

Adaptive Ratchets and the Evolution of Molecular Complexity

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Biological systems have evolved to amazingly complex states, yet we do not understand in general how evolution operates to generate increasing genetic and functional complexity. Molecular recognition sites are short genome segments or peptides that can bind a cognate recognition target of sufficient sequence similarity, thereby inducing a target-dependent function. Such sites are simple, ubiquitous modules of sequence information, cellular physiology, and evolution. Here we show that recognition sites, if coupled to a time-dependent recognition target, can rapidly evolve to complex states with larger code length and smaller coding density than sites recognizing a static target. Such processes take place in a new mode of molecular evolution called an *adaptive ratchet*, characterized by asymmetric selection for code extensions and compressions. The underlying evolutionary model contains selection for recognition depending on the site-target binding affinity and mutations with a separation of timescales between changes of binding function and complexity changes. We show that ratchet evolution increases the number of mutational paths available for adaptive code changes, thereby accelerating the response to moving targets and facilitating refinement and innovation of recognition functions. We apply these results to the recognition of fast-evolving antigens by the human immune system. Our analysis shows how molecular complexity can evolve as a collateral to selection for function in a dynamic environment.

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I. INTRODUCTION

Darwin's principle states that evolution progresses by mutations and selection. This principle describes a dynamical pathway to organisms of high fitness, the efficacy of which is modulated by other, stochastic evolutionary forces. In microbial systems, for example, fitness can increase drastically even on timescales of laboratory evolution experiments; these processes are carried by multiple mutations and occur repeatedly in parallel-evolving populations [1]. In a similar way, increasing complexity appears to be a ubiquitous feature of biological evolution. Already the onset of evolution requires the complexity of biopolymers to grow above a minimum required for autocatalytic replication [2,3]. In macro-evolution, a prominent example is the increase of genome and regulatory complexity in the transition from prokaryotic to multicellular eukaryotic organisms [4]. Nevertheless, the evolutionary forces behind complexity have remained controversial [5]. From a classical Darwinian perspective, organisms evolve complex functions because they adapt to complex environments; that is, complexity increases by positive selection. For example, complex gene regulatory networks have been argued to evolve in response to complex environments in adaptive,

incremental steps [6]. However, producing and maintaining complex features requires a physiological machinery. This generates a fitness cost of complexity, the relative weight of which is often unknown. Recent genomic and functional experiments show that molecular complexity can arise by neutral evolution and be maintained by selection, even if there is no discernible fitness benefit [7–15]. On much shorter timescales, humans and other vertebrates evolve complex antibody repertoires during their lifetime; these antibodies encode a memory of past infections and serve in the immune defense against future infections by similar antigens [16,17]. Is there a universal force that drives evolving systems to higher complexity, much as selection acts to increase fitness, or are the explanations for complex outcomes as complex as the subject matter? This question has no satisfactory answer to date. We lack a general definition of complexity and a unifying theory to predict when complexity is expected to evolve.

In this paper, we study the evolution of molecular complexity for recognition sites contained, for example, in DNA or proteins. By binding a cognate molecular target, such sites perform specific regulatory, enzymatic, or signaling tasks that feed into larger functional networks of the cell. Recognition sites are simple, ubiquitous, and often quantitatively understood units of molecular function, making them an ideal subject of our study. Most importantly, a single molecular phenotype, the binding affinity to the recognition target, characterizes the functionality of a site and is the target of natural selection. At the sequence level, recognition sites are approximately digital units of molecular information. The binding specificity of the target is often summarized in a sequence motif that lists the preferred nucleotides or amino acids at

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each position of the recognition site. Functional sites are biased towards sequences matching the target, which typically generates a relative information, or entropy loss compared to background sequence, of order one bit per unit of functional sequence [18,19].

The code length of a recognition site can serve as a measure of its molecular complexity. This measure is quite variable across different classes of recognition sites. Strong transcription factor binding sites in prokaryotes have typical lengths ~ 10 – 20 base pairs; these sites are compact functional units close to the minimal code length required for function. This lower bound is comparable to the algorithmic complexity of a computer program predicting recognition of the target [20,21]. Weak binding sites, often acting together with adjacent sites, spread this information to larger code length and lower coding density, defined as the number of matches per unit of length. Prokaryotic RNA polymerase binding sites contain about 40 base pairs, a subset of which carries a highly conserved recognition code [22,23]. More complex transcription units containing low-affinity sites are common in eukaryotes [24]. Similarly, human immune recognition can be carried by compact T cell receptors binding to antigenic peptides of code length < 10 amino acids; more complex immune responses can involve multiple antibody lineages interacting with their cognate antigenic sites [25]. Immune recognition systems have recently been discussed as targets of host-pathogen coevolution and eco-evolutionary control [26–28]. These examples contain the main question of this paper. When is recognition encoded in sites close to minimal code length needed for specificity? Conversely, when do we find longer and fuzzier sites, where higher molecular complexity is associated with the same function?

