Evolutionary Biology (2018) 45:156–169
https://doi.org/10.1007/s11692-017-9440-9

RESEARCH ARTICLE

Population Size Affects Adaptation in Complex Ways: Simulations on Empirical Adaptive Landscapes

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Received: 30 June 2017 / Accepted: 13 November 2017 / Published online: 22 November 2017

Abstract
Do large populations always outcompete smaller ones? Does increasing the mutation rate have a similar effect to increasing the population size, with respect to the adaptation of a population? How important are substitutions in determining the adaptation rate? In this study, we ask how population size and mutation rate interact to affect adaptation on empirical adaptive landscapes. Using such landscapes, we do not need to make many ad hoc assumption about landscape topography, such as about epistatic interactions among mutations or about the distribution of fitness effects. Moreover, we have a better understanding of all the mutations that occur in a population and their effects on the average fitness of the population than we can know in experimental studies. Our results show that the evolutionary dynamics of a population cannot be fully explained by the population mutation rate $N\mu$; even at constant $N\mu$, there can be dramatic differences in the adaptation of populations of different sizes. Moreover, the substitution rate of mutations is not always equivalent to the adaptation rate, because we observed populations adapting to high adaptive peaks without fixing any mutations. Finally, in contrast to some theoretical predictions, even on the most rugged landscapes we study, small population size is never an advantage over larger population size. These result show that complex interactions among multiple factors can affect the evolutionary dynamics of populations, and simple models should be taken with caution.

Keywords Population size · Rate of adaptation · Fitness landscape · Transcription factor binding site

Introduction

How do mutation rate and population size interact on different landscape topographies to affect a population's adaptation? Answering this question can be important for predicting the evolutionary dynamics of different kinds of populations, such as those of pathogens or endangered species. There are many factors affecting the adaptation of organisms, including the presence or absence of genetic recombination; the structure of the fitness landscape (Wright 1932), e.g. its shape and size; DNA mutation rates; the distribution of fitness effects of mutations; and effective population size (Allen 2000; McDonald and Linde 2002; Wilke 2004; Desai and Fisher 2007; Desai et al. 2007; Handel and Rozen 2009; Jain et al. 2011; Lourenço et al. 2013). We focus on two of these factors; namely, effective population size N_e (Charlesworth 2002; Luikart et al. 2010) and mutation rate μ, to better understand their role in adaptation on empirical adaptive landscapes. Specifically, we would like to know at which mutation rates and levels of landscape ruggedness smaller or larger populations have an evolutionary advantage. Do smaller populations outcompete larger ones when landscape ruggedness increases? What is the role of mutation rate in the adaptation of populations of different sizes?

Population size has a major impact on evolutionary dynamics. Under some circumstances, it is advantageous for a population to be larger. The reason is that natural selection is more effective in removing weakly deleterious mutations

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11692-017-9440-9) contains supplementary material, which is available to authorized users.

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and fixing weakly beneficial mutations (Ohta 1992). Consequently, the beneficial mutations go to fixation more frequently in larger populations, and deleterious mutations go to fixation less frequently (Lanfear et al. 2013; Akashi et al. 2012). Additionally, when the product of population size and mutation rate ($N\mu$) is large enough, an evolving population can cross fitness valleys through a process called stochastic tunneling (Komarova et al. 2003; Iwasa 2004; Weinreich and Chao 2005; Weissman et al. 2010; Altland et al. 2011). Specifically, such a population is more likely to produce double mutants that do not experience the deleterious effect of a single mutant, which may allow it to cross a fitness valley (Szendro et al. 2013).

Producing more mutations is not always an advantage. When several beneficial mutations are simultaneously present in an asexual population, they compete with each other for fixation. This slows the time to fixation of a beneficial mutation. This phenomenon is called clonal interference (Gerrish and Lenski 1998), and it can slow down the rate of adaptive substitutions in a population (Charlesworth and Eyre-Walker 2006). Producing fewer mutations per generation, smaller populations are less likely to be affected by clonal interference, and they may thus adapt faster (Gerrish and Lenski 1998; Szendro et al. 2013). Furthermore, genetic drift is stronger in smaller populations. In a rugged landscape, where achieving a higher fitness likely requires passing through fitness valleys, strong genetic drift facilitates valley crossing (Handel and Rozen 2009; Jain et al. 2011). Moreover, some fitness valleys for large populations become flat for smaller populations, because any fitness difference between two mutations smaller than $1/N$ becomes invisible to selection (Ohta 1992; Jain et al. 2011; Szendro et al. 2013; Lachapelle et al. 2015).

The many factors affecting evolutionary dynamics often interact in non-intuitive ways to define the evolutionary outcome of a population. Therefore, most previous theoretical studies include simplifying assumptions to model the role of one or a few of these factors (Desai and Fisher 2007; Desai et al. 2007; Campos and Wahl 2010; Lourenço et al. 2013; Lachapelle et al. 2015). Examples include epistatic interactions among mutations (Cordell 2002; de Visser et al. 2011), and the distribution of fitness effects (Cowperthwaite et al. 2005; Eyre-Walker and Keightley 2007; Tamuri et al. 2012), which define the ruggedness of a fitness landscape. For example, Handel and Rozen (2009) used randomly generated fitness landscapes to study the effect of population size on the evolution of microbes; and Jain et al. (2011) used a three-locus model with arbitrary fitness values for each genotype to study the advantage of small populations on rugged landscapes. Another example is an assumed distribution of fitness effects with rare beneficial mutations to predict the association between the substitution rate of beneficial mutations and the population size (Lanfear et al. 2013). Whether beneficial mutations are rare depends on the proximity of a population to a fitness peak. Violation of such assumptions can lead to dramatically different evolutionary outcomes (Lanfear et al. 2013). In experimental studies, where realistically complex fitness landscapes are examined (Rozen et al. 2008; Kryazhimskiy et al. 2012), researchers have inevitably limited knowledge about, and control over, underlying evolutionary mechanisms, such as the distribution of fitness effects and the mutational trajectories of a population. This is because such fitness landscapes are usually large, and the possibilities to replicate experiments and to vary parameters are limited.

For these reasons, some studies make contradictory observations about the effect of population size on adaptation. For example, the rate of adaptation, defined as the number of beneficial substitutions, has been predicted to increases with effective population size N_e (Lanfear et al. 2013). However, this prediction only holds when beneficial mutations are rare. The frequency of beneficial mutations, in turn, depends on the location of a population on a fitness landscape and on the topology of the landscape (Lanfear et al. 2013). Thus, some studies have found associations between the N_e and rate of adaptation (Dey et al. 2013), while others have not (Bachtrog 2008; Karasov et al. 2010; Gayral et al. 2013). Our study tries to fill the gap between theoretical and experimental studies, using a system where we have more knowledge about, and control over, important factors such as population mutation rates, evolutionary trajectories, and the identity of substituted genotypes, than experimental systems. At the same time, we need to make fewer ad hoc assumptions than most previous theoretical studies. One of these assumptions is the distribution of fitness effects. In an empirical landscape, this distribution changes as a population approaches a fitness peak. For example, when a population gets closer to a peak, beneficial mutations become rarer, without the need to make ad hoc assumptions about their frequency.

