

# 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 were surprisingly robust to isolation by distance. 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

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## 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 to be  $N_W = 4\pi\rho\sigma^2$ , where  $\sigma$  is the mean parent-  
110 offspring distance and  $\rho$  is population density. This can be thought of as proportional to the average  
111 number of potential mates for an individual (those within distance  $2\sigma$ ), or the number of potential  
112 parents of a randomly chosen individual. Empirical estimates of neighborhood size vary hugely  
113 across species – even in human populations, estimates range from 40 to over 5,000 depending on the  
114 population and method of estimation (Table 1).

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

129 To avoid hard-to-evaluate approximations, we here used forward-time, individual-based simulations  
130 across continuous geographical space. The question of what regulates real populations has a  
131 long history and many answers (e.g., Lloyd 1967; Antonovics and Levin 1980; Crawley 1990), but it is  
132 clear that populations must at some point have density-dependent feedback on population size, or  
133 else they would face eventual extinction or explosion. In the absence of unrealistic global population  
134 regulation, this regulation must be local, and there are many ways to achieve this (Bolker *et al.* 2003). In  
135 our simulations, each individual’s probability of survival is a decreasing function of local population  
136 density, which shifts reproductive output towards low-density regions, and produces total census sizes  
137 that fluctuate around an equilibrium. This also prevents the population clumping seen by Felsenstein  
138 (1975) (Supplemental Figure S1)). Such models have been used extensively in ecological modeling  
139 (Durrett and Levin 1994; Bolker and Pacala 1997; Law *et al.* 2003; Fournier and Méléard 2004; Champer  
140 *et al.* 2019) but rarely in population genetics, where to our knowledge implementations of continuous  
141 space models before their availability through SLiM (Haller and Messer 2019) have focused on a small  
142 number of genetic loci (e.g., Slatkin and Barton 1989; Barton *et al.* 2002; Robledo-Arnuncio and Rousset

143 2010; Rossine 2014; Jackson and Fahrig 2014), which limits the ability to investigate the impacts of  
 144 continuous space on genome-wide genetic variation as is now routinely sampled from real organisms.  
 145 By simulating chromosome-scale sequence alignments and complete population histories we are able  
 146 to treat our simulations as real populations and replicate the sampling designs and analyses commonly  
 147 conducted on real genomic data.

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

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

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  $\sigma$  in both the  $x$  and  $y$  coordinates. Each offspring is produced  
 158 with a mate selected randomly from those within distance  $3\sigma$ , with probability of choosing a neighbor  
 159 at distance  $d$  proportional to  $\exp(-d^2/2\sigma^2)$ .

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

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

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

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

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

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

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

183 We stored the full genealogy and recombination history of final-generation individuals as tree  
 184 sequences (Kelleher *et al.* 2018), as implemented in SLiM (Haller *et al.* 2019). Scripts for figures and  
 185 analyses are available at <https://github.com/kern-lab/spaceness>.

186 We ran 400 simulations for the spatial and random-mating models on a square landscape of width  
 187  $W = 50$  with per-unit carrying capacity  $K = 5$  (census  $N \approx 10,000$ ), average lifetime  $L = 4$ , genome

size  $10^8$ bp, recombination rate  $10^{-9}$  per bp per generation, and drawing  $\sigma$  values from a uniform distribution between 0.2 and 4. To speed up the simulations and limit memory overhead we used a mutation rate of 0 in SLiM and later applied mutations to the tree sequence with msprime's `mutate` function (Kelleher *et al.* 2016). Because msprime applies mutations proportionally to elapsed time, we divided the mutation rate of  $10^{-8}$  mutations per site per generation by the average generation time estimated for each value of  $\sigma$  (see 'Demographic Parameters' below) to convert the rate to units of mutations per site per unit time. We verify that this procedure produces the same site frequency spectrum as applying mutations directly in SLiM in Figure S3, in agreement with theory (Ralph *et al.* 2019b). Simulations were run for 1.6 million timesteps (approximately  $30N$  generations).

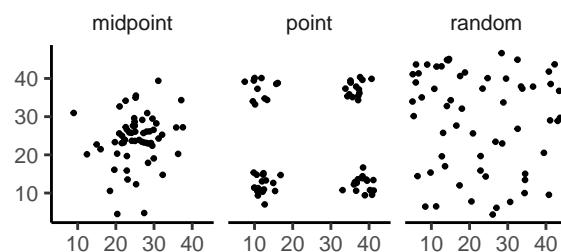
We also compared our model's output to a commonly-used approximation of continuous space, the stepping-stone model, which we simulated with msprime (Kelleher *et al.* 2016). These results are discussed in detail in the Appendix, but in general we find that the demographic structure of a stepping-stone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

#### 204 Demographic Parameters

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

#### 217 Sampling

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



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

225    **Summary Statistics**

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

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

251    The effects of sampling on summary statistic estimates were summarized by testing for differences  
252    in mean (ANOVA, (R Core Team 2018)) and variance (Levene's test, (Fox and Weisberg 2011)) across  
253    sampling strategies for each summary statistic.

254    **Demographic Inference**

255  
256    To assess the impacts of continuous spatial structure on demographic inference we inferred pop-  
257    ulation size histories for all simulations using two approaches: stairwayplot (Liu and Fu 2015) and  
258    SMC++ (Terhorst *et al.* 2016). Stairwayplot fits its model to a genome-wide estimate of the SFS, while  
259    SMC++ also incorporates linkage information. For both methods we sampled 20 individuals from all  
260    spatial simulations using random, midpoint, and point sampling strategies.

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

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

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

275 **Association Studies**

276 To assess the degree to which spatial structure confounds GWAS we simulated four types of nongenetic  
277 phenotype variation for 1000 randomly sampled individuals in each spatial SLiM simulation and  
278 conducted a linear regression GWAS with principal components as covariates in PLINK (Purcell *et al.*  
279 2007). SNPs with a minor allele frequency less than 0.5% were excluded from this analysis. Phenotype  
280 values were set to vary by two standard deviations across the landscape in a rough approximation  
281 of the variation seen in height across Europe (Turchin *et al.* 2012; Garcia and Quintana-Domeque  
282 2006, 2007). Conceptually our approach is similar to that taken by Mathieson and McVean (2012),  
283 though here we model fully continuous spatial variation and compare GWAS output across a range of  
284 dispersal distances.

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

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

305 We ran linear-model GWAS both with and without the first 10 principal components as covariates  
306 in PLINK and summarized results across simulations by counting the number of SNPs with  $p$ -value  
307 below 0.05 after adjusting for an expected false positive rate of less than 5% (Benjamini and Yekutieli  
308 2001). We also examined  $p$  values for systematic inflation by comparing to the values expected from  
309 a uniform distribution (because no SNPs were used when generating phenotypes, well-calibrated  
310  $p$ -values should be uniform).

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

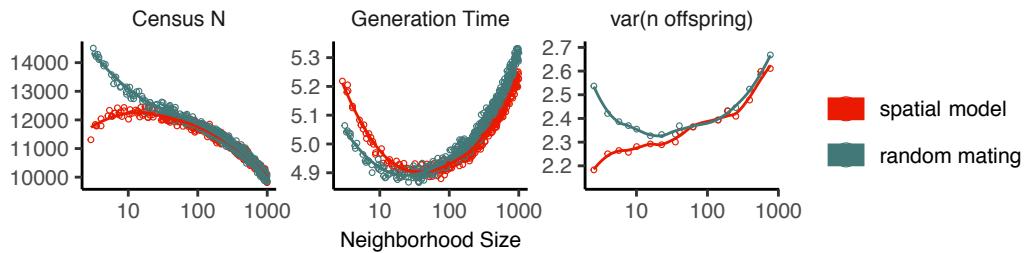
313 **Results**

314 **Demographic Parameters and Run Times**

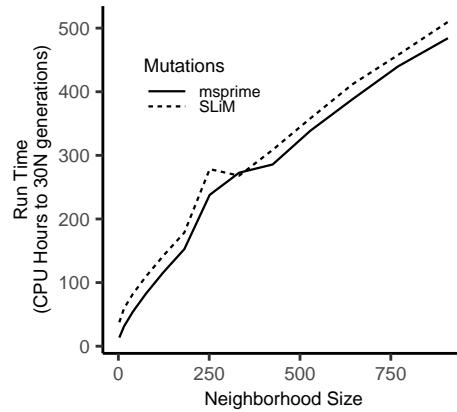
315 Adjusting the spatial dispersal and interaction distance,  $\sigma$ , has a surprisingly large effect on de-  
316 mographic quantities that are usually fixed in Wright-Fisher models – the generation time, census  
317 population size, and variance in offspring number, shown in Figure 2. Because our simulation is  
318 parameterized on an individual level, these population parameters emerge as a property of the inter-  
319 actions among individuals rather than being directly set. Variation across runs occurs because, even  
320 though the parameters  $K$  and  $L$  that control population density and mean lifetime respectively were  
321 the same in all simulations, the strength of stochastic effects depends strongly on the spatial interaction  
322 distance  $\sigma$ . For instance, the population density near to individual  $i$  (denoted  $n_i$  above) is computed  
323 by averaging over roughly  $N_W = 4\pi K\sigma^2$  individuals, and so has standard deviation proportional to  
324  $1/\sqrt{N_W}$  – it is more variable at lower densities. (Recall that  $N_W$  is Wright's neighborhood size.) Since  
325 the probability of survival is a nonlinear function of  $n_i$ , actual equilibrium densities and lifetimes differ

326 from  $K$  and  $L$ . This is the reason that we included *random mating* simulations – where mate choice and  
 327 offspring dispersal are both nonspatial – since this should preserve the random fluctuations in local  
 328 population density while destroying any spatial genetic structure. We verified that random mating  
 329 models retained no geographic signal by showing that summary statistics did not differ significantly  
 330 between sampling regimes (Table S2), unlike in spatial models (discussed below).

331 There are a few additional things to note about Figure 2. First, all three quantities are non-monotone  
 332 with neighborhood size. Census size largely declines as neighborhood size increases for both the spatial  
 333 and random mating models. However, for spatial models this decline only begins for neighborhood  
 334 size  $\geq 10$ . Spatial and random mating models are indistinguishable from one another for neighborhood  
 335 sizes larger than 100. Census sizes range from around 14,000 at low  $\sigma$  in the random mating model  
 336 to 10,000 for both models when neighborhood sizes approach 1,000. The scaling of census sizes in  
 337 both random-mating and spatial models appears to be related to two consequences of the spatial  
 338 competition function: the decline of fitness at range edges, which effectively reduces the habitable area  
 339 by one  $\sigma$  around the edge of the map and so results in a smaller habitable area at high  $\sigma$  values; and  
 340 variation in the equilibrium population density given varying competition radii. Furthermore, census  
 341 size increases in spatial models as neighborhood size increases from 2 to 10. This may reflect an Allee  
 342 effect (Allee *et al.* 1949) in which some individuals are unable to find mates when the mate selection  
 343 radius is very small.



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



**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.

344 Generation time similarly shows complex behavior with respect to neighborhood sizes, and varies

345 between 5.2 and 4.9 timesteps per generation across the parameter range explored. Under both the  
346 spatial and random mating models, generation time reaches a minimum at a neighborhood size of  
347 around 50. Interestingly, under the range of neighborhood sizes that we examined, generation times  
348 between the random mating and spatial models are never quite equivalent – presumably this would  
349 cease to be the case at neighborhood sizes higher than we simulated here.

350 Last, we looked at the variance in number of offspring – a key parameter determining the effective  
351 population size. Surprisingly, the spatial and random mating models behave quite differently: while  
352 the variance in offspring number increases nearly monotonically under the spatial model, the random  
353 mating model actually shows a decline in the variance in offspring number until a neighborhood size  
354 of around 10 before it increases and eventually equals what we observe in the spatial case.

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

### 361 **Impacts of Continuous Space on Population Genetic Summary Statistics**

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

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

380 One of the most commonly used summaries of variation is Tajima’s summary of nucleotide diversity,  
381  $\theta_\pi$ , calculated as the mean density of nucleotide differences averaged across pairs of samples. As can  
382 be seen in Figure 4B,  $\theta_\pi$  in the spatial model is inflated by up to three-fold relative to the random  
383 mating model. This pattern is opposite the expectation from census population size (Figure 2), because  
384 the spatial model has *lower* census size than the random mating model at neighborhood sizes less than  
385 100. Differences between these models likely occur because  $\theta_\pi$  is a measure of mean time to most recent  
386 common ancestor between two samples, and at small values of  $\sigma$ , the time for dispersal to mix ancestry  
387 across the range exceeds the mean coalescent time under random mating. (For instance, at the smallest  
388 value of  $\sigma = 0.2$ , the range is 250 dispersal distances wide, and since the location of a diffusively  
389 moving lineage after  $k$  generations has variance  $k\sigma^2$ , it takes around  $250^2 = 62500$  generations to  
390 mix across the range, which is roughly ten times larger than the random mating effective population  
391 size).  $\theta_\pi$  using each sampling strategy approaches the random mating expectation at its own rate, but  
392 by a neighborhood size of around 100 all models are roughly equivalent. Interestingly, the effect of  
393 sampling strategy is reversed relative to that observed in Tajima’s *D* – midpoint sampling reaches  
394 random mating expectations around neighborhood size 50, while random sampling is inflated until  
395 around neighborhood size 100.