Recognition sites and their complexity are the outcome of long-term evolution. These dynamics have three kinds of elementary steps. Point mutations of the recognition site update individual letters and may change the coding density; site extensions and compressions also change the code length. Moreover, in most instances, the target of recognition evolves itself. Target changes can be independent of the recognition function, which is often the case for proteins with multiple functions. They can also be directed to change recognition; for example, the evolution of transcription factors can update regulatory networks [29–35]. On shorter timescales, a prominent example is escape mutations of antigens from immune recognition by their hosts. Natural selection acts on the recognition function, which depends on the target binding affinity ΔG , but is blind to details of site and target sequence. This has an important consequence: at a given value of ΔG , the molecular complexity of sites does not carry any direct fitness benefit. Moving targets generate time-dependent selection and, if the rates of change are mutually in tune, induce a continual adaptive evolution of their cognate sites. Coevolving sites can maintain recognition, albeit at a lower affinity ΔG than for static targets, because the adaptation of the site sequence always lags behind the evolution of the target [36]. Together, these dynamics shape sequence architecture, information content, and functionality of recognition sites.

Here we describe the coevolution of recognition sites and their target in a minimal, analytically solvable, yet biophysically realistic model that links binding energetics to fitness.

We first analyze stationary states of evolving recognition sites interacting with evolving recognition targets, which act as driving force for changes in code and site architecture. Two key parameters, the driving rate and the cost of complexity, delineate different modes of evolution and set recognition code length and coding density. We find two evolutionary pathways towards molecular complexity: sites become long and fuzzy when the cost of complexity is low or when the target of recognition is moving sufficiently fast. In both cases, the evolution of complex sites does not follow the classical Darwinian scenario of adaptation in a static fitness landscape. Building on this analysis, we establish a central result of this work: adaptive ratchets, a new evolutionary mechanism for the growth of molecular complexity fueled by sustained driving and continuous shifts of the adaptive landscape. Finally, we discuss an example of complexity-dependent fitness in immune recognition and epitope evolution.

II. MODEL FOR RECOGNITION FUNCTION

Recognition sites work in a complex molecular environment where functional binding competes with spurious interactions in a heterogeneous background. A central requirement for functionality is therefore discrimination: accidental binding to off-target sequences must be sufficiently rare. This imposes a lower bound on the information content of recognition motifs, which can be defined as the Kulback-Leibler distance between the ensemble of target-bound sites and background sequence; see Appendix B. In addition, functional sites must be target-bound at physiological concentrations of the target molecule, which sets a lower bound on the target binding affinity ΔG . These general biophysical constraints motivate the minimal recognition model used throughout this study [37]: sites have a code length $\ell \gtrsim 10$ and a sequence alphabet of q equiprobable letters (with $q = 4$ for nucleotides and $q = 20$ for amino acids); each sequence position has one matching letter and $(q - 1)$ mismatches with a reduced free energy difference $\Delta\Delta G/(k_B T) = \epsilon_0 \sim 1$. In this model, we can characterize recognition sites by just two summary variables: the code length ℓ and the number of matches, k , or equivalently, the coding density $\gamma = k/\ell$.

Under conditions of thermodynamic equilibrium, recognition of the target depends on the reduced binding affinity, or free energy gap, $\Delta G/(k_B T)$. Equilibrium binding is a justified assumption whenever the target binding kinetics relaxes fast compared to the physiological timescales of target density variation [38]. The recognition probability R is then a standard Hill function,

$$R(\gamma, \ell) = H(\Delta G - \Delta G_{50}), \quad (1)$$

as given in Appendix A. The functionality threshold (half-binding point) ΔG_{50} is set by the requirement of sufficient discrimination of functional sites from off-target sites in background sequence. For simplicity, we assume that background sequence can be modeled as equi-distributed random sequence, and we measure binding affinity with respect to a zero point of average random sequence. In the minimal model, the reduced binding affinity then takes the simple form $\Delta G/(k_B T) = (\gamma - \gamma_0)\ell\epsilon_0$, where $\gamma_0 = 1/q$ is the density of matches in random sequence. In line with empirical data, we

use a threshold value $\Delta G_{50}^{\min} \sim 10 k_B T$ for compact binding sites under physiological conditions. In the minimal model, functional sites have at least $k_0 \equiv \Delta G_{50}^{\min} / (\epsilon_0 k_B T)$ matches more than average random sites, which requires a minimal code length $\ell_{\min} = k_0 / (1 - \gamma_0)$. In a heterogeneous sequence background, ΔG_{50} acquires a weak dependence on the site length ℓ , as derived in Appendix A. We use a ℓ -independent approximation in the analytical calculations and the full form $\Delta G_{50}(\ell)$ in the numerical analysis.

