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Abstract:	In his 1931 monograph, Sewall Wright predicted genetic drift would overwhelm selection in very small populations, and selection would dominate in large ones, but also concluded drift could facilitate selection in populations of intermediate size. The idea that drift and selection would act together in smaller populations has not been evaluated using analytical or numerical approaches even as empirical evidence of rapid evolution associated with population bottlenecks has continued to accumulate. I used forward-time simulations with random mating and discrete generations to test the hypothesis that drift can facilitate selection in small populations. I find evidence of drift facilitation of selection as increases in levels of Δq in small populations ($N<100$) when selection is weak ($s<0.2$) and when allele frequencies are low ($q<0.5$). Fixation of beneficial mutations is accelerated by drift facilitation in small populations for recessive and codominant alleles, and less so for dominant alleles. Drift facilitation accelerated fixation of beneficial mutations in small populations compared to predictions from diffusion equations, while fixation time was longer than predicted in large populations. Drift facilitation increases the probability of fixation of new mutations in small populations. Accumulation of beneficial mutations (fixation flux) over several thousand generations was high in small populations and declined rapidly for large populations, which accumulated large amounts of standing genetic variation. Even though selection is more efficient in large populations, the increased time for allele replacement and lack of drift facilitation can result in substantially slower rates of adaptive evolution. Small populations were more susceptible to the accumulation of drift load, while larger populations maintained higher levels of segregation load. These results indicate that drift facilitation in small populations promotes purging of genetic load and accelerated fixation of beneficial mutations, and may account for the large number of observations of rapid adaptation during population bottlenecks.

Testing Wright's Intermediate Population Size Hypothesis – When Genetic Drift is a Good Thing

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Abstract – In his 1931 monograph, Sewall Wright predicted genetic drift would overwhelm selection in very small populations, and selection would dominate in large ones, but also concluded drift could facilitate selection in populations of intermediate size. The idea that drift and selection would act together in smaller populations has not been evaluated using analytical or numerical approaches even as empirical evidence of rapid evolution associated with population bottlenecks has continued to accumulate. I used forward-time simulations with random mating and discrete generations to test the hypothesis that drift can facilitate selection in small populations. I find evidence of drift facilitation of selection as increases in levels of Δq in small populations (N<100) when selection is weak ($s<0.2$) and when allele frequencies are low ($q<0.5$). Fixation of beneficial mutations is accelerated by drift facilitation in small populations for recessive and codominant alleles, and less so for dominant alleles. Drift facilitation accelerated fixation of beneficial mutations in small populations compared to predictions from diffusion equations, while fixation time was longer than predicted in large populations. Drift facilitation increases the probability of fixation of new mutations in small populations. Accumulation of beneficial mutations (fixation flux) over several thousand generations was high in small populations and declined rapidly for large populations, which accumulated large amounts of standing genetic variation. Even though selection is more efficient in large populations, the increased time for allele replacement and lack of drift facilitation can result in substantially slower rates of adaptive evolution. Small populations were more susceptible to the accumulation of drift load, while larger populations maintained higher levels of segregation load. These results indicate that drift facilitation in small populations promotes purging of genetic load and accelerated fixation of beneficial mutations, and may account for the large number of observations of rapid adaptation during population bottlenecks.

Impact Summary – After the recognition of Gregor Mendel's contributions to our understanding of the inheritance of genetically-determined traits around 1900, there was confusion as to whether the type of variation Mendel studied could account for evolution by natural selection, as described by Charles Darwin. This controversy was resolved when three theoreticians (Ronald Fisher, Sewell Wright, and J.B.S. Haldane) published books that integrated Mendelian genetics with evolution. Their contributions (referred to as the Modern Synthesis), focused on evolutionary processes occurring within and among populations of a species, and established a mathematical foundation for our understanding of evolutionary biology. The mathematical models developed by the three architects of the modern synthesis, and those who followed, predicted that the effects of natural selection would be overwhelmed by random genetic changes (referred to as Genetic Drift) in small populations, and that genetic drift would be minimal, while selection would be most effective in large populations. Even though one of Wright's major conclusions was that genetic drift and selection would work together (Drift Facilitation) to promote adaptive evolution in intermediate-sized populations, this idea has been almost completely ignored since it was first introduced in 1931. In this study, I use simulations of evolution in natural populations to evaluate the potential for drift facilitation to promote evolution in

small populations. My work largely confirms Wright's predictions; the removal of deleterious mutations and promotion of adaptive evolution are enhanced in population sizes ranging from about 10 to 100. These results indicate that our paradigm for our understanding of evolution within populations needs refinement to emphasize the importance of drift facilitation in small populations, and to recognize that periods of reduced population size are opportunities for enhanced levels of adaptive evolution.

Keywords: Adaptative evolution, conservation genetics, drift facilitation, drift load, inbreeding load, population bottleneck, purging, segregation load, simulations.

"In a population of intermediate size … there is continual random shifting of gene frequencies … which leads to a relatively rapid, continuing, irreversible, and largely fortuitous, but not degenerative series of changes, even under static conditions." Wright 1931, Page 157.

Introduction

The mathematical models for population genetics developed during the Modern Synthesis (Fisher 1930; Wright 1931; Haldane 1932) and beyond (Kimura 1983; Crow 1987; Ewens 2004; Charlesworth and Charlesworth 2017; Charlesworth 2020) have provided a foundation for a general understanding of evolutionary processes accounting for divergence among populations, adaptation, and diversification among lineages. A fundamental aspect of this framework is the dichotomy between the roles of genetic drift and selection; specifically, that drift governs the accumulation of neutral and degenerative variation while only selection can promote the purging of deleterious mutations and the fixation of beneficial mutations (Crow and Kimura 1970; Kimura 1983). The general prediction is that genetic drift will dominate the dynamics of allele frequency change in small populations when selection is weak (i.e. when $s < 1/4N_e$; Charlesworth 2009), resulting in the accumulation of genetic load and limited potential for adaptive evolution. In contrast, selection is expected to overwhelm the effects of drift in large populations leading to fixation of beneficial mutations and the purging of genetic load (Charlesworth 2002; Gossmann et al. 2012; Lanfear et al. 2014; Charlesworth and Charlesworth 2017). However, in his 1931 monograph, Wright emphasized the idea that the rate of fixation of beneficial mutations would be much slower in large populations and that drift could overwhelm selection in very small populations, but there was potential for genetic drift and selection to act together to promote rapid adaptation in populations of "intermediate" size. This idea was intrinsic to his Shifting Balance Theory of adaptive evolution where he predicted that adaptive variants were more likely to become fixed in small populations, and subsequently spread by gene flow (Wright 1932). These predictions were premised on the idea that there was a range of population sizes where both drift and selection are effective enough to allow "fortuitous" shifts in allele frequencies to facilitate selection by generating larger numbers of high-fitness genotypes resulting in accelerated accumulation of adaptive genetic variation. This idea (hereafter referred to as "drift facilitation") was seized upon by George Gaylord Simpson (1944), who proposed small population size was a likely explanation for the lack of fossilization during rapid transitions that resulted in the origin of novel forms, including new genera and families. This idea of "quantum evolution" (i.e. periods of rapid evolution when selection is facilitated by genetic drift; Simpson 1944) inspired a number of evolutionary biologists, who subsequently identified a wide range of examples of adaptation associated with reduced population size (Mayr 1954; Lewis and Raven 1958). Verne Grant (1963, 1981) expanded on the idea of quantum evolution to include rapid speciation events in small, isolated populations at the periphery of a widespread species' range (budding or peripatric

speciation; Mayr 1954). While interest in the idea of quantum evolution has waned, evidence of rapid evolution associated with population bottlenecks continues to accumulate (e.g., Carvalho et al. 1996; Rosenblum et al. 2007; Tepolt et al. 2009; Marsico et al. 2011; Cruzan 2019; Rego et al. 2019; Chaturvedi et al. 2021; Cruzan et al. 2021; Mahrt et al. 2021; Yin et al. 2021; van der Zee et al. 2022; Appendix 3). Surprisingly, there have been no attempts to assess the potential for drift facilitation of selection in populations of small or intermediate size using analytical or numerical approaches.

In this study, I use forward-time, individual-based simulations of populations to evaluate the potential for drift facilitation to promote evolution in intermediate-sized (small) populations. I evaluate the effects of selection (s), dominance (h), and population size (N) on changes in allele frequencies between generations ($\Delta q = q_{n+1} - q_n$) and its consequences for the loss or accumulation of deleterious mutations, and the time to fixation and probability of fixation for beneficial mutations. I specifically focus on short time-frames to account for the numerous observations of rapid evolution within a few hundred generations during population bottlenecks. In all cases, loci are bi-allelic and segregate independently, generations are discrete, populations are dioecious with equal sex ratios, and mating is random (i.e. $N = N_e$). I first characterize the effects of population size, selection, and dominance on Δq for beneficial alleles going to fixation, and then examine the consequences of selection and population size for the accumulation of genetic load and beneficial mutations. For genetic load, I examine the proportion of loci that became fixed for deleterious mutations (drift load), and the proportion of deleterious mutations that remain segregating after a number of generations (segregation load). In separate simulations, I examine the probability of fixation for beneficial mutations, the time to fixation, and fixation flux (the rate at which beneficial alleles accumulate; Otto and Whitlock 1997). My results demonstrate the effects of drift facilitation as elevated levels of Δq in small populations ($N \leq 100$) when allele frequencies are low and selection is weak. I find that drift facilitation of selection promotes rapid purging of genetic load and the accumulation of beneficial mutations in small populations while large populations accumulate greater levels inbreeding load and standing genetic variation for beneficial mutations.

Methods

Forward-time, individual-based simulations were conducted using the SimuPOP module (Peng and Kimmel 2005) in Python and the COEUS High-Performance Computing Cluster at Portland State University. A set of N individuals, each homozygous for a single locus, were generated using the sim.Population simulator and mutations introduced ($a \rightarrow a'$) that were affected by varying levels of selection (s) and dominance (h). Transmission of alleles between generations was governed by the sim. MendelianGenoTransmitter. Selection was imposed at the time of mating by defining the fitness of each diploid genotype using MapSelector in SimuPOP (all Python scripts are available in Appendix 1). Depending on the goals of the simulation, mutations were either introduced once in the first generation, or at a per-generation rate determined by μ. Each population size of N individuals was replicated k times. For each replication, the proportion of individuals with fixed mutations (q_{fixed}), and the average allele frequency across individuals ($\overline{q}$) were extracted after g generations. Two sets of simulations were run; the first set examined levels of drift and segregation load, and the second set focused on beneficial mutations by evaluating the proportion of loci fixed for mutations, the rate of fixation of individual mutations, and the fixation flux over g generations.

Testing for Drift Facilitation of Selection in Small Populations – I tested for the effects of drift facilitation of selection by comparing Δq ($\Delta q = q_{n+1} - q_n$) in small ($N = 50$) and large ($N = 1000$) populations across generations as beneficial mutations increased from their initial frequency of $q = 1/2N$ to fixation. Mutations were repeatedly generated and their allele frequencies tracked until they were fixed or lost (Python script available in Appendix 1.A). For mutations that fixed, Δq for each of the n generations was calculated along with its mean and standard deviation across generations. Mean Δq for mutations that fixed was compared for a range of selection coefficients ($s = 0.01, 0.04, 0.12,$ and 0.20) across population sizes ranging from $N = 4$ to $N = 3000$ for recessive, additive, and dominant mutations ($h = 0.0, 0.5,$ and 1.0). I evaluated the effects of the effects of the strength of selection ($s = 0.01$ to 0.20) in a small ($N = 100$) and large ($N = 1000$) population on the mean and standard deviation of Δq ($k = 10{,}000$ replications) for beneficial mutations going to fixation (Appendix 1B).

