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Tuning environmental timescales to evolve and maintain generalists

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Natural environments can present diverse challenges, but some genotypes remain fit across many environments. Such "generalists" can be hard to evolve, outcompeted by specialists fitter in any particular environment. Here, inspired by the search for broadly neutralizing antibodies during B cell affinity maturation, we demonstrate that environmental changes on an intermediate timescale can reliably evolve generalists, even when faster or slower environmental changes are unable to do so. We find that changing environments on timescales comparable with evolutionary transients in a population enhance the rate of evolving generalists from specialists, without enhancing the reverse process. The yield of generalists is further increased in more complex dynamic environments, such as a "chirp" of increasing frequency. Our work offers design principles for how nonequilibrium fitness "seascapes" can dynamically funnel populations to genotypes unobtainable in static environments.

evolution | time-varying environments | broadly neutralizing antibodies | bnAbs | generalists

Evolutionary outcomes are driven by environmental pressures, but environments are rarely static (1). In a changing environment, some genotypes—termed generalists—maintain a uniformly high fitness over time, even if they are not globally fit at any particular instant. A striking example is that of broadly neutralizing antibodies against HIV and other viruses—these antibodies maintain potency against the large diversity of viral strains that may arise in an infected individual over time (2–4). It is desirable for the immune system to select for generalist antibodies during B cell affinity maturation, a rapid evolutionary process (5), but generalists are often outcompeted by specialists that only bind particular viral strains.

Recent work has suggested that sequential vaccination with different viral antigens, rather than a single cocktail of those antigens, can better select for generalist antibodies during affinity maturation (6–9). This result is consistent with the broader idea that a time-varying environment can drive evolution out of equilibrium and into genotypes unevolvable in static environments (10–14). However, the broader principles underlying generalist selection by dynamic environments remain unknown. In particular, the interplay of environmental and evolutionary timescales and choices of correlated antigens generates a high-dimensional space of possible vaccination protocols. Hence, guiding principles are needed to find optimal protocols for evolving generalist genotypes.

Here, we take a phenomenological approach to design dynamic environments that select generalists. We analyze situations in which generalists are entropically disfavored or isolated by fitness valleys, and thus unevolvable in a static environment. We find that a dynamic environmental protocol can maximize the yield of generalists if the environment changes on the same timescale as the evolutionary transients of the population (i.e., on the timescale for allele frequencies to reach steady state. Consequently, switching antigens before antibody (Ab) populations have evolved to a steady state can dynamically funnel finite populations from specialists to generalists, even when faster or slower switching is unable to do so.

We understand these results in terms of a kinetic asymmetry between generalists and specialists. Environmental dynamics at the right timescale perturb specialist populations while leaving generalists relatively undisturbed. This asymmetry favors evolution from specialists to generalists without enhancing the time-reversed process. In contrast, faster or slower environmental dynamics may be cast into effective static fitness landscapes (15) and are thus unable to maintain a strong kinetic asymmetry between specialists and generalists. In this sense, the intermediate cycling mechanism studied here exploits a truly nonequilibrium evolutionary "seascape" (11, 13) with no static analog.

Our framework proposes protocols for evolving generalists, such as a "chirp" where the environment is cycled at an increasing frequency, and predicts optimal correlations between antigens to be used. Since we use a sufficiently abstracted model of B cell affinity maturation, our analysis might be adapted for other temporal evolution protocols [e.g., to avoid antibiotic resistance (16–18) and for cancer treatments (19, 20)].

Numerous works have studied evolution in time-varying environments, including in the context of evolving generalists (21–30). Relatively fewer works (15, 31–33) have analyzed the case of intermediate timescales where the environment changes before populations reach steady state, although these works do not consider the high-dimensional genotypic space and correlated environments studied here. In this broader sense,

Significance

Generalists, or jacks-of-all-trades, that are fit across diverse environments can be difficult to evolve since they may not be as fit as a specialist in any particular environment. Such generalists are sought in immunology, where broadly neutralizing antibodies that can detect a broad variety of strains of a rapidly changing virus like HIV are often hard to evolve. Here, we find that generalists are most easily evolved in the most poorly understood regime of evolution—where the environment changes are neither fast nor slow but on the same timescale as evolutionary response of the population. Our methods let us propose temporal vaccination protocols, such as a chirp, that exploit this highly dynamic regime of evolution.

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our work is a step toward a theory of evolution in time-varying environments with no separation of timescale between the evolutionary response of populations and environmental changes.

