



# Larger Numbers Can Impede Adaptation in Asexual Populations despite Entailing Greater Genetic Variation

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## Abstract

Periodic bottlenecks play a major role in shaping the adaptive dynamics of natural and laboratory populations of asexual microbes. Here we study how they affect the ‘Extent of Adaptation’ (*EoA*), in such populations. *EoA*, the average fitness gain relative to the ancestor, is the quantity of interest in a large number of microbial experimental-evolution studies which assume that for any given bottleneck size ( $N_0$ ) and number of generations between bottlenecks ( $g$ ), the harmonic mean size ( $HM = N_0g$ ) will predict the ensuing evolutionary dynamics. However, there are no theoretical or empirical validations for *HM* being a good predictor of *EoA*. Using experimental-evolution with *Escherichia coli* and individual-based simulations, we show that *HM* fails to predict *EoA* (i.e., higher  $N_0g$  does not lead to higher *EoA*). This is because although higher  $g$  allows populations to arrive at superior benefits by entailing increased variation, it also reduces the efficacy of selection, which lowers *EoA*. We show that *EoA* can be maximized in evolution experiments by either maximizing  $N_0$  and/or minimizing  $g$ . We also conjecture that  $N_0/g$  is a better predictor of *EoA* than  $N_0g$ . Our results call for a re-evaluation of the role of population size in predicting fitness trajectories. They also aid in predicting adaptation in asexual populations, which has important evolutionary, epidemiological and economic implications.

**Keywords** Population size · Experimental evolution · Extent of adaptation · Population bottlenecks · Adaptive size

## Introduction

Population size is a key demographic parameter that affects several ecological and evolutionary processes including the rate of adaptation (Desai et al. 2007; Desai and Fisher 2007; Gerrish and Lenski 1998; Lanfear et al. 2014; Samani and Bell 2010; Wilke 2004), efficacy of selection (Petit and Barbadilla 2009), organismal complexity (LaBar and Adami 2016), fitness decline (Katju et al. 2015), repeatability of evolution (Lachapelle et al. 2015; Szendro et al. 2013; Vogwill et al. 2016), etc. Interestingly though, what constitutes

a useful measure of population size for predicting evolutionary outcomes often depends on the ecological/evolutionary question being addressed and the population-genetics quantity in question (Charlesworth 2009). Consequently, it is crucial to use the relevant measure of population size while constructing or empirically validating any evolutionary theory.

Experimental evolution using asexual microbes has been one of the key tools in validating several tenets of evolutionary theory (Kassen 2014, reviewed in Kawecki et al. 2012). Most such studies deal with populations that face regular and periodic bottlenecks during their propagation (Kawecki et al. 2012). The absolute population size keeps changing regularly because of these periodic bottlenecks. Therefore, in order to make predictions and claims based on population size in such experiments, it is important to define a proper measure of population size depending upon the question of interest (Charlesworth 2009; Kawecki et al. 2012; Lanfear et al. 2014; Wang et al. 2016).

Previous theoretical studies have shown that the harmonic mean of population size over time acts as the measure of population size that can explain and predict the fixation

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probabilities of beneficial mutations in such systems (Patwa and Wahl 2008; Wahl and Gerrish 2001). Specifically, if a population grows from size  $N_0$  to  $N_f$  via binary fissions within a growth phase, and is diluted back periodically to  $N_0$  by random sampling at the end of the growth phase, then the relevant population-size measure for fixation probabilities is given by  $N_e \approx N_0 * \log_2(N_f/N_0) = N_0g$ , where  $g$  refers to the number of generations between successive bottlenecks and  $N_0g$  is the harmonic mean size (Lenski et al. 1991). From an evolutionary perspective, periodic bottlenecks play two opposite roles in such experiments. On the one hand, harsher bottlenecks (entailed by higher  $g$ ) reduce the probability that a given beneficial mutation would fix due to sampling errors during the bottleneck. On the other hand, higher values of  $g$  also imply an increase in  $N_f$ , which causes an increase in mutational opportunities (binary fissions) during exponential growth (Wahl et al. 2002). This is expected to increase the total supply of mutations that would survive drift, which in turn should increase the raw material available for evolution. It has been predicted that exponential growth between  $N_0$  and  $N_f$  influences fixation probabilities more than the elimination by sampling (Heffernan and Wahl 2002). More nuanced and complex measures of population size (Campos and Wahl 2009, 2010) also suggest that adaptation rates in terms of fixation probabilities would have a positive relationship with  $N_0$  and  $g$ , the two population size parameters amenable to experimental manipulation.

Unfortunately, most experimental evolution studies with serially bottlenecked asexual populations do not focus on the fixation probabilities of beneficial mutants. Instead, they are interested in the average amount of fitness gained with respect to the ancestor at a given time (we call this quantity the extent of adaptation, *EoA*) during the course of evolution (De Visser and Rozen 2005; Desai et al. 2007; Lachapelle et al. 2015; Lenski et al. 1991; Rozen et al. 2008; Samani and Bell 2010). Several experimental studies, dealing with quantities akin to *EoA* for periodically bottlenecked asexual populations, have used the harmonic mean ( $=N_0g$ ) for quantifying the evolutionarily relevant (i.e., predictive of the magnitude of evolutionary response) population size (De Visser and Rozen 2005; Desai et al. 2007; Lachapelle et al. 2015; Lenski et al. 1991; Rozen et al. 2008; Samani and Bell 2010). However, to the best of our knowledge, there is no theoretical basis or empirical justification (Raynes et al. 2014) for this usage of the harmonic mean. Here we use a combination of agent-based simulations and long-term evolutionary experiments using *Escherichia coli* to investigate the interplay of  $N_0$  and  $g$  in shaping the *EoA* of asexual populations. Since the harmonic mean has been widely used by experimentalists in the context of *EoA*-like quantities, we begin by testing the suitability of the harmonic mean as a predictor of *EoA*. We show that populations with similar values of  $N_0g$  can have

markedly different *EoA* trajectories, and this result applies to both real (bacterial) as well as simulated populations. Secondly, we demonstrate that although increasing the value of  $g$  (making the periodic bottleneck harsher) promotes adaptation through an increased supply of variation, it also reduces the efficacy of selection which impedes adaptation by restricting the spread of large-effect beneficial mutations. When these two opposing aspects of bottlenecks are considered together, counterintuitively, *EoA* turns out to have a negative relationship with  $g$ . Thirdly, we show that populations with similar harmonic mean ( $=N_0g$ ) can not only have different fitness trajectories but can also differ markedly in terms of how frequency-distribution of fitness amongst individuals changes during adaptation. Finally, we show that, for a given mutation rate,  $N_0/g$  can be a better predictor of *EoA* trajectories, i.e., populations with similar  $N_0/g$  have similar fitness trajectories and populations with higher  $N_0/g$  adapt faster. Our findings thus introduce a new way of thinking about the relationship between population size and adaptive trajectories.