Consequences of Drift Facilitation for Genetic Load – The frequency of fixed deleterious mutations (drift load) across loci and of the proportion of loci segregating for deleterious mutations after g generations (segregation load) was assessed for population sizes ranging from N = 2 to 5000 (Python script available in Appendix 1C). For each of $k = 1000$ replications for each population size, s was randomly generated from a Poisson distribution (range: 0.0 – 0.25; $\bar{s}$ = 0.07), and h was generated from a uniform distribution between 0.0 and 0.2 (assuming that deleterious mutations are generally recessive with $\bar{h}$ = 0.10; Willis 1999; García-Dorado and Caballero 2000; Agrawal and Whitlock 2011; Ruzicka et al. 2021). Fitness of the *aa, aa',* and *a'a'* genotypes were set to 1.0, *1-hs*, and *1-s*, respectively. The initial allele frequency of *a'* was set at $q = 0$ and mutations were allowed to accumulate for $g = 1000$ generations with $\mu = 1x10^{-3}$ This assumes that each locus consists of thousands of base pairs (i.e. if the per bp/generation mutation rate is on the order of $1x10^{-9}$), but the same qualitative results are obtained for lower mutation rates over longer timeframes.

The proportion of loci fixed for deleterious mutations (q_{fixed}) and selection (s) for each mutation were used to estimate the fitness effects of fixed deleterious mutations (drift load) for each replication of each population size as the reduction in fitness due to the selection coefficient weighted by the proportion of loci fixed for the mutation;

$$w_{fixed} = 1 - sq_{fixed}$$

Segregation load was assessed as the inbreeding effects on fitness for segregating mutations (inbreeding depression; Charlesworth and Willis 2009; Willi et al. 2013) for 1000 replicates for each population size after 1000 generations. Inbreeding depression was estimated based on the mean fitness of hypothetical progeny that would be the result of self-fertilization compared to progeny from random outcrossing. Fitness after selfing was estimated as,

$$\overline{w}_s = 2\overline{p}\overline{q}(1 - (0.25 + 0.5hs + 0.25s)^F);$$

where $\overline{p}$ and $\overline{q}$ are the mean frequencies of the normal (a) and mutant (a') alleles across segregating loci (individuals), and F is the number of segregating sites at the end of 1000 generations. I calculated the expected fitness of progeny from random outcrossing within each population as,

$$\overline{w}_o = 1 - \left(\overline{p}^2 + hs2\overline{p}\overline{q} + s\overline{q}^2\right)^F$$

Segregation load was then estimated for each *N* as inbreeding depression for selfed progeny compared to outcrossed progeny (Charlesworth and Willis 2009) as,

$$\delta_{s/o} = 1 - \frac{\overline{w}_s}{\overline{w}_o}$$

These estimates of drift load and segregation load assume independent fitness effects across unlinked loci.

Consequences of Drift Facilitation for Adaptation – I examined the processes of adaptation in populations using simulations to evaluate the probability of fixation of beneficial mutations, the rate of fixation of individual alleles, and the fixation flux for a range of population sizes (*N*), selection (*s*), and dominance (*h*; Appendix 1D). For beneficial mutations (*a* → *a'*), the fitness of the *aa, aa',* and *a'a'* genotypes were set to *1-s*, *1-hs*, and *1.0*, respectively.

The first set of simulations compared the fixation of beneficial alleles across a range of initial allele frequencies from *q = 0.01* to *0.50* when *Ns = 2* for population sizes of *N = 100* (*s = 0.02*) and *N = 1000* (*s = 0.002*). Initial frequency of the *a'* allele was set at *q = 1/2N* ($\mu = 0$) and the proportion of loci fixing for the mutant allele was compared to the diffusion prediction for the probability of fixation (Φ_q) derived by Kimura (1957; 1962). Alleles were codominant in simulations (*h = 0.5*) to match the assumptions of the diffusion approximation;

$$\Phi_q = \frac{1 - \exp(-2Nsq)}{1 - \exp(-2Ns)}$$

A second set of simulations examined the number of generations required for fixation over population sizes ranging from *N = 4* to *3000* with *s* randomly drawn from a Poisson distribution (*s = 0.0* to *0.25*) and *h* drawn from a uniform distribution (*h = 0.0* to *1.0*). Initial frequency of the *a'* allele was set at *q = 1/2N* ($\mu = 0$) and the proportion of loci fixing for the mutant allele was recorded after 10,000 generations. I compared the number of generations required for fixation for beneficial alleles ($\bar{t}_{gens}$ across *k = 10* replicates per population size) to the predicted time to fixation ($\bar{t}$) from the diffusion approximation (Ewens 1979; Otto and Whitlock 2013);

$$\bar{t} \approx \int_{1/2N}^{0.999} \frac{(e^{4Nsx} - 1)(e^{4Ns(1-x)} - 1)}{sx(1-x)(e^{4Ns} - 1)} dx$$

This integral is undefined when the upper limit is 1.0, and the approach to fixation is asymptotic, so an arbitrary upper limit of 0.999 was chosen for comparison to fixation frequencies from the simulations.

While this approach describes fixation times for individual alleles, it does not account for the contribution of the number of mutations entering populations each generation based on population size for the accumulation of beneficial mutations over time (fixation flux; Otto and Whitlock 1997). We

expect flux to be affected by the probability of fixation for individual mutations and the number of mutations entering the population each generation. I evaluated the probability of fixation across a range of population sizes (*N = 4* to *3000*) by introducing random mutations (*s = 0.0* to *0.2* following a Poisson distribution, and *h = 0.0* to *1.0* following a flat distribution) to 1000 loci in the first generation and tracking the proportion that fix after 10,000 generations (100 replications per population size).

We expect that more mutations would occur in larger populations and consequently flux should increase with population size. On the other hand, fixation will take longer in large populations because of the time required for replacement of the original allele in all individuals. Since the goal of this study is to understand conditions leading to rapid adaptation to a novel environment, simulations were limited to a few thousand generations without a prior burn-in period to allow for the accumulation of standing genetic variation. I examined fixation flux for population sizes ranging from *N = 4* to *3000* with $\mu = 1x10^{-4}$, *Ns = 2* (*s = 2/N*), and *h = 0.5* with 1000 loci and 10 replicates per population size for time periods of *g = 1000, 2000,* and *4000* generations (Appendix 1E). Fixation flux under selection was compared to fixation with only genetic drift (*s = 0*) over 1000 generations across the same range of population sizes. In addition, I estimated the mean and standard deviation of *Δq* across generations for beneficial mutations that ultimately fixed in populations across a range of populations sizes (*N = 4* to *3000*) and selection coefficients (*s = 0.01* to *0.20* for *N = 100* and *1000*).

Results

Drift Facilitation of Selection in Small Populations – Drift facilitation was evident as elevated levels of *Δq* when population size was small (*N = 50*) compared to large populations (*N = 1000*; Fig. 1). The effects of drift facilitation were strongest when selection was weak (*s < 0.2*), mutations were recessive (*h = 0.0*), and allele frequencies were low, but there were strong effects of drift facilitation for codominant mutations (Fig. 1), and weaker effects when mutations were completely dominant (Fig. S1). There was a general trend towards the strongest effects of drift facilitation in small populations with weaker selection and lower dominance. Mean *Δq* increased with the strength of selection (Fig. S2) and remained consistently higher in small (*N = 100*) compared to large (*N = 1000*) populations. The standard deviation of *Δq* was consistently higher for small populations and remained fairly constant across a range from *s = 0.01* to *0.20* (Fig. S3).

Consequences for Genetic Load – Fixation frequencies of deleterious mutations were highest for small populations (< 100), and was effectively zero for population sizes larger than *N* = 3000 (Fig. 2A). Mutations fixing in populations when *N > 100* had negligible fitness effects so that drift load was near zero when population size was greater than *N = 100* (Fig. 2B). Larger proportions of loci continued to segregate for deleterious mutations after 1000 generations in populations of less than *N* = 10 and more than *N* = 100, while segregation load was lower when *N > 10* and *N < 100*; Fig. 3).

Consequences for Adaptive Evolution – There was a close fit between the diffusion approximation for the probability of fixation across initial allele frequencies (Φ_q) and the proportion of loci fixed for beneficial mutations with population sizes of 100 and 1000 with *Ns = 2* and codominance (*h = 0.5*; Fig. S4).

The time to fixation of individual alleles ($\bar{t}_{gens}$) was similar to the diffusion approximation ($\bar{t}$) when selection was stronger (*s = 0.1* and *0.2*) and population size was less than *N = 100*, but was slower than

predicted when $N > 100$. Time to fixation was substantially faster when $s = 0.04$ and 0.02 when population sizes were greater than $N = 10$ and less than $N = 100$ (Fig. 4). The probability of fixation for beneficial mutations was highest for small populations ($N < 100$), and remained lower for large populations (Fig. 5). Fixation of random beneficial mutations was positively associated with the strength of selection ($t = 18.79$; $P < 0.0001$) and negatively associated with dominance ($t = -8.87$; $P < 0.0001$) and population size ($t = -2.71$; $P = 0.0072$). Fixation flux for beneficial mutations with $Ns = 2$ was greater after 4000 generations compared to 2000, and 1000 generations (Fig. 6A). Fixation flux and the loss of beneficial mutations was high when $N < 100$, but generally declined with increasing population size ($h = 0.5$; Figs. 6A, B) due to the limited time-frame (i.e. < 4000 generations) and the number of generations required for allele replacement in large populations. The number of mutations that remained segregating in large populations was not strongly affected by the length of the time for the accumulation of mutations, and was consistently low when $N < 100$, and increased rapidly when $N > 100$ (Fig. 6C).

Discussion

Forward-time simulations of natural populations indicate that drift facilitation of selection elevates Δq in small populations ($N \leq 100$) resulting in lower segregation load, accelerated times to fixation, and higher probability of fixation for beneficial mutations. Drift load was relatively high for small populations, but was negligible for populations larger than $N = 100$, while segregation load was lowest for population sizes between 10 and 100. Results of simulations for beneficial mutations were consistent with diffusion approximations of the probability of fixation when $h = 0.5$ (Kimura 1957; Kimura 1962). Observed times to fixation matched the diffusion approximation ($\bar{t}$) fairly well when selection was strong ($s \geq 0.10$ and $h = 0.5$), but fixation times were substantially faster when $s < 0.10$ for population sizes between $N = 10$ and 100, and slower for population sizes of $N > 100$. Drift facilitation in smaller populations led to higher probabilities of fixation and high levels of fixation flux of beneficial mutations across a broad range of small population sizes while flux rapidly declined for populations larger then $N = 100$ after 1000 generations, and $N = 300$ after 4000 generations, which was apparently due to the extended time to fixation in larger populations. While we expect that more beneficial mutations will occur in large populations, the lack of genetic drift reduces the chances of fixation and increases the time to fixation compared to small populations. These results suggest the that drift facilitation provides some explanation for observations of rapid adaptation during periods of reduced population size by increasing purging of deleterious alleles and promoting the acquisition of adaptive genetic variation.