Results

We study evolution in fitness landscapes with multiple fitness peaks in antibody sequence space as shown in Fig. 1. During affinity maturation, each antigen (Ag) defines a distinct "environment" and thus, a distinct fitness function with distinct fitness peaks. In general, "specialist" fitness peaks for one antigen are not fitness peaks for other antigens. However, we assume one of these fitness peaks is approximately in the same location for all antigens. We first study evolution in the vicinity of this "generalist" fitness peak and ignore the larger landscape. True generalists are found at the intersection of these peaks across environments; the challenge in evolving such generalists is primarily entropic. We then consider evolution on the full landscape with multiple fitness peaks; now, fitness valleys can prevent the evolution of generalists. By exploiting mathematical constructions from spin glass theory, we systematically study the impact of the relative placement of fitness peaks or equivalently, correlation of features across antigens. In both cases, we model populations (e.g., the population of B cells across all germinal centers in an organism). We explain our results in terms of the rate at which a population of specialists evolves generalists in time-varying environments relative to the rate of the time-reversed processes from generalists to specialists.

Both models here have been used in the context of affinity maturation (9, 34–38), corresponding to different molecular models of antigen–antibody binding. While more extensive antibody–antigen binding assays (39–41) can clarify the situation for a particular virus like HIV, we remain agnostic to the issue here and study both cases since they might be relevant in different evolutionary contexts.

Entropically Disfavored Generalists. A basic difficulty in evolving generalists is that generalists are often far fewer in number than specialists. This is schematically shown in Fig. 2*A*, where specialists in each environment form a connected set of genotypes of similar fitness. The relatively few generalists, found at the intersection of such sets, can easily mutate into the more numerous specialists in any fixed environment.

We study the problem quantitatively in a simplified molecular model of antigen–antibody binding, as used for affinity maturation against HIV antigens. Antibodies bind to a single epitope, partially conserved across antigens $\eta = 1, 2$. An (binary) antibody sequence $\mathbf{x}$ binds to an epitope sequence $\mathbf{h}^{\eta}$ with an affinity given by an additive sum-of-sites model: $\mathbf{x} \cdot \mathbf{h}^{\eta}$. Antibodies that bind above a threshold T are assigned fitness $s(\epsilon - 1) > 0$, while those that bind weaker have fitness $-s < 0$. We take $1 < \epsilon < 2$, such that the average fitness of an antibody across antigens is negative.

Since the epitope is relatively but not entirely conserved across antigens, $\mathbf{h}^{\eta}$ values for different antigens are assumed to share a conserved region of length $L_c = 12$ but have a variable region of length $L_v = 7$ (9) (*SI Appendix*, Fig. S3 shows other choices). While based on a simple model of molecular binding, our results below apply broadly to the phenomenological description of specialists as connected islands of relatively uniform fitness, with no fitness barriers separating the generalists.

We simulate a finite population ($N \sim 500$) of antibodies in an environment that switches between antigens 1 and 2 on a timescale τ_{epoch} using a birth–death model (*SI Appendix*, section IC1), working in the limit of frequent mutations ($\mu N > 1$). Initializing a monoclonal population in a random specialist state for antigen $\eta = 1$, we monitor the fraction of generalists in the populations at late times (Fig. 2*D*), systematically varying the timescale of switching τ_{epoch}. Averaging over many simulation, we find that neither fast nor slow cycling is able to reliably elicit generalists in the population; however, an intermediate timescale of switching is able to do so (Fig. 2*B*).

We sought to understand the origin of this nonmonotonic behavior by examining population dynamics in the limits of fast and slow cycling. For fast cycling (i.e., small τ_{epoch}), the initial specialist population is repeatedly confronted with an antigen it cannot bind to. Without enough time to mutate into a generalist, purifying selection drives the population to extinction (Fig. 2 *D*, *i*). Consequently, the fraction of trials in which specialists evolve into generalists, $\chi_{s\to g}$, is low (Fig. 2*C*).

In fact, in this limit the dynamics of the population are effectively described by a static, average landscape, where the

