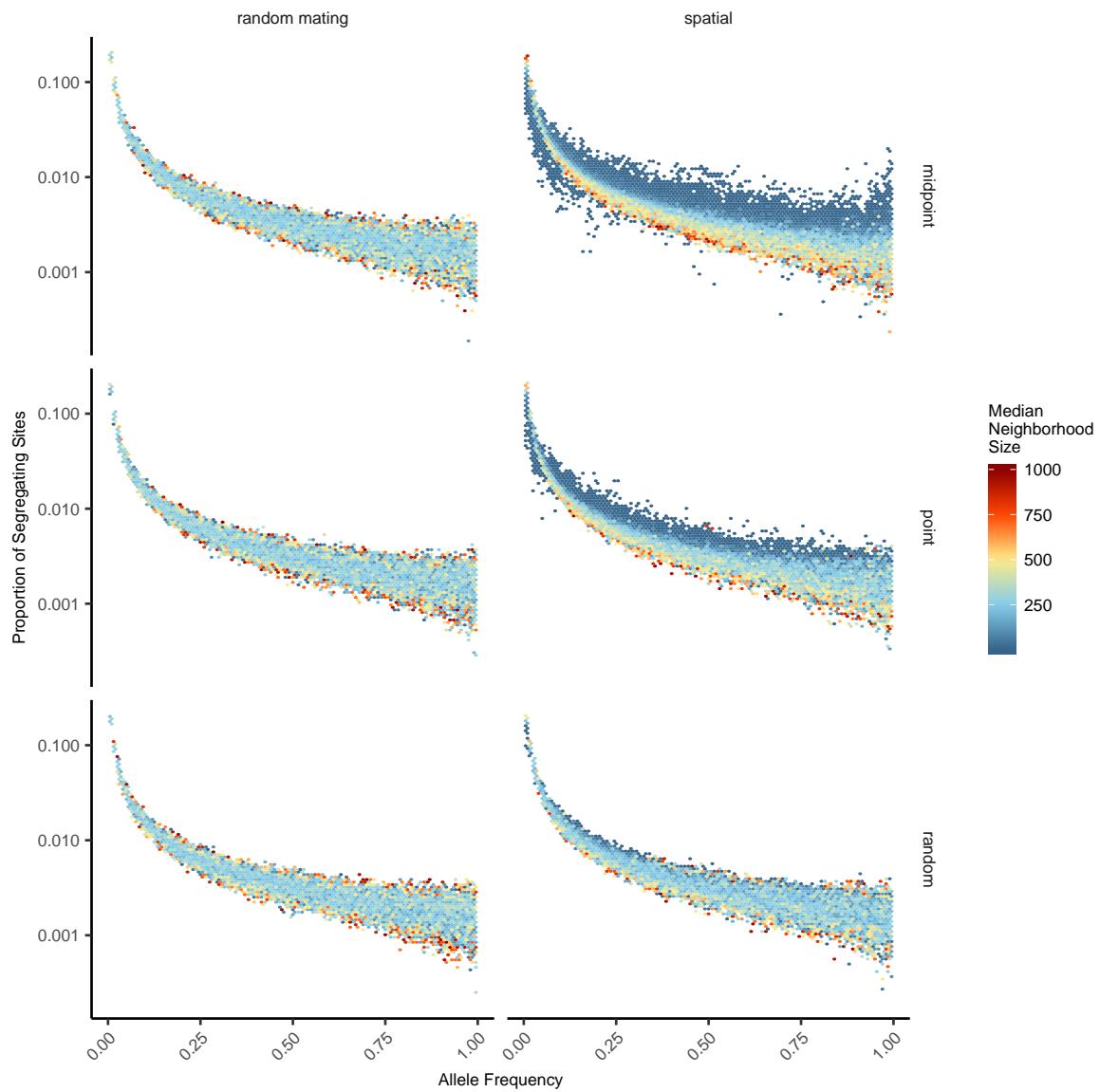


Figure S5 Site frequency spectra for random mating and spatial SLiM models under all sampling schemes.