III. FITNESS MODEL

In a biophysical fitness model, the recognition-dependent fitness of a site is taken to be proportional to the recognition probability, $F_r(\gamma, \ell) = f_0 R(\gamma, \ell)$. This type of fitness model has been broadly used for regulatory binding sites, protein interaction sites, and protein folding [12,39–45]. Importantly, selection in a biophysical fitness landscape acts on the recognition code only through an intermediate biophysical phenotype, here the binding free energy ΔG . This is an important prerequisite for the evolution of complexity: functionality of recognition imposes a lower bound on the code length, $\ell \geq \ell_{\min} \sim \Delta G_{50}^{\min}$ as discussed above, but does not generate an upper bound. The map from phenotype to fitness, which is determined by Eq. (1), has specific nonlinearities (epistasis) that can be traced in the evolutionary statistics of recognition sites [46,47].

Other components of cell physiology, including genome replication and competing molecular functions, are expected to constrain the code length of recognition sites. Here we describe this constraint by a minimal model with a linear fitness cost of genomic real estate, $F_c = -c_0 \ell$.

Together, recognition fitness and cost of complexity define our minimal fitness model,

$$F(\gamma, \ell) = F_r(\gamma, \ell) + F_c(\ell) = f_0 R(\gamma, \ell) - c_0 \ell. \quad (2)$$

This model has two system-specific parameters: the fitness effect of recognition, f_0 , and the complexity cost parameter c_0 , which will be used to discriminate modes of complexity evolution. The fitness landscape $F(\gamma, \ell)$ determines the selection acting on changes of the recognition site sequence. For example, a mutation from k to $(k+1)$ matches at constant code length has the selection coefficient $s_\gamma = F_r(\gamma + 1/\ell, \ell) - F_r(\gamma, \ell)$. As will become explicit in the following, most functional recognition sites are found on the upper branch of the Hill function just above the half-binding point (Fig. 1). In this region, we can approximate the Hill function (1) by an exponential function, which shows that selection is proportional to the recognition error, $s_\gamma = \epsilon_0 f_0 \Delta R$ with $\Delta R = 1 - R$.

IV. EVOLUTIONARY DYNAMICS

We study the evolution of recognition sites in the low-mutation regime, where site sequences are updated sequentially by substitutions in an evolving population. The rates of these substitutions depend on the corresponding mutation rates and on selection coefficients scaled by an effective population size N , as given by Haldane's formula [48] (Appendix A). Our minimal model contains three types of