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

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

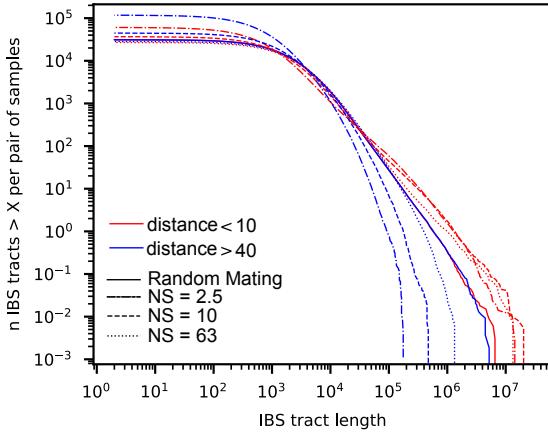
407 **IBS tracts and correlations with geographic distance** We next turn our attention to the effect of  
408 geographic distance on haplotype block length sharing, summarized for sets of nearby and distant  
409 individuals in Figure 5. There are two main patterns to note. First, nearby individuals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

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

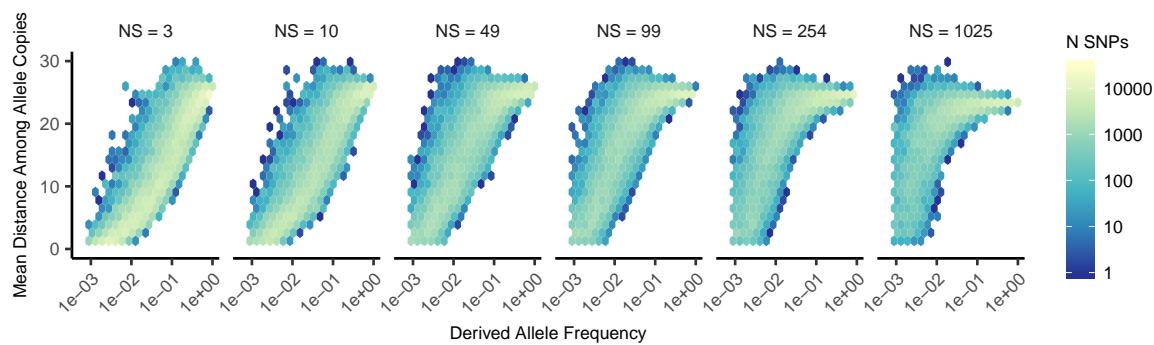
428 **Spatial distribution of allele copies** Mutations occur in individuals and spread geographically over  
429 time. Because low frequency alleles generally represent recent mutations (Sawyer 1977; Griffiths *et al.*  
430 1999), the geographic spread of an allele may covary along with its frequency in the population. To  
431 visualize this relationship we calculated the average distance among individuals carrying a focal  
432 derived allele across simulations with varying neighborhood sizes, shown in Figure 6. On average  
433 we find that low frequency alleles are the most geographically restricted, and that the extent to which  
434 geography and allele frequency are related depends on the amount of dispersal in the population.  
435 For populations with large neighborhood sizes we found that even very low frequency alleles can be  
436 found across the full landscape, whereas in populations with low neighborhood sizes the relationship  
437 between distance among allele copies and their frequency is quite strong. This is the basic process  
438 underlying Novembre and Slatkin’s (2009) method for estimating dispersal distances based on the  
439 distribution of low frequency alleles, and also generates the greater degree of bias in GWAS effect sizes  
440 for low frequency alleles identified in Mathieson and McVean (2012).

#### 441 **Effects of Space on Demographic Inference**

442 One of the most important uses for population genetic data is inferring demographic history of popu-  
443 lations. As demonstrated above, the site frequency spectrum and the distribution of IBS tracts varies  
444 across neighborhood sizes and sampling strategies. Does this variation lead to different inferences of  
445 past population sizes? To ask this we inferred population size histories from samples drawn from our



**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 simulated populations with two approaches: stairwayplot (Liu and Fu 2015), which uses a genome-  
447 wide estimate of the SFS, and SMC++ (Terhorst *et al.* 2016), which incorporates information on both the  
448 SFS and linkage disequilibrium across the genome.

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

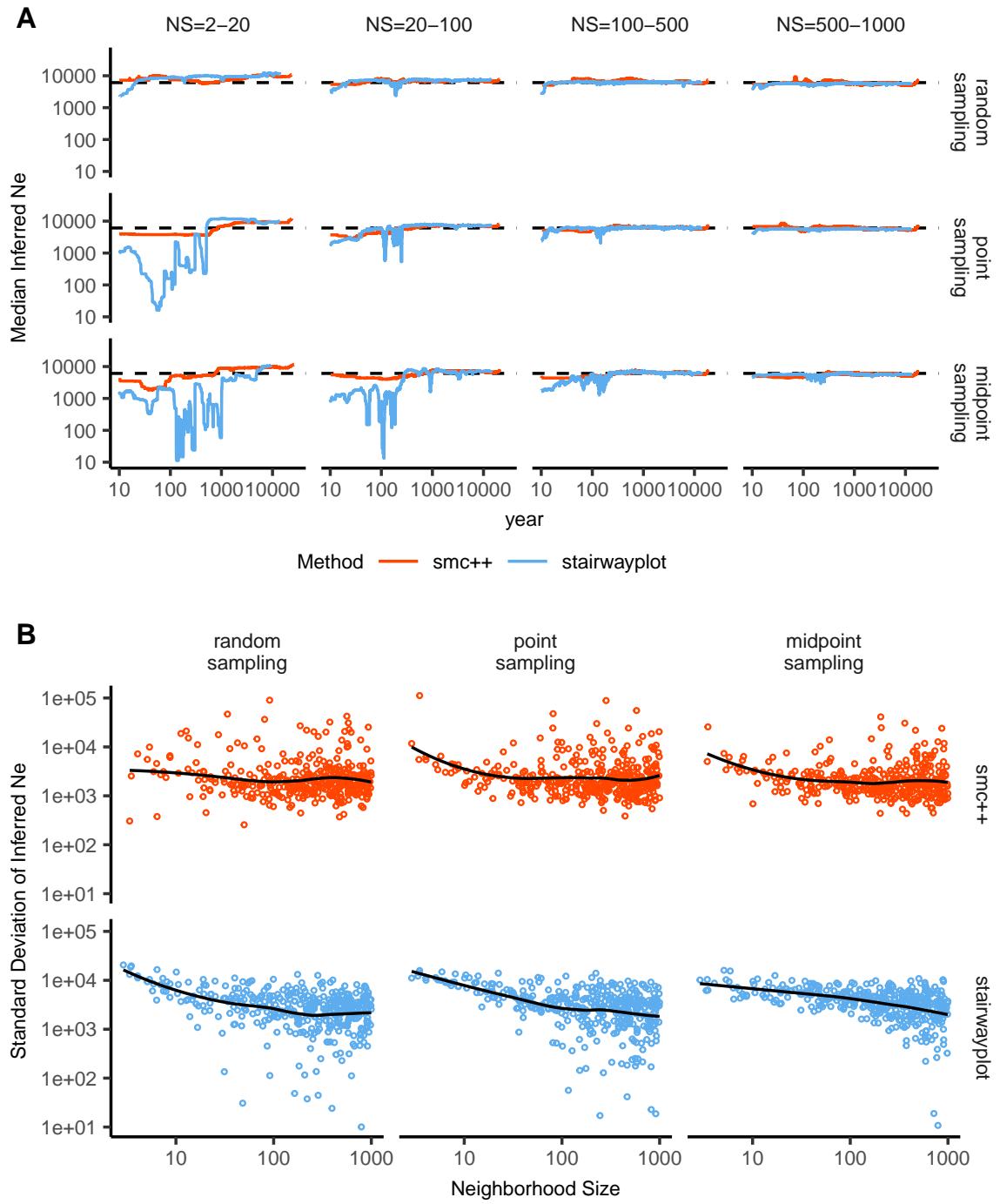
461 However, individual simulations were often inferred to have turbulent demographic histories, as  
462 shown by the individually inferred histories (shown in Figure S7). Indeed, the standard deviation of  
463 inferred  $N_e$  across time points (shown in Figure 7B) often exceeds the expected  $N_e$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

#### 469 **GWAS**

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

482 The confounding effects of geographic structure are well known, and it is common practice to  
483 control for this by including principal components (PCs) as covariates to control for these effects. This  
484 mostly works in our simulations – after incorporating the first ten PC axes as covariates, the vast  
485 majority of SNPs no longer surpass a significance threshold chosen to have a 5% false discovery rate  
486 (FDR). However, a substantial number of SNPs – up to 1.5% at the lowest dispersal distances – still  
487 surpass this threshold (and thus would be false positives in a GWAS), especially under “corner” and  
488 “patchy” environmental distributions (Figure 8C). At neighborhood sizes larger than 500, up to 0.31%  
489 of SNPs were significant for corner and clinal environments. Given an average of 132,000 SNPs across  
490 simulations after MAF filtering, this translates to up to 382 false-positive associations; for human-sized  
491 genomes, this number would be much larger. In most cases the  $p$  values for these associations were  
492 significant after FDR correction but would not pass the threshold for significance under the more  
493 conservative Bonferroni correction (see example Manhattan plots in figure S8).

494 Clinal environments cause an interesting pattern in false positives after PC correction: at low  
495 neighborhood sizes the correction removes nearly all significant associations, but at neighborhood  
496 sizes above roughly 250 the proportion of significant SNPs increases to up to 0.4% (Figure 8). This  
497 may be due to a loss of descriptive power of the PCs – as neighborhood size increases, the total



**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.

498 proportion of variance explained by the first 10 PC axes declines from roughly 10% to 4% (Figure  
499 8B). Essentially, PCA seems unable to effectively summarize the weak population structure present in  
500 large-neighborhood simulations, but these populations continue to have enough spatial structure to  
501 create significant correlations between genotypes and the environment. A similar process can also be  
502 seen in the corner phenotype distribution, in which the count of significant SNPs initially declines as  
503 neighborhood size increases and then increases at approximately the point at which the proportion of  
504 variance explained by PCA approaches its minimum.

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

## 514 Discussion

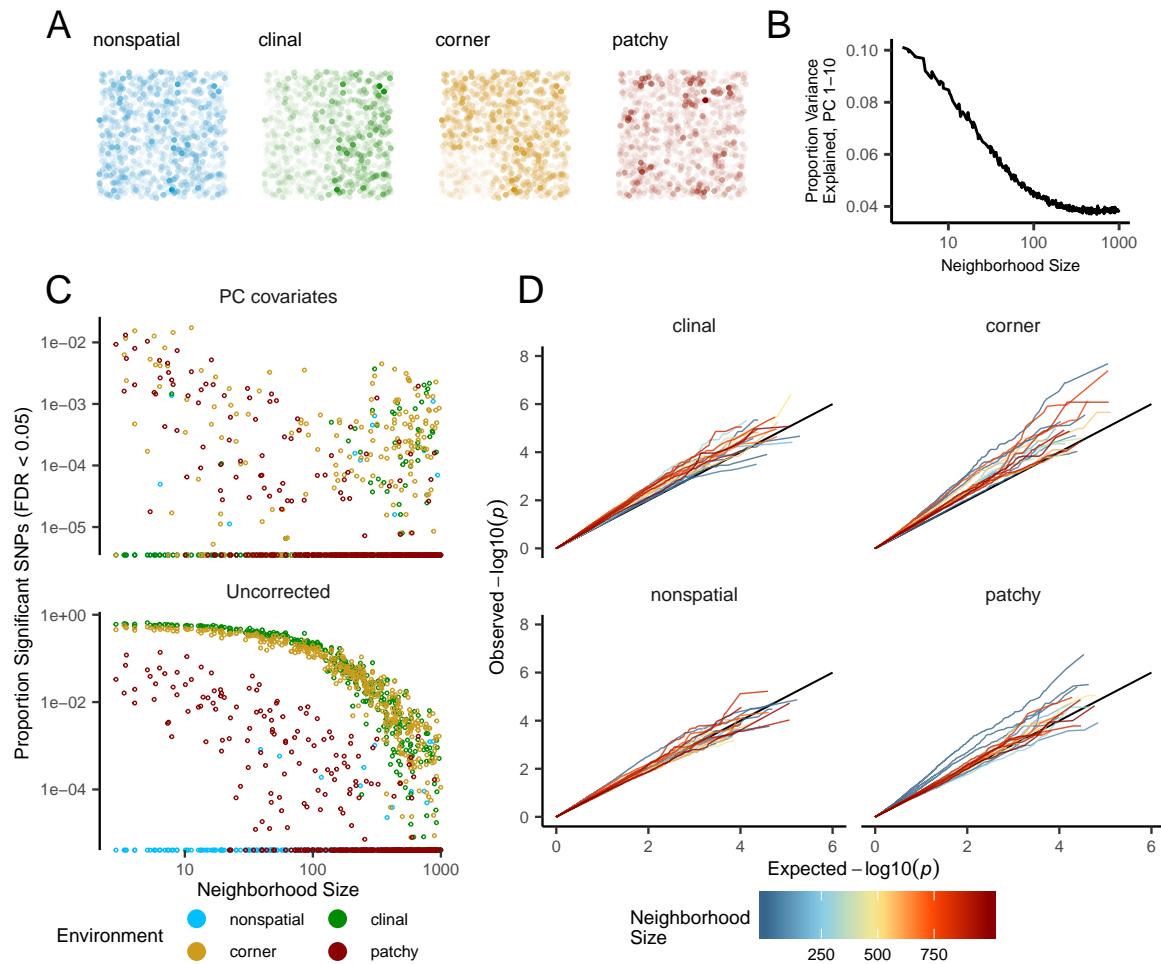
515 In this study, we have used efficient forward time population genetic simulations to describe the  
516 myriad influence of continuous geography on genetic variation. In particular, we examine how three  
517 main types of downstream empirical inference are affected by unmodeled spatial population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