We consider 957 empirical adaptive landscapes (Aguilar-Rodríguez et al. 2017). Each landscape encompasses the binding affinity of a transcription factor to all of its cognate DNA sequences (i.e., binding sites). These binding affinities are derived from protein binding microarrays in the form of an enrichment score (E-score), which describes the relative binding preference of a transcription factor to all possible DNA sequences of length eight (Berger et al. 2006). The topographies of these landscapes have recently been characterized in rich detail (Aguilar-Rodríguez et al. 2017), which provides an opportunity to study how the topographies of empirical adaptive landscapes interact with N and μ to affect the adaptation rate of an evolving population. Transcription factor binding affinity is an important molecular phenotype, because it can affect gene expression. For example, increasing the affinity of an activating transcription factor's binding

site will decrease the factor's disassociation rate, thereby increasing the rate of transcription of the downstream gene. If increased expression is selectively advantageous in a given environment (e.g., an antibiotic resistance gene in the presence of an antibiotic), then increased binding affinity may confer increased fitness. The importance of high binding affinity transcription factor binding sites is evidenced by their signature of positive selection in microbes and humans (Mustonen and Lässig 2005, 2009), as well by their proximity to actively transcribed genes in the embryo of *Drosophila melanogaster* (Li et al. 2008). We therefore use binding affinity as a proxy for fitness.

Using these empirical adaptive landscapes, we do not make any ad hoc assumptions about the distributions of fitness effects, the structure of the landscape, or epistatic interaction among mutations, because such information is implicitly present in the landscapes. We simulate populations with a range of mutation rates μ and population mutation rates $N\mu$, and analyze all mutational trajectories of populations during their evolution. We find that mutation rate μ and population mutation rate $N\mu$ are not always sufficient parameters to predict the adaptation rate of populations on these landscapes. Population diversity and the extent of landscape exploration, rather than the substitution rate of mutations, can affect the adaptation rate.

Methods

Genotype Network Construction and Analysis

Genotype networks were constructed as described in Payne and Wagner (2014) and Aguilar-Rodríguez et al. (2017). The data for these networks come from in vitro studies that assess the binding affinity of a transcription factor (Latchman 1997) to all possible DNA sequences of length 8 using protein binding microarrays (Berger et al. 2006; Berger and Bulyk 2009). The total genotype space consists of 32,896 sequences ($(4^8 - 4^4)/2 + 4^4$), where the factor 1/2 accounts for the merging of sequences with their reverse complement. The number 4^4 accounts for palindromic sequences, which are identical to their reverse complement and therefore cannot be merged (Aguilar-Rodríguez et al. 2017). Reference (Aguilar-Rodríguez et al. 2017) constructed and analyzed 1137 binding affinity landscapes from 129 different eukaryotic species and 62 DNA binding domain structural classes. For each transcription factor, a protein binding microarray measures the binding affinity of all 8-mers to the factor. The affinity is represented as a rank-based enrichment score (E-score), which is a variant of the Wilcoxon–Mann–Whitney statistic (Berger et al. 2006). This E-score ranges between − 0.5 (lowest affinity) to 0.5 (highest affinity). We use the E-score as a proxy for binding affinity, and consider only sequences whose E-score is above 0.35 bound by a transcription factor (Aguilar-Rodríguez et al. 2017). We use this threshold because it has yielded a false discovery rate below 0.001 in 104 mouse transcription factors (Badis et al. 2009a). After identifying a set of sequences that bind each transcription factor, we constructed genotype networks for each transcription factor. The nodes of the network are DNA sequences. Two nodes are connected by an edge if they differ by a single mutation. The single mutations considered are either point mutations or single nucleotide insertions/deletions. We characterized graph-theoretical properties of these networks using the iGraph library (version 0.7.1) (Csardi and Nepusz 2006) for Python. We used Gephi (version 0.9.1) (Bastian et al. 2009) for network visualization.

Population Evolution Model

Each landscape only includes sequences bound by a single transcription factor. However, the total number of sequences of length 8 used in the study (32,896 sequences, either bound to a transcription factor or not bound to any of factors), comprises a bigger network, which we call the network of all possible mutations. For simulations on each landscape, we initialized evolving populations with sequences of low binding affinity, because we wanted to explore the dynamics of populations evolving towards high binding affinity. Specifically, we started each simulation by choosing an arbitrary sequence from the bottom 5% of sequences, according to their E-scores, as the starting sequence of the simulation. Our simulations are limited to the dominant component within each landscape. We initialized a population of N individuals with the same initial sequence. For each set of parameters, we performed 100 simulation replicates, and for each replicate we simulated 1000 generations of mutation and selection. At each generation, we determined how many mutations each sequence would experience by drawing from a Poisson distribution with a mean equal to the mutation rate μ of the population. If a sequence was to experience one mutation, we chose randomly one of its neighbors in the landscape. If it was to experience two mutations, we first randomly chose one of its neighbors, and then randomly chose one of the neighbors of the neighbor as the mutant, excluding the original sequence (thus prohibiting back mutations), and likewise for any additional mutations. After the mutation step, we assigned a value l to each sequence by assigning a random number defined as its E-score $\pm$ Δ, where Δ is a parameter specific to each landscape, which defines a threshold to call two E-scores different in a protein binding microarray experiment, E-scores of each sequence are measured by two replicates, and Δ is the residual standard error of the linear regression between the E-scores of all bound sequences in the two replicate measurements (Aguilar-Rodríguez et al. 2017). Finally, as the selection

step, we randomly sampled exactly N sequences from all the sequences with replacement, where the probability of sampling each sequence was weighted by its value of l. We note that with this selection method, population sizes remain constant every generation.

Neutral Neighborhood Size Calculation

For each landscape, we considered the binding affinity of all neighbors of each of a landscape's sequences. If the binding affinity difference between the sequence and its neighbor was smaller than $1/N$, the neighbor is part of the neutral neighborhood of the sequence. We report the fraction of neutral neighbors of all sequences in each landscape.

Computing Population Diversity

We computed two measures of population diversity. The first measure corresponds to the number of unique sequences at the last generation in each simulation. We report its average across 100 simulation replicates. The second measure is the total number of unique sequences that were visited by a population across all generations, averaged over 100 simulation replicates.