The results presented here generally support Wright's (1931) predictions about the potential for interactions between drift and selection to promote rapid fixation of beneficial alleles, but the population sizes where drift facilitation of selection is effective are smaller than he predicted. Wright specifically defined intermediate populations as ranging between $Ns = 2.5$ and 20 (page 148). Simpson (1944) also assumed that the dynamics described by Wright would apply to very large population sizes (i.e. up to $N = 25{,}000$) compared to the range of population sizes where drift facilitation was found to be effective in the simulations presented here. In contrast to the predictions of Wright and Simpson, higher rates and probabilities of fixation of beneficial mutations were observed in simulations for $N < 100$. The results presented here indicate that we cannot assume there is a linear tradeoff between the effects of population size and the strength of selection when population sizes small (i.e. for $N < 100$). This is an important result because Ns has been used to represent the compensatory effects between genetic drift

and selection in analytical models including diffusion equation analyses of population genetic processes (Kimura 1957; Kimura 1962; Ewens 2004). Given the results presented above, more careful evaluation of the separate effects of selection and population size may be warranted when small populations are being considered.

While population genetic models have mostly ignored the potential for selection in small populations, empirical evidence for the effectiveness of selection during population bottlenecks continues to accumulate. For example, a search of Web of Science using the keywords "rapid and (evolution or adaptation) and bottleneck" yielded 153 publications since 1992 on a wide variety of organisms after removing reviews, editorials, non-biological studies, and studies focused on cancer (details in Appendix 3). A sample of the reported cases of rapid adaptation to novel conditions during establishment and bottlenecks includes artificial introductions of Trinidadian guppies (Carvalho et al. 1996; van der Zee et al. 2022), Eastern fence lizards on novel substrates in New Mexico (Rosenblum et al. 2007), European green crabs on the west coast of North America (Tepolt et al. 2009), the cactus-feeding moth, *Cactoblastis cactorum*, in Florida (Marsico et al. 2011), steelhead trout in Lake Michigan (Willoughby et al. 2018), the invasive grass, *Brachypodium sylvaticum*, in Oregon (Cruzan 2019; Marchini et al. 2019), during evolutionary rescue in the seed beetle *Callosobruchus maculatus* (Rego et al. 2019), in founding populations of *Daphnia* (Chaturvedi et al. 2021), and after migration into Lake Champlain by the Atlantic sea lamprey (Yin et al. 2021). In one case, there was rapid evolution of antibiotic resistance in *Pseudomonas aeruginosa* even when population bottlenecks were severe and selection was weak (Mahrt et al. 2021). Rapid acquisition of adaptive genetic variants also seems to have occurred in a case of recent budding speciation (peripatric speciation; Mayr 1954) in a range-restricted species of buttercup (Cruzan et al. 2021). In all of these cases, adaptation to novel conditions appears to have occurred quickly during periods of reduced population size and suggests drift facilitation of selection may be a widespread phenomenon.

One of the more surprising results of simulations was the observation of higher rates of fixation flux of beneficial mutations when $N < 300$, suggesting drift facilitation can enhance rates of adaptation in small populations. This result is in consistent with diffusion analyses, which predicts flux estimated as $4NsU$ (where U is the genome-wide mutation rate) will remain constant across population sizes if Ns is held constant (Otto and Whitlock 1997; Ewens 2004), but the decline in flux when $N > 300$ is due to the slow accumulation of standing genetic variation. The simulations conducted here assumed transitions to novel conditions or colonization events so adaptation from novel mutations would only be for those occurring after the transition. The rate of flux may have been equally high for large populations if substantial "burn-in" periods (> 10,000 generations) had been implemented to allow the accumulation of standing genetic variation. However, the rate of adaptation from standing genetic variation in large populations will depend on initial allele frequency, and even for alleles that are at mid-frequencies (e.g., Lai et al. 2019), the time to fixation would be substantially longer than for small populations.

The observations of elevated Δq, reduced time to fixation, and increased probability of fixation of beneficial mutations in small populations are consistent with some previous analyses that predicted genetic drift and selection could interact to increase rates of divergence among populations (Cohan 1984; Lynch 1986), and with selection experiments in maize and simulations where enhanced rates of fixation of small effect mutations were observed when subjected to high genetic drift (Desbiez-Piat et al.

2021). It's notable that predictions from diffusion analyses are often evaluated only for a range of population sizes that are much larger than what was considered here (e.g., for N > 1000; Charlesworth 2020), but many species have historically experienced severe population bottlenecks. While diffusion analyses have led to numerous insights for evolution in large populations, alternative approaches may be required when population sizes less than $N = 300$ are considered.

Previous studies that have found evidence of drift facilitation did not identify its causes (e.g., Cohan 1984; Lynch 1986), but the evaluation of Δq across allele frequencies and population sizes sheds some light on the mechanisms responsible for enhancement of reduced times to fixation in small populations. The mean and standard deviation of Δq increased in small populations when selection was weak ($s < 0.2$) and mostly for allele frequencies of $q < 0.5$. For mutations at low frequencies, it appears that "fortuitous" fluctuations due to drift generate larger numbers of high-fitness genotypes (i.e. homozygotes when $h = 0$, and heterozygotes as well when $h = 0.5$). Consequently, the effects of drift facilitation are greatest for recessive and codominant mutations as drift facilitation elevates Δq and generates a "ratcheting effect" that accelerates changes in allele frequencies. This idea is supported by the observation that the mean of Δq is positively associated with selection, and is consistently higher in small compared to large populations. The effects of drift facilitation are apparently different when allele frequencies are close to $q = 0$ (for deleterious mutations) or fixation (i.e. $q = 1$ for beneficial mutations). Previous analyses indicate that diffusion equations do not perform well when allele frequencies are close to fixation when alleles under selection are completely recessive or dominant (Charlesworth 2020). Simulations performed here indicate that higher levels of drift in smaller populations increase the probability for random fluctuations to result in absorption, while minimal drift in larger populations results in persistent segregation. Consequently, larger populations appear to accumulate higher levels of segregation load and standing variation for beneficial mutations, while having lower drift load lower probabilities of fixation for beneficial mutations compared to smaller populations (i.e. for $N < 100$).

The observation that large populations maintain higher levels of segregation load and standing variation for beneficial mutations suggests that episodes of reduced population size may be disproportionally important for rapid evolution in novel or changing environments. While it remains true that more beneficial mutations are likely to occur in large populations, the number of generations required for fixation may be too great to keep pace with current rates of climate change. On the other hand, large populations possess higher levels of standing genetic variation so bottlenecks would have enhanced potential for the fixation of beneficial mutations (e.g., Chaturvedi et al. 2021). Moreover, reductions in population size due to bottlenecks and founder events are likely to be associated with increased environmental stress, and consequently, we expect selection to often increase during population bottlenecks (Fowler and Whitlock 2002; Matute 2013). Higher frequencies of segregating mutations along with drift facilitation may be responsible for elevated levels of purging following population bottlenecks (Bouzat 2010), and may help explain the substantial number of cases of rapid adaption during episodes of reduced population size (e.g., Carvalho et al. 1996; Rosenblum et al. 2007; Tepolt et al. 2009; Marsico et al. 2011; Cruzan 2019; Rego et al. 2019; Cruzan et al. 2021; Mahrt et al. 2021; Yin et al. 2021; van der Zee et al. 2022; Appendix 3). On the other hand, we expect population bottlenecks and founder events to result in elevated accumulation of drift load (Lynch et al. 1995; Willi et al. 2013), but the simulations presented here indicate the potential for purging of segregation load and the fixation of deleterious mutations depends on the severity of the reduction in population size. It is notable that the

standard guideline of a minimal effective population size of *N = 50* (i.e. the 50/500 rule; Jamieson and Allendorf 2012) for conservation efforts is too small to prevent the accumulation of drift load, but is in the right range for drift facilitation of beneficial mutations. Further analyses may help resolve more specific effects of changes in population size for adaptation to novel conditions and for the purging of deleterious mutations.

Conclusions

The results of individual-based, forward-time simulations are consistent with a wide range of observations from natural populations, but contrast with many of the predictions from models based on diffusion equations (e.g., Ewens 2004) when population sizes are small. In general, it appears that Wright's (1931) intuitive predictions about the potential for genetic drift to facilitate selection were correct, but the population sizes where drift facilitation is most effective are smaller than he suggested. Furthermore, while Wright saw the potential for weak deleterious mutations to fix in small populations, he did not anticipate that drift facilitation could lead to more effective purging of segregation load. One of the most striking results from these simulations is that the probability of fixation and flux of beneficial mutations are substantially high in small populations ($N < 100$) and is similar to flux in larger populations under the assumption that Ns remains constant. The assumption that selection is stronger in small populations may generally hold true; especially under conditions where population declines are precipitated by increased levels of environmental stress. Moreover, the time to fixation for beneficial mutations was lower in small populations when selection was weak ($s < 0.2$), so the prediction of rapid evolution due to drift facilitation is not dependent on the assumption of increased selection in small populations.

The potential for rapid evolution in small populations may be further enhanced as large populations experience reductions in population size. While it is generally assumed population bottlenecks will result in decreased fitness due to the accumulation of genetic load, and that there will be limited potential for adaptive evolution, this is not always true (Bouzat 2010). The simulation results presented here indicate drift facilitation of selection in small populations leads to effective removal of segregating deleterious mutations, and increases the rate and probability of fixation for beneficial mutations. While there has been some speculation and evidence for enhanced evolution in small populations (Simpson 1944; Grant 1981; Cohan 1984; Lynch 1986), no previous study has provided a detailed characterization of the potential for drift facilitation to promote rapid evolution.

Results from these simulations emphasize the importance of the adaptive evolution potential in small populations. Furthermore, more attention should be paid to the consequences of deleterious mutations as they depend heavily on population size; a clear distinction needs to be made between the fixation of deleterious mutations contributing to drift load in small populations, and the persistence of deleterious mutations in large populations resulting in inbreeding depression due to segregation load. The results of these simulations highlight genetic drift as a generative, beneficial process that promotes evolution in small populations. Alternatively, the lack of drift in large populations effectively stalls purging of segregation load and the fixation of beneficial mutations. While further analyses of drift facilitation are needed, the results presented here give more weight to Wright's (1931, 1932) suggestion that adaptive evolution is more likely to occur in small populations (Wade and Goodnight 1998), then to Fisher's (1930) argument that large populations are more important for fixation of beneficial mutations (Coyne

et al. 1997). Further work on evolution in small populations from modeling and empirical perspectives will clarify the potential for drift facilitation to promote evolution, and ultimately determine whether the current paradigm for our understanding of population genetic processes of evolution needs to be refined.

Acknowledgements

I thank Michael Wade and Michael Whitlock for suggestions on the development of simulations and relevant parameters to focus on. Karla de Lima Berg, Elizabeth Hendrickson, Jaime Schwoch, and Ariana Walczyk provided suggestions to improve the manuscript and presentation of results. Thanks to Bo Peng for assistance with coding in simuPop, and to Jim Stapleton and the PSU OIT for assistance with configuration of Coeus HPC for simulations in simuPop. This research was supported by NSF-DEB award 2051235.

Author Contributions

MBC is solely responsible for the development of the SimuPOP simulations in Python, data analyses, and writing of the manuscript.

Data Accessibility

All Python scripts are available in Appendix 1 and will be made available on Github.

Conflict of Interest

The author declares no conflicts of interest.

Literature Cited

Agrawal, A. F. and M. C. Whitlock. 2011. Inferences about the distribution of dominance drawn from yeast gene knockout data. Genetics 187:553-U274. (http://doi.org/10.1534/genetics.110.124560).

Bouzat, J. L. 2010. Conservation genetics of population bottlenecks: the role of chance, selection, and history. Conserv. Genet. 11:463-478. (http://doi.org/10.1007/s10592-010-0049-0).