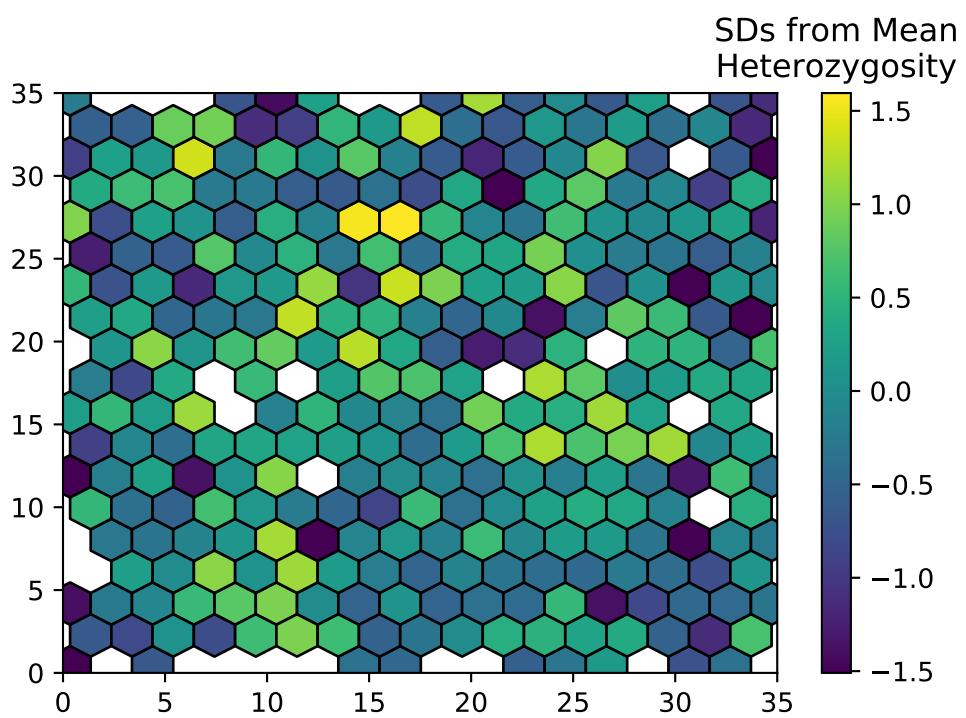


Figure S6 Variation in observed heterozygosity (i.e. proportion of heterozygous individuals) in hexagonal bins across the landscape, estimated from a random sample of 200 individuals from the final generation of a simulation with neighborhood size ≈ 25 . Values were Z-normalized for plotting.