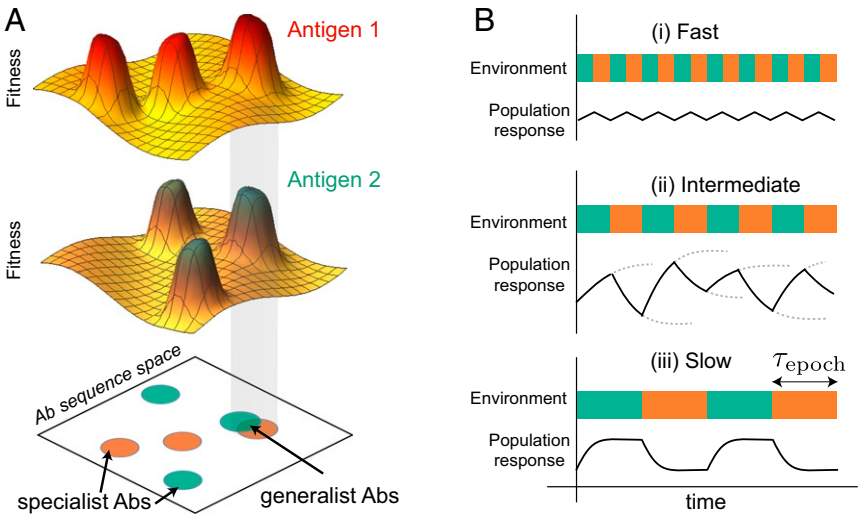

Fig. 1. Time-varying environments on intermediate timescales can dynamically funnel specialists to generalists. (*A*) Generalist antibodies that bind multiple antigens can be hard to evolve during B cell affinity maturation as compared with specialists that only bind one antigen. Specialists for an antigen can constitute a single (Fig. 2) or multiple islands (Fig. 4) in antibody sequence space. (*B*) We consider time-varying selection pressure on timescales (*i*) fast, (*ii*) intermediate, or (*iii*) slow relative to evolutionary transients. In the intermediate regime, the selection pressure (e.g., antigen) changes before evolutionary transients (dashed lines) are complete and a steady state is reached.

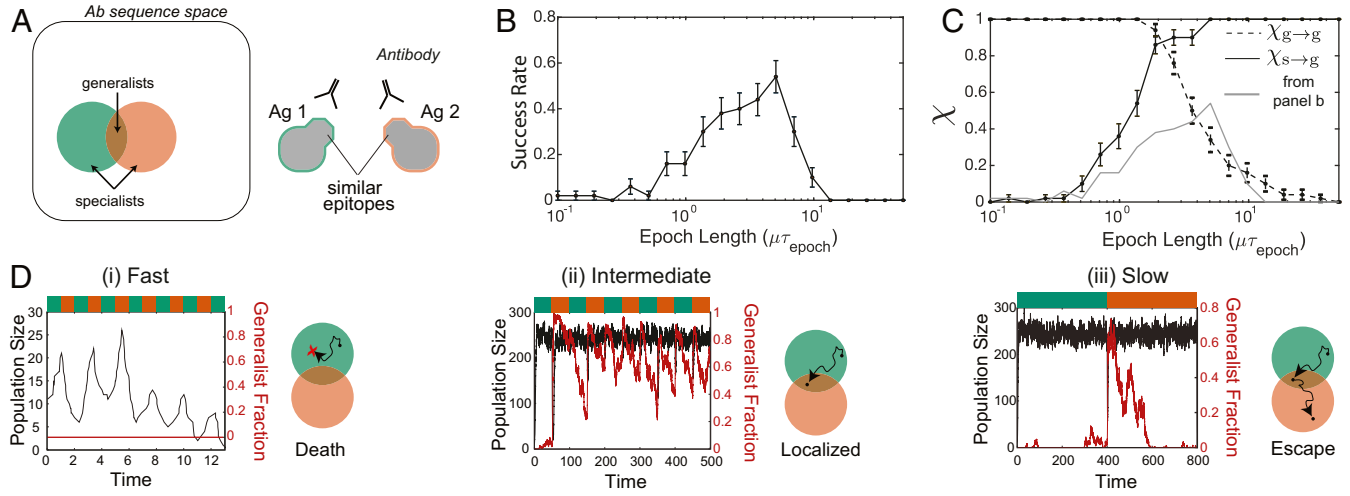

Fig. 2. Intermediate timescale cycling of antigens strikes a balance between evolving and maintaining rare generalist antibodies. (*A*) We assume that many specialist antibodies can bind each antigen at a partially conserved epitope; the text discusses the model. Generalists and specialists have similar fitness. (*B*) Cycling antigens at an intermediate timescale τ_{epoch} most reliably yields generalists in repeated $K = 500$ population simulations. (*C*) An initially specialist population is more likely to evolve generalists (higher $\chi_{s\to g}$) with slower cycling since (*D*, *i*) fast cycling typically leads to death of the entire population before any generalists are evolved. (*D*, *iii*) In contrast, slow cycling allows generalists to specialize; the probability of an initially generalist population that remains generalists, $\chi_{g\to g}$, falls with τ_{epoch}. (*D*, *ii*) Intermediate timescale switching allows sufficient time for generalists to evolve from specialists without providing enough time for generalists to specialize.

specialist has fitness $s(\epsilon - 2) < 0$. In this regime, we find that purifying selection drives the population to extinction when $s > \mu \log N$; *SI Appendix*, section IE has derivation and discussion of alternative cases where purifying selection is reduced.

On the other hand, for very slow cycling (large τ_{epoch}), any generalists that arise have enough time to specialize again by mutational drift (Fig. 2 *D*, *iii*). As a result, the fraction of an initially generalist population that stays generalists over an environmental cycle, $\chi_{g\to g}$, falls with τ_{epoch}, as seen in Fig. 2*C*.