Carvalho, G. R., P. W. Shaw, L. Hauser, B. H. Seghers, and A. E. Magurran. 1996. Artificial introductions, evolutionary change and population differentiation in Trinidadian guppies (Poecilia reticulata:Poeciliidae). Biol. J. Linn. Soc. 57:219-234. (http://doi.org/10.1111/j.1095-8312.1996.tb00310.x).

Charlesworth, B. 2002. Effective population size. Curr. Biol. 12:R716-R717. (http://doi.org/10.1016/S0960-9822(02)01244-7).

Charlesworth, B. 2009. Effective population size and patterns of molecular evolution and variation. Nature Reviews Genetics 10:195-205. (http://doi.org/10.1038/nrg2526).

Charlesworth, B. 2020. How long does it take to fix a favorable mutation, and why should we care? The American Naturalist 195:753-771. (http://doi.org/10.1086/708187).

Charlesworth, B. and D. Charlesworth. 2017. Population genetics from 1966 to 2016. Heredity 118:2-9. (http://doi.org/10.1038/hdy.2016.55).

Charlesworth, D. and J. H. Willis. 2009. The genetics of inbreeding depression. Nat. Rev. Genet. 10. (http://doi.org/10.1038/nrg2664).

Chaturvedi, A., J. Zhou, J. A. M. Raeymaekers, T. Czypionka, L. Orsini, C. E. Jackson, K. I. Spanier, J. R. Shaw, J. K. Colbourne, and L. De Meester. 2021. Extensive standing genetic variation from a small number of founders enables rapid adaptation in Daphnia. Nature Communications 12:4306. (http://doi.org/10.1038/s41467-021-24581-z).

Cohan, F. M. 1984. Can uniform selection retard random genetic dif=vergence between isolated conspecific populations? Evolution 38:495-504. (http://doi.org/10.2307/2408699).

Coyne, J. A., N. H. Barton, and M. Turelli. 1997. A critique of Sewall Wright's shifting balance theory of evolution. Evolution 51:643-671.

Crow, J. F. 1987. Population genetics history: A personal view. Annu. Rev. Genet. 21:1-22. (http://doi.org/10.1146/annurev.ge.21.120187.000245).

Crow, J. F. and M. Kimura. 1970. An Introduction to Population Genetics Theory. Harper & Row, New York.

Cruzan, M. B. 2019. How to make a weed - the saga of the slender fasle brome invasion in the North American west and lessons for the future. Bioscience 69:469-507. (http://doi.org/https://doi.org/10.1093/biosci/biz051).

Cruzan, M. B., P. G. Thompson, N. A. Diaz, E. C. Hendrickson, K. R. Gerloff, K. A. Kline, H. M. Machiorlete, and J. M. Persinger. 2021. Weak coupling among barrier loci and waves of neutral and adaptive introgression across an expanding hybrid zone. Evolution 75:3098-3114. (http://doi.org/https://doi.org/10.1111/evo.14381).

Desbiez-Piat, A., A. Le Rouzic, M. I. Tenaillon, and C. Dillmann. 2021. Interplay between extreme drift and selection intensities favors the fixation of beneficial mutations in selfing maize populations. Genetics 219. (http://doi.org/10.1093/genetics/iyab123).

Ewens, W. J. 1979. Mathematical Population Genetics. Springer-Verlag, Berlin.

Ewens, W. J. 2004. Mathmatical Population Genetics, Volume I: Theoretical Foundations. Springer-Verlag, Berlin.

Fisher, R. A. 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford.

Fowler, K. and M. C. Whitlock. 2002. Environmental stress, inbreeding, and the nature of phenotypic and genetic variance in Drosophila melanogaster. Proceedings. Biological sciences / The Royal Society 269:677-683. (http://doi.org/10.1098/rspb.2001.1931).

García-Dorado, A. and A. Caballero. 2000. On the average coefficient of dominance of deleterious spontaneous mutations. Genetics 155:1991-2001. (http://doi.org/10.1093/genetics/155.4.1991).

Gossmann, T. I., P. D. Keightley, and A. Eyre-Walker. 2012. The effect of variation in the effective population size on the rate of adaptive molecular evolution in eukaryotes. Genome Biol Evol 4:658-667. (http://doi.org/10.1093/gbe/evs027).

Grant, V. 1963. The Origin of Adaptations. Columbia University Press, New York.

Grant, V. 1981. Plant Speciation. Columbia University Press, New York.

Haldane, J. B. S. 1932. The Causes of Evolution. Princeton University Press, Princeton.

Jamieson, I. G. and F. W. Allendorf. 2012. How does the 50/500 rule apply to MVPs? Trends Ecol. Evol. 27:578-584. (http://doi.org/https://doi.org/10.1016/j.tree.2012.07.001).

Kimura, M. 1957. Some problems of stochastic processes in genetics. The Annals of Mathematical Statistics 28:882-901, 820.

Kimura, M. 1962. On the probability of fixation of mutant genes in a population. Genetics 47:713-719. (http://doi.org/10.1093/genetics/47.6.713).

Kimura, M. 1983. The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge.

Lai, Y.-T., C. K. L. Yeung, K. E. Omland, E.-L. Pang, Y. Hao, B.-Y. Liao, H.-F. Cao, B.-W. Zhang, C.-F. Yeh, C.-M. Hung, H.-Y. Hung, M.-Y. Yang, W. Liang, Y.-C. Hsu, C.-T. Yao, L. Dong, K. Lin, and S.-H. Li. 2019. Standing genetic variation as the predominant source for adaptation of a songbird. Proceedings of the National Academy of Sciences 116:2152-2157. (http://doi.org/doi:10.1073/pnas.1813597116).

Lanfear, R., H. Kokko, and A. Eyre-Walker. 2014. Population size and the rate of evolution. Trends Ecol. Evol. 29:33-41. (http://doi.org/http://dx.doi.org/10.1016/j.tree.2013.09.009).

Lewis, H. and P. H. Raven. 1958. Rapid evolution in *Clarkia*. Evolution 12:319-336. (http://doi.org/10.2307/2405854).

Lynch, M. 1986. Random drift, uniform selection, and the degree of population differentiation. Evolution 40:640-643. (http://doi.org/10.2307/2408585).

Lynch, M., J. Conery, and R. Burger. 1995. Mutational meltdowns in sexual populations. Evolution 49:1067-1080. (http://doi.org/10.2307/2410432).

Mahrt, N., A. Tietze, S. Kunzel, S. Franzenburg, C. Barbosa, G. Jansen, and H. Schulenburg. 2021. Bottleneck size and selection level reproducibly impact evolution of antibiotic resistance. Nature Ecology & Evolution 5. (http://doi.org/10.1038/s41559-021-01511-2).

Marchini, G. L., C. A. Maraist, and M. B. Cruzan. 2019. Functional trait divergence, not plasticity, determines the success of a newly invasive plant. Ann. Bot. 123:667-679. (http://doi.org/https://doi.org/10.1093/aob/mcy200).

Marsico, T. D., L. E. Wallace, G. N. Ervin, C. P. Brooks, J. E. McClure, and M. E. Welch. 2011. Geographic patterns of genetic diversity from the native range of Cactoblastis cactorum (Berg) support the documented history of invasion and multiple introductions for invasive populations. Biol. Invasions 13:857-868. (http://doi.org/10.1007/s10530-010-9874-9).

Matute, D. R. 2013. The role of founder effects on the evolution of reproductive isolation. J. Evol. Biol. 26:2299-2311. (http://doi.org/https://doi.org/10.1111/jeb.12246).

Mayr, E. 1954. Change of genetic environment and evolution *in* J. Huxley, ed. Evolution as a Process. Allen and Unwin, London.

Otto, S. P. and M. C. Whitlock. 1997. The probability of fixation in populations of changing size. Genetics 146:723-733. (http://doi.org/10.1093/genetics/146.2.723).

Otto, S. P. and M. C. Whitlock. 2013. Fixation Probabilities and Times. eLS.
Peng, B. and M. Kimmel. 2005. simuPOP: a forward-time population genetics simulation environment. Bioinformatics 21:3686-3687. (http://doi.org/10.1093/bioinformatics/bti584).
Rego, A., F. J. Messina, and Z. Gompert. 2019. Dynamics of genomic change during evolutionary rescue in the seed beetle Callosobruchus maculatus. Mol. Ecol. 28:2136-2154. (http://doi.org/10.1111/mec.15085).
Rosenblum, E. B., M. J. Hickerson, and C. Moritz. 2007. A multilocus perspective on colonization accompanied by selection and gene flow. Evolution 61:2971-2985. (http://doi.org/10.1111/j.1558-5646.2007.00251.x).
Ruzicka, F., T. Connallon, and M. Reuter. 2021. Sex differences in deleterious mutational effects in Drosophila melanogaster: combining quantitative and population genetic insights. Genetics 219. (http://doi.org/10.1093/genetics/iyab143).
Simpson, G. G. 1944. Tempo and Mode in Evolution. Columbia University Press, New York.
Tepolt, C. K., J. A. Darling, M. J. Bagley, J. B. Geller, M. J. Blum, and E. D. Grosholz. 2009. European green crabs (Carcinus maenas) in the northeastern Pacific: genetic evidence for high population connectivity and current-mediated expansion from a single introduced source population. Divers. Distrib. 15:997-1009. (http://doi.org/10.1111/j.1472-4642.2009.00605.x).
van der Zee, M. J., J. R. Whiting, J. R. Paris, R. D. Bassar, J. Travis, D. Weigel, D. N. Reznick, and B. A. Fraser. 2022. Rapid genomic convergent evolution in experimental populations of Trinidadian guppies (Poecilia reticulata). Evolution Letters 6:149-161. (http://doi.org/10.1002/evl3.272).
Wade, M. J. and C. J. Goodnight. 1998. The theories of Fisher and Wright in the context of metapopulations: When nature does many small experiments. Evolution 52:1537-1553.
Willi, Y., P. Griffin, and J. Van Buskirk. 2013. Drift load in populations of small size and low density. Heredity 110:296-302. (http://doi.org/10.1038/hdy.2012.86).
Willis, J. H. 1999. Inbreeding load, average dominance and the mutation rate for mildly deleterious alleles in *Mimulus guttatus*. Genetics 153:1885-1898.
Willoughby, J. R., A. M. Harder, J. A. Tennessen, K. T. Scribner, and M. R. Christie. 2018. Rapid genetic adaptation to a novel environment despite a genome-wide reduction in genetic diversity. Mol. Ecol. 27:4041-4051. (http://doi.org/https://doi.org/10.1111/mec.14726).
Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97 - 159.
Wright, S. 1932. The roles of mutation, inbreeding, crossbreeding and selection in evolution. Proceedings of the Sixth International Congress of Genetics 1:356-366.
Yin, X. S., A. S. Martinez, M. S. Sepulveda, and M. R. Christie. 2021. Rapid genetic adaptation to recently colonized environments is driven by genes underlying life history traits. BMC Genomics 22. (http://doi.org/10.1186/s12864-021-07553-x).