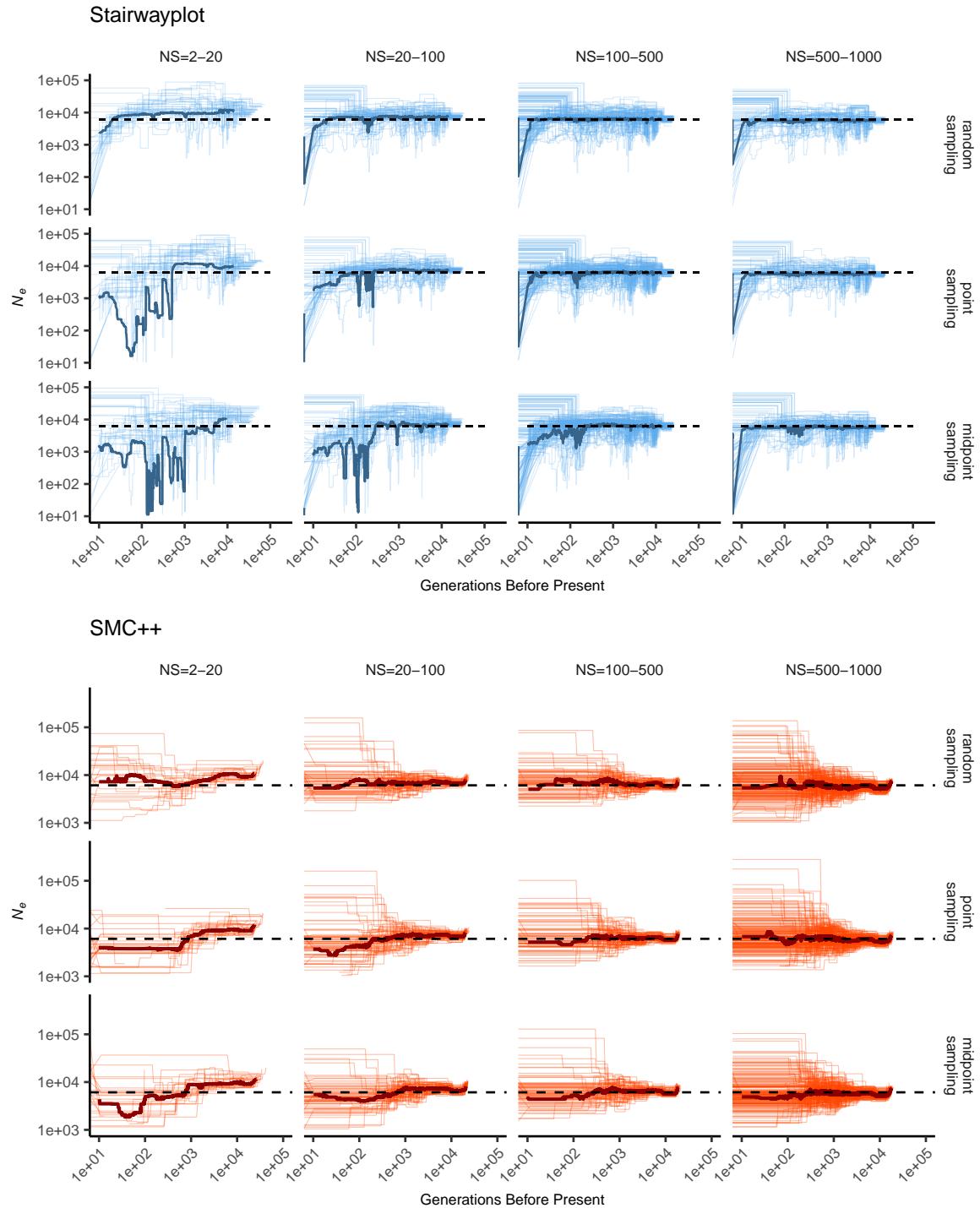


Figure S7 Inferred demographic histories for spatial SLiM simulations, by sampling scheme and neighborhood size (NS) range. Thick lines are rolling medians across all simulations in a bin and thin lines are best fit models for each simulation. Dashed horizontal lines are the average N_e across random-mating SLiM models estimated from θ_π .