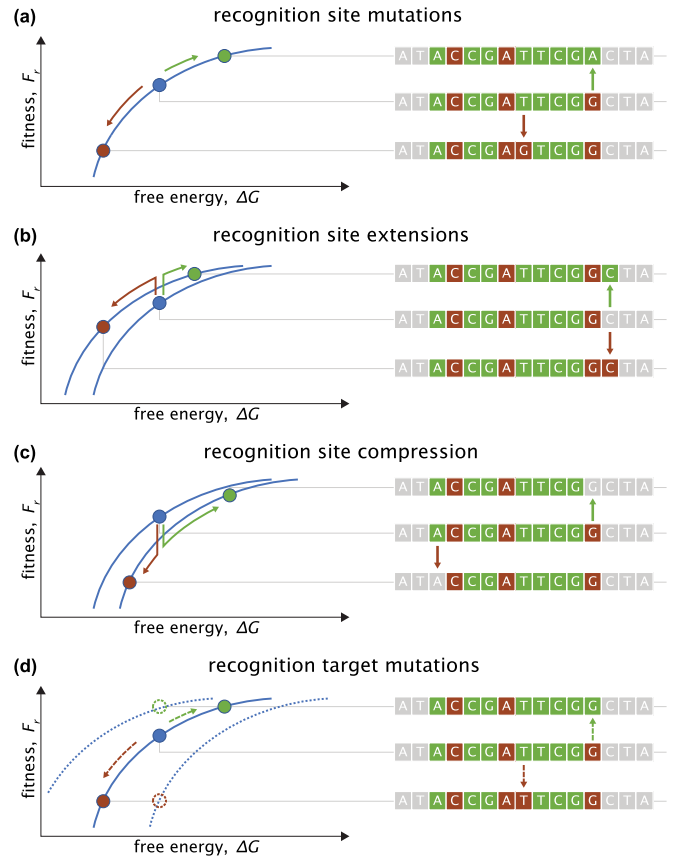


FIG. 1. Evolution of molecular recognition. Evolutionary steps change the recognition code (green squares: target matches, purple squares: mismatches). These changes affect the free energy of recognition, ΔG , and the recognition fitness, F_r (green arrows: beneficial changes, purple arrows: deleterious changes). (a) Point mutations of a recognition site add or remove one letter matching the target, $k \rightarrow k \pm 1$, at constant code length ℓ . (b) Site extensions and (c) compressions add or remove one unit of length, $\ell \rightarrow \ell \pm 1$. (d) Recognition target mutations make the fitness of a given recognition site explicitly time-dependent (dotted lines) and act as effective site mutations with neutral rates (dashed arrows).

updating steps. First, point mutations of the site sequence occur at a homogeneous rate μ per unit of length [Fig. 1(a)]. Hence, a recognition site with k matches and $\ell - k$ mismatches has a total rate $\mu_+(\gamma, \ell) = \mu \ell \gamma \gamma_0 / (1 - \gamma_0)$ of beneficial mutations and a total rate $\mu_-(\gamma, \ell) = \mu \ell (1 - \gamma)$ of deleterious mutations (Appendix A). These changes have selection coefficients $\pm s_\gamma(\gamma, \ell)$; the resulting substitution rates $u_\pm(\gamma, \ell) = \pm s_\gamma(\gamma, \ell) \mu_\pm(\gamma, \ell)$ depend on the fitness landscape $F(\gamma, \ell)$ introduced above.

Second, the recognition target sequence can change by extension and compression steps, which include one unit of sequence into the recognition site or exclude one unit from recognition [Figs. 1(b) and 1(c)]. These changes, which affect the architecture of recognition, are assumed to occur at a much lower rate than point mutations, $\nu \ll \mu$, and to be generated by external factors independent of the recognition function. Both of these features will emerge as essential for the evolutionary dynamics of complexity emerging in this system. Extensions add a random letter of background sequence to

the site, whereas compressions exclude a random letter of the existing site sequence. In the minimal model, beneficial and deleterious extensions occur with total rates $v_{++}(\gamma, \ell) = v\gamma_0$ and $v_{+-}(\gamma, \ell) = v(1 - \gamma_0)$, respectively; beneficial and deleterious compressions occur with total rates $v_{-+}(\gamma, \ell) = v(1 - \gamma)$ and $v_{--}(\gamma, \ell) = v\gamma$. The basic rate parameter v is assumed to be length-independent and the same for extensions and compressions. This is without loss of generality, because any bias between extension and compression rates can be absorbed in an effective cost parameter c_0 . Furthermore, the evolutionary dynamics of any realistic receptor-target interface is subject to multiple steric constraints. Such constraints are strongest for internal positions of the recognition site. An extension or compression step at an internal position will induce mismatches at adjacent positions, generating a large fitness cost. In the minimal model, we account for these constraints by restricting changes to the flanks of the recognition target. The compression and extension rates, together with the corresponding selection coefficients $s_{\pm\pm}(\gamma, \ell)$ given by the fitness landscape $F(\gamma, \ell)$, determine the total substitution rates for extensions and compressions, $v_+(\gamma, \ell)$ and $v_-(\gamma, \ell)$ (Appendix A).

Third, the recognition target sequence changes at a basic rate $\rho = \kappa\mu$ per unit of length [Fig. 1(d)]. This rate is assumed to be comparable to the point mutation rate μ and to be generated by external factors independent of the recognition function. Target changes generate a *fitness seascape* describing explicitly time-dependent fitness of a given recognition site sequence [49]. In the low mutation regime, target changes act as additional effective mutations that change the free energy ΔG and the recognition R with respect to the moving target. Therefore, the selective effects of target mutations are again given by the fitness landscape $F(\gamma, \ell)$ but their rates are not: beneficial and deleterious changes occur at neutral rates $\rho_+(\gamma, \ell) = \rho\ell\gamma\gamma_0/(1 - \gamma_0)$ and $\rho_-(\gamma, \ell) = \rho\ell(1 - \gamma)$, respectively, generating a net degradation of recognition and driving the adaptive evolution of recognition sites. Examples for recognition target sequence mutations are mutations in DNA binding domains of transcription factors. Experiments have shown that the effects of mutations in the DNA binding domain of LacI in *E. coli* can be reduced to a change in binding energy, similar to mutations in the DNA itself [50].