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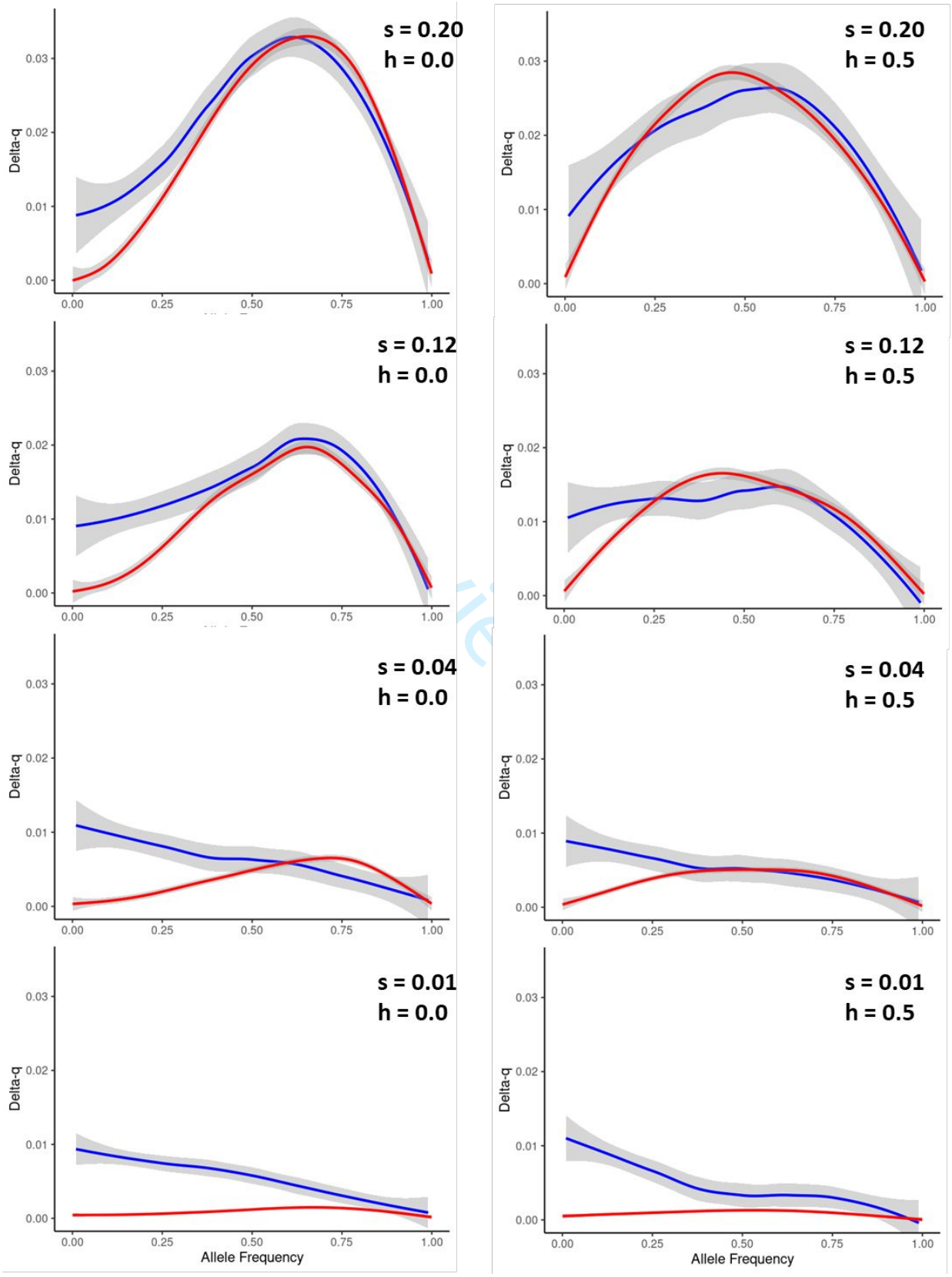
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Delta-q
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1.00

601

Fig. 1. Mean *Δq* across allele frequencies from *q = 1/2N* to *q = 1.0* for beneficial alleles in a small (*N = 50;* blue line) compared to a large (*N = 1000*) population for recessive (*h = 0.0*; left column) and codominant (*h = 0.5*; right column) mutations with selection ranging from *s = 0.01* to *s = 0.20.*

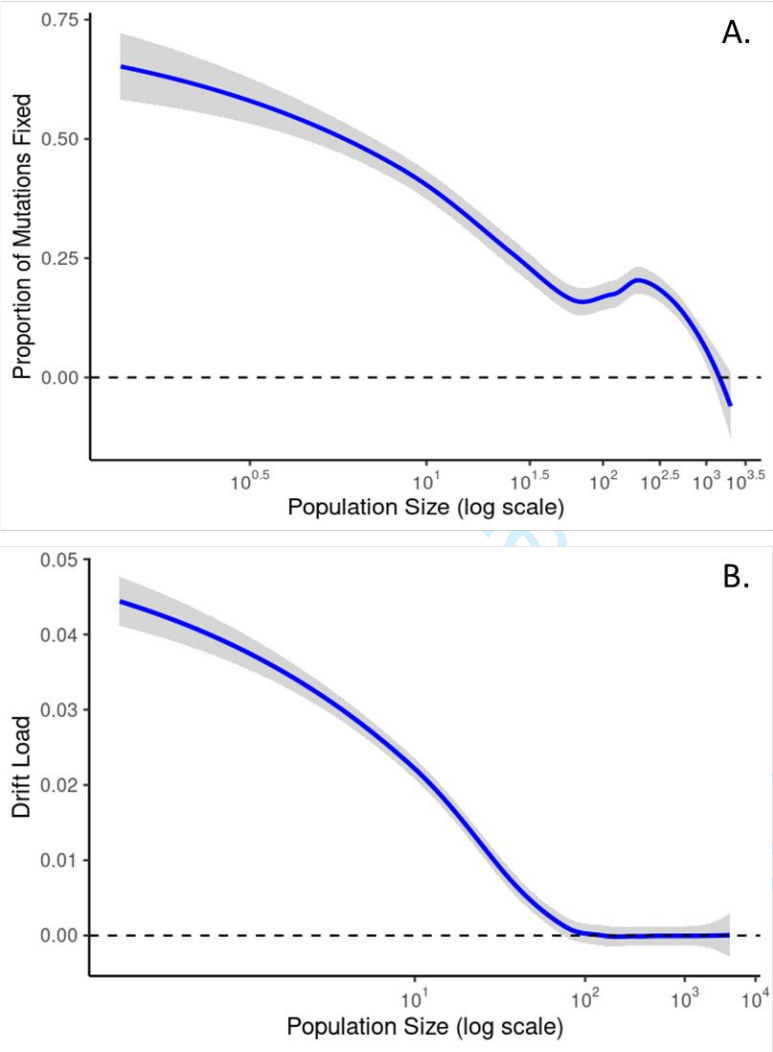

Fig. 2. The proportion of deleterious mutations fixing (A) and the levels of drift load (B) across population sizes of *N = 2* to 4000 after 1000 generations ($\mu = 1x10^{-3}$). Selection on mutations ranged from 0.0 to 0.25 and were drawn randomly from a Poisson distribution, and dominance ranged from 0.0 to 0.2 and were drawn randomly from a uniform distribution. The blue lines represent the loess-smoothed means from 1000 replications for each *N*, and gray shading indicate 95% confidence intervals.

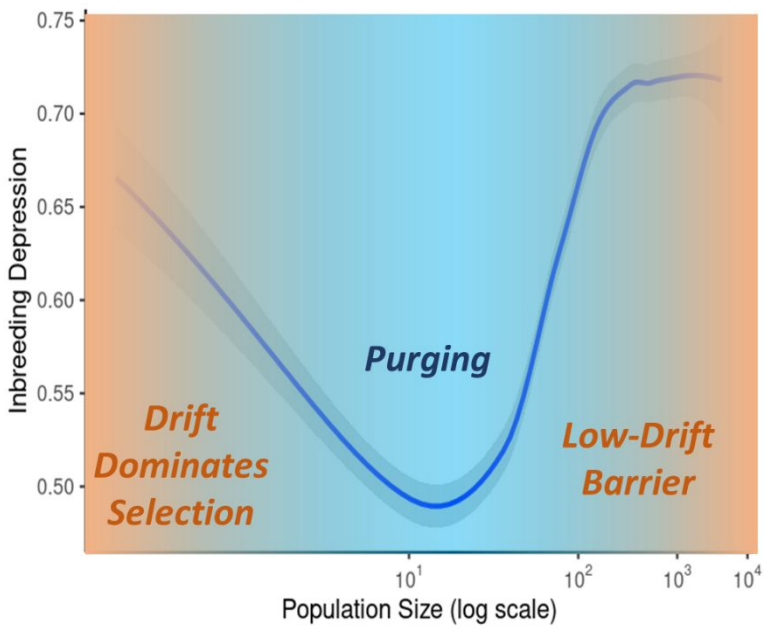

Fig. 3. Levels of inbreeding load due to loci segregating for deleterious mutations across population sizes of $N = 2$ to 4000 after 1000 generations ($\mu = 1x10^{-3}$). Selection on mutations ranged from 0.0 to 0.25 and were drawn randomly from a Poisson distribution, and dominance ranged from 0.0 to 0.2 and were drawn randomly from a uniform distribution. The blue line represents the loess-smoothed means from 1000 replications for each N, and gray shading indicate 95% confidence intervals.

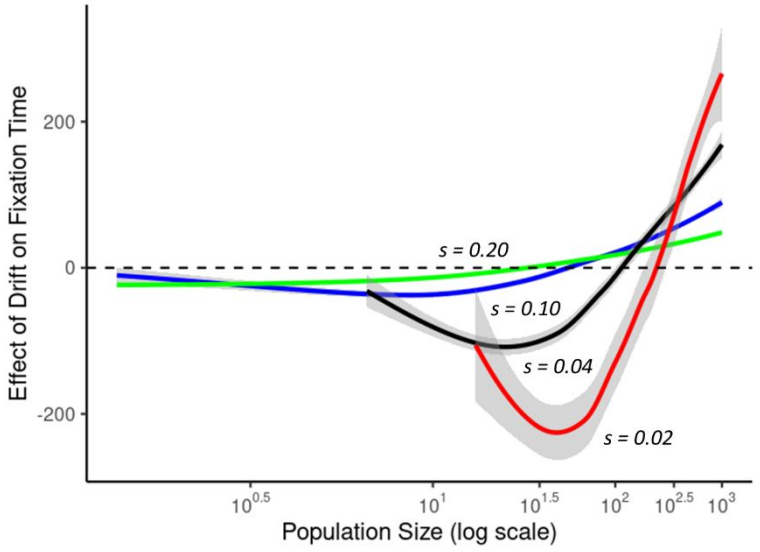

Fig. 4. Effects of population size on number of generations for fixation of beneficial alleles from simulations ($h = 0.5$) compared to the diffusion approximation averaged across 10 replicates per population size ($\bar{t} - \bar{t}_{gens}$) for $s = 0.02$ (red), 0.04 (black), 0.10 (blue), and 0.20 (green). The lines represent the loess-smoothed means across 100 replications for each N, and gray shading indicates 95% confidence intervals.

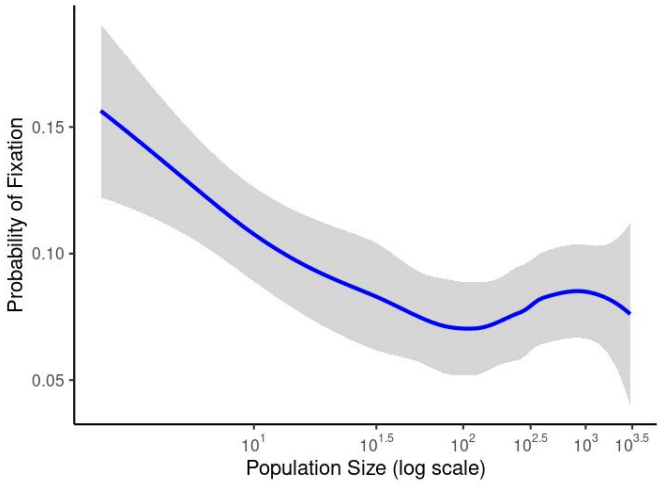

Fig. 5. The proportion of loci fixing for random beneficial mutations introduced in the first generation with *s = 0.0* to *0.2* (Poisson distribution) and *h = 0.0* to *1.0* (flat distribution) after 10,000 generations for population sizes ranging from *N = 4* to *3000*.