Consequently, we find that intermediate timescale cycling strikes a balance: providing enough time for specialists to evolve into generalists (high $\chi_{s\to g}$) but not enough time for generalists to switch back to specialists again (high $\chi_{g\to g}$). In *SI Appendix*, section IE, we determine this regime to be

$$\tau_{\min} \sim \frac{1}{\mu} d_{\text{init}\to g} < \tau_{\text{epoch}} < \tau_{\max} \sim \frac{1}{\mu} \log(\Omega_g N), \qquad [1]$$

where $d_{\text{init}\to g}$ and Ω_g are the mutational distance of the initial naive repertoire from generalists and the number of generalist genotypes, respectively (*SI Appendix*, sections IA and IE).

Notably, an intermediate regime—that is, a cycling time τ capable of eliciting generalists—only exists when the number of generalists, Ω_g, is sufficiently large: $\log \Omega_g N > d_{\text{init}\to g}$. In contrast, when the number of specialists is large compared with the number of generalists and population sizes are small, it takes longer for generalists to evolve from specialists than to specialize again. In this regime, the entropic bias in sequence space driving generalists to specialists is large, and even fixed frequency cycling may not produce generalists.

Hence, we propose a dynamic protocol—a chirp—that can alleviate this tension between evolving generalists from specialists ($\chi_{s\to g}$), which requires slower cycling, and the ability to maintain a population of generalists ($\chi_{g\to g}$), which requires faster cycling. A chirp, shown in Fig. 3, starts with slow cycling and increases the cycling frequency over time. Such highly dynamic chirp protocols outperform any fixed frequency cycling protocol (Fig. 3*C*).

Generalists Isolated by Fitness Valleys. We now consider a more general case where fitness valleys separate viable genotypes, and specialists and generalists form disconnected sets in sequence space. Such models have been used to describe antibodies for influenza and malaria (34, 35, 37, 38) as well as describe RNA molecular fitness landscapes (42, 43). Rugged landscapes are relevant whenever mutations can act nonadditively: that is, when epistasis is present. Indeed, epistasis has been broadly observed for molecular phenotypes and was quantified recently for antigen–antibody binding interactions (44). In the affinity maturation context, such a model with multiple fitness peaks naturally arises if each antigen has multiple epitopes, with one epitope shared across antigens (37).

Here, we take a phenomenological approach that is agnostic to molecular details. Exploiting the construction of Hopfield (45) [or more generally, Gardner (46)], we construct fitness landscapes for each antigen with fitness islands around sequences corresponding to each epitope. In particular, consider P epitopes on each antigen $\eta = 1, 2$ that bind to antibody sequences $\mathbf{h}^{\eta}_{\alpha}$ ($\alpha = 1, \dots P$). The fitness of an antibody with sequence $\mathbf{x}$ confronted by antigen η is chosen to be $F^{\eta} \propto s \sum_{\alpha} \kappa_{\alpha} (\mathbf{x} \cdot \mathbf{h}^{\eta}_{\alpha})^{p}$ where we set $p = 2$ (the Hopfield model). This minimal construction produces fitness landscapes with peaks at the specified epitopes $\mathbf{h}^{\eta}_{\alpha}$, provided P is sufficiently small compared with sequence length L (47). Larger p creates more sharply defined fitness peaks. Finally, the weights κ_{α} are used to reduce the fitness of generalists relative to specialists in any one environment.

By making different choices for the epitopes $\mathbf{h}^{\eta}_{\alpha}$, we may construct fitness landscapes with arbitrary amounts of correlation between them. We begin by studying the minimal case where one epitope is shared between the two antigens, $\mathbf{h}^{1}_{1} = \mathbf{h}^{2}_{1}$, with the other epitopes being uncorrelated. Later, we relax this assumption. For our theoretical analysis, we assume that selection is strong and beneficial mutations are rapidly fixed, $sN \gg \mu N$, $sN \gg 1$; hence, fitness valleys between islands play a significant role.

We simulate a finite population of antibodies evolving via Moran dynamics. Initializing a monoclonal population at a specialist, we once again carried out simulations at different antigen