### 522 Effects of Dispersal

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

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

542 The genealogical patterns introduced by limited dispersal are particularly apparent in the distribu-  
543 tion of haplotype block lengths (Figure 4). This is because identical-by-state tract lengths reflect the  
544 impacts of two processes acting along the branches of the underlying genealogy – both mutation and  
545 recombination – rather than just mutation as is the case when looking at the site frequency spectrum or  
546 related summaries. This means that the pairwise distribution of haplotype block lengths carries with  
547 it important information about genealogical variation in the population, and correlation coefficients



**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.

548 between moments of the this distribution and geographic location contain signal similar to the correlations  
549 between  $F_{ST}$  or  $D_{xy}$  and geographic distance (Rousset 1997). Indeed this basic logic underlies  
550 two recent studies explicitly estimating dispersal from the distribution of shared haplotype block  
551 lengths (Ringbauer *et al.* 2017; Baharian *et al.* 2016). Conversely, because haplotype-based measures of  
552 demography are particularly sensitive to variation in the underlying genealogy, inference approaches  
553 that assume random mating when analyzing the distribution of shared haplotype block lengths are  
554 likely to be strongly affected by spatial processes.

### 555 **Effects of Sampling**

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

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

577 We observed the largest sampling effects using “midpoint” sampling. This model is meant to  
578 reflect a bias in sampling effort towards the middle of a species’ range. In empirical studies this  
579 sampling strategy could arise if, for example, researchers choose to sample the center of the range  
580 and avoid range edges to maximize probability of locating individuals during a short field season.  
581 Because midpoint sampling provides limited spatial resolution it dramatically reduces the magnitude  
582 of observed correlations between spatial and genetic distances. More surprisingly, midpoint sampling  
583 also leads to strongly positive Tajima’s  $D$  and an inflation in the proportion of heterozygous individuals  
584 in the sample – similar to the effect of sampling a single deme in an island model as reported in Städler  
585 *et al.* (2009). This increase in observed heterozygosity appears to reflect the effects of range edges,  
586 which are a fundamental facet of spatial genetic variation. If individuals move randomly in a finite  
587 two-dimensional landscape then regions in the middle of the landscape receive migrants from all  
588 directions while those on the edge receive no migrants from at least one direction. The average number  
589 of new mutations moving into the middle of the landscape is then higher than the number moving  
590 into regions near the range edge, leading to higher heterozygosity and lower inbreeding coefficients  
591 ( $F_{IS}$ ) away from range edges. Though here we used only a single parameterization of fitness decline at  
592 range edges we believe this is a general property of non-infinite landscapes as it has also been observed  
593 in previous studies simulating under lattice models (Neel *et al.* 2013; Shirk and Cushman 2014).

594 In summary, we recommend that empirical researchers collect individuals from across as much  
595 of the species’ range as practical, choosing samples separated by a range of spatial scales. Many  
596 summary statistics are designed for well-mixed populations, and so provide different insights into  
597 genetic variation when applied to different subsets of the population. Applied to a cluster of samples,  
598 summary statistics based on segregating sites (e.g., Watterson’s  $\theta$  and Tajima’s  $D$ ), heterozygosity, or  
599 the distribution of long haplotype blocks, can be expected to depart significantly from what would be

600 obtained from a wider distribution of samples. Comparing the results of analyses conducted on all  
601 individuals versus those limited to single individuals per locality can provide an informative contrast.  
602 Finally we wish to point out that the bias towards intermediate allele frequencies that we observe may  
603 mean that the importance of linked selection, at least as is gleaned from the site frequency spectrum,  
604 may be systematically underestimated currently.

605 **Demography**

606 Previous studies have found that population structure and nonrandom sampling can create spurious  
607 signals of population bottlenecks when attempting to infer demographic history with microsatellite  
608 variation, summary statistics, or runs of homozygosity (Chikhi *et al.* 2010; Städler *et al.* 2009; Ptak  
609 and Przeworski 2002; Mazet *et al.* 2015). Here we found that methods that infer detailed population  
610 trajectories through time based on the SFS and patterns of LD across the genome are also subject to this  
611 bias, with some combinations of dispersal and sampling strategy systematically inferring deep recent  
612 population bottlenecks and overestimating ancient  $N_e$  by around a factor of 2. We were surprised  
613 to see that both stairwayplot and SMC++ can tolerate relatively strong isolation by distance – i.e.,  
614 neighborhood sizes of 20 – and still perform well when averaging results across multiple simulations.  
615 Inference in populations with neighborhood sizes over 20 was relatively unbiased unless samples were  
616 concentrated in the middle of the range (Figure 7). Although median demography estimates across  
617 many independent simulations were fairly accurate, empirical work has only a single estimate to work  
618 with, and individual model fits (Figure S7) suggest that spuriously inferred population size changes  
619 and bottlenecks are common, especially at small neighborhood sizes. As we will discuss below, most  
620 empirical estimates of neighborhood size, including all estimates for human populations, are large  
621 enough that population size trajectories inferred by these approaches should not be strongly affected by  
622 spatial biases created by dispersal in continuous landscapes. In contrast, Mazet *et al.* (2015) found that  
623 varying migration rates through time could create strong biases in inferred population trajectories from  
624 an  $n$ -island model with parameters relevant for human history, suggesting that changes in migration  
625 rates through time are more likely to drive variation in inferred  $N_e$  than isolation by distance.

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

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

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

651 **GWAS**

652 Spatial structure is particularly challenging for genome-wide association studies, because the effects of  
653 dispersal on genetic variation are compounded by spatial variation in the environment (Mathieson  
654 and McVean 2012). Spatially restricted mate choice and dispersal causes variation in allele frequencies  
655 across the range of a species. If environmental factors affecting the phenotype of interest also vary over  
656 space, then allele frequencies and environmental exposures will covary over space. In this scenario an  
657 uncorrected GWAS will infer genetic associations with a purely environmental phenotype at any site  
658 in the genome that is differentiated over space, and the relative degree of bias will be a function of the  
659 degree of covariation in allele frequencies and the environment (i.e., Figure 8C, bottom panel). This  
660 pattern has been demonstrated in a variety of simulation and empirical contexts (Price *et al.* 2006; Yu  
661 *et al.* 2005; Young *et al.* 2018; Mathieson and McVean 2012; Kang *et al.* 2008, 2010; Bulik-Sullivan *et al.*  
662 2015; Berg *et al.* 2018; Sohail *et al.* 2018).

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

676 In this work we simulated a single chromosome with size roughly comparable to one human  
677 chromosome. If we scale the number of false-positive associations identified in our analyses to a  
678 GWAS conducted on whole-genome data from humans, we would expect to see several thousand weak  
679 false-positive associations after PC corrections in a population with neighborhood sizes up to at least  
680 1000 (which should include values appropriate for many human populations). Notably, very few of the  
681 spurious associations we identified would be significant at a conservative Bonferroni-adjusted *p*-value  
682 cutoff (see Figure S8). This suggests that GWAS focused on finding strongly associated alleles for traits  
683 controlled by a limited number of variants in the genome are likely robust to the impacts of continuous  
684 spatial structure. However, methods that analyze the combined effects of thousands or millions of  
685 weakly associated variants such as polygenic risk scores (Khera *et al.* 2018) are likely to be affected  
686 by subtle population structure. Indeed as recently identified in studies of genotype associations for  
687 human height in Europe (Berg *et al.* 2018; Sohail *et al.* 2018), PC regression GWAS in modern human  
688 populations do include residual signal of population structure in large-scale analyses of polygenic traits.  
689 When attempting to make predictions across populations with different environmental exposures,  
690 polygenic risk scores affected by population structure can be expected to offer low predictive power, as  
691 was shown in a recent study finding lower performance outside European populations (Martin *et al.*  
692 2019).

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

703 regression.

704 We suggest a straightforward alternative for species in which the primary axes of population  
 705 differentiation is space (note this is likely not the case for some modern human populations): run a  
 706 GWAS with spatial coordinates as phenotypes and check for *p*-value inflation or significant associations.  
 707 If significant associations with sample locality are observed after correcting for population structure,  
 708 the method is sensitive to false positives induced by spatial structure. This is essentially the approach  
 709 taken in our “clinal” model (though we add normally distributed noise to our phenotypes). This  
 710 approach has recently been taken with polygenic scores for UK Biobank samples in Haworth *et al.*  
 711 (2019), finding that scores are correlated with birth location even in this relatively homogenous sample.  
 712 Of course, it is possible that genotypes indirectly affect individual locations by adjusting organismal  
 713 fitness and thus habitat selection across spatially varying environments, but we believe that this  
 714 hypothesis should be tested against a null of stratification bias inflation rather than accepted as true  
 715 based on GWAS results.

**Table 1 Neighborhood size estimates from empirical studies.**

Species	Description	Neighborhood Size	Method	Citation
<i>Ipomopsis aggregata</i>	flowering plant	12.60 - 37.80	Genetic	(Campbell and Dooley 1992)
<i>Borreria frutescens</i>	salt marsh plant	20 - 30	Genetic+Survey	(Antlfinger 1982)
<i>Oreamnos americanus</i>	mountain goat	36 - 100	Genetic	(Shirk and Cushman 2014)
<i>Homo sapiens</i>	Gainj- and Kalam-speaking people, Papua New Guinea	40 - 213	Genetic	(Rousset 1997)
<i>Formica sp.</i>	colonial ants	50 - 100	Genetic	(Pamilo 1983)
<i>Astrocaryum mexicanum</i>	palm tree	102 - 895	Genetic+survey	(Eguiarte <i>et al.</i> 1993)
<i>Spermophilus mollis</i>	ground squirrel	204 - 480	Genetic+Survey	(Antolin <i>et al.</i> 2001)
<i>Sceloporus olivaceus</i>	lizard	225 - 270	Survey	(Kerster 1964)
<i>Dieffenbachia longispatha</i>	beetle-pollinated colonial herb	227 - 611	Survey	(Young 1988)
<i>Aedes aegypti</i>	Yellow-fever mosquito	268	Genetic	(Jasper <i>et al.</i> 2019)
<i>Homo sapiens</i>	Gainj- and Kalam-speaking people, Papua New Guinea	410	Survey	(Rousset 1997)
<i>Quercus laevis</i>	Oak tree	> 440	Genetic	(Berg and Hamrick 1995)
<i>Drosophila pseudoobscura</i>	fruit fly	500 - 1,000	Survey+Crosses	(Wright 1946)
<i>Homo sapiens</i>	POPRES data NE Europe	1,342 - 5,425	Genetic	(Ringbauer <i>et al.</i> 2017)
<i>Bebicium vittatum</i>	intertidal snail	240,000	Survey	(Rousset 1997)
<i>Bebicium vittatum</i>	intertidal snail	360,000	Genetic	(Rousset 1997)

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

717 For how much of the tree of life do spatial patterns circumscribe genomic variation? In Table 1 we  
 718 gathered estimates of neighborhood size from a range of organisms to get an idea of how strongly

local geographic dispersal affects patterns of variation. This is an imperfect measure: some aspects of genetic variation are most strongly determined by neighborhood size (Wright 1946), others (e.g., number of segregating sites) are more strongly determined by global  $N_e$  or by the ratio of the two. In addition, these empirical examples are likely biased towards small-neighborhood species (because few studies have quantified neighborhood size in species with very high dispersal or population density). However, from the available data we find that neighborhood sizes in the range we simulated are fairly common across a range of taxa. At the extreme low end of empirical neighborhood size estimates we see some flowering plants, large mammals, and colonial insects like ants. Species such as this have neighborhood size estimates small enough that spatial processes are likely to strongly influence inference. These include some human populations such as the Gainj- and Kalam-speaking people of Papua New Guinea, in which the estimated neighborhood sizes in Rousset (1997) range from 40 to 410 depending on the method of estimation. Many more species occur in a middle range of neighborhood sizes between 100 and 1000 – a range in which spatial processes play a minor role in our analyses under random spatial sampling but are important when sampling of individuals in space is clustered. Surprisingly, even some flying insects with huge census population sizes fall in this group, including fruit flies (*D. melanogaster*) and mosquitoes (*A. aegypti*). Last, many species likely have neighborhood sizes much larger than we simulated, including the recent ancestors of modern humans in northeastern Europe (Ringbauer *et al.* 2017). For these species demographic inference and summary statistics are likely to reflect minimal bias from spatial effects as long as dispersal is truly continuous across the landscape. While that is so we caution that association studies in which the effects of population structure are confounded with spatial variation in the environment are still sensitive to dispersal even at these large neighborhood sizes.