Clearly, the minimal evolutionary model is a broad approximation to the evolutionary dynamics of any specific receptor-target interface. The model neglects many details of actual molecular evolution processes that are not important for conclusions of this paper. Three model features turn out to be crucial for what follows. First, the sequence mutation dynamics does not introduce any bias towards higher complexity. Second, we assume a separation of mutational timescales: architectural changes in the recognition interface occur at a much lower rate than point mutations in the recognition sequence or its target ($v \ll \mu$). This reflects the fact that changes in site length or modular architecture require insertions, deletions, or duplications; all these changes have lower mutation rates than point mutations and are often deleterious. Third, because selection on the recognition function depends only the binding affinity phenotype ΔG , the model does not introduce any explicit fitness benefit of sequence complexity. We will show that complexity can emerge nevertheless:

as a collateral of selection for function in a nonequilibrium dynamical pathway—an evolutionary ratchet. We will first characterize the complexity of stationary states in different parameter regimes of the evolutionary model, then derive specific dynamical pathways towards these states.

V. STATIONARY STATES OF RECOGNITION EVOLUTION

In an evolutionary steady state, recognition sites keep catching up with a moving target and maintain time-independent ensemble averages of binding affinity and recognition probability. In general, non-equilibrium processes in a nonlinear fitness landscape are quite complicated. Remarkably, however, the steady state of the minimal recognition model can be computed analytically for arbitrary target evolution rates $\rho = \kappa\mu$. We write the steady-state distribution of coding density and code length in an ensemble of parallel-evolving populations as the exponential of an evolutionary potential, $Q(\gamma, \ell) = \exp[\Psi(\gamma, \ell)]$. Furthermore, by weighting the distribution $Q(\gamma, \ell)$ with the recognition function $R(\gamma, \ell)$, we can define a distribution of functional codes, $Q_f(\gamma, \ell)$.

Because recognition site mutations occur at a much higher rate than extensions or compressions, the coding density relaxes to a stationary state between any two code length changes and the potential takes the form $\Psi(\gamma, \ell) = \Psi(\gamma|\ell) + \Psi(\ell)$. Given this separation of timescales, the analytical solution proceeds in two steps. First, we build the coding density component $\Psi(\gamma|\ell)$ from ratios of substitution rates for beneficial code changes and their reverse deleterious changes at constant length, $[u_+(\gamma, \ell) + \rho_+(\gamma, \ell)]/[u_-(\gamma + 1/\ell, \ell) + \rho_-(\gamma + 1/\ell, \ell)]$. Given an approximately Gaussian form of $Q(\gamma|\ell)$, we evaluate conditional averages in saddle point approximation, $\bar{\gamma}(\ell) \approx \gamma^*(\ell) = \arg \max_{\gamma} Q(\gamma|\ell)$ (see Appendix B, Fig. 9). Second, we write the length component $\Psi(\ell)$ in terms of effective substitution rates for site extensions and their reverse compressions, $\bar{v}_+(\ell)/\bar{v}_-(\ell + 1)$, evaluated at the conditional saddle point $\gamma^*(\ell)$. The stationary average of the code length is then evaluated from the global maximum point (γ^*, ℓ^*) . The full analytic solution is given in Appendix B, Eqs. (B1)–(B8).

A. Weak- and strong-driving regimes

Stationary distributions $Q(\gamma, \ell)$ for different cost parameters and driving rates are shown in Figs. 2(a) and 2(c); global maximum points (γ^*, ℓ^*) are marked by dots for various sets of complexity costs and driving rates. These plots display three evolutionary modes: First, recognition sites coupled to a static target at unit cost of complexity ($\kappa = 0$, $2Nc_0 \sim 1$) evolve in a compact sequence state. Second, sites coupled to a static target ($\kappa = 0$) evolve increasing code length and decreasing coding density with decreasing cost of complexity, $2Nc_0$ [Fig. 2(a)]. Third, recognition sites coupled to a dynamic target at unit cost ($2Nc_0 = 1$) evolve increasing molecular complexity with increasing driving rate, κ [Fig. 2(c)]. The analytical distributions $Q(\gamma, \ell)$ [Figs. 2(a) and 2(c)] are in accordance with time averages over individual trajectories obtained by simulation [Figs. 2(b) and 2(d)].