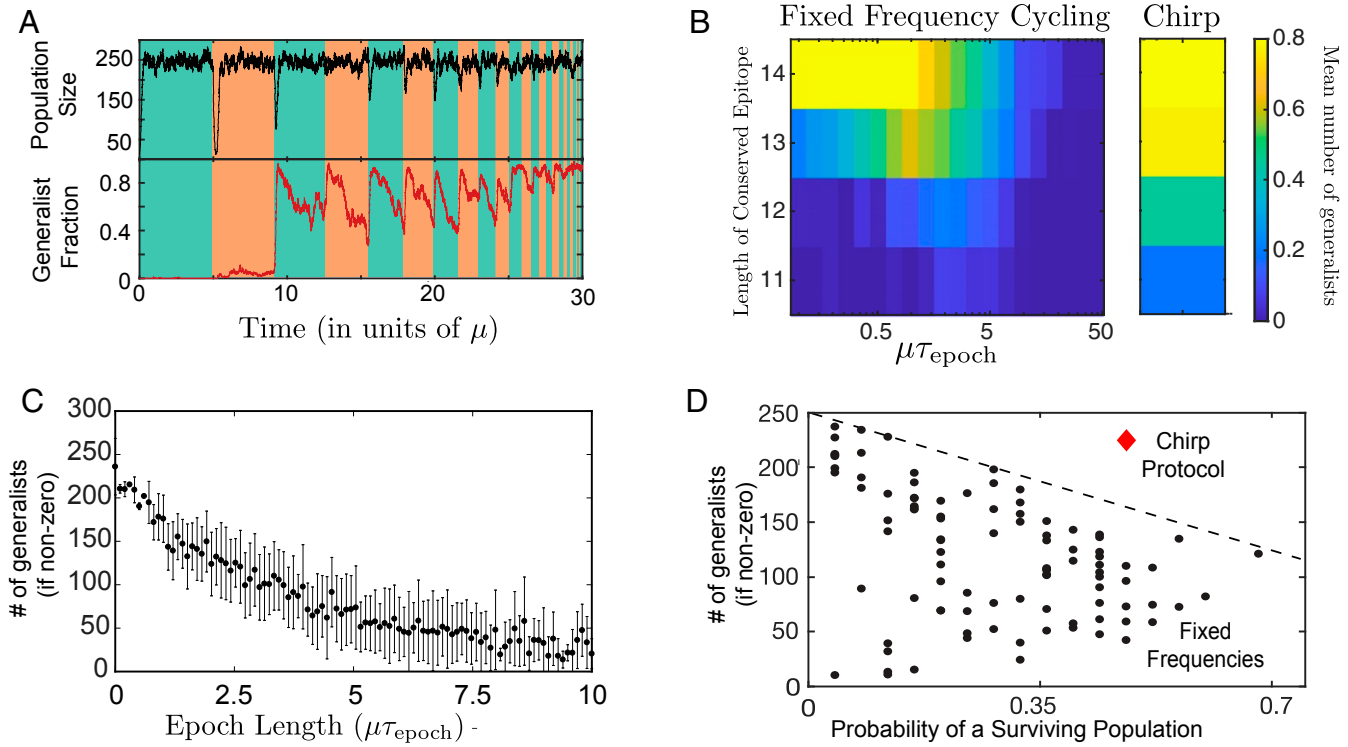

Fig. 3. Chirped cycling yields generalist populations more robustly than fixed frequency cycling. (*A*) We consider chirped protocols, where the cycling frequency is increased over time, $\tau_{\text{epoch}} \to \frac{5}{6}\tau_{\text{epoch}}$, after each epoch. (*B*) When the number of generalists Ω_g is reduced by reducing the length of the conserved epitope, the range of τ_{epoch} that yields generalists decreases. Chirped cycling, however, continues to recover generalists with little parameter tuning. (*C*) Fixed frequency cycling results in a tension between high number of generalists if the population survives (high for fast cycling) and population survival (high for slow cycling), resulting (*D*) in a tradeoff along a Pareto front. Chirped cycling breaks the tradeoff since slow cycling initially ensures population survival and fast cycling later on ensures that a high fraction of the surviving population is generalists.

switching times, τ_{epoch}, and quantified the fraction of generalists in the population at long times. As seen in Fig. 4*B*, an intermediate timescale of switching elicits generalists in the population. This is reminiscent of the entropic model above but for different underlying reasons.

Here, fast switching fails to produce generalists because populations stay confined to their initial position (48) (Fig. 4*D*). Rapid switching can be approximated by the averaged fitness landscape if the switching is fast enough and each individual has a fitness given by its fitness averaged over environments experienced in its lifetime. In such cases, new fitness peaks and valleys can be created as shown before for the spin glass-like fitness functions used here (47). Consequently, the population remains segregated away from the generalist genotypes by valleys of low fitness, and generalist acquisition, $\chi_{\text{s}\to\text{g}}$, is small. In practice, such populations stuck in a specialist genotype for extended time can go extinct in the presence of multiple antigens (9).

In contrast, at slower switching times, evolution in each environment can shift the population away from its initial position in the prior environment (Fig. 4*D*). As shown in *SI Appendix*, section IID, this requires at least time $\tau_{\min} \sim d_{12}/\mu$, where d_{12} is the typical mutational distance separating specialists across environments. Consequently, the population is forced to continually traverse genotype space. This continual evolution is by necessity stochastic (Fig. 4*F*), contingent on the random order of mutations that arise, as well as on any potential population variance. This cycling-induced mobility, augmented by stochasticity, allows the population to widely explore genotype space and find the generalist, and hence, $\chi_{\text{s}\to\text{g}}$ rises (Fig. 4*D*).