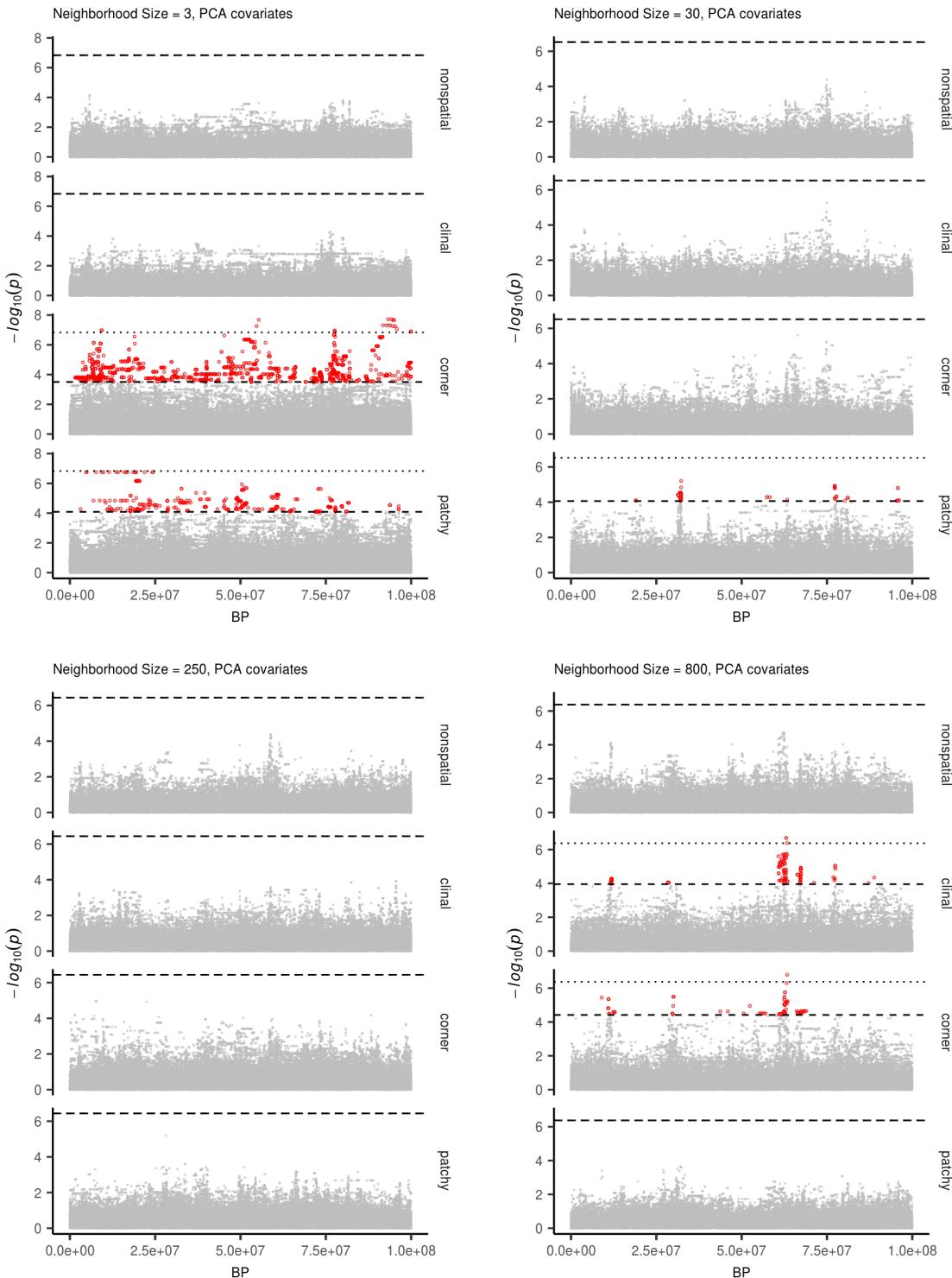


Figure S8 Manhattan plots for a sample of simulations at varying neighborhood sizes. Labels on the right of each plot describes the spatial distribution of environmental factors (described in the methods section of the main text). Points in red are significantly associated with a nongenetic phenotype using a 5% FDR threshold (dashed line). For runs with significant associations the dotted line is a Bonferroni-adjusted cutoff for $p = 0.05$.

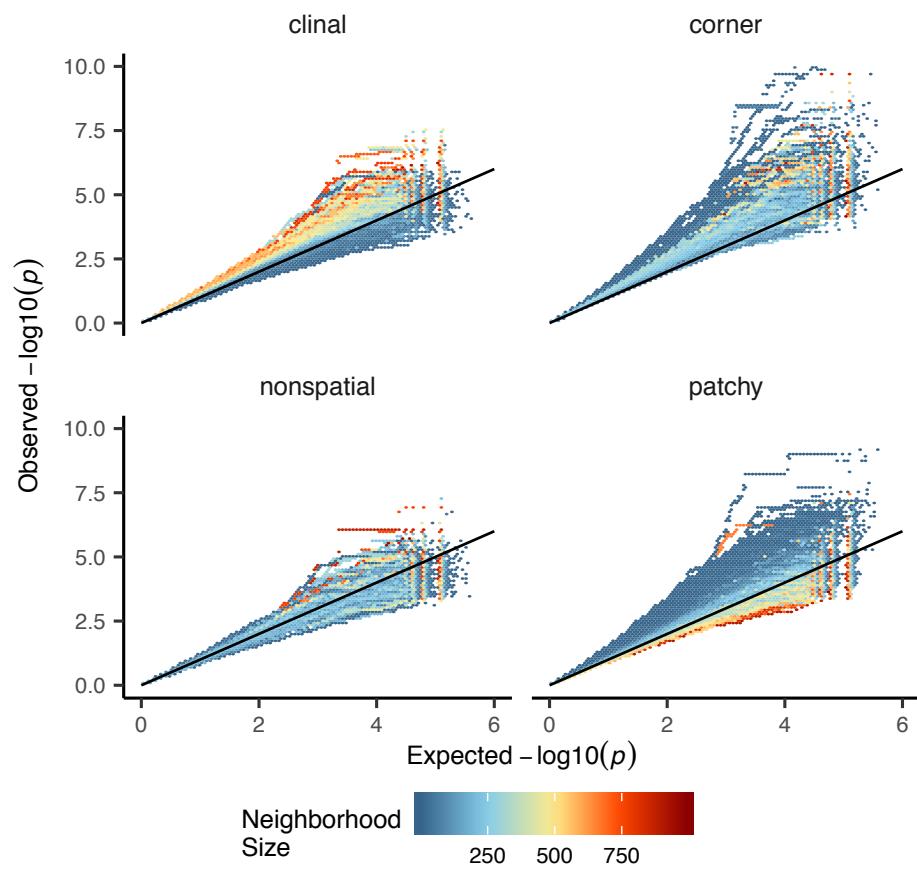


Figure S9 Quantile-quantile plots showing observed $-\log_{10}(p)$ for PC-corrected GWAS run on simulations with varying neighborhood sizes and environmental distributions. Hexagonal bins are colored by the average neighborhood size of simulations with points falling in a given region of quantile-quantile space. Qqplots for a subset of these simulations are shown as lines in Figure 8D.

Table S1 Summary statistics calculated on simulated genotypes.