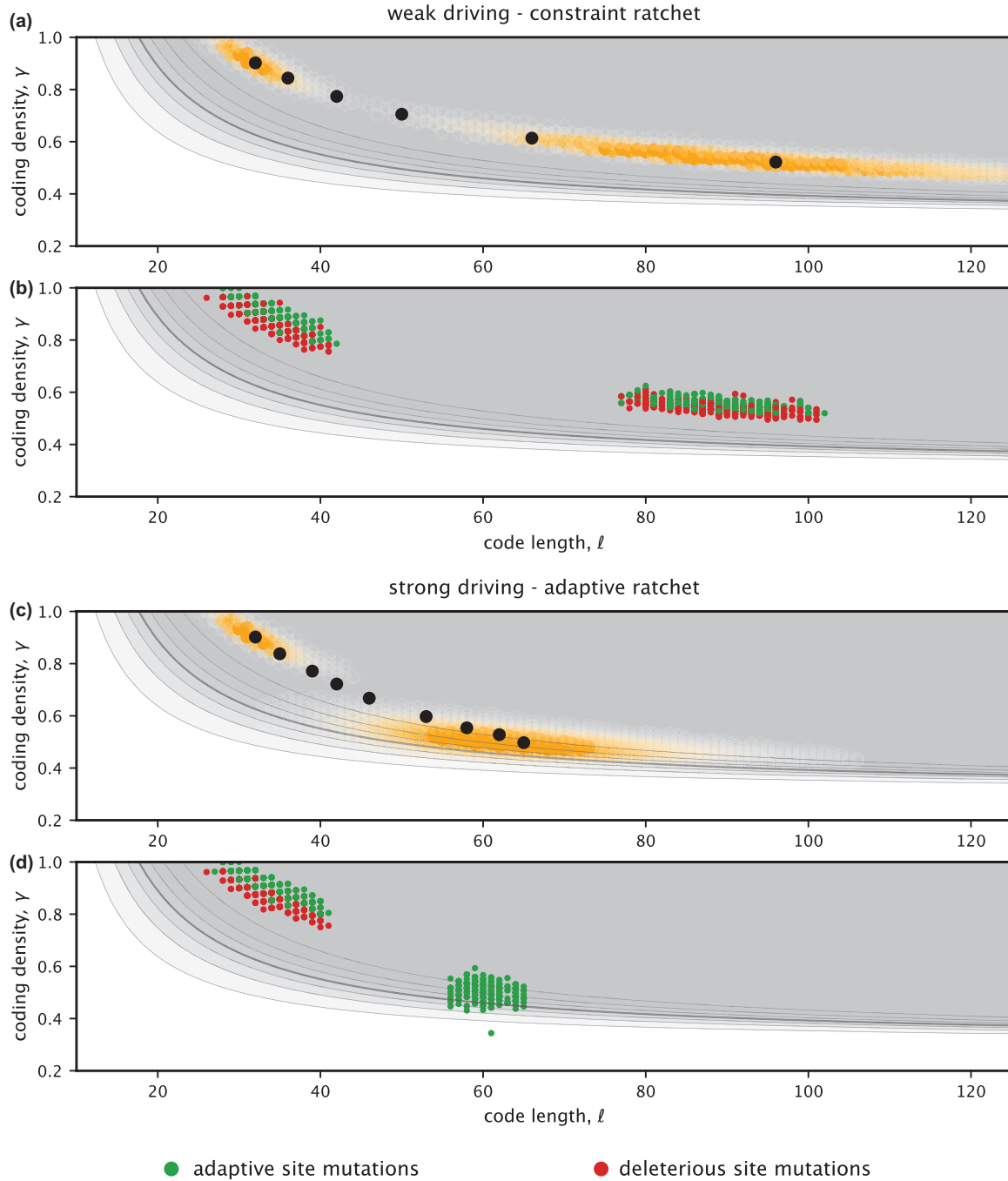


FIG. 2. Statistics and evolutionary trajectories of recognition sites. (a), (c) Joint stationary distributions of code length and coding density for functional sites, $Q_f(\gamma, \ell)$, for different values of the scaled complexity cost, $2Nc_0$, and the driving rate, $\kappa = \rho/\mu$. Contours show the recognition fitness landscape $F_r(\gamma, \ell)$, which is steepest at half-binding, $\gamma = \gamma_{50}(\ell)$ (thick contours). (a) Weak-driving regime: distributions $Q(\gamma, \ell)$ for $2Nc_0 = 1$ (left) and $2Nc_0 = 0.1$ (right) at $\kappa = 0$, maximum points $(\gamma^*, \ell^*)(\kappa=0, 2Nc_0)$ (black dots) for $2Nc_0 = 1, 0.7, 0.5, 0.35, 0.2, 0.14, 0.1$ (left to right). (c) Crossover to the strong driving regime: distributions $Q(\gamma, \ell)$ for $\kappa = 0$ (left) and $\kappa = 15$ (right) at $2Nc_0 = 1$, maximum points $(\gamma^*, \ell^*)(\kappa, 2Nc_0=1)$ (black dots) for $\kappa = 0, 0.2, 0.5, 1, 2, 5, 10, 15, 30$ (left to right). (b), (d) Recognition site mutations (adaptive: green, deleterious: red) on individual evolutionary paths $(\gamma, \ell)(t)$ for (b) $2Nc_0 = 1$ (left) and $2Nc_0 = 0.1$ (right) at $\kappa = 0$, and (d) $\kappa = 0$ (left) and $\kappa = 30$ (right) at $2Nc_0 = 1$. Zooms into these paths are shown in Appendix C, Fig. 11. Other evolutionary parameters: $\gamma_0 = 1/4$, $k_0 = 10$, $\epsilon_0 = 0.5$, $2Nf_0 = 1000$, $v/\mu = 0.01$, $N = 10^5$.

The full phase diagram of these evolutionary modes is shown in Fig. 3(a). In this diagram, black contour lines give the stationary code length ℓ^* as a function of the model parameters $(\kappa, 2Nc_0)$. The two evolutionary modes of complex sites are distinguished by the relative excess of beneficial over

deleterious substitutions, $\alpha = (u_+ - u_-)/(u_+ + u_-)$. For static or slow-moving targets, the stationary state has approximate detailed balance of beneficial and deleterious code changes [$\alpha^* \approx 0$; cf. Fig. 2(b)], indicating evolution close to equilibrium. At strong driving, most code changes are