#### 741 ***Other demographic models***

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

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

769 **Future Directions and Limitations**

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

779 One facet of spatial variation that we did not address in this study is the confounding of dispersal  
780 and population density implicit in the definition of Wright's neighborhood size. Our simulations were  
781 run under constant densities, but Guindon *et al.* (2016) and Ringbauer *et al.* (2017) have shown that  
782 these parameters are identifiable under some continuous models. Similarly, though the scaling effects  
783 of dispersal we show in Figure 4 should occur in populations of any total size, other aspects such as  
784 the number of segregating sites are also likely affected by the total landscape size (and so total census  
785 size). Much additional work remains to be done to better understand how these parameters interact to  
786 shape genetic variation in continuous space, which we leave to future studies.

787 Though our simulation allows incorporation of realistic demographic and spatial processes, it  
788 is inevitably limited by the computational burden of tracking tens or hundreds of thousands of  
789 individuals in every generation. In particular, computations required for mate selection and spatial  
790 competition scale approximately with the product of the total census size and the neighborhood  
791 size and so increase rapidly for large populations and dispersal distances. The reverse-time spatial  
792 Lambda–Fleming–Viot model described by Barton *et al.* (2010) and implemented by Kelleher *et al.*  
793 (2014) allows exploration of larger population and landscape sizes, but the precise connection of these  
794 models to forward-time demography is not yet clear. Alternatively, implementation of parallelized  
795 calculations may allow progress with forward-time simulations.

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

802 **Data Availability**

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

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1026 **Comparisons with Stepping-Stone Models**

1027

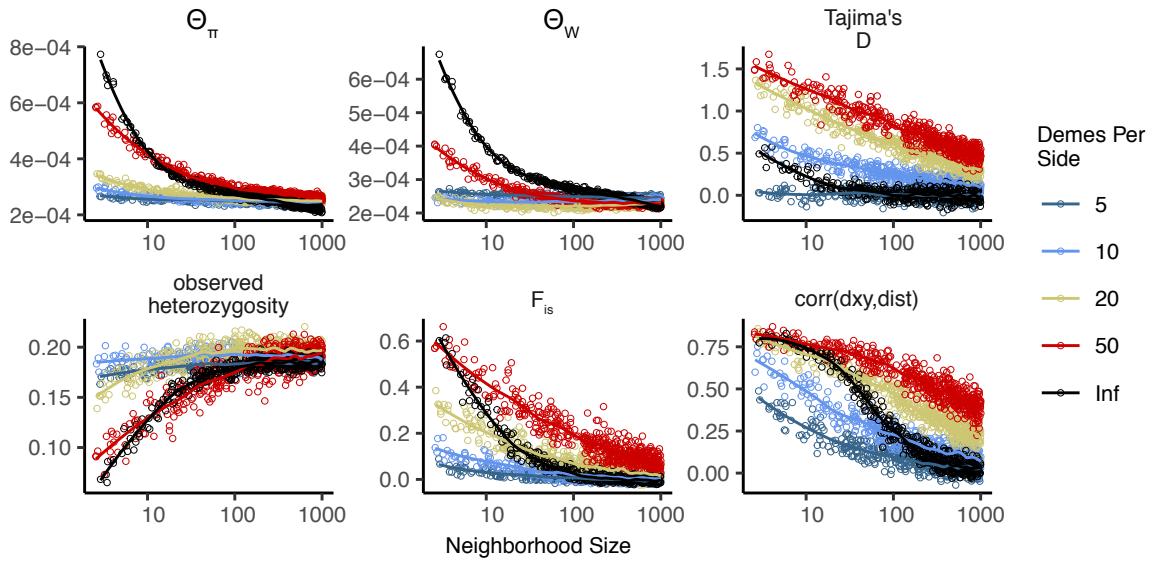
1028 We also compared our model results to a regular grid of discrete populations, which is commonly  
 1029 used as an approximation of continuous geography. An important reason that this approximation  
 1030 is often made is that it allows more efficient, coalescent simulations; we implemented these using  
 1031 `msprime` (Kelleher *et al.* 2016). In this class of models we imagine an  $n \times n$  grid of populations  
 1032 exchanging migrants with neighboring populations at rate  $m$ . If these models are good approximations  
 1033 of the continuous case we expect that results will converge as  $n \rightarrow \infty$  (while scaling  $m$  appropriately  
 1034 and keeping total population size fixed), so we ran simulations while varying  $n$  from 5 to 50 (Table  
 1035 A1). To compare with continuous models we first distributed the same “effective” number of  
 1036 individuals across the landscape as in our continuous-space simulations ( $\approx 6100$ , estimated from  $\theta_\pi$   
 1037 of random-mating continuous-space simulations). We then approximate the mean per-generation  
 1038 dispersal distance  $\sigma$  given a total landscape width  $W$  as the product of the probability of an individual  
 1039 being a migrant and the distance traveled by migrants:  $\sigma = 4m(W/n)$ . This means that  $m$  in different  
 1040 simulations with the same  $\sigma$  scales with  $\sqrt{n}$ . We ran 500 simulations for each value of  $n$  while  
 1041 sampling  $\sigma$  from  $U(0.2, 4)$ . We then randomly selected 60 diploid individuals from each simulation  
 1042 (approximating diploidy by combining pairs of chromosomes with contiguous indices within demes)  
 1043 and calculated a set of six summary statistics using the scripts described in the summary statistics  
 1044 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

1045 In general we find many of the qualitative trends are similar among continuous and stepping-stone  
 1046 models and that, at low neighborhood sizes, many (but not all) statistics from stepping-stone models  
 1047 approach the continuous model as the resolution of the grid increases. For example,  $\theta_\pi$  is inflated at low  
 1048 neighborhood sizes (i.e., low  $m$ ), and the extent of the inflation increases to approach the continuous  
 1049 case as the resolution of the landscape increases. Similar patterns are observed for  $F_{IS}$  and observed  
 1050 heterozygosity. However,  $\theta_W$  behaves differently, showing a non-monotonic relationship with grid  
 1051 resolution. This results in an increasingly positive Tajima’s  $D$  in grid simulations at small neighborhood  
 1052 sizes, to a much greater extent than seen in a continuous model. In contrast to  $\theta_\pi$ , increasing the  
 1053 resolution of the grid causes Tajima’s  $D$  to deviate *more* from what is seen in the continuous model.

1054 These differences relative to our continuous model mainly reflect two shortcomings of the reverse-  
 1055 time stepping stone model. If we simulate a coarse grid with relatively large populations in each  
 1056 deme, we cannot accurately capture the dynamics of small neighborhood sizes because mating within  
 1057 each deme remains random regardless of the migration rate connecting demes. This likely explains  
 1058 the trends in  $\theta_\pi$ , observed heterozygosity, and  $F_{IS}$ . However increasing the number of demes while  
 1059 holding the total number of individuals constant results in small within-deme populations for which  
 1060 even the minimum sample size of 1 approaches the local  $N_e$  (Table A1). This results in an excess  
 1061 of short terminal branches in the coalescent tree, which decreases the total branch length and leads  
 1062 to fewer segregating sites, deflated  $\theta_W$ , and inflated Tajima’s  $D$ . Overall, the stepping-stone model  
 1063 reproduces important features of spatial structure in our continuous space model, such as a decline in  
 1064  $\theta_\pi$  and correlations between spatial and genetic distance with increasing migration, but introduces  
 1065 artifacts caused by binning the landscape into discrete demes.



**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  $\sigma$  as described above.

## Demographic model

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

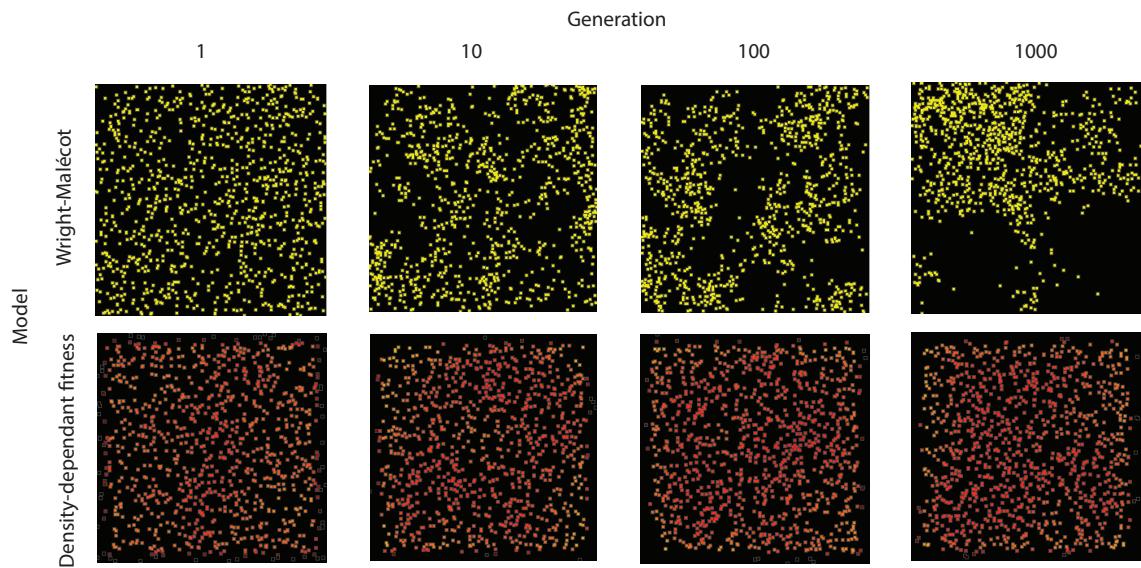
$$p = \min \left( 0.95, \frac{1}{1 + n/(K(1 + L))} \right). \quad (3)$$

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

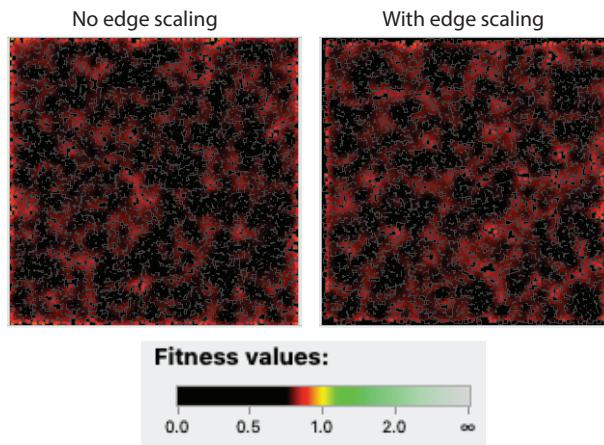
$$n = K \frac{L + 1}{L - 1} \quad (4)$$

individuals per unit area. At this density, the per-capita death rate is  $1/L$ , so the mean lifetime is  $L$ . This equilibrium density is *not*  $K$ , but (since  $L = 4$ ) is two-thirds larger. However, in practice this model leads to a total population size which is around  $K$  multiplied by total geographic area (but which depends on  $\sigma$ , as discussed above). The main reason for this is that since offspring tend to be near their parents, individuals tend to be “clumped”, and so experience a higher average density than the “density” one would compute by dividing census size by geographic area (Lloyd 1967). To maintain a constant expected total population size would require making (say)  $K$  depend on  $\sigma$ ; however, typical local population densities might then be more dissimilar.