Our approach differs from previous studies in two important ways. First, unlike many studies (Campos and Wahl 2009, 2010; Heffernan and Wahl 2002; Wahl and Gerrish 2001) we focus on how *EoA* (and not long-term fixation probabilities) is shaped by bottleneck size ( $N_0$ ) and bottleneck ratios ( $N_0/N_f$ ). This makes our study directly relevant to a rich body of microbial experimental evolution literature (De Visser and Rozen 2005; Desai et al. 2007; Lachapelle et al. 2015; Lenski et al. 1991; Rozen et al. 2008; Samani and Bell 2010, reviewed in Kawecki et al. 2012). Second, many previous theoretical studies on periodically bottlenecked systems (where  $N_f = N_02^g$ ), assume that the culture volume (and therefore  $N_f$ ) is a constant, and then go on to explore what value of  $N_0$  or  $g$  leads to the minimum loss of variation during bottlenecks and/or in the long run (Campos and Wahl 2009, 2010; Heffernan and Wahl 2002; Wahl et al. 2002; Wahl and Gerrish 2001; Wahl and Zhu 2015). In our simulations, we remove this restriction and seek to compare loss of variation in those cases where both  $N_0$  and  $N_f$  can be different (e.g. between a population grown in 50 ml of medium versus one grown in (say) 1 ml of medium). Clearly, it is possible to have two populations with very different  $N_0$  and  $N_f$  values that can nevertheless have similar values of  $N_0g$ . One of the questions that we investigate is whether such populations have similar fitness trajectories or not. Thus, our results make it possible to compare the expected *EoA* across experimental studies that employ similar environments but different culture volumes, which is a rather common scenario in experimental evolution studies (Lachapelle et al. 2015; Raynes et al. 2012, 2014; Rozen et al. 2008; Samani and Bell 2010).

## Methods

### Experimental Evolution, Assays and Statistical Analysis

Here we present a brief description of the experimental protocol, relegating the details to Supplementary Methods. Our primary aim was to investigate if a commonly used measure of population size in experimental evolution, namely harmonic mean ( $N_{og}$ ), could predict *EoA* trajectories. We also wanted to see if populations with similar values of  $N_f$  have similar *EoA*. To this end, we experimentally evolved three different population regimens (LL, SL, and SS) in Nutrient Broth containing a sub-lethal cocktail of three antibiotics (Norfloxacin, Rifampicin and Streptomycin) for ~380 generations in batch culture. The first letter in the name refers to the harmonic mean size and the second letter refers to  $N_f$ ; L means ‘large’ and S means ‘small’. Each regimen consisted of 8 independently evolving replicate populations, all of which were started from a single *E. coli* MG 1655 colony. The three population regimens were propagated at different bottleneck sizes: LL faced lenient bottlenecks ( $1/10$ ), whereas SS ( $1/10^4$ ) and SL ( $1/10^6$ ) experienced much harsher bottlenecks. LL and SL were grown at larger culture volumes (100 ml, culture in flasks) than SS (1.5 ml, culture in 24 well-plates). Thus, in terms of  $N_f$ ,  $LL = SL \gg SS$  but in terms of  $N_{og}$   $LL \gg SL = SS$  (see Table S1 for the values of these parameters). The *EoA* trajectories of the three population types were reconstructed by assaying the maximum population-wide growth rates ( $R$ ) and carrying capacities ( $K$ ) of each replicate at different time-points during evolution following standard protocols (Karve et al. 2015, 2016), the details of which can be found in Supplementary Methods.  $K$  of a population was defined as the maximum OD value attained over a period of 24 h (the highest value in the sigmoidal growth curve) (Karve et al. 2016; Novak et al. 2006).  $R$  was estimated as the maximum slope of the growth curve over a running window of four OD readings (each window spanning 1 h) (Karve et al. 2015, 2016; Vogwill et al. 2016).

To analyze the data, we performed separate repeated measures ANOVA for  $K$  and  $R$ . “Regimen-type” (LL/SL/SS) was treated as the categorical factor, and TIME (nine time-points) as the repeated measures factor. We also included an interaction of Regimen-type and TIME in our model to determine if the fitness trajectories of the regimes were significantly different from each other. Furthermore, to compare the fitness values at each time point, we used a nested-design ANOVA with “regimen-type” (SS, SL or LL, fixed factor) and “replicate-line” (1–8, random factor, nested in regimen-type). We used Holm–Šidák correction

(Abdi 2010) for controlling the family-wise error rates. For all ANOVAs where there was a significant effect of “Regimen-type” after the Holm–Šidák correction, we used Tukey’s HSD to compare pairwise differences between LL, SL, and SS (See Supplementary Methods for details and rationale).

### Simulations of Microbial Evolution

Any difference between the three regimens in our experiment can, in principle, be due to some idiosyncratic properties of the experimental organism (*E. coli*) or potential differences between the selection environments in flasks and plates. In order to account for that possibility and enhance the generalizability of our results, we used an individual based model to simulate bacterial growth under resource-limited conditions (Wahl et al. 2002). Except for differences in the amount of resources, our model contained no other parameters specific to *E. coli* or related to differences in culture conditions. Thus, in terms of differences between the *EoA* of the regimens, if the model output matched the empirical observations then our results were likely to be applicable for other asexual systems. Treating our experiment as a case-study, we used our model to investigate if our results were generalizable.

Our simulations start with a nearly clonal distribution of fitness effects. In our model, an individual bacterium was characterized by three principal parameters: efficiency, threshold, and body-mass. The simulation (coded in the C programming language) began with a fixed amount of resources available in the environment, utilized by the bacteria for growth. A typical individual was represented by an array that specified three principal parameters: (1) bodymass, (2) efficiency, and (3) threshold. Efficiency and threshold were the only two evolvable parameters. Bacteria consumed resources in an iterative and density-dependent manner. The parameter  $Bodymass_i$  of the  $i$ th individual represented how big that individual was during a given iteration. Its efficiency ( $K\_eff_i$ ) specified how much food it assimilated per iteration. If  $population\ size / K\_eff_i < 1$ ,  $10(1 - (population\ size / K\_eff_i))$  units were added to  $Bodymass_i$ . Otherwise,  $Bodymass_i$  remained unchanged.  $Bodymass_i$  increased with cumulative assimilation. When  $Bodymass_i$  becomes greater than or equal to  $thres_i$  (its threshold parameter), the individual  $i$  underwent binary fission and divided into two equally sized daughter individuals. Each fission event had a fixed probability of giving rise to mutations based on a mutation rate that remained constant for all individuals in the population.  $K\_eff_i$  and  $thres_i$  mutate independently, and were the only two parameters that could undergo mutation. The mutated value was drawn from a static normal distribution with the frequency of deleterious mutations being much higher than that of beneficial mutations, which is in

line with experimental observations (see Table S3; Kassen and Bataillon 2006; Eyre-Walker and Keightley 2007). The distribution of mutational effects remained fixed throughout the simulation (Kassen and Bataillon 2006) due to which, *EoA* was expected to eventually approach a plateau. When the population ran out of resources (once the amount of body-mass accumulated per unit time by the population went below a pre-decided threshold so that the sigmoidal curve reached a plateau), it was sampled according to the sampling ratio being studied. The above process was repeated for 400 generations, where each generation represented twofold growth in population size (see Supplementary Methods for a more detailed description of the model). We also checked if our model met several intuitive theoretical predictions that had not been coded directly (see Supplementary Data (SD2); also see Figs. S1, S2, and S3).