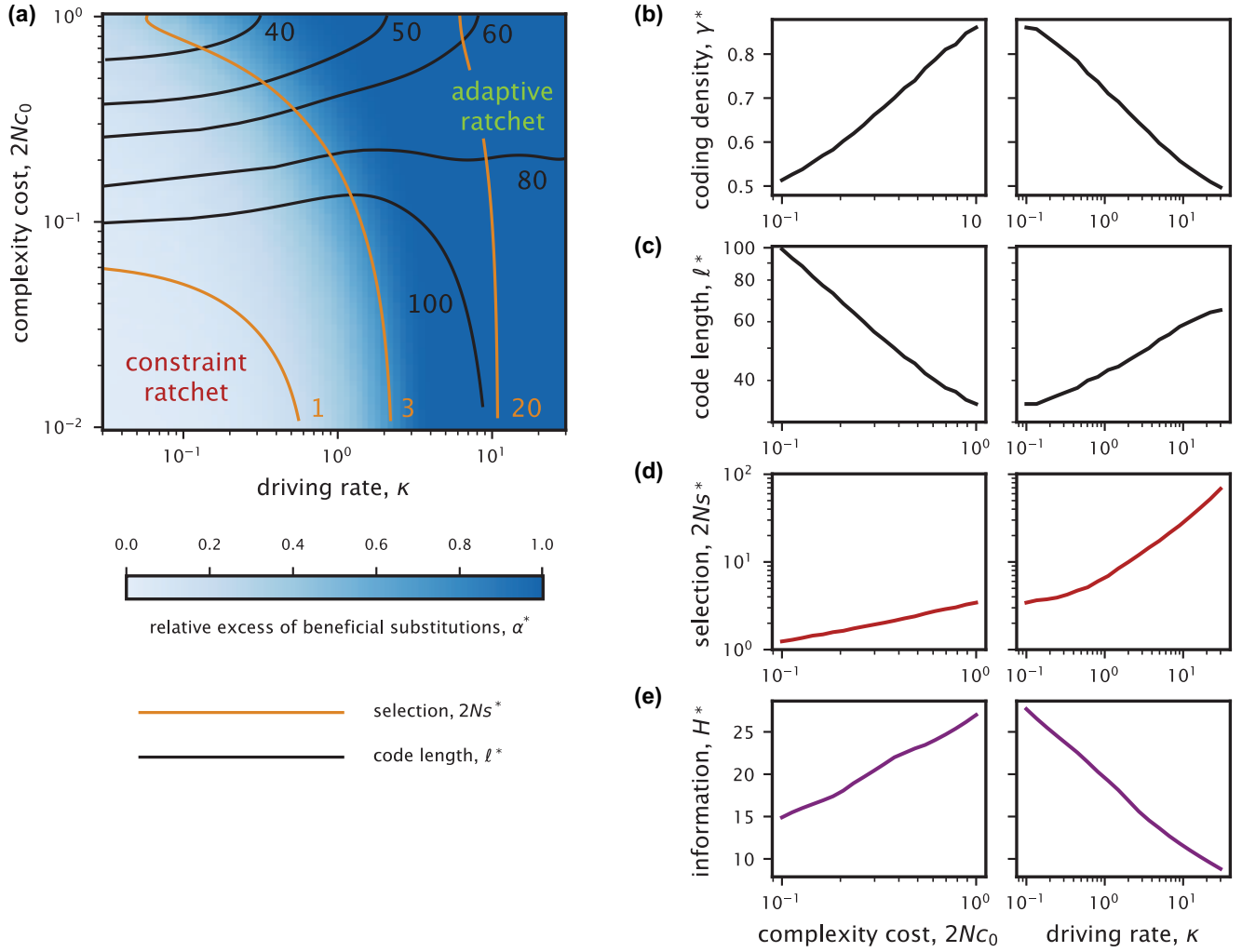


FIG. 3. Modes of evolution at stationarity. (a) Phase diagram of evolutionary modes. This diagram shows three stationary-state observables as functions of the model parameters ($\kappa, 2Nc_0$): the code length ℓ^* as a measure of molecular complexity (black contours), the selection on code changes, $2Ns^*$ (orange contours), and the relative excess of beneficial code substitutions, α^* (color shading). We distinguish three evolutionary modes: compact sites ($\kappa \lesssim \tilde{\kappa}(2Nc_0)$, $2Nc_0 \sim 1$), constraint ratchets ($\kappa \lesssim \tilde{\kappa}(2Nc_0)$, $2Nc_0 \ll 1$), and adaptive ratchets ($\kappa \gtrsim \tilde{\kappa}(2Nc_0)$). Compact sites and constraint ratchets evolve under weak driving and are close to evolutionary equilibrium, which is characterized by an approximate balance of beneficial and deleterious substitutions ($\alpha^* \ll 1$); adaptive ratchets evolve under strong driving and far from equilibrium ($\alpha^* \gtrsim 1$). The crossover to adaptive ratchets involves an increase of selection that freezes out deleterious code changes; the cost-dependent crossover line $\tilde{\kappa}(2Nc_0)$ can be estimated from the condition $2Ns^* \sim 3$. (b)–(e) Left: crossover to complexity at weak driving; ($\kappa = 0$, decreasing cost $2Nc_0$). Right: crossover to complexity at unit cost (increasing driving rate κ , $2Nc_0 = 1$). Stationary-state observables: (b) coding density, γ^* ; (c) code length, ℓ^* ; (d) scaled selection coefficient, $2Ns^*$; (e) information content, H^* . Other evolutionary parameters: $\gamma_0 = 1/4$, $k_0 = 10$, $\epsilon_0 = 0.5$, $2Nf_0 = 1000$, $v/\mu = 0.01$, $N = 10^5$.