Counting the Incidence of Deleterious, Neutral, and Beneficial Mutations

To calculate the incidence of deleterious, neutral, and beneficial mutations in each population, we tracked every mutation. If the binding affinity difference of sequence and its mutant (whose affinity is given by l defined above, a random number in the range E-score $\pm$ Δ) was more than $1/N$, we considered the mutation non-neutral; it would be beneficial or deleterious depending on whether the binding affinity had increased or decreased, respectively.

Number of Substitutions

We considered any sequence different from the ancestral sequence as a sequence that has become fixed if it ever reached a population frequency exceeding 90% (a common practice in simulating populations (Desai and Fisher 2007; Vatsiou et al. 2016) to limit computational costs). Strictly speaking, fixation means an allele is present in 100% of the population. If a sequence passed the 90% threshold and dropped below this threshold more than once, we considered it as fixed only once.

Results

Structure of Binding Affinity Landscapes

From the 1137 landscapes studied in Aguilar-Rodríguez et al. (2017), we simulated the evolution of populations on those 957 landscapes that had at least 100 sequences. We then chose nine of these landscapes for a more detailed analysis. The nine landscapes differ in their ruggedness, as measured by their number of peaks. A peak is defined as a set of sequences whose affinity is larger than that of all their neighboring sequences (Khalid et al. 2016). Table 1 lists the names of these nine transcription factors, their DNA binding domains, the species they belong to, and their number of peaks.

Some landscapes have multiple connected components, i.e. sets of nodes (sequences) that are reachable from one another through a sequence of single step mutations. We

Table 1 Landscapes in our study

TF name	Species	Number of components	Network size	Size of the dominant genotype network	Number of peaks	Study
NCU03110	*Neurospora crassa*	1	1064	1064	1	Weirauch et al. (2014)
TIFY2B	*Arabidopsis thaliana*	1	1050	1050	1	Weirauch et al. (2014)
NCU06990	*Neurospora crassa*	1	1038	1038	2	Weirauch et al. (2014)
AZF2	*Arabidopsis thaliana*	1	1051	1051	3	Weirauch et al. (2014)
Six6	*Mus musculus*	3	658	656	6	Badis et al. (2009b)
NCU00445	*Neurospora crassa*	4	589	586	7	Weirauch et al. (2014)
KDM2B	*Homo sapiens*	6	634	629	9	Weirauch et al. (2014)
FBXL19	*Tetraodon nigroviridis*	7	730	724	13	Weirauch et al. (2014)
kdm2aa	*Danio rerio*	13	513	499	36	Weirauch et al. (2014)

Each column describes the following information: *TF name* name of the transcription factor to which the sequences bind; *species*: the species in which the transcription factor occurs; *number of components* number of connected components within each network, i.e., components in which sequences are accessible from one another through a path of one or more edges; *network size* total number of sequences in landscape; *Size of the dominant genotype network* number of sequences in the largest connected component; *number of peaks* number of peaks in the landscape (see "Methods"); *study* the study from which data were retrieved for constructing the landscape

call the largest of these components the dominant component and limit our simulations to these dominant components. The single step mutations we consider are either point mutations, or single base pair insertions/deletions (Payne and Wagner 2014; Aguilar-Rodríguez et al. 2017). The landscapes comprise between 513 and 1064 sequences, and have between 1 and 13 connected components (Table 1). Figure 1 shows one of the landscapes used in this study, that of the *Arabidopsis thaliana*'s transcriptional repressor AZF2. Each circle represents a sequence and edges connect sequences that differ by a single mutation.

The evolutionary dynamics of a population on an adaptive landscape depends in part on the average fraction of neutral neighbors of its genotypes. When genotypes in a population have larger neutral neighborhoods, the population may be able to explore a larger fraction of the landscape without facing deleterious mutations. Hence, it may more easily discover beneficial mutations and new phenotypes (Ancel and Fontana 2000). Neutral neighborhood size is a function of effective population size N_e (Hartl and Clark 1997), which equals consensus population size N in our simulations, because our simulated populations experience no population size fluctuations. We analyzed the size of each neutral neighborhood in different landscapes and with different population sizes. We consider the fitness difference of any two neighboring sequences neutral if it is smaller than $1/N$ (Kimura 1962; Ohta and Gillespie 1996). Figure S1 shows the fraction of neutral neighbors among all nodes in a landscape, for all nine different landscapes and different population sizes. As expected, neutral neighborhood size decreases with increasing population size, which makes it more difficult for larger populations to evolve neutrally and cross fitness valleys (Ancel and Fontana 2000).

We used a variation of the Wright–Fisher model (see "Methods") to evolve populations on our landscapes for 1000 generations of mutation and selection, which favors increases in binding affinity. We performed 100 replications for each simulation. Since we are interested in analyzing the effect of population size N and mutation rate μ on the adaptation of populations, we systematically explored a range of mutation rates ($0.001 \leq \mu \leq 1$) and population mutation rates ($0.01 < N\mu < 10$) with seven population sizes ($10 < N < 640$). We chose a maximum population size of 640 based on the size of the landscapes, so that even in a high mutation regime, only a fraction of the landscape would be occupied by a population.

Landscape Ruggedness Strongly Affects Adaptation

We initially determined whether the measurement of ruggedness in these landscapes, namely the number of peaks, affects evolutionary dynamics. To that end, we simulated evolution on all of the 957 landscapes (Aguilar-Rodríguez et al. 2017). We analyzed correlations between the mean final affinity of simulated populations, normalized by the maximum binding affinity in each landscape, and the number of peaks in each landscape, and at different mutation rates. In line with our expectation, populations in more rugged landscapes have lower mean population affinity at the end of simulations (i.e. generation 1000) (Table S1). In more rugged landscapes, populations are more likely to get trapped on local optima, and this may be a bigger problem for larger populations, because drift is weaker for them compared to smaller populations. These observations hold for all mutation rates ($\mu = 0.001$–$\mu = 1$).

We also asked whether the size of (number of sequences in) the global peak of each landscape correlates with the mean final affinity of the populations. We found strong and positive correlations (Table S2): the larger the size of the global peak of a landscape, the higher the mean final affinity of a population. This indicates that larger peaks are easier to find.

Adaptive Evolution Under Varying Mutation Rate μ

We first investigated how interactions between different mutation rates μ and population sizes N affect population adaptation, using a range of mutation rates between $\mu = 0.001$ and $\mu = 1$.