Density-dependent growth, clonal interference, the presence of deleterious mutations, the presence of variable fitness effects of mutations, etc. are some key features that are instrumental in shaping the adaptive dynamics of periodically bottlenecked asexual populations (Patwa and Wahl 2008; Sniegowski and Gerrish 2010). Unfortunately, the complex interactions of so many features are difficult to capture in analytical models (Sniegowski and Gerrish 2010). Consequently, previous theoretical studies have been forced to make simplifying assumptions like the absence of deleterious mutations (Desai and Fisher 2007; Wahl and Gerrish 2001), constancy of beneficial mutational effects (Desai and Fisher 2007), constancy of  $N_f$  (Campos and Wahl 2009, 2010; Wahl and Gerrish 2001; Wahl and Zhu 2015), the presence of discrete generations (Campos and Wahl 2009, 2010; Desai and Fisher 2007), etc. (see Table S2 for details). Our model avoids these simplifying assumptions, which might explain why some of the features captured by our model have not been reported earlier. Moreover, our

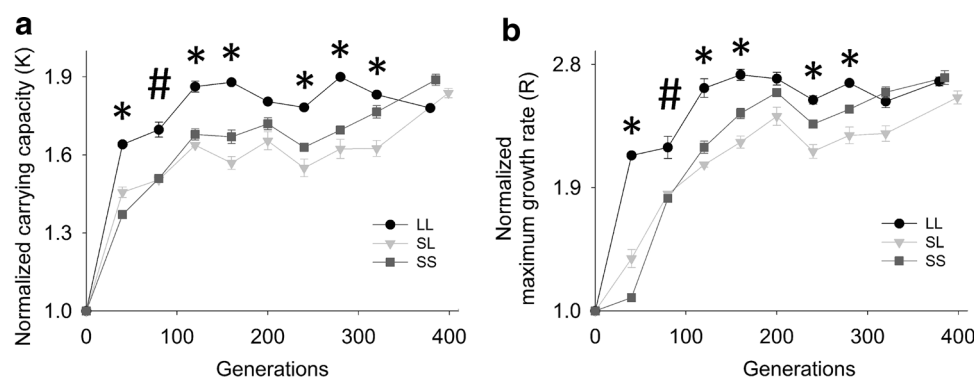
study is in the context of *EoA*, while most of the earlier studies have investigated fixation probabilities.

## Results

### Harmonic Mean Failed to Predict and Explain the *EoA* Trajectories of Experimental Populations

Repeated measures ANOVA on all three regimens indicated a significant Regimen-type  $\times$  TIME interaction for both  $K$  ( $F_{16, 168} = 5.72$ ;  $p < 0.000001$ ) and  $R$  ( $F_{16, 168} = 7.306$ ;  $p < 0.000001$ ). However, in principle, this interaction could be driven by the fact that the LL populations had a much larger increase in  $K$  and  $R$  compared to the SL and SS populations. Since our primary interest was to check whether the SL and SS populations differed in terms of these two fitness measures, we performed the repeated measures ANOVA for only these two regimens and again found a significant Regimen-type  $\times$  TIME interaction for both  $K$  ( $F_{8, 112} = 2.070$ ;  $p = 0.0446$ ) and  $R$  ( $F_{8, 112} = 3.594$ ;  $p = 0.000948$ ). Since the interaction term was significant, we chose not to interpret the main effects of regimen-type or TIME.

Individual ANOVAs showed that the *EoA* of SS was greater than that of SL at 5/6 and 4/5 time-points which had significant difference in terms of  $K$  (Fig. 1a) and  $R$  (Fig. 1b). The  $p$ -values and the  $F$ -values (with corresponding  $df$ ) for each time-point for  $K$  and  $R$  are presented in Tables S4 and S5 respectively. Thus, particularly during the last two-thirds of the evolution experiment, the *EoA* of SS was consistently higher than that of SL. The effect sizes [Cohen's  $d$  (Cohen 1988)] of *EoA* differences between SL and SS were found to be either medium or large (with the majority being large effects; see Table S6) for several points on the *EoA* trajectory. Thus, similar harmonic mean can give rise to fairly



**Fig. 1** Experimental *EoA* trajectories in terms of carrying capacity and maximum growth rate. **a** *EoA* of carrying capacity ( $K$ ). **b** *EoA* of maximum growth rate ( $R$ ). Data points show mean  $\pm$  SEM for eight replicates. Asterisk refers to cases when all three pairwise differences (LL–SL, LL–SS, and SL–SS) are significant (Tukey post hoc

$p < 0.05$ ). # refers to significant difference across LL–SL and LL–SS, but not SL–SS (see Tables S4 and S5). SS and SL have markedly different adaptive trajectories despite having similar harmonic mean population sizes



different adaptive trajectories. This observation is consistent with recent empirical findings that question the validity of harmonic mean as an evolutionarily relevant population size (Raynes et al. 2014). Surprisingly, SS had a larger overall *EoA* than SL despite having lower  $N_f$ . Interestingly, despite having similar  $N_f$ , LL typically had much larger extent of adaptation than SL, which is explainable by the fact that the latter regimen suffered more severe bottlenecks. This shows that similar  $N_f$  does not lead to similar extents of adaptation if the bottleneck ratios are different.

In summary, the harmonic mean failed to predict the adaptive trajectories of our experimental populations as, in spite of having similar values of  $N_0g$ , the SL and SS regimens had markedly different adaptive trajectories for  $K$  (Fig. 1a) as well as  $R$  (Fig. 1b).

### Simulations Also Revealed That the Harmonic Mean Fails to Predict Adaptive Trajectories

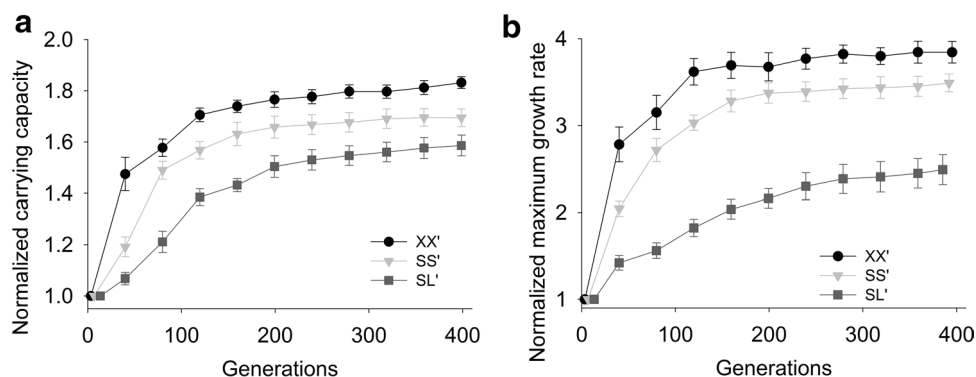
We simulated evolution in populations with identical harmonic mean sizes but with different values of  $N_0$  and  $g$ , such that the product ( $N_0g$ ) remained constant. If the harmonic mean ( $=N_0g$ ) were a good predictor of how much a population is expected to adapt, then these three treatments were expected to show similar *EoA*. This was not found to be the case for both  $K$  (Fig. 2a) and  $R$  (Fig. 2b), which was consistent with our experimental observations of *EoA* trends in SL and SS (Fig. 1; also see Supplementary Methods and Fig. S4). The simulated populations with identical harmonic mean sizes (XX', SS', and SL') were also found to be remarkably different in terms of the adaptive increase in average efficiency of individuals (Fig. S4a). Interestingly, populations with similar harmonic mean were also found to differ in terms of the frequency distributions of

the efficiency parameters amongst their constituent individuals (Fig. S5). To determine why  $N_0g$  could not explain *EoA* trajectories, we determined how *EoA* varied with  $N_0$  and  $g$ , independently.