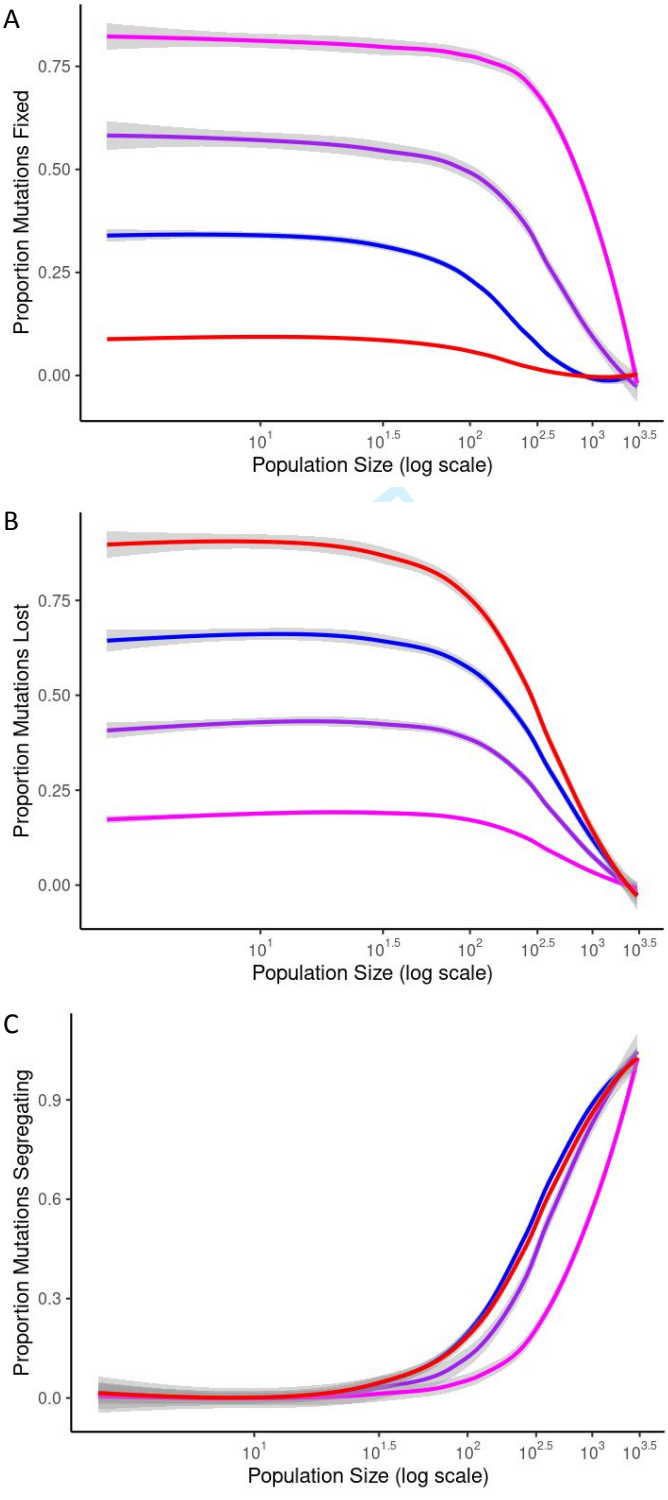

Fig. 6. Accumulation of beneficial mutations (A; fixation flux), mutations lost (B), and the proportion continuing to segregate (C) after 1000 (blue), 2000, (purple), and 4000 (magenta) generations with *Ns = 2*, and over 1000 generations with *s = 0* (red) with no prior burn-in. The lines represent the loess-smoothed means across 100 replications for each *N*, and gray shading indicates 95% confidence intervals.

Appendix 2. Supplemental Figures.

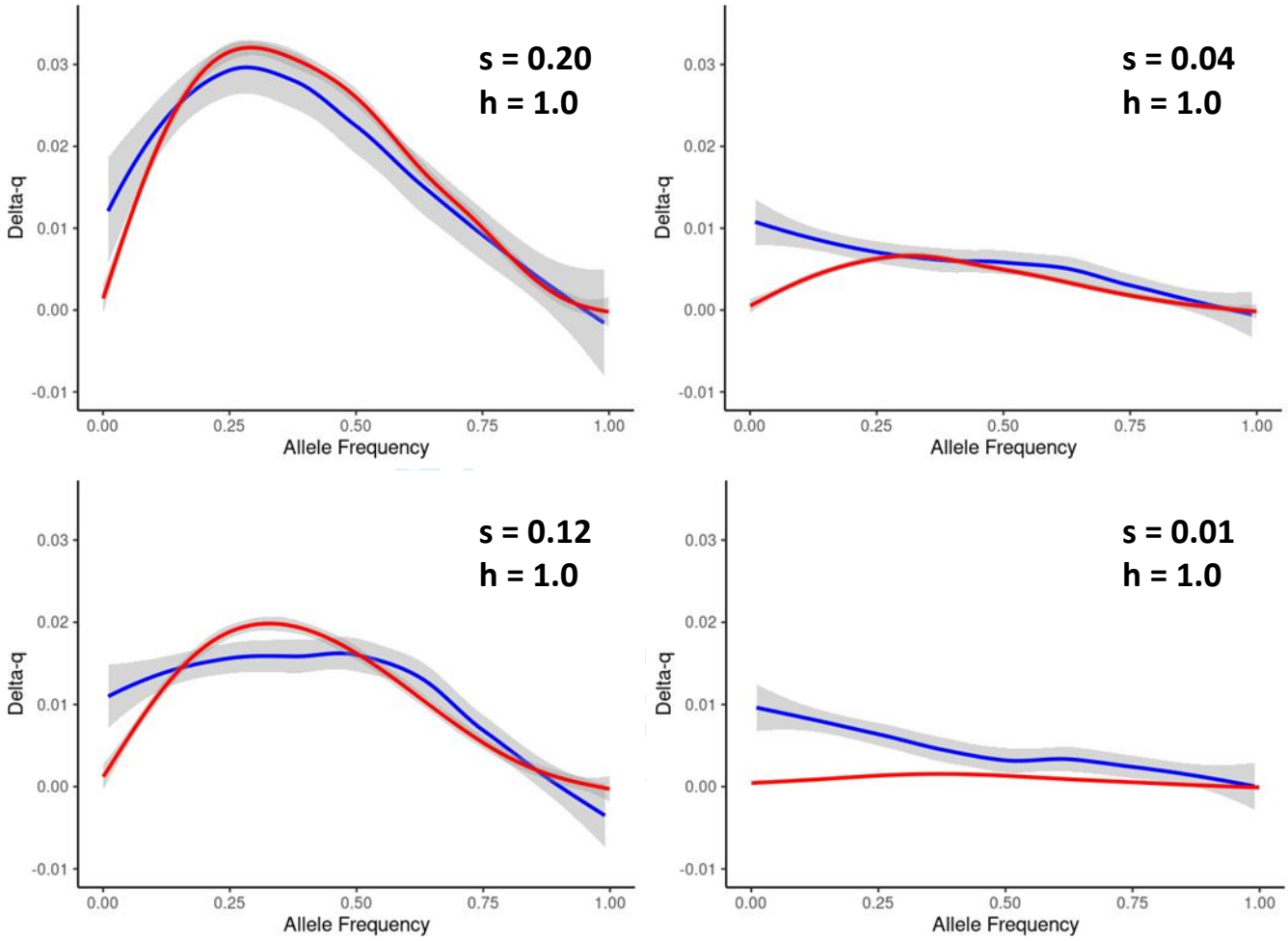

Fig. S1. Mean *Δq* across allele frequencies from *q = 1/2N* to *q = 1.0* for beneficial alleles in a small (*N = 50;* blue line) compared to a large (*N = 1000*; red line) population for dominant mutations (*h = 1.0*) with selection ranging from *s = 0.01* to *s = 0.20.*

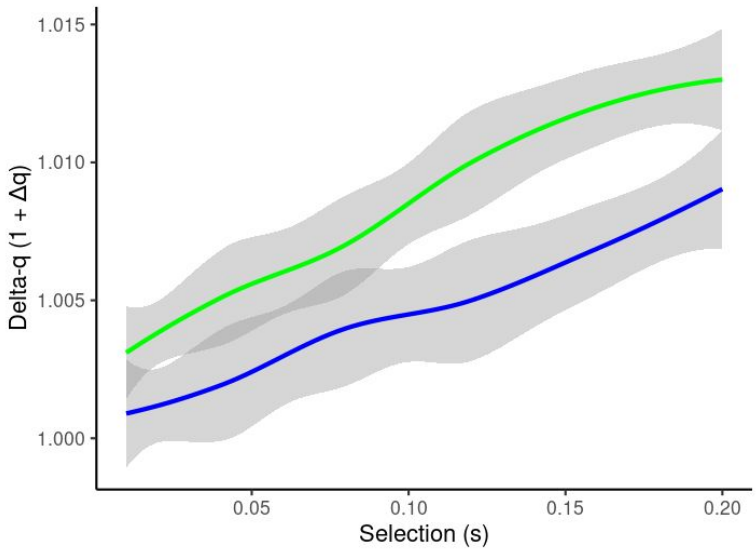

Fig. S2. Mean *Δq* for beneficial mutations going to fixation from an initial frequency of *1/2N* across selection coefficients from *s = 0.01* to 0.20 for *N = 100* (green line) and *N = 1000* (blue line). The line represents the loess-smoothed means across 100 replications for each *N*, and gray shading indicates the 95% confidence interval.

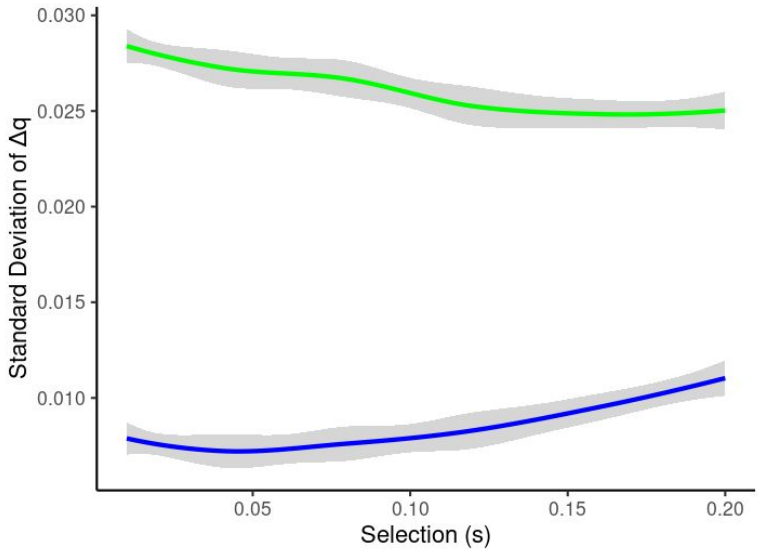

Fig. S3. Standard deviation of *Δq* for beneficial mutations going to fixation from an initial frequency of *1/2N* across selection coefficients from *s = 0.01* to 0.20 for *N = 100* (green line) and *N = 1000* (blue line). The line represents the loess-smoothed means across 100 replications for each *N*, and gray shading indicates the 95% confidence interval.