Importantly, upon evolving into generalists, environmental cycling no longer disturbs the population, as the fitness of generalist sequences does not appreciably change over time. Thus, cycling breaks the symmetry between specialists and generalists and enhances $\chi_{\text{s}\to\text{g}}$ without enhancing $\chi_{\text{g}\to\text{s}}$. Intuitively, intermediate cycling selectively "warms up" (i.e., increases stochasticity) specialist parts of sequence space, naturally leading the population to collect in "cooler" generalist sequences.

Cycling significantly slower than $\tau_{\min}$ is counterproductive. The cycling-induced leaks from specialists to generalists only occur due to environmental switches; hence, unnecessarily long τ_{epoch} only adds dead time with no additional population divergence.

In the meantime, as shown in *SI Appendix*, section IID, escape from generalists to specialists becomes significant on timescales of $(1/\mu)e^{\Delta F_g N}$ where ΔF_g is the fitness of the generalist relative to the fitness valley separating it from specialists; N is the population size. Refs. 48–50 have calculations of valley crossing rates in other parameter regimes. These considerations limit intermediate timescales favorable for evolving generalists:

$$\tau_{\min} \sim d_{12}/\mu \quad < \quad \tau_{\text{epoch}} \quad < \quad \tau_{\max} \sim (1/\mu)e^{\Delta F_g N}. \qquad [2]$$

As in the earlier model, if $\tau_{\min} > \tau_{\max}$, fixed frequency cycling may fail. In *SI Appendix*, Fig. S5, we find that chirped cycling can continue to recover generalists, even in these regimes. Chirp protocols produce generalists by alleviating the tension between $\chi_{\text{s}\to\text{g}}$ and $\chi_{\text{g}\to\text{g}}$ and do not require fine-tuning of parameters, as before in our models of entropically disfavored generalists.

Correlation between specialists. The effectiveness of this theoretical cycling mechanism depends on the correlation between specialists of $F^{(1)}$ and $F^{(2)}$, as demonstrated in a recent study of generalist evolution in tunably correlated landscapes (51): if specialists of $F^{(1)}$ and $F^{(2)}$ are similar or well within each other's