### *EoA* Varied Positively with $N_0$ but Negatively with $g$

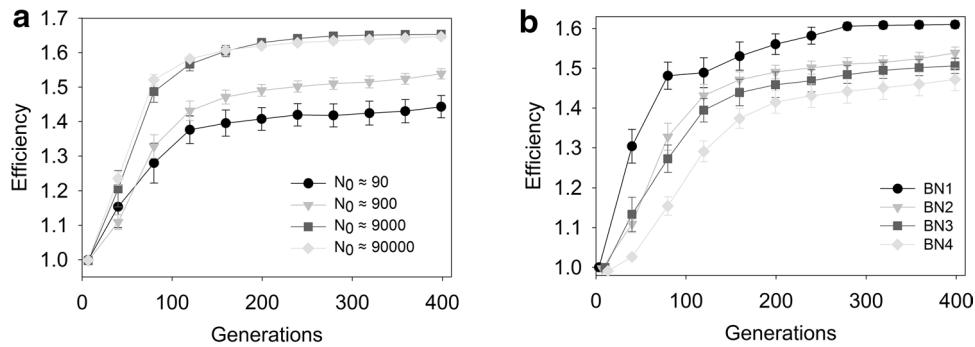
If  $N_0g$  were a good measure of the population size that has a positive relationship with *EoA*, then increasing either  $N_0$  or  $g$  or both should lead to greater *EoA*. We tested this intuitive prediction via simulations using several combinations of  $N_0$  and  $g$ , spanning four orders of magnitude for both  $N_0$  and the sampling ratio ( $N_0/N_f$ ). Although *EoA* was found to increase with greater  $N_0$  (Fig. 3a, also see Fig. S6), the relationship between *EoA* and  $g$  turned out to be negative (Fig. 3b; also see Fig. S7) which was reflected in terms of both individual-level (Fig. 3b—in terms of efficiency) and population-level (Fig. S8—in terms of  $R$ ) fitness parameters. The latter result implied that larger values of  $N_f$  impeded adaptation in populations when the population size during the bottleneck ( $N_0$ ) was held constant. The nature (sign) of this relationship between *EoA* and  $g$  was found to be robust to changes in mutation rate over a 100-fold range in our simulations (Fig. S10).

A negative relationship between the extent of adaptation and  $g$  is particularly surprising because, in populations with similar  $N_0$ , increase in  $g$  is expected to lead to an increase in the available variation. All else being equal, this should have led to greater adaptation. Since that was not the case, we went on to check if these slowly adapting populations (with similar  $N_0$  but higher  $g$ ) were limited, qualitatively and/or quantitatively, by the availability of variation.



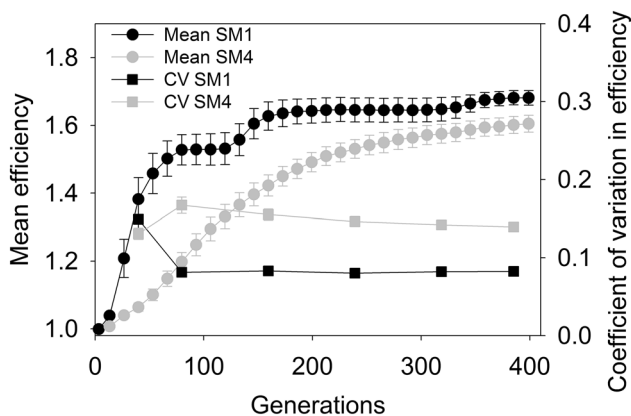
**Fig. 2** Simulations: adaption in three populations with similar harmonic mean size. Data points show mean *EoA*  $\pm$  SEM for eight replicates. **a** Adaptation in terms of normalized carrying capacity ( $K$ ). **b** Adaptation in terms of normalized maximum growth rate ( $R$ ). XX', SS' and SL' had similar harmonic mean sizes and represent lenient,

medium and harsh bottlenecks with  $N_0 \approx 3.6 \times 10^3$ ,  $1.8 \times 10^3$ ,  $9 \times 10^2$  and bottleneck ratio of  $1/10$ ,  $1/10^2$ ,  $1/10^4$  respectively. These simulations suggest that populations with similar harmonic mean size can have markedly different *EoA* trajectories



**Fig. 3** Simulations: the relationship of *EoA* (expressed in terms of efficiency) with  $N_0$  and  $g$ . Data points show mean  $\pm$  SEM; eight replicates. The populations shown in **a** had the same bottleneck ratio ( $1/10^2$ ) but different bottleneck sizes ( $=N_0$ ). *EoA* varies positively with  $N_0$ . On the other hand, the populations shown in **b** had identi-

cal bottleneck size ( $=N_0$ ) but different bottleneck ratios reflected by different values of  $g$ . Bottleneck ratios: BN1:  $1/10$  ( $g=3.32$ ); BN2:  $1/10^2$  ( $g=6.64$ ); BN3:  $1/10^3$  ( $g=9.96$ ); BN4:  $1/10^4$  ( $g=13.28$ ). *EoA* varies negatively with  $g$ . Also see Figs. S6, S7 and S8



**Fig. 4** Simulations: trajectories of efficiency in terms of across-population mean and within-population coefficient of variation. The within-populations coefficient of variation (CV) was computed for each replicate population across its constituent individuals using discrete frequency distributions. The error bars represent SEM (eight replicates). Both SM1 and SM4 had similar bottleneck size ( $N_0 \approx 900$ ). SM1 experienced a periodic bottleneck of  $1/10$  whereas SM4 experienced a periodic bottleneck of  $1/10^4$ . SM4 had a consistently lower *EoA* than SM1 despite having consistently more variation