$\mu = 0.001$

At this low mutation rate, the population mutation rate is $N\mu << 1$ for all population sizes. Larger populations consistently achieve higher mean binding affinity at the end of simulated evolution (Fig. 2a). Larger populations have several advantages to help them find adaptive peaks better than smaller populations, even at mutation rates this small. First, since larger populations have a higher population mutation rate $N\mu$, they are slightly more diverse at any generation (Fig. 2b). Second, and consequently, larger populations visit more unique sequences (Fig. 2c). They are therefore better at exploring the landscape, which gives them more opportunities for identifying adaptive peaks. Third, and in line with the second observation, larger populations fix more mutations, most of which are beneficial (Fig. S2). This is because they experience more mutations, and because selection is more effective in larger populations (Jain et al. 2011; Szendro et al. 2013; Lachapelle et al. 2015).

$\mu = 0.01$

At a mutation rate of $\mu = 0.1$, we still find that larger populations have higher mean binding affinity at the end of the evolutionary simulations than smaller populations, although the difference between larger populations is smaller than at

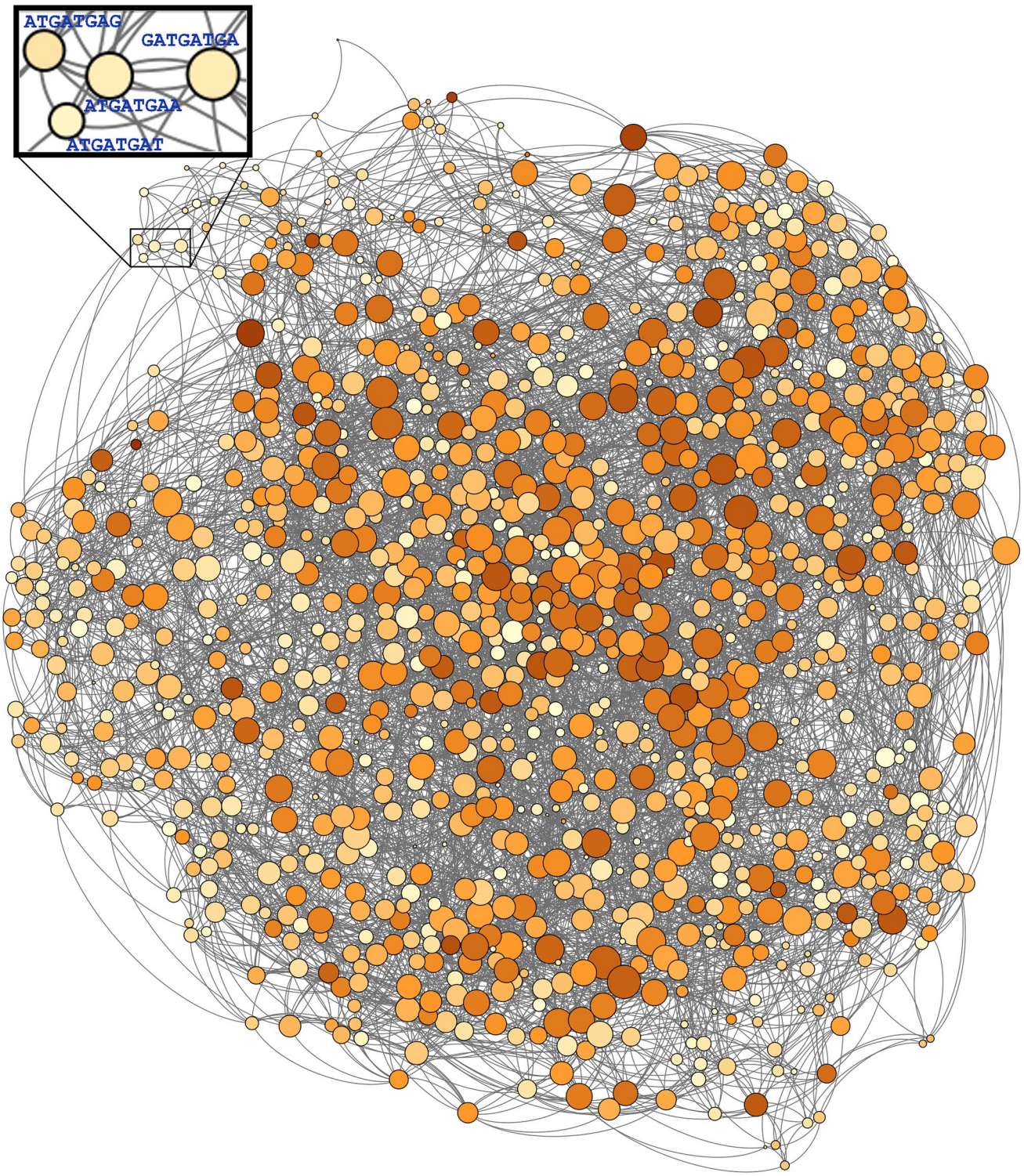

Fig. 1 The adaptive landscape of the AZF2 transcription factor. Each node corresponds to a DNA sequence. Two nodes are connected if they differ by a single point mutation or a single indel. Node color corresponds to the affinity of the sequence (darker = higher), and node size corresponds to the number of neighbors of the node (bigger = more). The inset shows that two nodes are connected if they differ by a single mutation. Our display allows for overlapping nodes, so the actual number of nodes may be greater than the number of nodes that are visible. (Color figure online)