Statistic	Description
Θ_{pi}	Mean of the distribution of pairwise genetic differences
Θ_W	Effective population size based on segregating sites
Segregating Sites	Total number of segregating sites in the sample
Tajima's D	Difference in Θ_{pi} and Θ_W over its standard deviation
Observed Heterozygosity	Proportion of heterozygous individuals in the sample
F_{IS}	Wright's inbreeding coefficient $1 - H_e / H_o$
$var(D_{xy})$	Variance in the distribution of pairwise genetic distances
$skew(D_{xy})$	Skew of the distribution of pairwise genetic distances
$mean(IVS)$	Mean of the distribution of pairwise identical-by-state (IBS) tract lengths taken over all pairs.
$var(IVS)$	Variance of the distribution of pairwise identical-by-state (IBS) tract lengths taken over all pairs.
$skew(IVS)$	Skew of the distribution of pairwise identical-by-state (IBS) tract lengths taken over all pairs.
$nIBS$	Mean number of IBS tracts with length > 2bp across all pairs in the sample.
$nIBS > 1e6$	Mean number of IBS tracts over 1×10^6 bp per pair across all pairs in the sample.
$corr(D_{xy}, dist)$	Pearson correlation between genetic distance and $\log_{10}(spatial\ distance)$
$corr(mean(IVS), dist)$	Pearson correlation between the mean of the IBS tract distribution for each pair of samples and $\log_{10}(spatial\ distance)$
$corr(nIBS, dist)$	Pearson correlation between the number of IBS tracts for each pair of samples and $\log_{10}(spatial\ distance)$
$corr(IVS > 1e6, dist)$	Pearson correlation between the number of IBS tracts > 1×10^6 bp for each pair of samples and $\log_{10}(spatial\ distance)$
$corr(skew(IVS), dist)$	Pearson correlation between the skew of the distribution of pairwise haplotype block lengths for each pair of samples and $\log_{10}(spatial\ distance)$

Table S2 Anova and Levene's test p values for differences by sampling strategy. Bolded values are rejected at $\alpha = 0.05$

variable	model	p(equal means)	p(equal variance)
segsites	random mating	0.998190	0.980730
$\Theta\pi$	random mating	0.997750	0.996450
Θ_W	random mating	0.998190	0.980730
Tajima's D	random mating	0.879690	0.188770
observed heterozygosity	random mating	0.531540	0.433230
F_{IS}	random mating	0.474790	0.785730
$mean(D_{xy})$	random mating	0.997770	0.996510
$var(D_{xy})$	random mating	0.283630	0.647240
$skew(D_{xy})$	random mating	0.958320	0.260750
$corr(D_{xy}, dist)$	random mating	0.601980	0.000000
$mean(IBS)$	random mating	0.997960	0.997730
$var(IBS)$	random mating	0.486450	0.399490
$skew(IBS)$	random mating	0.117980	0.069770
$nIBS$	random mating	0.997680	0.996570
$nIBS > 1e6$	random mating	0.834870	0.888730
$corr(mean(IBS), dist)$	random mating	0.073270	0.308420
$corr(IBS > 1e6, dist)$	random mating	0.268440	0.002100
$corr(skew(IBS), dist)$	random mating	0.396920	0.000620
$corr(nIBS, dist)$	random mating	0.581090	0.000000
segsites	spatial	0.000000	0.000000
$\Theta\pi$	spatial	0.026510	0.013440
Θ_W	spatial	0.000000	0.000000
Tajima's D	spatial	0.000000	0.000000
observed heterozygosity	spatial	0.000000	0.000000
F_{IS}	spatial	0.000000	0.000120
$mean(D_{xy})$	spatial	0.025390	0.012910
$var(D_{xy})$	spatial	0.004970	0.006230
$skew(D_{xy})$	spatial	0.000000	0.000000
$corr(D_{xy}, dist)$	spatial	0.000000	0.000000
$mean(IBS)$	spatial	0.272400	0.114250
$var(IBS)$	spatial	0.000000	0.000000
$skew(IBS)$	spatial	0.000000	0.000000
$nIBS$	spatial	0.033920	0.016640
$nIBS > 1e6$	spatial	0.000000	0.000000
$corr(mean(IBS), dist)$	spatial	0.000000	0.590540
$corr(IBS > 1e6, dist)$	spatial	0.000000	0.000000
$corr(skew(IBS), dist)$	spatial	0.000000	0.000000
$corr(nIBS, dist)$	spatial	0.000000	0.000000

To the Editor(s) –

We are writing to submit another revised version of our manuscript, “Space is the Place: Effects of Continuous Spatial Structure on Analysis of Population Genetic Data”. We have addressed reviewer comments below and made a number of changes to better explain our demographic parameters and alternate factors affecting GWAS transferability. Included here is a combined PDF including the revised text and response to reviewers, and a diff file comparing this revision and our previous submission (note that page and line numbers in the response to reviewers refers to the revised text, not the diff file). Thank you for your time and efforts.