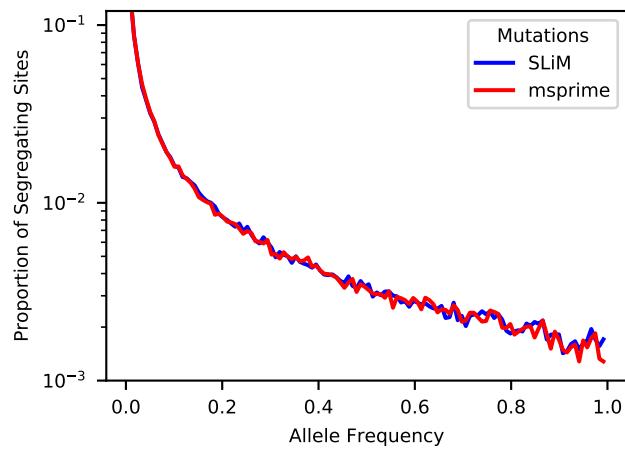
<sup>1088</sup> **Supplementary Figures and Tables**



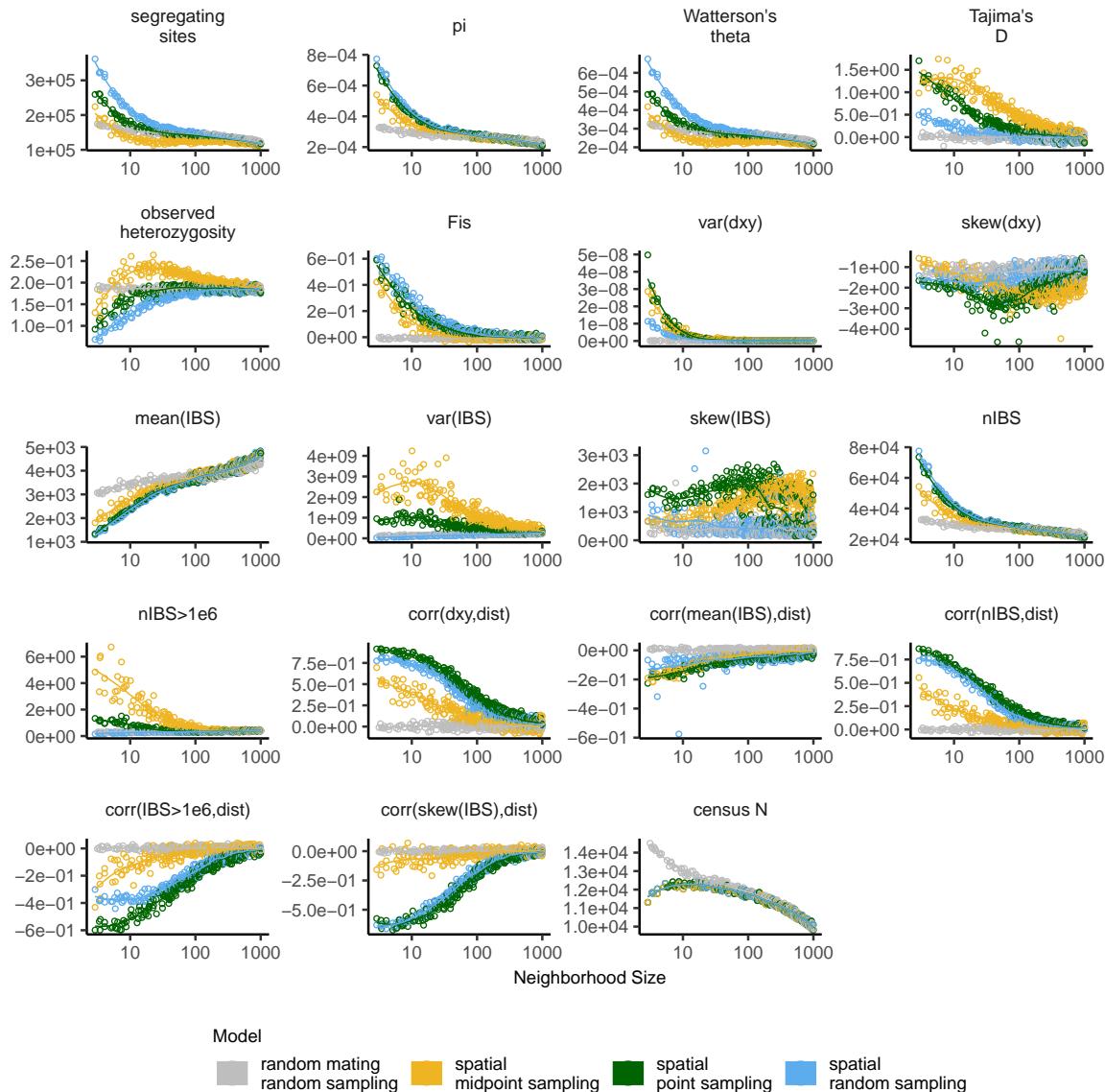
**Figure S1** Maps of individual locations in a continuous-space Wright-Malécot model with independent dispersal of all individuals (top) and under our continuous space model incorporating density-dependant fitness (bottom). The clustering seen in the top row is the “Pain in the Torus” described by Felsenstein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



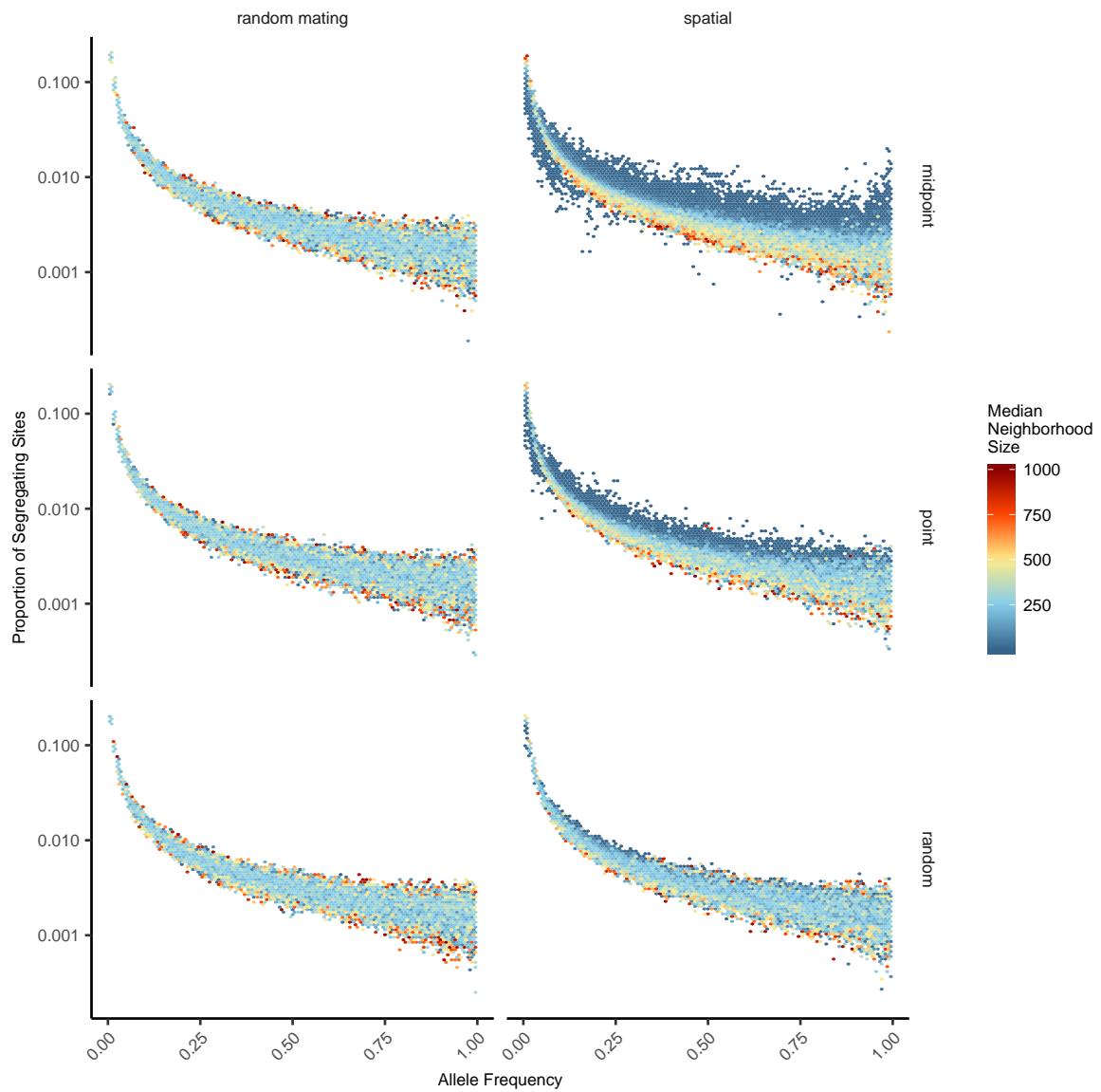
**Figure S2** Comparison of individual fitness across the landscape in simulations with (right) and without (left) a decline in fitness approaching range edges. Note the slight excess of high-fitness individuals at edges on the left, which is (partially) counteracted by the scaling procedure.



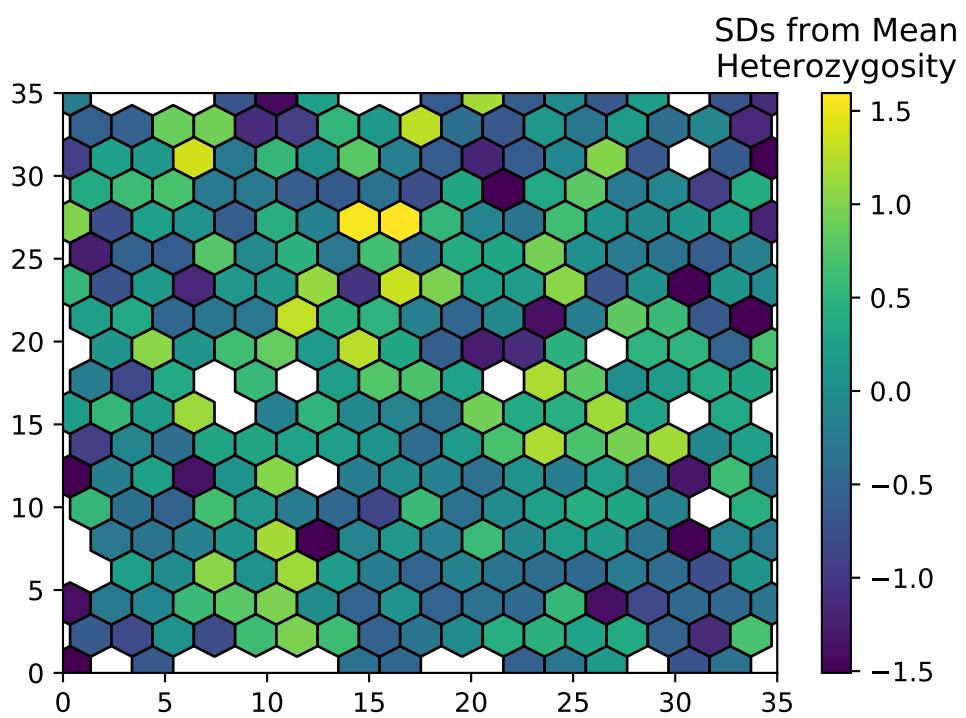
**Figure S3** Site frequency spectra from a simulation with neighborhood size = 12.5 when mutations are recorded directly in SLiM (blue line) or applied later in msprime (red line).