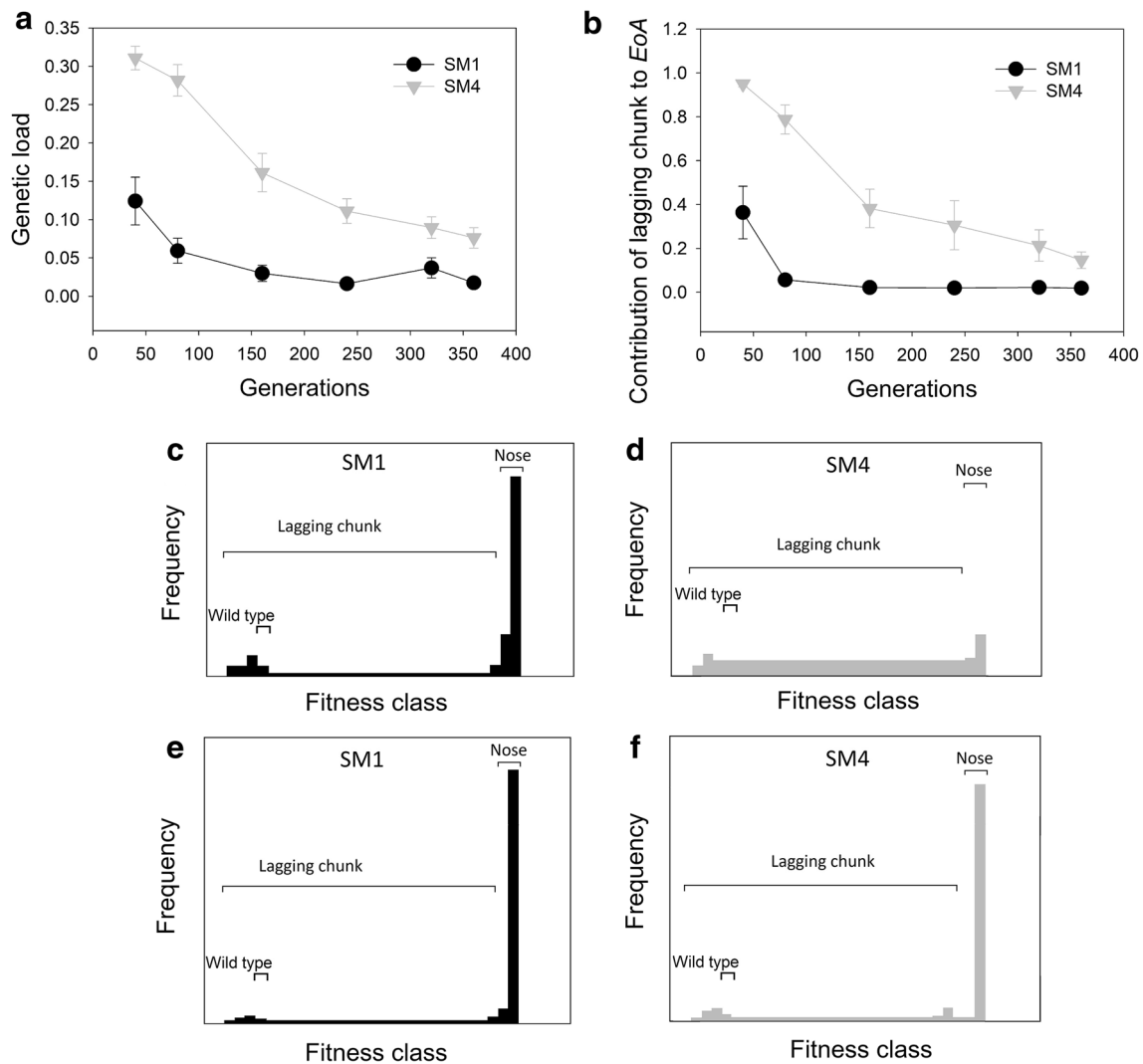
### The Quantitative Availability of Beneficial Traits Could Not Explain Why *EoA* Varied Negatively with $g$

To determine why the extent of adaptation varied negatively with  $g$ , we probed population regimens that had similar starting population size ( $N_0$ ) after the first bottleneck but also had  $g$  values of 3.32 and 13.28 respectively (SM1 and SM4), where SM refers to sampling ratio, expressed in terms of  $\log(10)$  (see Figs. 4, 5). SM1 grew to a final size of  $10N_0$  in one growth phase (i.e., before bottleneck), while SM4 grew to  $10^4N_0$ . Consequently, SM1 faced a periodic bottleneck of  $1/10$  whereas SM4 was sampled  $1/10^4$ . Since SM4 experienced approximately 278 times more fission events than SM1 per

evolutionary generation, the former was expected to undergo more mutations and consequently show more variation. Moreover, SM4 was also expected to arrive at very large-effect benefits that were so rare that the probability of SM1 stumbling upon them was vanishingly low due to its lower mutational supply. As expected, compared to SM1, SM4 had a greater within-population coefficient of variation in terms of efficiency values (Fig. 4) and therefore was not limited by the supply of variation. To better understand the contributions of phenotypes of different magnitudes to the extent of adaptation, we classified the phenotypes into 50 discrete static classes. We found that SM4 also had a continuous access to highly fit genotypes (Fig. S11a) that were inaccessible to SM1 throughout the simulations. On the basis of these observations, the extent of adaptation can be expected to vary positively with  $g$  and thus SM4 was expected to be fitter than SM1 at a given point of time in general. However, counterintuitively, SM4 had a consistently lower extent of adaptation than SM1 (Fig. 4). Evidently, harsher periodic sampling impeded adaptation despite resulting in increased substrate for selection. We also found that although higher  $N_f$  allowed SM4 to arrive at extremely rare mutations with very large benefits, these mutations failed to survive the harsh periodic bottlenecks by rising to large enough frequencies (Fig. S12a). In other words, SM4 typically wasted the best mutation explored by it but SM1 almost always conserved it. This explains why arriving at these rare mutations with very large benefits did not make SM4 adapt more than SM1 in a sustained manner. However, this does not explain why the extent of adaptation of SM4 was consistently lower than that of SM1.

### The Negative Relationship Between *EoA* and $g$ Can Be Explained in Terms of the Efficacy of Selection

The efficacy of selection in eliminating deleterious mutations and spreading beneficial ones is an important factor



**Fig. 5** The efficacy of selection in SM1 was more than that in SM4. **a** SM1 consistently experienced a much lower genetic load than SM4 [the error bars represent SEM (eight replicates)]. **b** The lagging chunk was the major contributor to the Extent of Adaptation (*EoA*) in SM4 but not in SM1 [the error bars represent SEM (eight replicates)]. This also means that the contribution of the nose to the *EoA* [which equals  $(1 - \text{contribution of the lagging chunk})$ ] in SM1 was much more than that of the lagging chunk. **c, d** Schematic representations of the distribution of efficiency across individuals during adaptation

that influences the increase of the extent of adaptation. We quantified the inefficacy of selection in increasing *EoA* using the Genetic Load, which was defined as:  $\text{Genetic Load} = (\text{Best Efficiency} - \text{Average Efficiency}) / \text{Best Efficiency}$  (Crow 1958; Rice 2004). The term “Best Efficiency” refers to the highest efficiency value that succeeds in surviving the bottleneck. As discussed earlier, the magnitudes of the highest efficiency values explored by SM4 populations are much greater than those explored by SM1 (Fig. S11a). However, these high-fitness phenotypes of SM4 typically have such low frequencies that they almost always fail to

during the initial phases of evolution (before generation 80). Due to the high efficacy of selection in SM1, the majority of individuals were found in the nose (**c**). On the other hand, a relatively low efficacy of selection due to harsher bottlenecks in SM4 resulted in most individuals being found in the lagging chunk (please refer to the text for more details) (**d**). **e, f** During the later phases of evolution (around generation 360), the contributions of the nose to the overall *EoA* became relatively similar in SM1 and SM4

survive bottlenecks and thus do not contribute significantly to the overall extent of adaptation (Fig. S12a). Therefore, we defined the genetic load only in terms of the phenotypes that survived the bottlenecks. We found that the Best Efficiency (after bottlenecks) for SM1 was very similar to that of SM4 (Fig. S12b). We note here that the phenotypes that are fitter than the wild type but less fit than the best phenotype also contribute to the genetic load. Thus, consistently higher genetic load entails lower contribution of the best phenotype to the *EoA*. Furthermore, if these best phenotypes (with respect to which genetic load is defined) are similar across

populations being compared, consistently lower contribution of the best phenotype to  $EoA$  would in turn entail slower rise of the latter.

