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

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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

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**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 (1/2*N*) 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 (2*Nu*) 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).