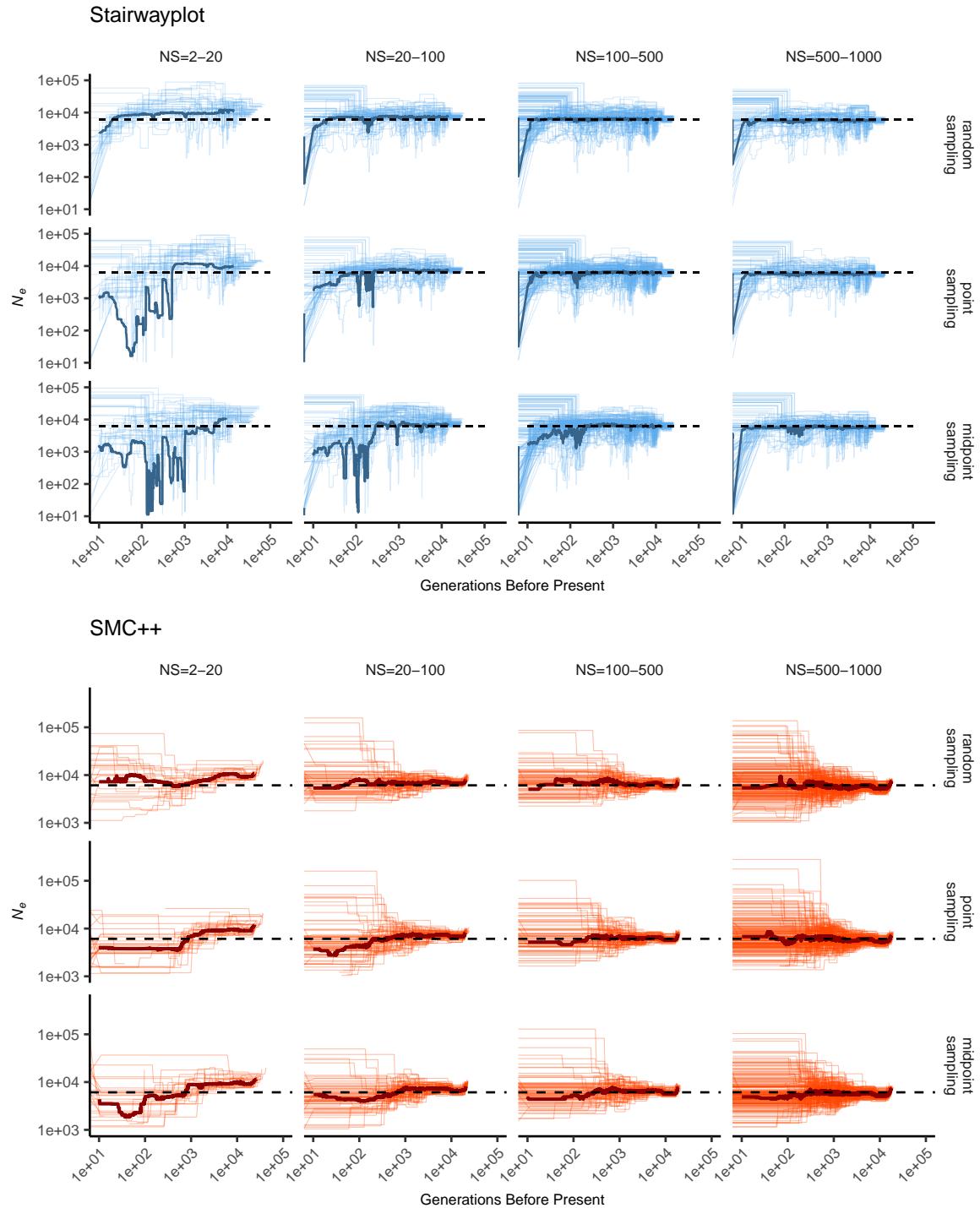
**Figure S4** Change in summary statistics by neighborhood size and sampling scheme calculated from simulated sequence data of 60 individuals.



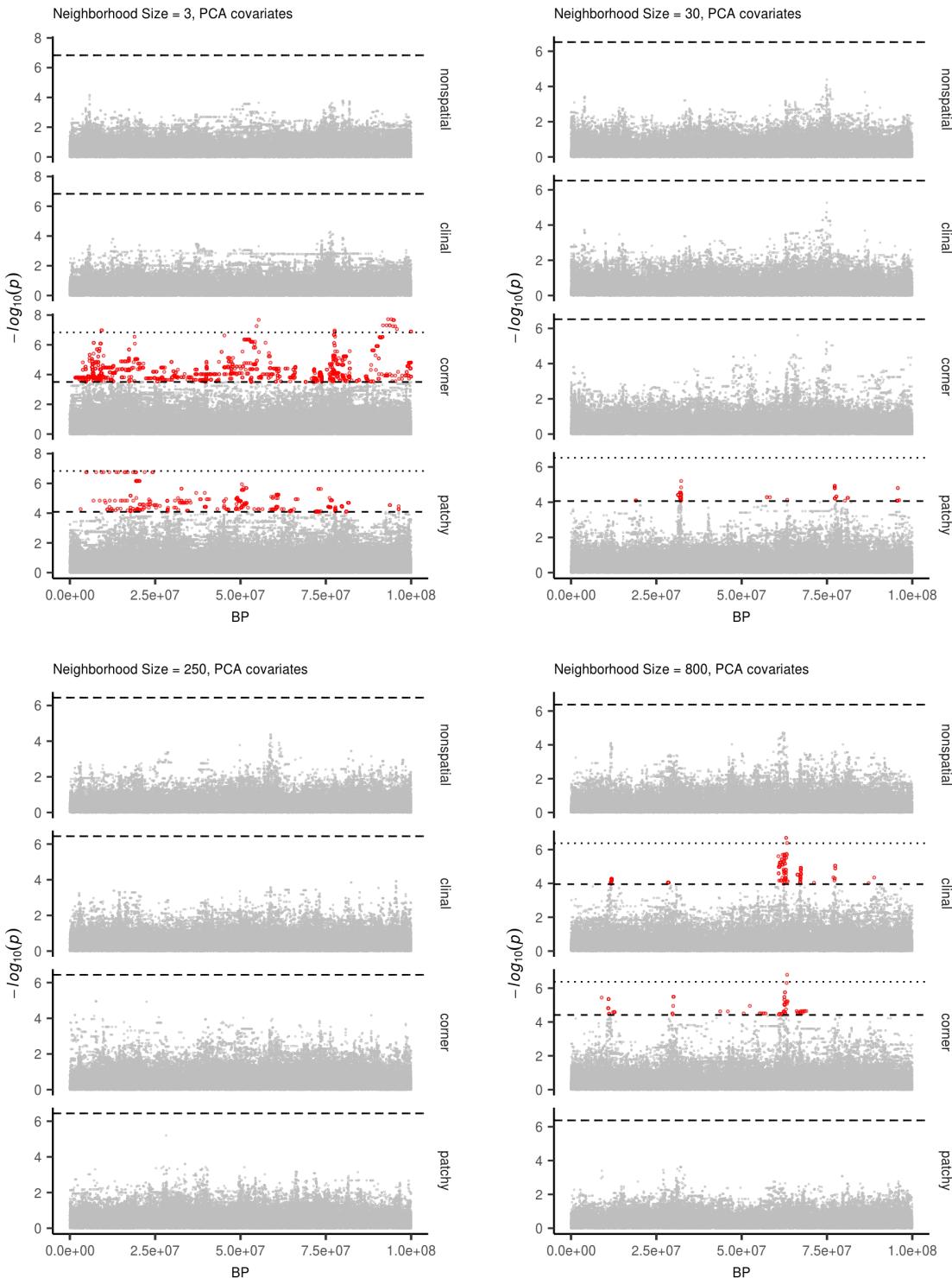
**Figure S5** Site frequency spectra for random mating and spatial SLiM models under all sampling schemes.



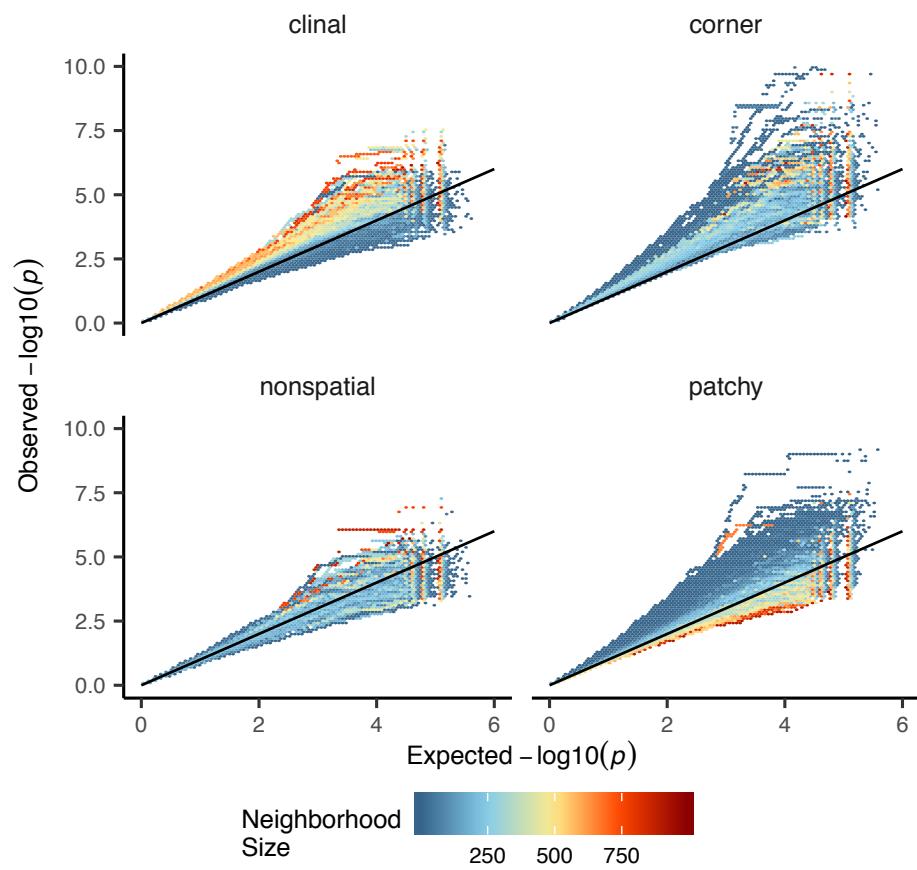
**Figure S6** Variation in observed heterozygosity (i.e. proportion of heterozygous individuals) in hexagonal bins across the landscape, estimated from a random sample of 200 individuals from the final generation of a simulation with neighborhood size  $\approx 25$ . Values were Z-normalized for plotting.



**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  $\theta_\pi$ .



**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
$\Theta_{pi}$	Mean of the distribution of pairwise genetic differences
$\Theta_W$	Effective population size based on segregating sites
Segregating Sites	Total number of segregating sites in the sample
Tajima's $D$	Difference in $\Theta_{pi}$ and $\Theta_W$ 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 $1 \times 10^6$ 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 > $1 \times 10^6$ 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
$\Theta_W$	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
$\Theta_W$	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 a revised version of our manuscript, “Space is the Place: Effects of Continuous Spatial Structure on Analysis of Population Genetic Data”. We thank the AE and reviewers for constructive comments, which we have addressed in detail, below. The main changes we have made have to do with our choice of model. We have added some more discussion of the biological motivation for the particular model of continuous space we use (with references to the ecological literature, where it appears) to the Methods (around (p. 3, l. 131)), and in the Discussion (at (p. 21, l. 744)). We have included more explanation of some of the more opaque consequences of a more realistic demographic model, around (p. 8, l. 343). Finally, we have added a substantial comparison to the discrete stepping-stone model, most of which is in the Appendix.

We have included a PDF of the revised version as well as a latex diff showing changes relative to the previous submission.

**Sincerely,**

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

## **Reviewer AE:**

The manuscript admirably explores a lot of consequences of isolation-by-distance in the context of a novel model that is easily amenable to forward simulation; however, given that this model may be used in a lot of future studies based on the precedent set here, there is some concern about the model and its support. Reviewers 2 and 3 highlight this in particular (it underlies the main 2 points of reviewer 2's review, and the core of Reviewer 3's comment), and I agree. Whatever can be done to strengthen the standing of this model, and/or connect it to more thoroughly studied models, will be helpful for the manuscript. The concern would be that there are peculiarities of this model that do not generalize well. A new supplemental section or opener to the results section establishing the model more thoroughly would make the strongest response.

This is a good point. There are many ways to go in the quest for demographic realism, and the fundamental question – what regulates population density in real populations – is still very much an open question (or rather, a question with a great many answers). As highlighted below, we have included further discussion of our particular choice of local, density-mediated control on mortality, along with pointers into the literature, at several places in the manuscript; see in particular in the Methods when we introduce the model (p. 3, l. 140), and (p. 21, l. 744).

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**(AE.1) Line 35:** Also cite Wilkins and Wakeley, *Genetics* 2002; Wilkins 2004

**Reply:** Done. (p. 1, l. 36)

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**(AE.2) (p. 3, l. 140)** “Such models have been used extensively in ecological modeling but rarely in population genetics”: Detailing these previous uses via citations and elaboration may help alleviate the major concern about the provenance of this model and its unique behaviors (see general comments above and R2 and R3 comments).

**Reply:** Good idea – we have added some historical discussion and a few more citations to this section, (p. 3, l. 140). Also see the additional discussion in the Discussion (p. 21, l. 744).

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**(AE.3) (p. 4, l. 187)** Please describe computation time needed per replicate

**Reply:** We have added a figure (Figure 3) and short discussion (p. 9, l. 360) of run times.

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**(AE.4) (p. 22, l. 806)** I read the acknowledgement to the Hearth and Falling Sky Brewing with a sense of familiarity in feeling of gratitude to my own favorite cafes and breweries, but I it's not a great precedent for Acknowledgements to be filled this way. Please cut.

**Reply:** Good point; we have done this.

---

**(AE.5) Figure 5:** Show random-mating expectation

**Reply:** This is done.

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**(AE.6) Figure 4A, S5:** Perhaps more revealing to show on log-log scale?

**Reply:** Good suggestion – the SFS in Fig 4A is now on a log-log scale, which shows the slight decrease in low frequency SNPs a little better. We've left Fig S5 as-is.

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**(AE.7) Figure S3:** Caption seems to be missing detail

**Reply:** Thanks for catching this - we have revised this caption to add details including the simulation parameters. (Supplemental Figure S6).