SM4 consistently experienced a heavier genetic load than SM1, particularly during the initial phases of evolution (Fig. 5a). This genetic load was constituted largely by phenotypes that are fitter than the wild type ancestor but less fit than the best phenotype (Fig. S13). We labelled the top five occupied fitness classes as the “nose” (sensu Desai and Fisher 2007) and all the classes inferior to the nose as the “lagging chunk.” During the early phases of evolution, the relative contribution of the lagging chunk to the extent of adaptation was much higher in SM4 than in SM1 (Fig. 5b). In other words, the nose accounted for most of the  $EoA$  in SM1 but not in SM4 (shown schematically in Fig. 5c, d). Thus, compared to SM1, the best phenotype of a typical SM4 population needed to outcompete many more phenotypes (present in sizable frequencies) that were superior to the wild-type but inferior to itself. This suggests that the efficacy of selection was higher in SM1 than in SM4, which in turn explains the faster increase of  $EoA$  in the former. As selection proceeded, the genetic load of SM4 reduced greatly by generation 360 (Fig. 5a). This resulted in similar contributions of the respective noses to the overall  $EoA$  in SM1 and SM4 (Fig. 5e, f).

The above observations suggest that during the early phases of evolution, populations with higher  $g$  (here SM4) can face greater impediment (genetic load), which translates into a reduced  $EoA$ .

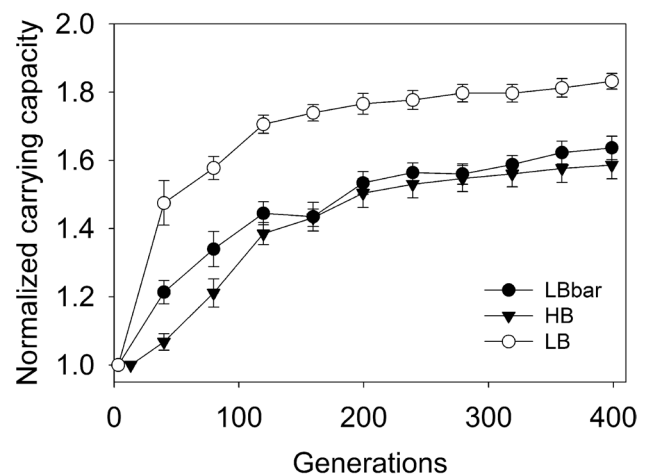
### $N_0/g$ is a Better Predictor of $EoA$ than $N_0g$

Since our simulations suggested that the extent of adaptation is positively related to  $N_0$  and negatively related to  $g$ , we went on to test if  $N_0/g$  is a better predictor of adaptive trajectories than  $N_0g$ . This turned out to be the case, not only in our simulations (Fig. 6; also see Fig. 2, S14), but also for our experiments. The  $N_0/g$  values of LL, SS and SL populations were approximately  $3.01 \times 10^9$ ,  $1.13 \times 10^4$ , and  $5.02 \times 10^3$ , respectively. This led to a predicted  $EoA$  trend of  $LL > SS > SL$ , which was observed in the experiments in terms of both the fitness measures ( $K$  and  $R$ ) (Fig. 1).

## Discussion

### Overview of the Main Results and What They Suggest

Most experimental studies with periodically bottlenecked asexual populations have used the harmonic mean as the measure of population size (De Visser and Rozen 2005; Desai et al. 2007; Lachapelle et al. 2015; Lenski et al.



**Fig. 6** Simulations:  $EoA$  trajectories in terms of  $K$ . Populations with similar  $N_0/g$  (LBbar and HB) match more closely in terms of mean adaptive trajectories than populations with similar  $N_0g$  (LB and HB). LB:  $N_0 \approx 3600$ , bottleneck ratio: 1/10; HB:  $N_0 \approx 900$ , bottleneck ratio: 1/10<sup>4</sup>; LBbar:  $N_0 \approx 225$ , bottleneck ratio: 1/10

1991; Rozen et al. 2008; Samani and Bell 2010; Vogwill et al. 2016) to investigate quantities akin to the  $EoA$ . Desai et al. (2007) stated that the enhancement in mean population fitness with respect to time (a quantity equivalent to  $EoA$ ) depends upon the harmonic mean of the population size in such populations (Desai et al. 2007). However, there has been no empirical or theoretical test for the validity of the harmonic mean as a predictor of the extent of adaptation. Therefore, as a starting-point, we performed evolutionary experiments on *E. coli* populations to test if the harmonic mean of population size ( $=N_0g$ ) can predict  $EoA$ . Our experiments revealed that  $N_0g$  does not predict  $EoA$  (Fig. 1). This observation could be interpreted in two ways. Either there was something wrong with harmonic mean in terms of predicting  $EoA$ , or there were some idiosyncratic properties of our experimental system (e.g. different kinds of containers) that masks the relationship between harmonic mean and  $EoA$ . Apart from their different numbers (whose effect we study here) and the fact that the LL/SL treatments are grown in flasks while the SS treatment is grown in tissue culture plates, there are no differences between the three treatments, and hence the corresponding selection pressures. Since they are grown with continuous shaking, aeration is unlikely to be a significant issue. To account for the possibility that some idiosyncrasies of our experiments were responsible for our results, and to test if the results of our experimental case-study were generalizable, we simulated the adaptive evolution of asexual populations that grow via fission. For this purpose, we used a very generic model that did not contain any *E. coli* specific functions or parameters. The idea here was that if the outcomes of the simulations matched the experiments, we could be reasonably confident that the



experimental results are not due to some peculiarities of the *E. coli* system or experimental protocols. The simulations also revealed no association between  $N_0g$  and  $EoA$  (Fig. 2) which strengthened the first interpretation that  $N_0g$  is not a good predictor of  $EoA$ .

It must be added here that conventionally, the harmonic mean has been treated as an evolutionarily relevant measure of population size only in terms of neutral mutations (Charlesworth 2009; Kimura 1983). However, at least in terms of fixation probabilities of beneficial mutations, it has been shown that population size measures similar to the harmonic mean can act as the relevant measure of population size (Campos and Wahl 2009, 2010; Heffernan and Wahl 2002).