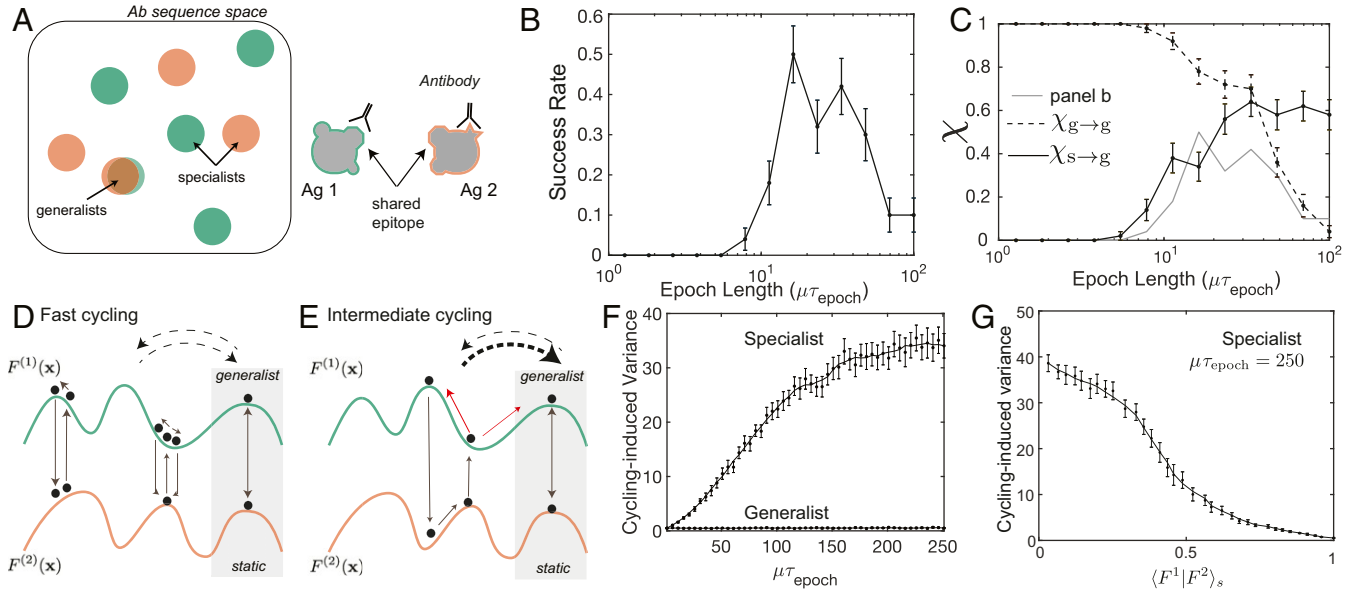

Fig. 4. Intermediate timescale cycling enhances specialist-to-generalist conversions across fitness valleys without enhancing the time-reversed process. (*A*) Antibodies that bind distinct epitopes on antigens (*Right*) form distinct specialist islands (*Left*) in sequence space, separated by fitness valleys. Generalists bind an epitope shared by antigens. (*B*) Cycling at intermediate τ_{epoch} most reliably yields generalists in a finite population $N = 100$ simulation. (*C*) Specialist-to-generalist transitions, $\chi_{s\to g}$, grow with τ_{epoch}, while the ability to retain generalists $\chi_{g\to g}$ falls (both measured after $n = 30$ cycles). (*D*) Fast cycling traps populations at fitness peaks near where they are initialized. (*E*) However, intermediate τ_{epoch} allows evolution between specialists. Such evolution introduces sequence variance even in initially monoclonal specialist populations (red arrows in *E*, quantified in *F*) but not for generalists. Such higher variance for specialists enhances specialists-to-generalists transitions but not the reverse process. (*G*) Cycling-induced variance is largest when specialists in $F^{(1)}, F^{(2)}$ are uncorrelated (low $\langle F^{(1)}|F^{(2)}\rangle_s$).

attractors, cycling will primarily cycle the population between specialists with minimal divergence into generalists. In contrast, given that generalists exist, least similarity between specialists of $F^{(1)}$ and $F^{(2)}$ would best enable reliable evolution of generalists. As shown in *SI Appendix*, section IIF, we can quantify relevant correlations by

$$\langle F^{(1)}|F^{(2)}\rangle_s \equiv \frac{c_{1,2}}{\sqrt{c_{1,1}\ c_{2,2}}},$$

where $c_{\eta,\gamma} = \frac{1}{LP}\sum_{\alpha,\beta\neq 1} \mathbf{h}_\alpha^\eta \cdot \mathbf{h}_\beta^\gamma$ excludes the generalist pattern $\mathbf{h}_1^1 = \mathbf{h}_1^2$. When $\langle F^{(1)}|F^{(2)}\rangle_s$ is high, cycling-induced variance is low (Fig. 4*G*). Consequently, the small asymmetry between $\chi_{s\to g}$ and $\chi_{g\to s}$ created by a single environmental cycle must be compounded by cycling multiple times; however, in practice, other considerations might limit the number of such cycles. Hence, our proposal requires specialists of $F^{(1)}$ and $F^{(2)}$ to be sufficiently uncorrelated (low $\langle F^{(1)}|F^{(2)}\rangle_s$).

Is cycling a practical strategy given correlations between specialist antibodies found during HIV infection and physiological parameters for population dynamics? We analyzed specialist and generalist antibody sequences collected from an HIV patient (39–41) (Fig. 5*A*). We constructed landscapes $F^{(1)}, F^{(2)}$ with fitness peaks at these observed specialist and generalist sequences following Gardner's construction (46); as detailed in *SI Appendix*, section IIA, we repeated the analysis for multiple choices of fitness functions and restriction of sequence data to variable regions.

Simulations of cycling environments $F^{(1)}, F^{(2)}$ constructed from the above sequence data evolved generalist antibodies, while simultaneous presentation of both antigens, a practical alternative to fast cycling (9), fails to produce such generalists (Fig. 5*B*). We then artificially shuffled antigen labels for antibodies, so that CH105 was considered an Ag2 specialist and CH186 an Ag1 specialist, and reconstructed $F^{(1)}, F^{(2)}$. This artificial shuffling significantly increased the correlation $\langle F^{(1)}|F^{(2)}\rangle_s = 0.78$ compared with the real data ($\langle F^{(1)}|F^{(2)}\rangle_s = 0.43$). Cycling is no longer effective in evolving generalists. We conclude that the low correlation between specialists in the real data is crucial