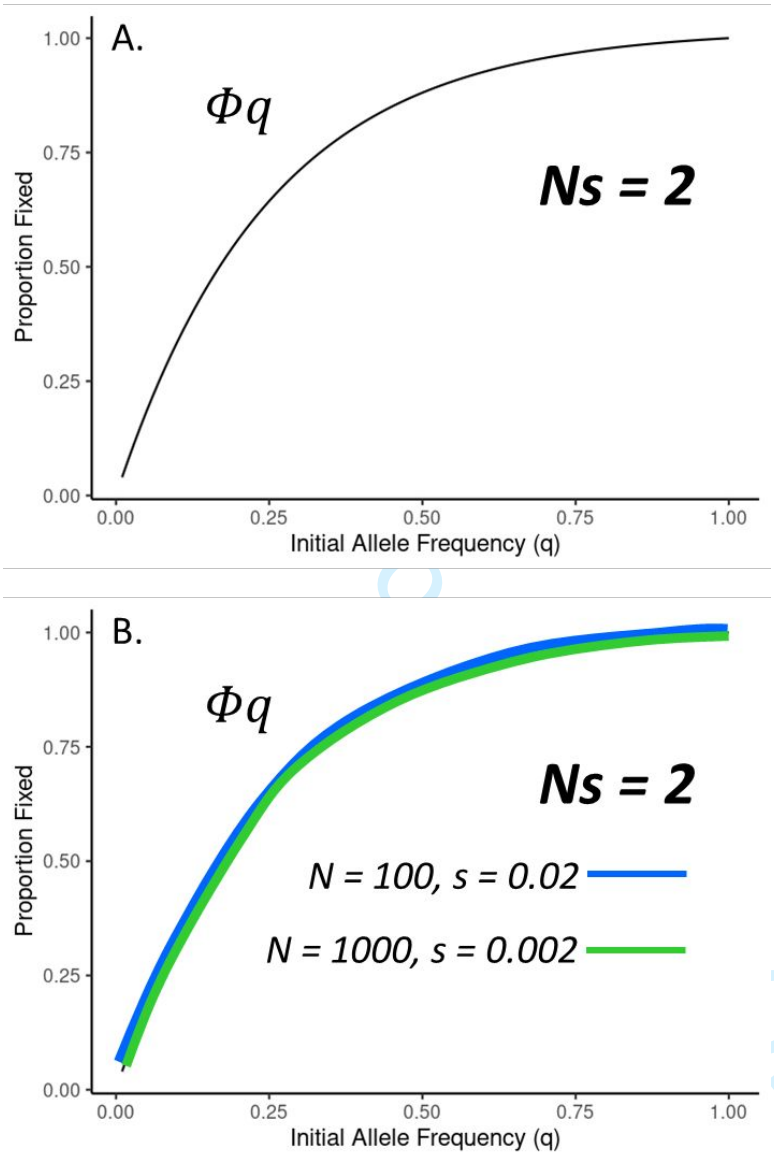

Fig. S4. The diffusion approximation for the probability of fixation (Φ_q) in response to initial allele frequency with *Ns = 2* (A), and the proportion of fixed alleles across 100 loci after 1000 generations for population sizes of *N = 100* and 1000 with *Ns = 2* (B; the relationship for Φ_q is obscured). Lines represent the loess-smoothed relationship of means for 20 replications per population size.

Dear Dr. Cruzan:

I'm writing to you regarding manuscript # EVL3-22-0119 entitled "Testing Wright's Intermediate Population Size Hypothesis – When Genetic Drift is a Good Thing", which you submitted to Evolution Letters. Your manuscript has been examined by an Associate Editor (AE) who then sent it to three external reviewers. As you will see, the reviewers have provided some very detailed and rigorous comments. The reviews are somewhat mixed - one reviewer is enthusiastic about the work, while the other two feel that it is not well suited to publication in Evolution Letters. Both of the less positive reviewers feel that the main findings are quite well established, and that analytical equations rather than simulations might have been the better approach. One of the reviewers feels that a considerable amount of relevant literature has been missed. The AE broadly shares these views. In view of the reservations voiced by the reviewers, which you will find at the bottom of this letter, we have decided not to accept your manuscript for publication in Evolution Letters. If you feel very strongly that the reviewers have got this badly wrong and that their comments could be addressed, we would consider a new submission, but I get the impression that these would be very difficult concerns to successfully rebut.

Thank you for considering Evolution Letters for the publication of your research. I hope the outcome of this specific submission will not discourage you from future submissions to our journal.

Sincerely,
Prof. Jon Slate
Editor in Chief, Evolution Letters
j.slate@sheffield.ac.uk

Associate Editor Comments to Author:

Associate Editor
Comments to the Author:
We have received three reviews of your manuscript. The reviewer assessments were very much split. One reviewer was very positive and suggested that this is an important study. The other two reviews were quite critical. The biggest point made by both are that the results presented here are known from existing analytical theory. I think the reviewers make a strong case for this. One of the critical reviewers also raised concerns about the validity of the more novel finding, that is the decrease in fixation flux at large N, arguing that this likely reflects an artefact of the simulations. While I appreciate the utility of simulations in theory and I think the paper is well written and that it addresses a topic of interest, I do not think the current version clearly demonstrates novel results. Thus, I don't think the paper is suitable for publication. I would however be willing to consider a revised manuscript that provides a strong rebuttal to the concerns of novelty and validity of the results.

- Thank you for these comments. It appears that the primary issues raised by the reviewers are a consequence of some misunderstandings of the goals of this manuscript. I apologize for not making this clear; in no way do I intend to challenge the substantial and detailed existing body of analytical theory. However, there is ample empirical evidence for rapid evolution during population bottlenecks that is not accounted for by current analytical approaches (see comments below). My intention is to evaluate the causes of this phenomenon using forward-time simulations to develop a better understanding of the underlying

processes causing this phenomenon of "drift facilitation of selection" in small populations. I clearly demonstrate the effects of drift facilitation as elevated levels of delta-q when selection is weak and allele frequencies are less than 0.5. I also show that this increase in delta-q reduces the time to fixation – this is a phenomenon that is not accounted by existing theory. This is an evolutionarily important phenomenon, and one that is particularly critical under our current circumstances of rapid climate change.

Reviewer(s)' Comments to Author:

Reviewer: 1
Copied from pdf file:

General comments
This paper attempts to establish that genetic drift facilitates selection in populations of intermediate size, primarily on the basis of extensive computer simulations. The author refers to remarks by Wright (1931), but never mentions the shifting balance theory, first sketched in Wright's 1932 paper, which Wright believed was the major important role of drift in evolution.

- Thank you for this comment. While Wright's shifting balance theory is not directly relevant to the current manuscript, I have added mention of it in the introduction.

This theory has, of course, been widely discussed in the literature. The features discussed by Cruzan were not the focus of Wright's attention.

- I find this statement confusing. In his 1931 monograph, Wright provides some lengthy discussions of the effects of selection and drift in intermediate population sizes. Indeed, in the section titled "Lability as a condition for evolution" (pages 147-151 including Figs. 18-21) he develops an argument that adaptive evolution is most likely in populations that are small enough to be subject to genetic drift and large enough so selection can be effective. He returns to the discussion of population size in the "Summary" section (page 155-158), and in the final paragraph of this section he concludes that rapid adaptive evolution is most effect in large population divided into smaller subpopulations subject to selection and drift. It is striking that the final section and the concluding paragraph of his monograph is devoted to the role of the interplay between selection and drift for adaptive evolution. The final paragraph in Wright's 1931 monograph establishes the scenario that is the focus of his 1932 paper, which describes the shifting balance process. As mentioned in the text, Wright's conclusions concerning the interplay between drift and selection to precipitate rapid evolution has been largely ignored, which is perhaps why this reviewer does not believe it was a focus of his attention.

The results that fixation probabilities and times to fixation of beneficial mutations (inappropriately referred to as "rates of fixation") increase with population size are not novel; they simply reflect the fact that variants inevitably take longer to spread to fixation in larger populations.

- I'm sorry that I did not make this clear. I discuss fixation time in the text associated with Fig. 6 and the rate of fixation (fixation flux) in Fig. 7. The confusion with Fig. 7 arises because reviewers assume the system has reached a state of equilibrium; it

has had enough time such that fixation flux is high even for very large populations. However, this assumption does not match biological conditions where populations are subject to novel selection regimes and potential adaptation over only short periods of time. Under these conditions, flux declines sharply in larger populations as illustrated in Fig. 7. It is striking that even after 4000 generations there is not enough time for large populations to reach equilibrium, so mutations occurring in a new environment would require extensive amounts of time before they become fixed. My simulations reflect realistic conditions of purging and adaptation in natural populations exposed to changing environments where a "burn-in" period to establish equilibrium conditions is irrelevant.

He makes no use of the ratios of these quantities to their values under neutrality, which are standard in the molecular evolution literature.

- I agree that the molecular evolution literature focuses on the ratio of non-synonymous to synonymous mutations (dN/dS), but this statistic is only useful for evolution processes occurring over many thousands of generations. The simulations described here are focused on much shorter time frames that are relevant to the many cases of rapid evolution that have been described in the literature.

Most people in the field would think that these are the best measures of the efficacy of selection; if so, then the standard wisdom that selection is more effective in large populations still holds. The major threat to this view would apparently come from Fig. 7, which seems to show that the rate of substitution of beneficial mutations tends to zero in large populations. As explained below, this is likely to be an artefact of the way the numbers are calculated.

- The decline in fixation flux for large populations in Fig. 7 is a consequence of the increased time to fixation in large populations and is not an artifact of the way flux is calculated. While the reviewer is focused on "threats" to the conclusion that selection is more effective in large populations, they ignore the results indicating that drift facilitation decreases the time to fixation and results in high rates of fixation flux in small population over time frames that are realistic for adaptive processes in natural populations.

A major problem with this paper is that the author seems to lack familiarity with the theoretical literature on mutation, selection and drift, in particular on work on mutational load in finite populations, which goes back to Kimura *et al.* (1963, Genetics 48:1303); for a recent study, see Charlesworth (2018, Mol Ecol 24:4991), who also studied inbreeding depression and heterosis.

- The Kimura et al. 1963 study examines genetic load across a broad range of effective population sizes. They define load based on the forward and reverse mutation rate, dominance, and selection. They conclude that genetic load will be higher in small populations, but they define load based on mutation rates and do not distinguish between drift and segregation load. My simulation results differ from their predictions by showing that purging is most effective in populations of intermediate size, that levels of drift load are high only for Ne < ~100 (Fig. 2), and that inbreeding load is higher for very small (< 10) and large populations (> 100), but

remains low for populations of intermediate size (Fig. 3). These observations are not addressed in either of the papers cited by the reviewer.

It is hard to see that Cruzan has added anything very useful to this topic, other than producing simulation results for very small populations, where diffusion approximations are likely to be somewhat inaccurate, although other studies have shown that they perform remarkably well, even in very small populations, e.g. Kimura & Ohta (1969, Genetics 61: 763).

- The difficulty with the current diffusion approximations are a consequence of the general use of the composite variable *Ns*, which assumes that there is a linear tradeoff between population size (the effects of genetic drift) and the strength of selection. The Kimura and Ohta 1969 paper examines time to fixation only in terms of Ns, and consequently does not make predictions based on population size alone. My simulations confirm that fixation time is much longer in large populations, but also include the unique result that under weak selection ($s < 0.1$), fixation time is accelerated for populations of intermediate size ($N > 10$ and < 300) and slower than the diffusion predictions for large populations (> 300; Fig 5). The shorter time to fixation in small populations with weak selection is due to drift facilitation as shown by elevated levels of delta-q. I have not found any analytical or numerical analysis that has documented the effects of drift facilitation.