To investigate why  $N_0g$  is an inappropriate measure for predicting  $EoA$ , we used our model to test how  $EoA$  varied with  $N_0$  and  $g$  independently, and found the counter-intuitive result that  $EoA$  varies negatively with  $g$  (Fig. 3b and S7). To explain this result, we probed the composition of our simulated populations as they evolved (Figs. 4, 5). We found that  $g$  plays a dual role in terms of determining  $EoA$ . Higher values of  $g$  positively affect  $EoA$  by increasing the supply of variation, but negatively affect  $EoA$  by decreasing the efficacy of selection, as reflected by a consistently greater genetic load. We found that this second effect of  $g$  on  $EoA$  overshadows the first, something that is underappreciated in the empirical literature. Since  $N_0$  and  $g$  have positive and negative relationships respectively with  $EoA$ , intuition suggests that a good predictor of  $EoA$  should also do the same. One such expression (of the many possible, taking into account that in most evolutionary experiments  $N_0 \gg g$ ) is  $N_0/g$ .  $N_0/g$  indeed turns out to be a better predictor of  $EoA$  in our simulations than  $N_0g$  (Fig. 6 and S14). We show below how both measures, (i.e.  $N_0g$  and  $N_0/g$ ) could lead to similar predictions about  $EoA$  under certain circumstances, and why is it important to consider the cases when this correspondence breaks down.

The rest of the discussion elaborates the various insights mentioned above (and some more) and their consequences.

### Periodic Bottlenecks Lead to Increased Variation But Reduced Adaptation

The growth of many natural asexual populations is punctuated by episodic bottlenecks caused by, for example, abrupt dissociation from hosts or spread of infections across hosts (reviewed in Abel et al. 2015), etc. Moreover, periodic sampling during sub-culturing is a common feature of most asexual populations propagated during experimental evolution studies (Kawecki et al. 2012; Lenski et al. 1991). Therefore, it is important to appreciate the complex role played by periodic bottlenecks in the evolutionary dynamics of asexual populations.

Most experimental evolution studies with asexual microbes are started with either genetically uniform/clonal replicate populations or a mixed inoculum of relatively small number of genotypes. In such populations, de novo beneficial mutations are the principal basis of adaptation (Barrick et al. 2009; Kawecki et al. 2012). That is why populations that experience greater number of binary fissions per generation are expected to generate more de novo beneficial variation and thus, to have a higher extent of adaptation. Now, the number of binary fissions per generation is given by  $N_0(2^g - 1)/g$ . This quantity varies positively with the number of generations before a bottleneck ( $g$ ) and also with the size of the population at the bottleneck ( $N_0$ ). Thus, all else being equal, the harmonic mean ( $\approx N_0g$ ) is expected to be a good predictor of the extent of adaptation.

However, the above line of reasoning disregards the fact that there can be a significant loss of variation during periodic bottlenecks. As  $g$  increases,  $N_0$  represents a smaller fraction of the final population size ( $N_f$ ) before bottleneck, which in turn increases the chances of loss of variation. For example, assume that there are two bacterial populations that have the same value of  $N_0$  ( $= 10^2$ ) but  $g$  values of 3.32 and 13.28, leading to  $N_f$  values of  $10^3$  and  $10^6$  respectively (Lenski et al. 1991). For a given value of  $N_0$ , increasing the value of  $g$  decreases the probability that a new beneficial mutation would survive the bottlenecks (Wahl et al. 2002; Wahl and Zhu 2015). All else being equal, this should reduce the extent of adaptation.

Thus, increasing  $g$  has opposite effects on supply and survival of mutations in a population. Several theoretical studies have investigated which of these two effects is more important for adaptive evolution in asexual populations. For example, it has been suggested that increasing  $g$  increases the probability of fixation of a beneficial mutation (Heffernan and Wahl 2002). This implies that the positive relationship between  $g$  and mutational supply can overcome the negative effect of increasing  $g$  on adaptation. Other theoretical studies have also shown a positive relationship between adaptively relevant population size and the product  $N_0g$  (Campos and Wahl 2009, 2010). Unfortunately, this rich body of theoretical predictions are not in the context of quantities (like  $EoA$ ) that are experimentally tractable, which was one of the motivations behind this study.

Our experiments (Fig. 1) and simulations (Fig. 2) showed that populations with similar values of  $N_0g$  can have very different adaptive trajectories, suggesting that  $N_0g$  is not a good predictor of  $EoA$ . Moreover, our simulations predicted the relationship between  $EoA$  and  $g$  to be negative (Fig. 3b and S7) and not positive. These two results disagree with a rather large body of existing literature, as outlined above. One way by which this can happen is if our model incorporates some atypical assumptions which lead to the observed counter-intuitive results. However, if that were to

be the case, then one would also expect our model to show other unintuitive results. Therefore, we first investigated whether various other predictions of our model matched those from the literature. Our model was able to replicate several intuitive theoretical predictions that had not been coded directly [See Supplementary Data (SD2)]. Firstly, as expected (Elena et al. 1996; Sniegowski and Gerrish 2010), very small populations showed discontinuous staircase-like (stepwise) trajectories of fitness increase whereas large populations showed smooth adaptive trajectories (Fig. S1). Secondly, *EoA* trajectories showed diminishing returns with time despite never hitting the explicitly coded wall of adaptive limit (Fig. 2, 3, 4, 6, S2a, S3, S4a, S6, S7, and S8) (Lenski et al. 1991; Tenaillon et al. 2016). Thirdly, as expected, we found a non-monotonous relationship between *EoA* and mutation rate (Fig. S10c) (Orr 2000). Fourthly, *EoA* showed a positive but saturating relationship with  $N_0$  (which is an unambiguous measure of absolute population size) (Fig. 3a) (Gerrish and Lenski 1998; Sniegowski and Gerrish 2010). All this was highly unlikely if our model incorporated unrealistic or atypical assumptions. Furthermore, for numerically similar populations (i.e. populations with similar  $N_0$  and  $g$ ) and identical time-frames, the results from our simulations were a very close match to the results of our experiments in terms of both  $K$  and  $R$  trajectories (Fig. S3). This again suggests that the IBM captures at least some features of the *EoA* of our *E. coli* populations. Finally, the *EoA* rank predictions generated for the three experimental populations based on our model agreed well with the empirical data (LL > SS > SL, Fig. 1). Therefore, it is reasonable to state that our model was generic and a good descriptor of evolving bacterial populations.

### ***EoA* Varies Negatively with $g$ Because Higher $g$ Makes Selection Less Effective**

In order to explain why *EoA* varies negatively with  $g$ , we simulated populations with similar values of  $N_0$  (i.e., bottleneck size) but different degrees of harshness of the bottlenecks, namely SM1 (lenient bottleneck ( $= 1/10$ ),  $g = 3.32$ ) and SM4 (harsh bottleneck ( $= 1/10^4$ ),  $g = 13.28$ ) (Figs. 4, 5).

Our results demonstrate that higher  $g$  decreases the efficacy of selection in terms of spreading beneficial mutations and purging deleterious ones (Fig. 5, also see Figs. S11 and S12). As shown in Fig. 5, very high-efficiency classes rise to very high frequencies in SM1 populations by generation 80. However, such classes fail to do so in SM4 populations. Owing to lenient bottlenecks (lower  $g$ ), selection operates so effectively in SM1 that its best efficiency class quickly converges with the modal class (Fig. S11b). This is also reflected by the proximity of the mean class with the modal class in SM1 (Fig. S11c). Thus, once a high-fitness class arises in an SM1 population, its rapid spread results in a

steep increase in the population's *EoA*. However, despite having the same bottleneck size ( $= N_0$ ) as SM1, SM4 populations exhibit a much slower rise in their *EoA*. This happens due to two reasons. As opposed to SM1, high-fitness genotypes in SM4 need to rise to much higher frequencies to survive the harsh periodic bottlenecks. This results in the removal (due to sampling) of several high-fitness classes from SM4 during the bottleneck (Fig. S12a). More importantly, the higher mutational supply rate of SM4 increases the genetic load (Fig. 5), which ultimately results in a much slower rise in the extent of adaptation of SM4.