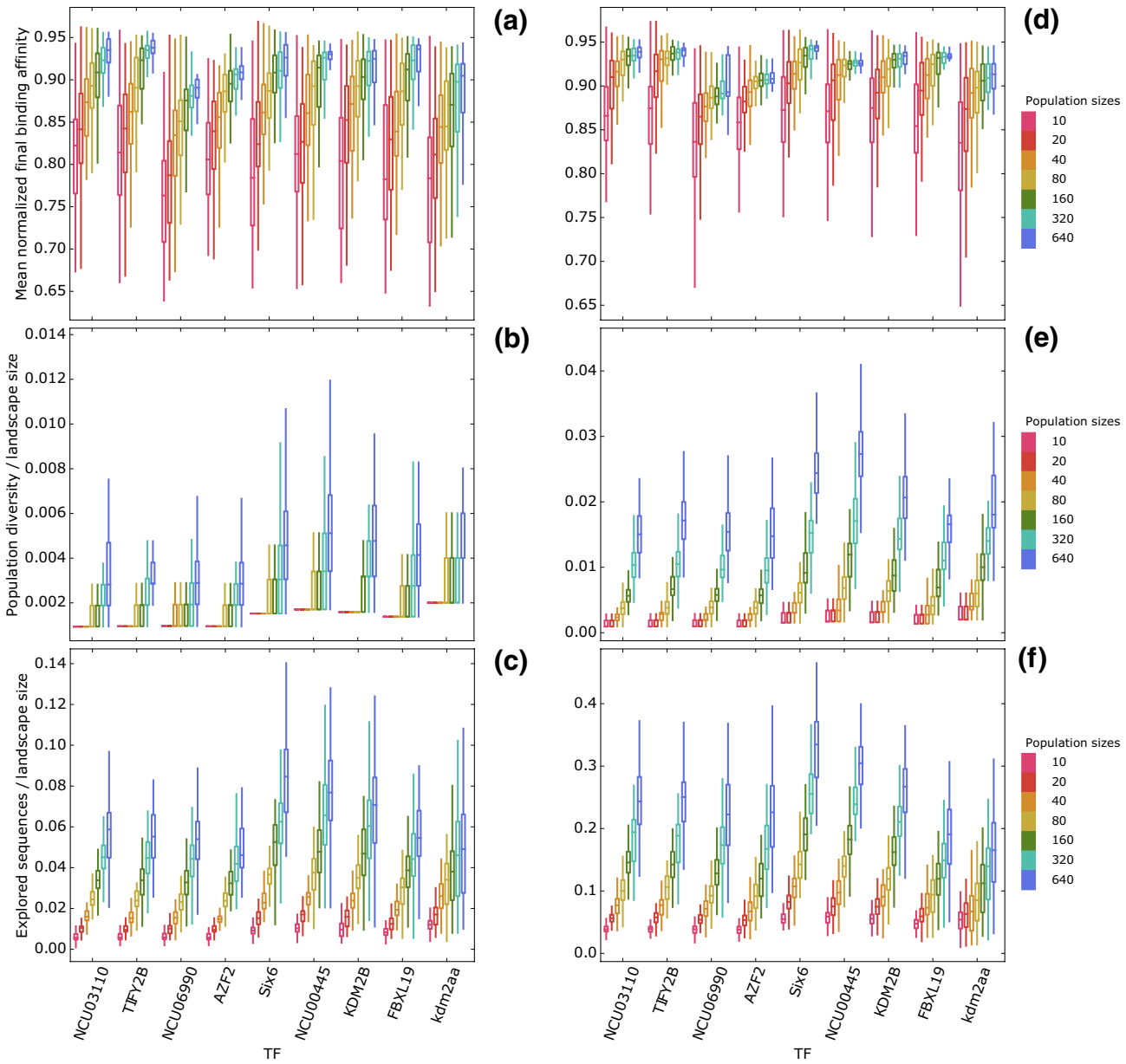

Fig. 2 Mean final binding affinity, sequence exploration and diversity of populations at $\mu = 0.001$ and 0.01. The figure shows **a**, **d** the mean population binding affinity at the end of the simulations for $\mu = 0.001$ and 0.01, respectively, **b**, **e** the population diversity at the end of the simulations, i.e. the number of unique sequences at generation 1000, for $\mu = 0.001$ and 0.01, respectively, and **c**, **f** the total number of unique sequences visited by a population during 1000 generations, for $\mu = 0.001$ and 0.01, respectively. Data in **a** and **d** are normalized by the maximum affinity value in each landscape, data in **b**, **c**, **e** and **f** are normalized by landscape size. Horizontal axes on all panels show different transcription factor affinity landscapes ordered from left to right in increasing order of ruggedness. We randomly selected a sequence of low binding affinity to initialize each simulation, and then simulated 1000 generations of mutation and selection. We performed 100 replicate simulations for each population size at a fixed mutation rate of $\mu = 0.001$ and 0.01 per sequence per generation (see "Methods"). Each box encloses the second and third quartiles of data from 100 replicates, the center line corresponds to the median, and the whiskers depict the minimum and maximum values obtained from any replicate, excluding outliers

$\mu = 0.001$ (Figs. 2d and S3). At this mutation rate, populations fall into two evolutionary regimes. Specifically, for four population sizes ($N = 10$, $N = 20$, $N = 40$, and $N = 80$) $N\mu < 1$, and for the other three ($N = 160$, $N = 320$, and $N = 640$) $N\mu > 1$. When there is more than one lineage harboring a beneficial mutation, these lineages compete with each other for fixation, resulting in slower fixation rates of either lineage, a phenomenon called clonal interference (Gerrish and Lenski 1998). When $N\mu > 1$, populations are polymorphic most of the time, which increases

the likelihood of clonal interference (Park and Krug 2007). We first tested whether we find clonal interference in these populations, and if it increases with population size. Figure 3 shows the average number of unique mutations that are simultaneously present in the population, and the effect of these mutations, i.e. beneficial, deleterious or neutral, relative to the ancestral sequence of the population. The average number of unique mutations at each generation, and the average number of beneficial unique mutations, increases with population size. Consistent with the existence of clonal interference, we find that the number of beneficial substitutions for most landscapes (all except FBXL19 and kdm2aa) is an increasing function of N when $N\mu < 1$ ($N = 10$, $N = 20$, $N = 40$, and $N = 80$), but a decreasing function of N when $N\mu > 1$ ($N = 160$, $N = 320$, and $N = 640$) (Fig. S4). Moreover, despite fixing no more or even fewer beneficial mutations than smaller populations due to increased clonal interference, larger populations reach higher mean final binding affinity. To explain this pattern, we pooled data from all simulations, and asked whether the mean final population binding affinity correlates with two measures of population diversity, i.e. the number of explored sequences during the evolutionary simulation and the amount of standing variation at the final generation. We found strong positive associations between both metrics of diversity and mean final binding affinity (Tables S4 and S5). Note that larger populations are both more diverse in the last generation (Fig. 2e) and explore more sequences during evolution (Fig. 2f). These observations suggest that, unsurprisingly, larger populations have more standing variation, which increases the prevalence of beneficial mutations (Fig. S5), which in turn is strongly associated with increased mean population binding affinity (Table S6). In sum, the mean final binding affinity of evolving populations is not completely determined by the number of beneficial substitutions, but also by the population diversity.

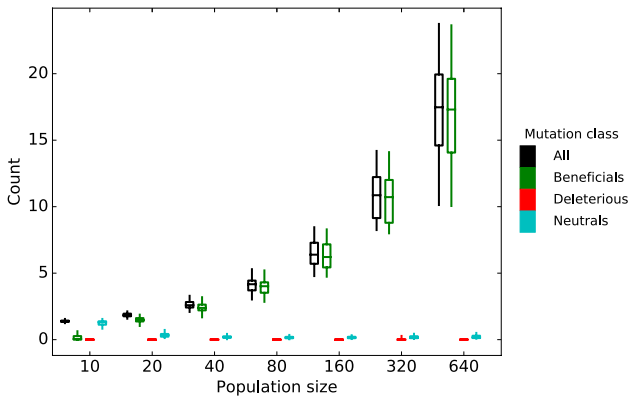

Fig. 3 More beneficial mutations coexist in larger populations evolving on the AZF2 landscape at constant $\mu = 0.01$. Boxplots summarize mean numbers of unique total, beneficial, deleterious, and neutral mutations that coexist per generation (color legend) for populations of different sizes (horizontal axis) evolved on the AZF2 landscape. When more than one beneficial mutation is present at the same time in a population, those mutations compete for fixation (clonal interference), resulting in longer fixation time for the mutation that finally fixes in the population. We determined the effect of each mutation compared to the ancestral sequence starting the population simulation. Effects smaller than $1/N$ are neutral. Each box encloses the second and third quartiles of data from 100 replicates, the center line corresponds to the median, and the whiskers depict the minimum and maximum values obtained from any replicate, excluding outliers. Population evolution was simulated in the same way as explained in the caption of Fig. 2, except that $\mu = 0.01$. (Color figure online)