Sincerely,

C. J. Battey, Peter Ralph, and Andrew Kern

Reviewer Decision Letter:

It is most important that you address the following in a revised manuscript:

1. in response to reviewer 3, add text to help clarify how the reproduction scheme / dispersal model differs from models that do not induce the bimodality in the distance distribution the reviewer points out (see my comments below as well);
2. Respond to the concern about the definitions of sigma and neighborhood size;
3. Reviewer 2 has several minor comments that would be straightforward and helpful to address/add depth into your discussion (e.g., comments 2, 4, 5).
4. The other minor comments from each reviewer.

Thanks very much for the useful input. The inaccuracy in describing dispersal under the model was our mistake, and we have added more careful discussion of this point (see below). We have modified the manuscript to incorporate all remaining feedback, as enumerated below, and think this has improved the manuscript.

Reviewer AE Comments:

The point of R3 on the bimodality in the realized dispersal distance in your model is interesting. You can make this more explicit in the paper and describe how there are realistic biological scenarios that motivate such a model (many/most plants) and demarcate how well you think you can generalize to others (the new appendix already helps in that regard). It strikes me your model has relevance for patrilocal/matrilocal systems where the paternal/maternal parent-offspring distances differ - though your model is hermaphroditic so it's an imperfect analogy. Perhaps, to explore more typical animal mating patterns, you can quickly check the impact for one or two outcomes of changing the simulation such that offspring are dispersed around the midpoint of the parental origins, as this would remove the bimodality. That said, please be sure we respect the work done is extensive and don't mean to invite/require a complete re-working. The core tension is between ensuring your results are generalizable if that is what you wish to claim; versus specific to a particular model that is biologically motivated. It's particularly key for the material on GWAS, where you are explicitly trying to address human GWAS results: those sections will be read and taken at face value by a large community even if this simulation scheme introduces some discrepancies from what might more realistically emerge. So at a minimum, some caveats directly in the GWAS discussion section would help.

Thank you for your comments. We have responded to each reviewer below. In response to reviewer 2 we have added several caveats to the GWAS section noting other sources of error such as allele frequency and LD variation among populations. Reviewer 3 raised two good points on our definition of neighborhood size and the interpretation of σ – first, that σ refers to one- rather than two-dimensional distances between parent and offspring (this is consistent with previous theoretical work, and we have corrected our definition in the text), and second, that the use of separate draws for mate selection and dispersal means the total distance traveled by a locus along a pedigree link is greater for “paternal” than “maternal” material (taking “maternal” to mean the parent from which dispersal is calculated). However, our simulated genome is autosomal and individuals non-selfing, so a locus will experience a mix of “maternal” and “paternal” transmission events along its genealogy. We see this as a scaling issue rather than one of a “bimodal” dispersal kernel per se – the expected axial displacement of a locus along a pedigree link is $\sqrt{3/2}\sigma$, rather than σ . (Note that although the parent-offspring displacement is a mixture of mean-zero Gaussians with respect to a bimodal distribution of standard deviations, the norm of any such mixtures is never itself bimodal.) We have added a paragraph discussing these points (while trying to keep out of the weeds), to (p. 5, l. 207).

Reviewer 1:

Only minor changes were needed, and they were made. This study neatly summarises many biases arising from unmodelled continuous space (pertinent for real studies), and I would not extend it by further analyses. When polishing the final version, the authors can make some of their messages stronger. For example, Figure 7 and the accompanying text may give the impression that we are doing pretty well with smc++. It is only when panel B is seen one can appreciate the degree of uncertainty. So, in a way, Figure S7 is more telling as to how much we can trust our results in real analysis. After all, in reality, as authors mention, we only have one sample set, not replicates.

Thank you for your comments. While we agree that our analysis found problems with demographic inference under isolation by distance, since this manifests as higher variance in predictions rather than a straightforward bias as we expected (i.e. a reduction in recent inferred Ne), we have chosen to keep the somewhat more nuanced presentation of results in that section used in a previous version, although we added an additional note about the between-replicate variance to the discussion. (p. 18, l. 608)

Reviewer 2:

The authors have done a commendable job of responding to the reviewers' comments. The extended discussion and additional supplemental figures helps with the clarity of the manuscript. The results for the comparison with the discrete stepping-stone model were useful - though see below. The authors have also provided sufficient detail justifying their choice of model parameters.