### **Evolution of Carrying Capacity Can Feedback Into Adaptive Trajectories**

Both our experiments and simulations showed that carrying capacity ( $K$ ) can evolve during adaptation in asexual microbes (Figs. 1a, 2a respectively), which is consistent with previous results (Novak et al. 2006). Unfortunately, most models of asexual adaptation do not take into account such adaptive changes in the carrying capacity (Campos and Wahl 2009, 2010; Gerrish and Lenski 1998; Wahl and Gerrish 2001). Most evolution experiments keep the bottleneck ratio (represented by  $g$ ) constant (Kawecki et al. 2012; Lenski et al. 1991). This constancy of  $g$  ensures that any evolutionary change in carrying capacity would also change  $N_0$ . In other words, if  $K$  increases, a constant value of  $g$  throughout evolution would ensure an increase in  $N_0$ . Since higher values of  $N_0$  accelerate adaptation (Fig. 3a), the regularity of bottlenecks introduces a positive feedback during evolution if  $K$  increases adaptively. Stated differently, a larger value of  $N_0$  would make a population evolve higher  $K$ , which in turn would increase the next  $N_0$ , and so on. We think that this aspect of fitness should not be omitted from theoretical models of how microbes evolve, particularly under resource-limited conditions, which are a common feature of experimental evolution protocols (Kawecki et al. 2012; Lenski et al. 1991).

### **$N_0/g$ is a Better Predictor of the Extent of Adaptation Than $N_0g$**

As shown in Figs. 3b, 4 and S7, when selection is at work, the extent of adaptation decreases with increasing  $g$ . This suggests that a population size measure which is an increasing function of  $N_0$  but a decreasing function of  $g$  can be a better predictor of *EoA* than the conventional measure ( $N_0g$ ). For example, as shown in Fig. 6, we found that  $N_0/g$  is a better predictor of *EoA* than the harmonic mean size ( $= N_0g$ ). Admittedly, it is not possible to reason from this that the expression  $N_0/g$  will always be a good predictor of *EoA*, and we make no such claims. We simply submit this expression as a potential candidate for this purpose and hope that

future theoretical work will be able to validate this empirically derived quantity.

## Implications of Our Results

The adaptive dynamics of asexual populations depend on a delicate interplay of the rate at which variation is introduced in the population and the amount of variation lost periodically during bottlenecks. Luria and Delbrück (1943) showed that in periodically bottlenecked systems, each generation contributes equally to the total number of mutants, which, in turn, is proportional to  $N_0(2^g)\mu$ . Furthermore, ignoring the competition between distinct mutations, the per-generation rate of production of the mutants that would eventually survive the bottleneck is proportional to  $(1/2^g)N_0(2^g)\mu$  ( $=N_0\mu$ ). Thus, the only population size parameter that would determine the supply rate of mutations in the absence of mutational competition is  $N_0$ . However, ignoring mutational competition inevitably overestimates the supply of variation in the population. Moreover, we have shown that populations with the same  $N_0$  can have starkly different adaptive trajectories if they have different values of  $g$ , with the extent of adaptation varying negatively with  $g$  (Figs. 4, 5). If  $N_0$  is an overestimation of the mutational supply,  $N_0g$  (the harmonic mean size) is an even bigger overestimate. Our finding that  $N_0/g$  successfully predicts the adaptive trajectories of bottlenecked populations can thus potentially correct for such overestimations in the supply rate of mutations. It is possible to think of other theoretical expressions that can also capture the observed relationships between  $N_0$  and  $EoA$  (positive) or  $g$  and  $EoA$  (negative). A detailed theoretical investigation of what is the correct expression that incorporates these relationships will be the logical next step, but is outside the scope of the current study.

Most theoretical studies assume that the final population size attained in their study systems ( $N_f$ ) is constant (Campos and Wahl 2009, 2010; Gerrish and Lenski 1998; Heffernan and Wahl 2002; Wahl et al. 2002; Wahl and Gerrish 2001). Interestingly, if the experimental populations that are being compared have similar values of  $N_f$  (Desai et al. 2007; Raynes et al. 2014; Vogwill et al. 2016), then the populations with larger values of  $N_0g$  will typically also have larger values of any quantity that is an increasing function of  $N_0$  but a decreasing function of  $g$ . This is because of two reasons. First, if  $N_f$  is held constant, since  $N_f = N_02^g$ , increasing  $N_0$  necessarily decreases  $g$ . Second, in most empirical studies,  $N_0 \gg g$ . Consequently, if  $N_f$  is assumed to be the same the populations being compared, any prediction based on the relative values of  $N_0g$  will typically be similar to predictions based on  $N_0/g$  (Fig. S15). However, whenever  $N_f$  is not held constant (e.g., Fig. 2, 3, 4, 6 and S14, and studies like

Lachapelle et al. 2015; Ramsayer et al. 2013; Raynes et al. 2014; Rozen et al. 2002; Samani and Bell 2010),  $N_0/g$  predicts  $EoA$  much better than  $N_0g$ . The above observations can explain why  $N_0g$  has been widely used across several empirical studies despite failing to capture the effects of  $g$  on  $EoA$  accurately.

At very long time-scales, the high-fitness mutations accessible only to SM4 (but not to SM1) may end up surviving a harsh periodic bottleneck. A post-facto analysis of our SM4 simulations shows that mutations of this kind rise to a frequency between  $10^{-7}$  and  $10^{-8}$  in a typical growth phase just prior to bottlenecks in SM4. Since  $N_0$  is close to  $10^3$  in these populations, the above high-quality mutations would survive one bottleneck in every  $10^4$ – $10^5$  growth phases which roughly amounts to  $1.3 \times 10^5$ – $1.3 \times 10^6$  generations. However, to this date, there are no reported experimental evolution studies over this long a time-span. Therefore, we conclude that the observation that increasing  $g$  decreases  $EoA$  should be relevant for the time-scales most commonly employed in experimental evolution studies.

Our results can be used to compare the extents of adaptation in independent evolution experiments with similar environments but dissimilar demographic properties (differences in terms of  $N_0$  and/or  $g$  and/or  $N_f$ ). Such studies, which compare populations evolving in similar environments but with dissimilar demographic properties, are reasonably common in the field of experimental evolution (Desai et al. 2007; Lachapelle et al. 2015; Raynes et al. 2014; Rozen et al. 2002; Samani and Bell 2010; Vogwill et al. 2016).

Our study shows that in serially bottlenecked asexual populations, the destructive aspect of bottlenecks (reduction in efficacy of selection by harsher bottlenecks) can overshadow their constructive aspect (increase in supply of variation in harsher bottlenecks). This calls for a change in perspective about periodic bottlenecks and a substantial re-evaluation of the role of population size as a predictor of adaptive evolution.

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## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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