$\mu = 0.1$

At a mutation rate of $\mu = 0.1$, the population mutation rate is $N\mu > 1$ for all populations, and clonal interference is prevalent in all populations, but becomes stronger in larger populations (Fig. S6). The largest populations ($N = 160$, $N = 320$, and $N = 640$), therefore, have nearly no substitutions (Fig. S7). Still, they arrive at a higher mean binding affinity than smaller populations (Fig. 4a). The largest populations in some landscapes (FBXL19, NCU00445, and TIFY2B), however, do not differ in their mean final binding affinity.

Population diversity can help explain how larger populations reach higher mean binding affinity levels, despite fixing nearly no mutations. Larger populations explore more sequences than smaller populations, and the difference in this exploration ability between larger and smaller populations is greater at $\mu = 0.1$ (Fig. 4c). Similarly, the difference between the fraction of beneficial mutations among all mutations that occur in larger populations and in smaller populations is greater at $\mu = 0.1$ (compare Figs. S5 and S8).

$\mu = 1$

At this large mutation rate, where on average every sequence mutates in every generation ($N\mu >> 1$), we do not find striking differences between the mean final binding affinity at different population sizes (Fig. 4d). Only the two smallest populations ($N = 10$ and $N = 20$) have a slightly lower mean binding affinity than larger populations. More pronounced, however, is a drop in mean final binding affinity of all population sizes compared with $\mu = 0.1$ (compare Fig. 4a with d). This is because of the high fraction of mutant individuals that are created generation. When a population finds and moves to a sequence with a high binding affinity, it will not stay there, because at the next generation, most individuals mutate away from it. Therefore, the mean affinity of populations fluctuates around lower values and the highest possible mean affinities cannot be attained.

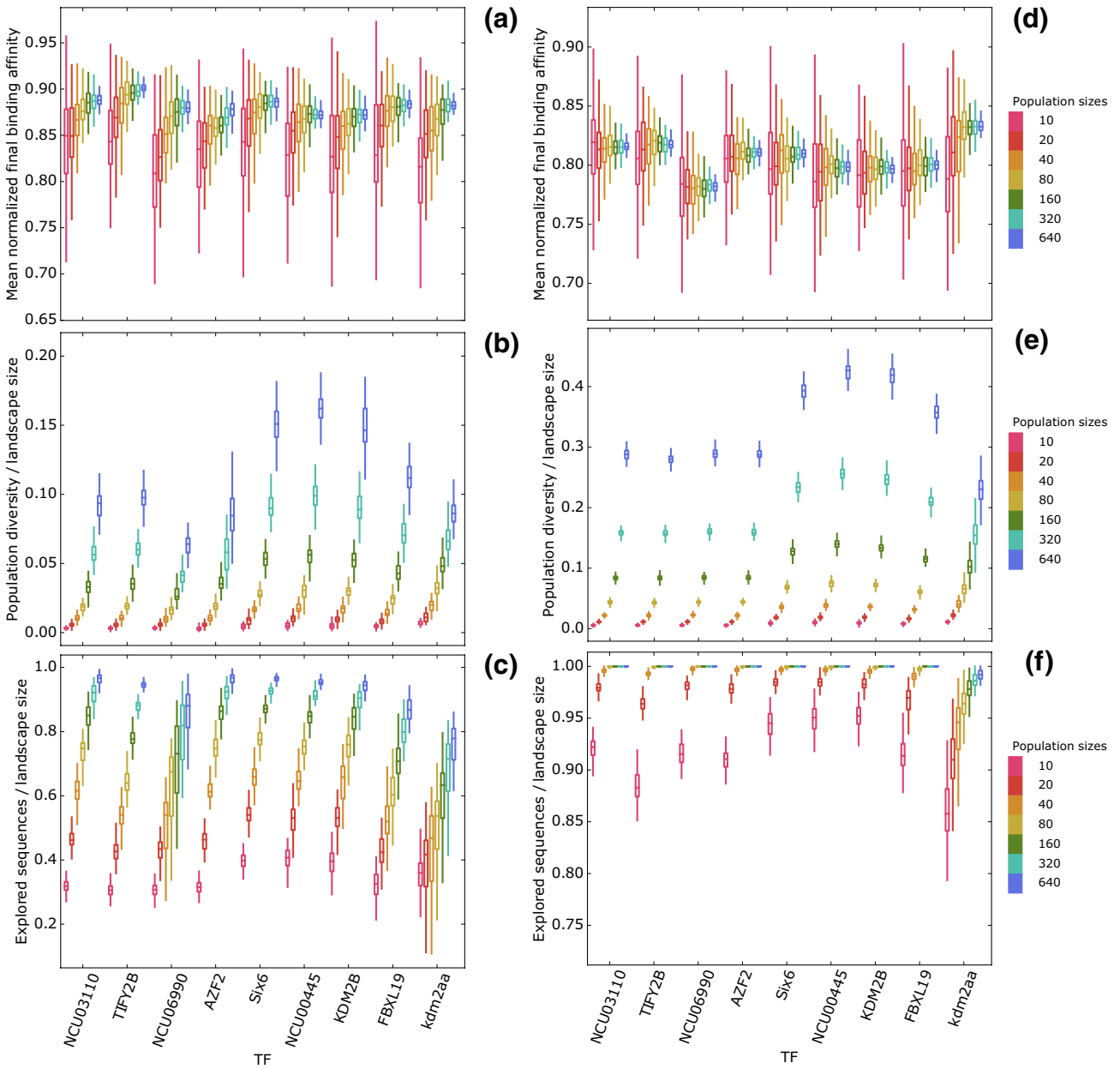

Fig. 4 Mean final binding affinity, sequence exploration and diversity of populations at $\mu = 0.1$ and 1. The figure shows **a** and **d** the mean population binding affinity at the end of the simulations for $\mu = 0.1$ and 1, respectively, **b** and **e** the population diversity at the end of the simulations, i.e. the number of unique sequences at generation 1000, for $\mu = 0.1$ and 1, respectively, and **c** and **f** the total number of unique sequences visited by a population during 1000 generations, for $\mu = 0.1$ and 1, respectively. Data in **a**, **d** are normalized by the maximum affinity value in each landscape, data in **b**, **c**, **e**, **f** are normalized by landscape size. Horizontal axes on all panels show different transcription factor affinity landscapes ordered from left to right in increasing order of ruggedness. Each box encloses the second and third quartiles of data from 100 replicates, the center line corresponds to the median, and the whiskers depict the minimum and maximum values obtained from any replicate, excluding outliers. Population evolution was simulated in the same way as explained in the caption of Fig. 2, except that $\mu = 0.1$ and 1

Adaptive Evolution Under Varying Population Mutation Rates $N\mu$

Another important quantity in population genetics is the population mutation rate $N\mu$. In the following sections, we will analyze the effect of $N\mu$ on adaptive evolution to find out whether it alone can explain the difference in adaptation between populations of different sizes.