It is, in fact, unnecessary to conduct simulations of very small populations, as these can be modelled exactly by the use of stochastic matrices (e.g. Ewens 2004, Mathematical Population Genetics.1. Theoretical Introduction; Springer, NY). With modern computers, results for population sizes of 500 or more can be generated with the matrix approach. There have been many recent applications of this method, e.g. Eyre-Walker & Keightley (2009 MBE 26:2097).

- I find this statement very confusing. The advantage of forward-time, individual-based simulations is that they are not subject to the constraints of assumptions from analytical approaches. The matrix approach is reasonable for evaluating the predictions of analytical analyses, but may not adequately reflect processes in natural populations. The cited paper evaluates the validity of measures of adaptive molecular evolution (alpha; based on MK and other tests) with deleterious mutations, varying levels of recombination, and changes in population size. They find little bias in estimates of alpha for constant population size, overestimation under population expansion, and underestimation under contraction. My results are unique because I find evidence that genetic drift facilitates adaptive evolution over short timeframes with weak selection when allele frequencies are low and for a wide range of dominance (clearly for $H <= 0.5$; Fig. 1, but some drift facilitation even when $h = 1$; Fig. S1). This result is due to consistently larger average delta-q values during fixation for smaller population sizes (Figs. 1 and S2) when selection is weak ($s < 0.2$). The only difference between small and large populations is an increased effect of genetic drift, which is direct evidence that genetic drift facilitates selection in smaller populations.

Of course, the inaccuracies introduced by the diffusion approximation are quantitative not qualitative, so the claim on l.289-299 that there something fundamentally wrong with the standard results cannot be correct.

- I agree that diffusion approximations are perfectly valid within the confines of their assumptions, but they may not always accurately reflect natural phenomena. In particular, there is a large body of empirical evidence for rapid evolution during population bottlenecks that is not predicted by current analytical theory. In this manuscript I make a start on evaluating this gap in our understanding of population genetic processes by conducting forward-time, individual-based simulations. It is confusing to me why this reviewer would completely disregard the results that I present in favor of diffusion approaches. It's my understanding that there should be an interplay between empirical evidence and theory, where each informs the other to improve our understanding of natural processes. Individual-based simulations provide an important bridge between these approaches as they adequately mimic natural conditions while allowing for the exploration of a much wider range of parameter space than would be possible using biological organisms.

Specific comments

The two diffusion equation results that he reproduces are for the special case of semidominance (h = 0.5); although not explicitly stated, it seems that his comparisons with diffusion equation predictions are based solely on the h=0.5 case, even when he is simulating models with different h values (e.g. Fig. 5). This is inappropriate, and presumably accounts for the apparent discrepancies with the simulations. Kimura and others have provided completely general expressions for arbitrary dominance coefficients for fixation and loss probabilities, as well as expected times to fixation or loss (see the relevant chapters of Ewens (2004). Analyses based on these formulas have been used in many publications since Kimura et al. (1963); for recent examples, see Vicoso & Charlesworth (2009, Evolution 63:2413) for fixation probabilities, Mafessoni & Lachmann (2015, Genetics 201:1581) and Charlesworth (2022, Genetics 221:iyac027) for times to fixation and loss.

- The reviewer is correct that this analysis assumes H=0.5. I have dropped this figure as it is not centrally relevant to the results and the effects of drift facilitation.

His Fig. 6 is puzzling, as the second of these other studies showed excellent agreement between simulations and diffusion predictions for a population size of 50, in contrast to Fig. 6. The formula on l.190 is the time conditioned on a fixation having occurred. I wonder if he has calculated the unconditional fixation time, which will be very different. He has also overlooked the very interesting effects of dominance on fixation and loss times described in these two papers.

- Fixation time in these simulations is defined as the number of generations required for an allele to increase frequency from 1/2N to 1.0.
- The simulations the reviewer is referring to apparently used probability functions to simulate allele frequency changes rather than following individuals in artificial populations as was done here. The difference in these approaches is critical because individual-based, forward-time simulations will mimic real populations more accurately.
- The effects of dominance on fixation or loss when the recessive allele is near 0.0 for deleterious alleles or near 1.0 for beneficial ones is to stall loss or fixation when populations are

large. My simulations demonstrate that drift facilitates purging of genetic load and fixation of adaptive alleles in small populations. These effects of drift facilitation are not characterized in the papers cited by the reviewer.

It is also hard to see what is new or very interesting about his claims concerning positive selection in small populations. I think most population geneticists understand that the fixation probability of a new mutation is larger in a small rather than a large population, regardless of the direction of selection on it. For the case of semi-dominance, shown in the equation on l.173 (for some reason, the equations are left unnumbered), a new mutation has a frequency 1/2N, so the term in the numerator reduces to $1 - \exp(-s)$. If $s > 0$, the numerator is positive, and the denominator is an increasing function of Ns, so that the fixation probability must decrease as N increases.

- Thank you for this. Please note that my simulations matched the predictions from this equation for h = 0.5.

For a completely recessive favorable mutation in a randomly mating population, the formula of Kimura (1957), extending old work by Haldane (1927), showed that the fixation probability is proportional to the square root of s/N, so that it becomes vanishing small as N increases, yielding the widely-discussed principle of "Haldane's sieve" against recessive beneficial mutations in large populations. These results simply reflect the fact that drift is more effective in causing fixations in small rather than large populations, for all types of mutation.

- Thank you for mentioning the work by Kimura. Unfortunately, Kimura points out that his equations do not provide adequate approximations of the probability of fixation when 2Ns is small (Kimura 1957; page 897), and his examples use only large populations (i.e. N=1000; page 898). Apparently, these equations do not perform well when population size is small (i.e. N < 100), which is the focus of my simulations.
- Actually, the estimate of sqrt(s/N) for the chance of fixation was obtained by Haldane, while Wright's estimate was sqrt(s/2N). Kimura's 1957 estimate falls between these two. All of these predict a negative relationship between N and the probability of fixation (a decelerating exponential curve), but they do not adequately account for the effects of drift. In contrast, my results for deleterious mutations show low purging at small and large populations (Fig. 3). Similarly, recessive beneficial mutations accumulate at the highest rate for populations of intermediate size (N > ~30 and N < 1000) after 1000 generations (Fig. 4).

However, this way of looking at the problem ignores the fact that the fixation probability of a beneficial mutation *relative* to that of a neutral mutation ($1/2N$) decreases with N, and vice-versa for a deleterious mutation, as repeatedly emphasized by Kimura. It therefore does not really correspond to "drift facilitating selection".

- I agree with the reviewer that selection is more effective in large populations because of the reduced effects of genetic drift. However, the time to fixation in large populations is excessively long so this prediction cannot account for the numerous observations of rapid evolution during population bottlenecks. My simulations provide some insights into the process of rapid

evolution due to the effects of drift facilitation on delta-q and reduced time to fixation in in small populations.

Similar considerations apply to the time to fixation of a beneficial mutation; explicit formulae for this quantity have been provided by Hermisson & Pennings (2005 Genetics 169:2335) and Charlesworth (2020, Am Nat 195:753) and others. Cruzan also discusses what Kimura and Ohta, following Haldane (1957), called the rate of substitution of mutations, and which has been widely studied in the molecular evolution literature, i.e., the product of the rate of total input of new mutations into the population ($2Nu$) and their fixation probability. He uses the term "fixation flux", which seems an unnecessary novelty. The rate of input of mutations is proportional to N, whereas the fixation probabilities of beneficial mutations decrease with N. For the semidominant case, if the equation on l.173 is multiplied by N, a simple differentiation of the product with respect to N shows that the "fixation flux" must always increase with N. This raises the question of why Cruzan's Fig.7A seems to show that the fixation flux decreases at the larger population sizes. I think this is an artefact of the way in which this has been calculated; the rate of substitution as applied in studies of molecular evolution uses the steady state rate at which mutations are fixed. His simulations run for a limited period of time; for large populations (where diffusion equations are known to be almost totally accurate, unless selection is very strong), the system will not have a approached the steady state. I thus don't think the results undermine a very solidly based theory.

- The term "fixation flux" has been used previously in the literature (Otto and Whitlock 1997).
- The goal of my simulations is to understand the process of rapid evolution during bottlenecks and transitions to novel environments. The text points out that the drop in flux in larger populations after 4000 generations or less is due to the lack of adequate standing genetic variation, but the point is understand processes contributing to rapid evolution in small populations. Even for mutations at intermediate frequencies in large populations will take much longer to fix than those in small populations due to allele replacement time and probably also due to the lack of drift in large populations.
- The reviewer's comments about flux increasing with N seems to ignore the fact that Ns was held constant in these simulations.

Reviewer: 2

Comments to the Author
In this paper, Cruzan uses and IBM to understand how drift and selection interact across different population sizes and selection intensities. I found this manuscript absolutely fascinating because it sheds some light on the examples of rapid adaptation that are becoming more commonplace in the literature. The model Cruzan designed was well described and appropriate for the phenomenon they set out to understand.

- Thank you for these comments.

I have no major criticisms but do provide two minor questions/comments:
1. In figure 6, why does the confidence interval shrink as s gets larger, irrespective of population size? I didn't understand if/how this was related to the ratcheting effect described around line 323.

- The new Fig. 1 shows the effects of drift facilitation on delta-q. So yes, the narrowing of confidence intervals is due to less genetic drift.

2. Should N in your models be interpreted as census or effective size? Throughout the manuscript I interpreted it as census but line 349 could indicate that effective size was correct. Clarification of line 349 or an explanation about effective size early in the model description would fix this issue.

- I have modified the text on line 103 to indicate that census and effective size are the same because mating is random.

Reviewer: 3

Comments to the Author
In the article "Testing Wright's Intermediate Population Size Hypothesis – When Genetic Drift is a Good Thing" the author conducted a series of simulations on the effect of drift in populations with varying population sizes. While in general I agree this is an interesting papers with a pressing question, I am unsure whether the displayed results are really novel. It appears to me that these are standard results, just nicely packaged.

- I have added new results for the effects of drift facilitation on delta-q (Fig. 1) that clearly show that the results presented are novel.

While the code is not available for review (no github or dryad address given), the simulation results look potentially correct. The only key problem I have is that fixation times, segregation times and loss times are all depended on N itself. That is not really mentioned in the MS. So for me the displayed results appear like they are standard popgen results, unless I missed something. Either I missed why this needs to be done by simulations rather than analytically. Or I missed the biological reason why it is reasonable to compare scenarios with different population sizes over the same number of generations. Unless there is a difference in generation time, one would look at two completely different time scales. For example if you look at Fig 2, where purging appears to lead to lowest inbreeding depression at size 10 - appears to me an effect of new mutations, not drift.

- All scripts are provided in Appendix 1 (sorry that this was not clear).
- Limited time frames were used to understand the processes leading to rapid evolution during population bottlenecks. The limited time frame of the simulations does have consequences for fixation probabilities in large populations, but it does not have consequences for the time to fixation (Fig. 5).

So from my point of view the reasoning behind the setup needs to properly explained, otherwise I feel uncomfortable recommending the paper for publication.

- I hope the revised manuscript has clear explanations.

Comments:
Shouldn't be on a log scale the orders be equally spaced?

- I'm not sure what this comment is referring to. The log scale for N is commonly used when evaluating a large range of population sizes.

Did you use a burn-in - if yes, how long?

- No burn-in used for the flux figure (Fig. 7).