I don't think I have any critical remaining issues or suggestions, but I did have a couple of thoughts. The authors might just want to think about them.

Thanks for the encouraging words and helpful points, as well as for your mindfulness of the burden of additional analyses.

(2.1) I appreciate the appendix comparing summary statistics in the continuous and stepping-stone models, although I must confess I am now even more uncertain than I was before about which models are more appropriate. I get that the coalescent models make assumptions that are not directly interpretable in terms of observable parameters (although are “interactions between geographically distant parts of the species range” really biologically unrealistic?). This forward model is more interpretable, in that sense, but does it actually produce more realistic data? I guess that’s a question for future work, but that question was one of the main things I came away from this paper with.

Reply: This is a good point. We have added additional discussion noting that while our continuous model has some advantages over discrete models, the best model for any empirical system will inevitably depend on the life history of the organism in question. (p. 22, l. 785)

(2.2) (p. 13, l. 494) “PCA is unable to capture”. Is this a sample size effect? i.e. the precision of the estimates of the PCs depends on sample size, so it’s possible that you just need a larger sample to estimate the PCs when you have weak structure. Or is it something else?

Reply: We have added the caveat “given the sample sizes tested” to the above statement. (p. 13, l. 494)

(2.3) (p. 19, l. 677) Is Khera et al (2018) really the right reference for the idea of polygenic scores? They have been around for a lot longer than that.

Reply: Yes this was an oversight. We now cite Wray et al. 2007 for individual disease risk prediction from GWAS estimates and the first, to our knowledge, PGS application (International Schizophrenia Consortium, 2009).

(2.4) (p. 19, l. 678) *I don't know that the results of Berg and Sohail et al. are indicating a fundamental issue with PC correction, rather than a problem with meta-analysis.*

Reply: We think Berg *et al.* (2018) shows that population structure leaves signals in GWAS that resemble those of polygenic selection, and given that this was most apparent in GIANT data employing PCA corrections, indicates a problem with the GWAS that make up the GIANT study at least as much as any issues with meta-analysis.

(2.5) (p. 19, l. 684) *Is the result of Martin et al. really due to population stratification? There seem to be a number of factors affecting transferability of PRS (LD differences, effect size heterogeneity, differences in causal variants etc.), but I don't know that anyone thinks residual structure is the major one, particularly for eg. UKB summary stats.*

Reply: This is a good point – differences in allele frequency and LD structure certainly have significant effects on PRS transferability (at least when the GWAS is based on SNP assays rather than whole-genome sequencing). We think the population structure issue is important to point to here because it will not be solved by simply generating more detailed genomic data from existing samples, but have added a caveat noting these other important potential sources of error at the head of that sentence. (p. 19, l. 684)

(2.6) "population genetics flavor" (p. 21, l. 747) *I don't understand what this means?*

Reply: We meant to contrast this sort of "single-species" model, common in population genetics, to a more "ecological" model involving explicit modeling of resources and/or other species. We've reworded this. (p. 21, l. 747)

(2.7) (p. 22, l. 801) *"process-driven descriptions of ancestry and/or more generalized unsupervised methods". Aren't these two things opposite? That's ok, but it sounds a bit like you have no idea what would help...*

Reply: They certainly entail research in somewhat different directions, but you are correct that we don't know which is the best way to go.

(2.8) Fix citation: Peter L. Ralph and Ashander ??? on pg. 5, line 216

Reply: corrected - thanks.

(2.9) 'there' written twice on pg. 27, line 1033

Reply: thanks for catching this, corrected.

Reviewer 3:

I am fairly pleased overall with the revised version of this article. I commend the authors for the substantial amount of work conducted. This contribution now convincingly demonstrates how important it is to properly account for population structure when individuals are distributed along a spatial continuum.

Thank you for your comments.

(3.1) A couple of minor points about the forward model are still puzzling me though. First of all, in one dimension, the variance of $L(o)$, the random variable corresponding to the offspring's spatial position, given $l(p)$, the parent's position, is equal to $E((L(o) - l(p))^2) := \sigma^2$. Hence, σ is here the square root of the expected squared Euclidean distance between parents and offspring. In general, assuming isotropic migrations, σ is the square root of the expected squared Euclidean distance taken along any dimension. It seems to me that this definition is distinct from the mean parent-offspring distance, as stated by the authors.