$N\mu = 0.01$ and $N\mu = 0.1$

At these low population mutation rates, populations of all sizes reach similar mean final binding affinity levels (Figs. 5a, b). Likewise, the extent of sequence exploration (Figs. S9 and S10) and population diversity in the last generation (Figs. S11 and S12) is similar among populations of all different sizes. This suggests that $N\mu$ may be adequate to explain evolutionary dynamics when $N\mu$ is not too large.

$N\mu = 1$ and $N\mu = 10$

At the moderate population mutation rate of $N\mu = 1$, we find that the smallest populations (i.e. $N = 10$, $N = 20$, and $N = 40$) are not reaching the same mean final binding affinity as larger populations (Fig. 5c). At the high population mutation rate $N\mu = 10$, this dependency of final fitness on population size is even stronger (Fig. 5d). In addition, there is a negative association between sequence exploration and population size (Figs. S13 and S14). This is likely due to larger neutral neighborhood that is characteristic of smaller populations (Fig. S1). Larger neutral neighborhoods mean that more neutral mutations are available to smaller populations (Figs. S15 and S16), which thus face fewer limitations exploring novel sequences. Such larger neutral neighborhoods also result in more neutral substitutions in smaller populations (Figs. S17 and S18). Larger populations experience (Fig. S19) and fix more beneficial mutations than smaller populations (Fig. S20) at $N\mu = 1$. At $N\mu = 10$, however, we observe a peak in the maximum fraction of beneficial mutations that the populations experience at intermediate population sizes (Fig. S21). All populations at $N\mu = 10$ fix fewer mutations than at $N\mu = 1$, but larger populations fix more beneficial mutations (Fig. S22). Two factors can explain the difference in mean final binding affinity between smaller and larger populations at constant

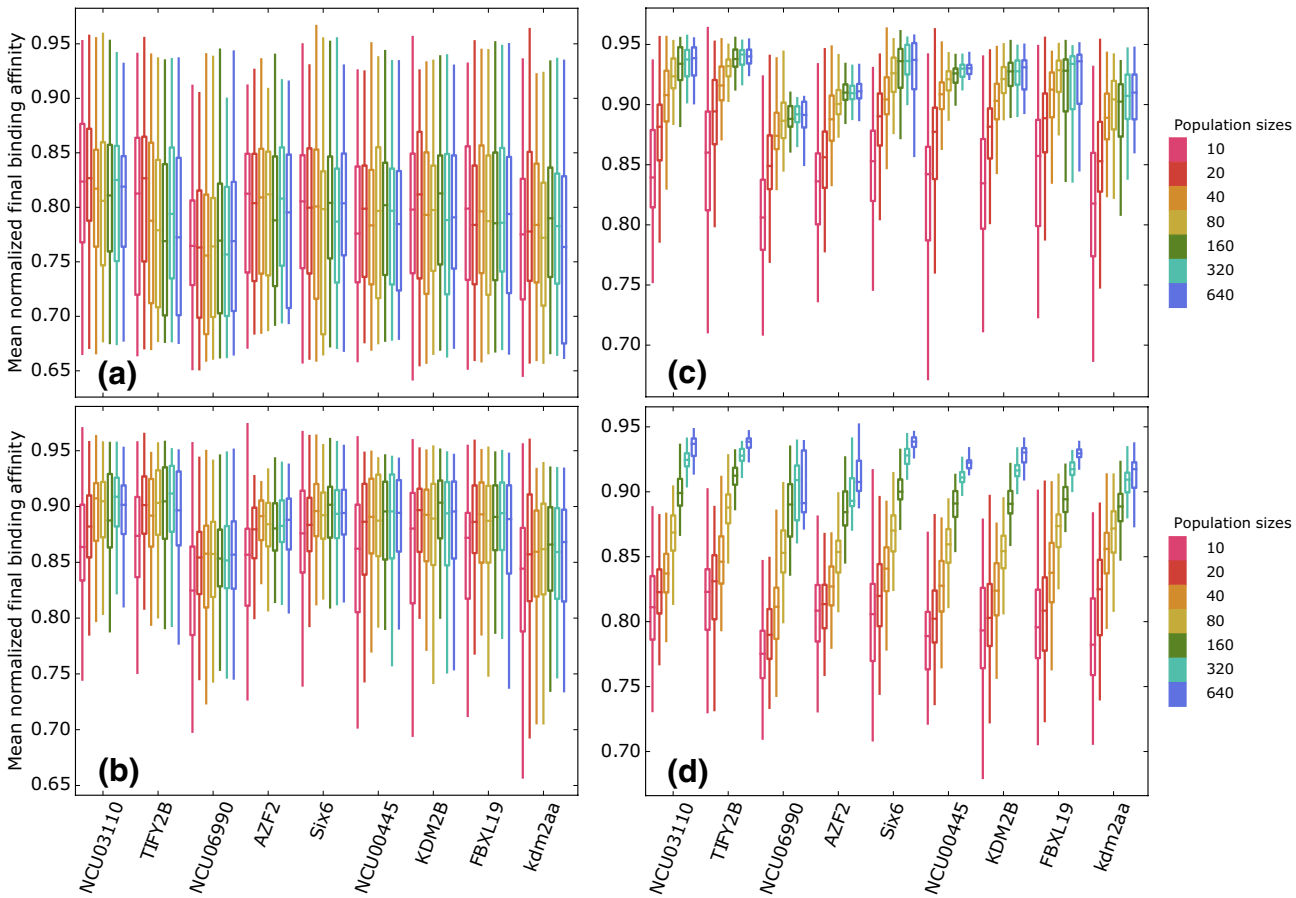

Fig. 5 Mean population binding affinity at the end of the simulations at constant $N\mu$. We randomly selected a sequence of low binding affinity to initialize each simulation, and then simulated 1000 generations of mutation and selection. We performed 100 replicate simulations for each population size at a fixed population mutation rate of **a** $N\mu = 0.01$, **b** $N\mu = 0.1$, **c** $N\mu = 1$, and **d** $N\mu = 10$ per sequence per generation (see "Methods"). Each box encloses the second and third quartiles of data from 100 replicates, the center line corresponds to the median, and the whiskers depict the minimum and maximum values obtained from any replicate, excluding outliers. Data are normalized by the maximum binding affinity in the landscape