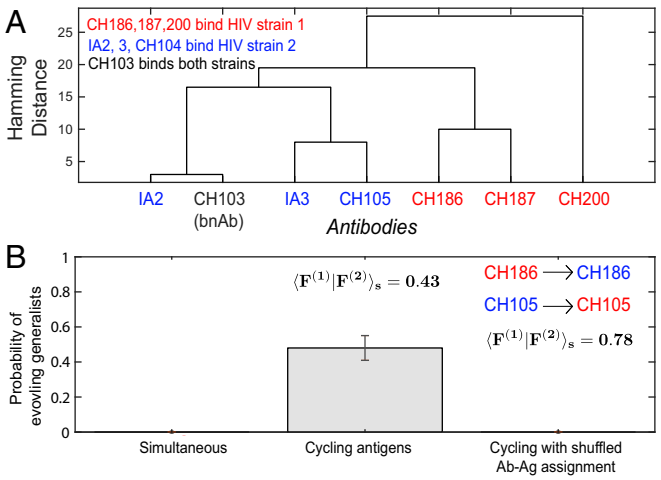

Fig. 5. Cycling between fitness landscapes constructed using antibody sequences from HIV patients yields generalists; however, cycling is less effective for artificially shuffled data with higher specialist correlation. (*A*) Sequence divergence of antibodies that bind two distinct strains (red and blue) of HIV. (*B*) Following Gardner (46), we constructed two fitness landscapes $F^{(1)}, F^{(2)}$ with peaks at red and blue sequences, respectively, and simulated evolution with realistic parameters (*SI Appendix*, section III). Generalists are evolved only if antigens are cycled. Cycling is less effective if we shuffle antibody–antigen assignment: CH105 now considered specialized for strain 2 (i.e., now red), CH186 for strain 1 (i.e., now blue). Shuffling artificially increases specialist correlation $\langle F^{(1)}|F^{(2)}\rangle_s$ from 0.43 to 0.78. See Dataset S1 for sequence and binding affinity data, taken from refs. 39–41.

for time-varying selection of generalists, in line with the result of ref. 51.

While our model here did not explicitly account for extinction, simultaneous presentation or fast cycling can cause most specialist B cells to perish, especially if many distinct antigens are used (*SI Appendix*, section IG). In this more realistic case, "chirped" cycling at increasing frequency as in Fig. 3 will alleviate the tension between $\chi_{s\to g}$ and $\chi_{g\to g}$ as demonstrated in Fig. 4*C*. That is, initial slow cycling allows the system to take advantage of cycling-induced stochasticity to find the generalist (the regime of high $\chi_{s\to g}$), while fast cycling toward the end forces the localization of the population to the generalist (high $\chi_{g\to g}$).

Discussion

We have shown that environmental changes on intermediate timescales can dynamically funnel populations from specialists to generalists. Alternative approaches to cycling antigens to vary selection pressures include "annealing" techniques in the selection pressure exerted on the germinal center to achieve breadth in antibody repertoires (30). Our quantitative framework suggests broad classes of experimental protocols such as "chirped cycling" that further enhance the evolution of generalists.

The relevant intermediate timescale here is that of evolutionary transients in a population—the environment must change slow enough for significant changes to accumulate but fast enough to prevent the population from settling to a steady-state distribution. This intermediate regime induces a highly dynamic fitness seascape with no effective static description (11). This dynamic regime has been relatively less explored (15) than limits where the environment changes much faster or slower than evolutionary transients and can be understood using effective static environments. Prior work has addressed the role of dynamic environments in crossing otherwise unpassable fitness barriers (25). Our work emphasizes the role of dimensionality, stochasticity, and correlations across environments in attaining generalists.

The principles developed here are broadly relevant whenever generalists are hard to evolve under simultaneous presentation of multiple selective pressures. For B cell affinity maturation, such a hurdle seems to result from specialists having negative fitness in such an averaged environment (9); thus, population death makes generalists hard to evolve. Other evolutionary contexts may not involve death; however, potential population death might be a necessary consequence of having sufficient purifying selection to eliminate specialists in favor of generalists. Such purifying selection is especially critical for processes like affinity maturation that terminate at finite population sizes; without death, such processes will terminate when specialists proliferate sufficiently and before generalists are evolved. *SI Appendix*, section IC2 has further discussion.

The simple models studied here ignore many ingredients present in B cell affinity maturation and other evolutionary processes in the natural world. For instance, affinity maturation starts from a specific naive antibody repertoire (52), and population response timescales can vary widely (53); our results require an ensemble of lineages to participate (9) and ignore clonal interference (54).

Nonetheless, our analysis has broad applicability since it relies only on a simple phenomenological characterization of how specialist and generalist genotypes are organized in sequence space. For example, while we used specific mathematical functions to model fitness landscapes, we related our results to phenomenological entropic and correlation measures of islands of high fitness. Further, our results are fundamentally linked to the fact that generalists experience less time variation of fitness than specialists, leading, for example, to higher stochasticity and mobility for the specialist parts of sequence space but not for the generalists. In this sense, the dynamic strategies presented here represent a broader class of nonequilibrium evolutionary strategies (11, 13) that can enhance the rate of transitions from specialists to generalists without enhancing the time-reversed processes.

Dynamic protocols have been investigated recently in other evolutionary contexts (e.g., in antibiotic resistance) where correlations in response to different antibiotics have been exploited to maximize cross-vulnerability (17, 55–57). While such cross-vulnerabilities have been primarily studied in the slow switching limit, switching antibiotics after a partial evolutionary response like that explored here might open a larger space of strategies.

While we have discussed dynamic environments as a prescriptive mechanism, natural environments are also dynamic (1). For example, coevolution of pathogens (58, 59), movement through spatially heterogeneous environments (60), and ecological changes (61, 62) can naturally create the intermediate timescale variations discussed here. The quantitative principles developed here suggest experiments to both understand and exploit this understudied regime of evolution with no separation of timescales between perturbation and response.

Data Availability Statement. All data discussed in the paper are available in *SI Appendix*, section IIJ1.

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