Reply: Good point here. We did intend to define σ as is usual in the literature, so have corrected the wording and now direct readers to (Rousset 1997) for discussion of how these parameters are defined in one- and two-dimensional habitats. (p. 3, l. 102)

(3.2) More importantly, I am still a bit confused about the reproduction scheme. My understanding is that, once mating takes place, the two parents involved (noted as $P(1)$ and $P(2)$) each produce a Poisson number of offspring. The location of each offspring is determined by a Gaussian distribution of covariance matrix $\sigma^2 I$, centered on the location of the parent that "produced" this offspring. Let $O(1, i)$ and $O(2, j)$ be the i -th and j -th offspring produced by $P(1)$ and $P(2)$ respectively. Now, if I am not mistaken, half of the genetic material of $O(2, j)$ comes from $P(1)$ (the other half coming from $P(2)$) while $O(1, i)$ receives one chromosome from $P(2)$ (and another one from its "true" parent, i.e., $P(1)$). Hence, when it comes to the parent-offspring distance as measured from their genetic material, half of the chromosomes have a "small" dispersal distance, equal to that of the parent-offspring distance, while the other half have a "large" distance roughly equal to the parent-offspring distance plus the distance between mates.

It is important that the authors clarify this last point because most results presented in this study rely on Wright's neighborhood size, which derives from a model where dispersal of individuals and chromosomes follow well-defined spatial dynamics. A sensible comparison of neighborhood sizes requires that dispersal (along with effective population density) is quantified in a coherent way across different models.

Additionally, the neighborhood size as defined by the authors (see (p. 5, l. 201)) is a function of the census density rather than the effective density of individuals. I am therefore not fully convinced that the range of values for this parameter is comparable to that given in Table 1.

Reply: You are entirely correct here; apologies for the confusion. To clarify the definition of σ and discuss these issues somewhat, we have added a new paragraph at (p. 5, l. 207). As we discuss there, we still calculate $N_W = 4\sigma^2\rho$, where σ is the mean mother-child dispersal distance (not including the distance between mates) and ρ is the census population density. This is not the quantity that is predicted to best describe geographic patterns of genetic variation, which would use *effective* versions of both σ and ρ . This does not bother us for a few reasons. First, we do not attempt to match statistics in our simulations to theoretical results (e.g., the Wright–Malecot formula). Instead, we use N_W as a proxy for "strength of spatial structure", and in these simulations, the "effective" N_W certainly has a monotonic relationship with the N_W we compute. The remaining question is whether, as the reviewer asks, the actual values of N_W are comparable to those computed in the wild. This depends, of course, on how those values were estimated in wild populations. In our opinion, a straightforward estimate of N_W using observed dispersal distances and census density or reproductive adults (similar to how we compute N_W for simulations) is likely more robust and reflective of modern demography than most methods to estimate "effective" versions.

Furthermore, our imprecisions roughly cancel out. As now noted in the text, an average of one and two dispersal distances is a better model for the mean parent-offspring distance; this would give us a value of $\sqrt{3/2}\sigma$, so by using only σ we have lowered N_W by a factor of about 2/3. However, effective population density is lower than census density by a factor of also around 2/3, which would cancel

the first. So, we are fairly confident that our values of N_W match the theoretical versions to within the precision we require (which is only order-of-magnitude, really).

(3.3) Furthermore, the neighborhood size estimates obtained for *Bebicium vittatum* derive from the analysis of a one-dimensional habitat. The product $\sigma^2 \rho$ is thus expressed in this particular case as a number of individuals *per unit of space* (in two dimensions, this product is expressed as a number of individuals). Hence, the unit of space matters a lot here, prohibiting the direct comparison with other figures in the same table or with the simulations conducted in this study.

Reply: Good point, we have removed *Bebicium vittatum* from table 1.

(3.4) Finally, Leblois, Estoup and Streiff (2006) *Molecular Ecology* conducted a large simulation studies with a focus very close to that of the present study. In particular, they investigated the impact of sampling on the inference of summary statistics commonly used in population genetics. It would probably be relevant to compare some of the results presented in Leblois et al. to that put forward here.

Reply: Thank you for bringing this study to our attention. We have added it to the citations in the demographic inference section.

Reviewer 4:

The authors have thoroughly addressed nearly all of my comments in their timely manuscript, and I am satisfied with their responses.

Thank you very much!

(4.1) Regarding Table S1, I would still suggest adding a column about interpretation for those variables and evaluated with respect to the simulated results.

Reply: Thank you for your comments. We chose to evaluate a few key statistics in detail in the main text and then provide an interpretation rooted in how the marginal genealogies of the tree sequence interact with space, and hope that this will assist readers when examining other statistics shown.