and large population mutation rates. First, selection is more effective at fixing beneficial mutation in larger population. Second, and more importantly, the constant high population mutation rate has a negative effect on the ability to reach high mean affinity for smaller populations, but not for larger populations. A value of $N\mu = 10$ means that an average of ten new mutations are introduced into a population each generation. For a population of size 10, this means that at every generation all individuals are mutated. In a population of size 20, half of all individuals are mutated, but in a population of size 640, only a fraction of 0.016 of individuals are mutated. The high number of mutations overwhelms selection in small populations, making it difficult for small populations to follow a gradual affinity-increasing path.

Discussion

To understand the rate and limitations of organismal adaptation is a central to evolutionary biology (Lynch and Lande 1998; Allen 2000; Franklin and Frankham 1998; Stockwell et al. 2003; de Visser and Rozen 2005; Barrick et al. 2009; Wiser et al. 2013). Efforts to increase our understanding in this area can be divided into two major classes based on their methodology. The first uses theoretical approaches (Desai and Fisher 2007; Desai et al. 2007; Campos and Wahl 2010; Lourenço et al. 2013). Due to the complex interactions between different factors, such as mutation rate, changes in effective population size, recombination rate, etc., these approaches usually make many simplifying assumptions, which may not always hold in biological populations. The second class uses experiments (Lenski et al. 1991; Lenski and Travisano 1994; Elena and Lenski 2003; Lachapelle et al. 2015), which can examine a biological system in its full complexity. However, they provide limited knowledge about the important evolutionary mechanisms, such as the effects of mutations on a population's trajectories, and a fitness landscape's structure. In addition, the ability to replicate experiments and to test different parameters in them is limited.

Here, we used a system that bridges these two approaches. We simulated evolving populations on 957 empirical adaptive landscapes of transcription factor binding sites, and analyzed the evolutionary dynamics on nine such landscapes (Aguilar-Rodríguez et al. 2017). We considered the binding affinity between transcription factor and DNA sequences as a proxy for fitness. With such landscapes, we did not have to make ad hoc assumptions about epistatic interactions between mutations, about the distribution of fitness effects, or about landscapes structures. Additionally, we could study the effects of all mutations, and could examine the and mutational trajectories of populations in detail. We found complex interactions between mutation rate and population size, as described below.

Firstly, we found that at any mutation rate, larger populations are better at increasing their mean final affinity (Fig. 4a). This is intriguing, because at high $N\mu$, due to increased clonal interference, large populations hardly fix any mutations (Fig. S7); and because the substitution rate, especially that of beneficial mutations, is commonly treated as a measure of adaptation rate (Park and Krug 2007; Campos and Wahl 2009, 2010; Gossmann et al. 2012; Lanfear et al. 2013; Pokalyuk et al. 2013; Wong and Seguin 2015). The likely reason that substitution rate does not always determine adaptation is this: Larger populations are more diverse at any given time, and thus explore more sequences in a landscape than smaller populations, which means that they can find beneficial mutations more easily. The presence of multiple beneficial mutations in a population helps the population increase its mean binding affinity, even if no mutation is fixed. This is akin to a soft selective sweep (Losos et al. 2013, p. 472), where multiple beneficial mutations occur and increase their frequency in a population without any of them being fixed (Hermisson and Pennings 2005; Pennings and Hermisson 2006).

Second, we found that even at constant $N\mu$ and for different population sizes, when $N\mu$ is large enough, smaller populations fail to find adaptive peaks as effectively as larger populations (Fig. 5d). The reason is that at constant population mutation rates, smaller populations have a higher mutation rate per genotype than the larger populations. This higher mutation rate overwhelms the small populations and prevents them from following an affinity-increasing path.

Third, we found that sequence exploration and population diversity almost always depend on population size N, even when population mutation rates $N\mu$ are constant (Fig. S14). The only exception is when the population mutation rate $N\mu$ is so low that all populations explore equally few sequences (Fig. S9).

In sum, we found that smaller populations have no adaptive advantage over larger ones, even when $N\mu$ is constant for populations at different sizes, because smaller populations do not have higher mean final affinity at the end of our simulations. This observation holds regardless of landscape ruggedness, because the landscapes we studied varied in their ruggedness (Table 1). In theory, smaller populations could have several advantages on rugged landscapes (Rozen et al. 2008), such as higher chances of escaping local optima, and larger neutral neighborhoods, which could help them explore more sequences, some of which could boost their adaptation. However, these advantages did not lead to better adaptation on the landscapes studied here.

Our study has limitations, which can be alleviated in future work. Firstly, we studied clonal populations with no recombination. It would be interesting to see how

populations adapt on our landscapes in the presence of recombination, because recombination can dramatically affect evolutionary dynamics (Muller 1932; Evans 1986; Ochman et al. 2000; Zhang et al. 2002; Otto and Gerstein 2006; Cooper 2007). Moreover, we used the number of peaks as a measure of landscape ruggedness. It would be interesting to compare the topology of these landscapes with random landscapes used in previous studies, where smaller populations do have an adaptive advantage over larger ones. For example, Handel and Rozen (2009) constructed random landscapes with different numbers of peaks (ruggedness). They simulated populations evolving on the landscapes, and observed that on landscapes with a minimum amount of ruggedness, smaller populations can reach a higher final fitness, because they do not get trapped on local peaks. The conditions that provide an advantage to smaller populations in such theoretical studies may also exist in other empirical landscapes. A third limitation is that we have assumed a one-to-one relationship between binding affinity to a transcription factor and its fitness. However, the exact relationship between affinity and fitness is not known. Changes in this relationship could result in major changes in the structure of landscape (its ruggedness, number of peaks, and accessibility), and thus affect the results we have obtained.

In sum, our results show that in empirical adaptive landscapes, there are complex interdependencies between population size and mutation rate that affect evolutionary dynamics, especially at high $N\mu$, suggesting that conclusions from simplified models should be taken with caution.

Acknowledgements AW acknowledges support by ERC Advanced Grant 739874, by Swiss National Science Foundation Grant 31003A_172887, as well as by the University Priority Research Program in Evolutionary Biology at the University of Zurich. We are indebted to Joshua L. Payne for data analysis and helps and suggestions on the paper.

Compliance with ethical standards

Conflict of interests The authors declare that they have no competing interests.

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