## Reviewer 1:

Overall, the authors explore an important but often neglected source of bias that can affect inference in many population-based studies (in medical genetics, evolutionary biology and ecology). This study can be of interest to a broader audience of readership, and I have only minor comments to improve clarity and increase accessibility for readers:

We thank the reviewer for their very constructive comments! Responses follow below:

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**(1.1)** When neighbourhood size is small (10-100), the mean number of IBS tracts  $> 2\text{bp}$  ( $n\text{IBS}$  as in Table S1) is elevated similar to Wright's inbreeding coefficient, but mean of the distribution of pairwise IBS ( $\text{mean}(IBS)$ ) is decreased. What could be the source of this discrepancy? How exactly  $\text{mean}(IBS)$  was calculated?

**Reply:** The mean IBS is simply the mean of the vector of lengths of IBS tracts (p. 6, l. 236). The short answer is that if one splits a chromosome up into more pieces (increasing  $n\text{IBS}$ ), the mean length of those pieces must necessarily be smaller. At low neighborhood sizes, spatially distant individuals tend to have longer coalescence times, leading to more (and hence smaller) IBS tracts. This can be seen in Supplemental Figure S4. Note that  $\text{skew}(IBS)$  and number of long ( $>1\text{e}6$ ) IBS tracts reflect other aspects of the distribution.

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**(1.2)** The authors use  $K$  to denote both carrying capacity (p. 4, l. 165) and population density (p. 4, l. 168). It might be better to use a different notation for these quantities since carrying capacity is fixed while density is an emergent quantity in the non-Wright-Fisher model. Use of  $K$  to denote carrying capacity and density is a bit confusing. For example, on (p. 7, l. 320) it is said that 'the "population density" ( $K$ ) and "mean lifetime" ( $L$ ) parameters were the same in all simulations'. Here  $K$  seems to indicate carrying capacity rather than density? The latter is an emergent quantity and varies across simulation runs?

**Reply:** We agree and have adjusted our language to underscore the point that  $K$  is a parameter that controls population density, rather than being equal to it, at (p. 4, l. 168) and (p. 5, l. 205) and (p. 7, l. 320).

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**(1.3)** Concerning the non-Wright-Fisher model used, it would be helpful to emphasize that some of the parameters are emergent in contrast to Wright-Fisher model. For example, on Page 11, lines 306-308, the author's goal was to look at census size variation and variation in other quantities. This would be better understood if to emphasize that these parameters are emergent properties in the non-Wright-Fisher model used.

**Reply:** We have added to the text at the beginning of the results to emphasize that this analysis is necessary because these parameters are emergent rather than fixed (p. 7, l. 319).

---

**(1.4) (p. 6, l. 255)** Perhaps 'Demographic Inference' might better reflect the content of this section.

**Reply:** Good suggestion – we have changed the section heading to "demographic inference" (p. 6, l. 255).

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**(1.5) (p. 7, l. 286)** This sentence with 'Gaussian noise with mean zero and standard deviation 10' is confusing since it was mentioned earlier that the modelled phenotype must vary as human height across Europe, and human height varies 2 standard deviations. Only after reading the whole paragraph it becomes clear that 'standard deviation 10' here refers to unit of height. Please consider rephrasing this sentence.

**Reply:** We have revised this sentence to clarify that we aim to produce a variation in mean phenotype of two standard deviations across the landscape (p. 7, l. 286).

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**(1.6) (p. 7, l. 308)** In the sentence, 'We also examined  $p$  values for systemic inflation' I think the authors meant 'systematic inflation'.

**Reply:** Whoops; thanks. Fixed.

---

**(1.7)** Please correct the legend in Figure 2: must be 'spatial model' and 'random mating' model.

**Reply:** Thanks for catching our confusing legend title placement! We have moved "model" to after "spatial" as suggested.

---

**(1.8)** Optional: a dashed line in Figure 2 that shows the total carrying capacity of  $50 \times 50 \times 5 = 12500$  would be helpful.

**Reply:** This is a good suggestion, but we decided to not include this as we don't have straightforward expectations for the other parameters shown.

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**(1.9) (p. 9, l. 369)** The phrase 'affect summaries of variation' is better to replace with 'summaries of genetic variation'.

**Reply:** Done. (p. 9, l. 369)

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**(1.10)** Please add or correct references to supplementary figures: For example, Figure S2 was probably meant to accompany Figure 3A, while Figure S1 Figure 3B, but references in the text are absent. In fact, the first reference is made to Figure S3 on page 15.

**Reply:** Thanks for catching this, should be fixed now.

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**(1.11)** There are also several typos and errors in the text. For example, (p. 7, l. 320); (p. 19, l. 656).

**Reply:** Thank you for noting these – they have been corrected.

## Reviewer 2:

Battey et al. use spatially explicit population genetic simulations to analyze the effects of spatial structure on (i) the estimation of key population genetic parameters, in turn used to (ii) make inferences about population history, and on (iii) confounding in genome-wide association studies (GWAS). I liked the paper a lot. It's interesting, well-written and addresses an important question - the effect of spatial population structure on population genetic statistics and inference-and I enjoyed reading it. The most positive aspects were:

1. It nice to actually see spatially explicit simulations and I'm happy that forward simulation is now fast enough that you can do this sort of thing.
2. The paper is very clear and well-written, easy to understand the motivation and most of the details. That's not always the case for this sort of paper.
3. I felt that the section about the effect on GWAS was the most interesting and novel part of the paper and gave me some intuition that I hadn't had before.

I don't have any major criticisms. There were a few aspects that I thought might warrant some additional discussion, and a few specific questions below. The general questions I had after reading it were:

Thanks very much for the encouraging words!

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(2.1) To what extent are any of the results dependent on the exact method of simulation. There are a number of choices about the exact details of the simulations (e.g. the way the overlapping generations are handled, the edge effects and, particularly, the form of Equation 1 - see below). It's not so much that these are non-standard (since I don't think there is a standard) and they all sort of make sense heuristically, and I was left wondering whether these sorts of choices actually make a difference. Do the authors have some thoughts/intuition/results about that? Given that the results in Figure 4 seem quite consistent with expectations, I suspect that on some level it doesn't make much difference but then there are intermediate results like Figure 2 which seem a bit counter-intuitive and I wonder if those aspects depend on the simulation scheme.

**Reply:** This is a good point also raised by other reviewers. We have added some more discussion of the choice of demographic model ((p. 3, l. 140) and (p. 21, l. 744)).

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(2.2) Related to the first point, to what extent are the results qualitatively different to those that would be obtained in a stepping-stone model? My interpretation is that they are actually very similar, but I didn't see whether that was explicitly discussed. In some sense, it's still easier to do large simulations in a stepping-stone model so it would be nice to be reassured that that's still ok.

**Reply:** We have added an appendix comparing our model to a reverse-time stepping stone simulation on several relevant summary statistics (p. 27, l. 1027). For some of these statistics (e.g.,  $\theta_\pi$ ), the behavior of the stepping stone model becomes more similar (although not identical) to the continuous model as the resolution of the landscape increases, but other statistics are more strongly affected by discretization. We are also curious how all the other analyses we perform would be affected using other simulation schemes, but hope to explore that aspect in future work. Furthermore, discrete-deme coalescent simulations are certainly faster, but come with significant issues either when neighborhood size is lower than the population of a deme, or when demes are small enough that the sample size approaches local  $N_e$ .

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(2.3) The source of equation (1) is not obvious to me. I sort of see how it makes sense, but a little but more intuition or a brief derivation or an illuminating either in the main text or the supplement, would be helpful.

**Reply:** Additional discussion of the model is now provided in the appendix (see "Demographic model") (p. 28, l. 1067)

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(2.4) The authors use a scaling factor in equation (2) to counteract the increase in fitness of individuals at the edges. Can they provide a figure showing that this is the case. What does "roughly" mean on line 164. Perhaps a heatmap of the fitness of individuals across the grid with and without the scaling factor?

**Reply:** Good suggestion – we have added a supplemental figure (Figure S2) to show the distribution of individual fitness across the landscape with and without our edge-scaling approach.

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(2.5) It would be helpful provide the figure showing that generating mutations during the forward simulations in SLIM is equivalent to applying mutations using msprime on pre-generated trees (p. 5, l. 194)? It sounds like this procedure would underestimate the variance in the number of mutations, since you remove the effect of random generation time. Is this effect small?

**Reply:** Theory says that any difference here would be extremely small and would affect only the variance, not the mean. Nonetheless, we have added a figure showing sample site frequency spectra generated from a subset of simulations run with SLiM mutations, and then using msprime to apply

mutations to the same tree sequences with our generation-time scaling approach (Figure S3). These approaches yield extremely similar spectra.

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**(2.6)** Can the authors provide a bit more intuition behind the patterns of variation seen in generation time, census population size, and variance in the number of offspring with respect to neighborhood size seen in Figure 2? For example, it is not obvious to me why the census population size, for example, should decline systematically with respect to neighborhood size. Presumably this isn't just due to the local demographic stochasticity. Could the authors briefly interpret the observed patterns or cite appropriate literature?

**Reply:** We agree that this is hard to intuit: there are a lot of factors at work. We have added some more discussion of these phenomena (p. 8, l. 343).

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**(2.7)** Figure 8D: I am surprised by the extent to which the observed values of  $-\log_{10}(p)$  fall below the  $y = x$  line. Particularly in the lower right panel for large neighbourhood sizes. I would expect that to be close to panmictic - why are the P-values underdispersed? That seems like a potential bug, or else something weird is going on.

**Reply:** We have checked the code to the best of our abilities and did not find a bug causing the underdispersion, but we have switched our visualization in Figure 8D to now show a subset of qqplots as lines, rather than the hexagonal binning over all simulations. This lets us see the density in addition to the range of values, and shows that relatively few points fall below the 1:1 lines. Those that do seem to reflect overcorrection in the regression when using PC coordinates as covariates – the PCA is capturing some information about the spatial genetic variation which itself covaries only weakly with the phenotype, and as a result we see anomalously low  $-\log_{10}(p)$  when regressing genotype against phenotype.

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**(2.8) (p. 20, l. 712)** It might be worth citing (Haworth et al. 2019), who do the proposed test (GWAS for birth location) in UK Biobank to illustrate the population structure.

**Reply:** Done - thank you for pointing us to this study. (p. 20, l. 712)

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**(2.9)** The analysis and discussion around the effect of GWAS is focused on PCA correction. Do mixed models help at all?

**Reply:** We are also very interested to know how mixed models perform here, but think that adding a second GWAS method would make this section too large for the current paper. We have added a note to (p. 19, l. 696) specifically citing mixed models as alternate methods that may perform better.

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**(2.10)** The github link to the code didn't work for me. I assume it will be made public later, but at this point I can't tell whether the code is available/useable.

**Reply:** We apologize – we had forgotten to make the repository public. This has been corrected.

### Reviewer 3:

The present study deals with a “hot topic” in spatial population genetics. Most inferential and descriptive methods in statistical spatial population genetic rely on a discrete approximation of space and it is not clear what impact this approximation may have when individuals migrate along a continuum instead. Spatial patterns in sampling is also another major issue which is often simply dismissed, mainly because of the paucity of statistical methods to deal with it. This work touches on these important issues in a timely manner.

We are glad you agree this is an important topic to explore!

Although I was enthusiastic about the topic, I was quite disappointed with the core of the study, i.e., the forward-in-time simulation of populations in continuous space. The field has been struggling with this issue for decades – examples of spectacular failures like the Wright-Malecot model (see Felsenstein’s “pain in the torus” article, 1975) or, more recently, the “mugration” or “discrete trait analysis” model in phylodynamics (see De Maio et al. 2015) have probably mostly harmed our research field – that one cannot make the economy of using a sound probabilistic model for generating geo-referenced genetic data. It does not seem to be the case here unfortunately.

We were surprised that the reviewer seems to be saying that local, density-mediated control of mortality doesn’t constitute a “sound probabilisitic model”, but hope that our additional discussion of the choice, and citations to the history of this sort of model, help to better motivate our choice.

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(3.1) *First, the simulation starts with individuals distributed uniformly at random in space. Is there any indication that the three-step algorithm used here maintains this distribution during the course of evolution? If it does not, then is there any stationary regime and how many generations does one need to wait before reaching it? I do appreciate that the competitive interaction term was introduced in order to avoid seeing the “clumping” of individuals that hampers the Wright-Malecot model. Yet, just because there are no such clusters does not mean that the spatial distribution of individuals reaches a stable regime and that the distribution reached, if any, is reasonable from a biological perspective.*

**Reply:** This is a natural question that we asked ourselves while developing the simulation using the built-in visualization tools of the SLiM GUI. We have now added a supplementary figure (Figure S1) showing the distribution of individuals in our density-dependant spatial model and a continuous-space Wright-Fisher simulation without density dependance. We encourage the reviewer to run our SLiM recipe using the GUI at different parameter values to see for themselves how quickly equilibrium is attained – we are tempted to include movies of this as supplementary material, but that seems excessive. Also note that we did *not* include competitive interaction to avoid clumping – we included it to make the model biologically realistic (so that, for instance, the population does not grow without bound), and lack of clumping is a *consequence* of this choice. We have clarified this in the text. (p. 3, l. 138)

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(3.2) *Second, the demographic process used here involves birth and death of individuals. Does the population survive asymptotically or, like any birth-death process, eventually dies with probability one? In fact, one needs to know a little about the dynamics of the population size to decide whether the corresponding process is reasonable from a biological standpoint.*

**Reply:** As in the last point, as part of sanity checking our models we monitored the population size over time while with SLiM. All simulations maintained asymptotic populations, and none of the runs we started ended because populations crashed. We agree that the population model will die out eventually with probability 1 (it is a Markov process with a single accessible absorbing state (death), and since it isn’t a branching process, doesn’t grow without bound). However, it will take an extremely long time to hit that state, so that extinction is not a practical consideration. We have added a clarifying note about this. (p. 3, l. 137)

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(3.3) *Third, it is not clear what the relationship between the expected lifespan and the probability of survival is. The expected lifespan,  $L$ , is first defined as the inverse of the expected number of offspring produced by a parent. The authors also define the probability of survival of a given individual at a given point in space,  $p_i$ . Hence, the expected lifespan at a point in space (and time) is the mean of a geometric distribution with parameter  $p_i$ , i.e.,  $1/p_i$ . Now, it is far from being obvious what the relationship between these two approaches for defining the expected lifespan actually is.*

**Reply:** We explained the relationship here, in a new Appendix ('Demographic Model'; (p. 28, l. 1067)).

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**(3.4)** Also, the web page <https://github.com/petrelharp/spaceness> does not seem to exist so that I was not able to experiment with the forward-in-time generator used here unfortunately.

**Reply:** We apologize – we had forgotten to make the repository public. Now it is.

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**(3.5)** All in all, more efforts need to be made here in my opinion to show that the forward-in-time simulations generate sensible outcomes. Sensible in terms of the behavior of the population demography at equilibrium (provided such equilibrium indeed exists) along with that of the spatial distribution of individuals. The authors could provide some guarantee of the good behavior of their model as evidenced from simulations using a broad range of parameter values for generating data. Alternatively, they could elect to use the spatial-Lambda-Fleming-Viot model for their simulations, which, in my opinion would seem the most sensible option given that (1) it is possible to run backward-in-time simulations under this model, thereby saving a lot of computation time and (2) it is a well-studied model with good mathematical and biological properties and (3) it is implemented in a publicly available software program (<https://github.com/jeromekelleher/discsim>).

**Reply:** Hopefully, the additional plots and discussion around demographic modeling (notably, the discussion at (p. 21, l. 744)) helps to increase the transparency in this model. We agree that demographic realism is a very important consideration, and due to the often hard-to-intuit nature of spatial demography, simulations need to be carefully sanity checked. (Furthermore, what is “realistic” for one species will not be for another!) We have added an Appendix comparing our model to a stepping-stone (p. 27, l. 1027), because we think these are the most familiar and widely used class of spatial models. We find that many features of our model are well approximated by stepping-stone models, and that for statistics like  $\theta_{\pi}$  the stepping stone model results approach our continuous space model as the number of demes used to describe the landscape increases.

We definitely think that a comparison to the spatial Lambda-Fleming-Viot would be an extremely useful thing to do, for the reasons the reviewer mentions, but this is beyond the scope of the current study. The SLFV model is only known to be an approximation of one particular biologically explicit model (of patchy extinction-recolonization) and is only conjectured to be a good approximation for spatial models more generally. To reiterate, the definition of the SLFV was explicitly motivated by a desire to approximate spatial models, especially Bolker-Pacala models like ours. Therefore, our study does important preliminary work for simulation study of the spatial Lambda-Fleming-Viot, since it works through some of the issues in simulating biologically realistic spatial models, and describes some of the patterns.

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**(3.6) Figure 2:** I do not understand why the neighborhood size varies to the same extent in the random mating model as it does for the spatial model. For the random mating model, I would have expected the neighborhood size to be equal to the census size since all individuals have the same probability of being a parent of any given offspring. From [the paragraph at (p. 5, l. 211)], it is clear that the spatial model would converge to the random mating model when the mean parent-offspring distance tends to infinity only if we were to ignore the impact of range edges. I am thus wondering whether the variation of neighborhood size one observes in Fig 2 for the random mating model is just a consequence of border effects. If that is the case, then the authors should state it clearly and try to justify it from a biological perspective.

**Reply:** It’s a good point that neighborhood size, as defined to be “number of possible parents”, should indeed be the census size. However, we are using  $N_W$  defined in the same way for both spatial and random mating simulations as a way of comparing spatial to “nonspatial” models keeping other aspects of demography (almost) the same. This is indeed part of what’s going on. We have added some discussion of the census size scaling at (p. 5, l. 211) and (p. 8, l. 343), but haven’t added a detailed discussion of whether or not most species experience decreased habitat quality at range edges, since that seems like it is getting too far into the weeds.

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**(3.7) (p. 21, l. 730)** “Many more species occur in a middle range of neighborhood sizes between 100 and 1000 - a range in which spatial processes play a minor role in our analyses [...]. Do the authors think that the spatial processes would still play a minor role when neighborhood sizes exceed 100-1000 if the habitat was larger than that taken in the present simulations? It would also probably be useful to mention that neighborhood sizes given in Table 1 should be compared with extreme caution since the size of the corresponding habitats vary across species. More generally, I suspect that the size of the habitat has a substantial impact on the vast majority of statistics examined in this study. Indeed, the mean parent-offspring distance, which is at the core of the definition of Wright’s neighborhood size, is only small or large relative to the size of the habitat.

**Reply:** This is a good point. Wright’s work (Wright 1943) suggests some aspects of genetic variation such as variance in allele frequencies and inbreeding coefficients can be estimated by looking only at what he would later (Wright 1946) call “neighborhood size”, but certainly other aspects like the number of segregating sites will also depend on total landscape size. We now note on (p. 21, l. 721) that we have evaluated only one landscape size, and have added a sentence to the discussion noting that exploration of these patterns in varying landscape sizes is an important avenue for further research (p. 22, l. 785).

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**(3.8) (p. 22, l. 781)** Please add a reference to Guindon, Guo and Welch (2016). This study clearly shows that population density and dispersal parameters are identifiable and can indeed be estimated in practice under the spatial Lambda-Fleming-Viot model.

**Reply:** Done. Thank you for pointing us to this study. (p. 22, l. 781)

#### Reviewer 4:

The manuscript by Battey et al explores the consequence of a well-known violation to population genetic models: the fact that populations are spatially structured and mate along a geographical cline, rather than randomly. This topic is important, particularly in light of recent work describing how spatially correlated genetic and environmental impacts can confound some population genetic insights, such as positive selection for height in Europe. The analyses and investigations presented here are thorough and sensible, and my comments are primarily intended to broaden accessibility for this interesting topic.

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**(4.1) Introduction.** The discussion is very clear, articulating the three primary goals of the project: the impact of failing to model spatial population structure on 1) population genetic summary statistics, 2) inference on demographic history from population genetic data, and 3) impacts on GWAS summary statistics. I found the discussion a bit easier to follow than the introduction and would suggest streamlining and introducing the topic a bit more. Since the paper follows the flow described in the discussion, it might help orient readers by introducing these topics in the same order.

**Reply:** Thank you for this suggestion. We have slightly revised the introduction and hope it is now clearer; however since we want to cover a little history and motivation for our continuous model vs stepping-stone approaches in the intro it does have a different flow from the discussion.

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**(4.2)** I agree that most modern work describes structure as discrete populations connected by migration. However, some methods/studies have explicitly modeled spatial structure, e.g. especially in ecology or using methods like dadi (diffusion approximations). Highlighting some examples of previously identified structure not possible to infer without modeling geography would be helpful to contextualize this work.

**Reply:** We have expanded our citations of some of the relevant ecology literature (p. 3, l. 131), which we hope helps to contextualize the study better. We haven’t cited dadi, which works with discrete populations.

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**(4.3)** There is some reference to spatial models using grids (e.g. Rousset 1997). Some additional discussion contextualizing more recent methods like EEMS that also construct demes and model migration through divergence between neighboring demes would be helpful and interesting.

**Reply:** We agree that not noting EEMS was an oversight. We have added the most recent EEMS paper to the citations at (p. 2, l. 39), and have added an Appendix giving a more thorough comparison with stepping-stone models.

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**(4.4)** Demographic modeling. Both approaches tested, stairwayplot and SMC++, are most sensitive to older demographic events, and consequently are very noisy and underestimate effect population sizes, especially in smaller neighborhood sizes. Models that consider haplotype structure are much better suited to this time period. It would be helpful to either 1) discuss the varying time sensitivities of different classes of demographic inference methods and how spatial patterns of genetic variation would influence these inferences, or 2) apply a method of this class (many options, e.g. DoRIS, IBDNe, Tracts, Globetrotter, etc) and show how it performs.

**Reply:** We now discuss haplotype methods in the relevant discussion section (p. 18, l. 629). However though these methods should be more accurate for recent events it is not clear that this will improve performance per se. The dips in recent inferred Ne from stairwayplot are not just prediction noise, but actually reflect an underlying genealogy in which terminal branches are shorter than expected from a constant-size random-mating population (see e.g. figure 4A and 5). The interpretation error is that these short branches are generated by spatial structure rather than changes in population size over time – a point also made in the (Mazet *et al.* 2015) paper we discuss in the introduction and discussion.

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**(4.5)** GWAS mixed models. To what extent can spatial signals (e.g. corner, patchy) be corrected with mixed models, e.g. with PCs and PC-adjusted GRM as in Conomos *et al.* 2016 using PC-AiR and PC-Relate? Is patchiness related to dispersal? I'm curious how this relates to the predictive ability of GWAS phenotypes with some spatial association that may or may not be associated with environmental effects.

**Reply:** Good question – we are also interested to know how mixed models perform here, but think that to properly test that we would want to change our design to generate phenotypes from simulated genotypes. This would allow us to evaluate false-negatives in addition to false-positives. This is important because, if mixed models do provide stronger control for stratification they are also likely to remove true signals of causal SNPs if those SNPs covary with spatial structure. We now point to these methods explicitly in the discussion (p. 19, l. 696), but think that incorporating that study here would make this paper too long and we plan to explore this timely issue in the future. We also think the PC results are still quite relevant as the method is quite population in current studies.

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**(4.6)** Code availability. This github link doesn't work, but is important to be able to evaluate for review: <https://github.com/petrelharp/spaceness>.

**Reply:** Apologies, it was accidentally set to private. The link should work now (and has been replaced by the primary link, <https://github.com/kern-lab/spaceness>).

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**(4.7)** Definitions and interpretations. There are quite a large number of metrics discussed in Figure 4, and it's a lot to take in. It might be helpful to have a table with a reminder of what the metric is, its interpretation, and how it is computed.

**Reply:** We have included a table describing the summary statistics in Table S1.

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**(4.8) (p. 4, l. 157)** Notation: "Offspring disperse a Gaussian-distributed distance away from the parent with mean zero and standard deviation  $\sigma$  in both the x and y coordinates. Each offspring is produced with a mate selected randomly from those within distance  $3\sigma$ , with probability of choosing a neighbor

*at distance  $x$  proportional to  $\exp(-x^2/2\sigma^2)$ ." I think  $x$  may be overloaded here, or I'm confused. Clarify?*

**Reply:** We have clarified the notation for the second instance (referring to the distance among individuals) to  $d$ . (p. 4, l. 157)

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**(4.9)** *When introducing the "spatial model" as opposed to this "random model," the more concrete illustration in Figure 1 hasn't yet been referenced, which makes it harder to follow. It would be helpful to introduce this figure with the model. Additionally, when Figure 1 is introduced, the order is from right to left (random, then point, then midpoint). It would be helpful to rearrange the figure to mirror what's in the text.*

**Reply:** We have rearranged the figure as suggested.

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**(4.10) (p. 7, l. 294)** *Not sure I follow this example: "Concretely, an individual at position  $(x, y)$  in a  $50 \times 50$  landscape has mean phenotype  $100 + 2x/5$ ."*

**Reply:** We have clarified by switching to "the phenotype  $p$  for an individual at location  $(x,y)$  is then  $p = 100 + 2x/5$ ". (p. 7, l. 294)

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**(4.11) (p. 7, l. 320)** *Minor typo (through vs though): This occurs because, even through the "population density" (K) and "mean lifetime" (L) parameters...*

**Reply:** Thanks, this sentence has been revised and fixed.

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**(4.12)** *Define NS abbreviation in Figure 6.*

**Reply:** Done.