adaptations to target mutations [$\alpha^* \approx 1$; cf. Fig. 2(d)]. Here enhanced code complexity is associated with strong deviations from evolutionary equilibrium, i.e., with an adaptive process. A cost-dependent driving rate, $\tilde{\kappa}(2Nc_0)$, marks the crossover between these modes.

Analytical approximations tailored for the weak- and strong-driving regime provide simple expressions for the stationary-state site architecture, (γ^*, ℓ^*) , which are derived in Appendix B. In the weak-driving regime, coding density and code length scale as powers of the cost, $\ell^*/k_0 \sim 1/(\gamma^* - \gamma_0) \sim \sqrt{(1 + \kappa)/(2Nc_0)}$. Hence, molecular complexity can be enhanced by a reduction in the long-term effective population size, for example, in the transition from prokaryotes to multicellular eukaryotes, as suggested previously for genome

evolution [9]. Similarly, in the strong-driving regime, (γ^*, ℓ^*) is the solution of a quadratic equation and depends only on the ratio $\kappa/(2Nc_0)$. In both regimes, the analytical closure is in accordance with the full solution (Appendix B).

In the following subsections, we describe several biological characteristics of stationary-state evolution in the weak- and strong-driving regime; analytical expressions for all of these observables are given in Appendix B.

B. Opposite evolution of code length and coding density

We recall that code length and coding density set the functionality of evolved sites, $R(\gamma, \ell)$, and determine the position on the fitness landscape $F_r(\gamma, \ell)$, as given by Eqs. (1) and

(2). As shown by our solution, the global stationary-state parameters (γ^* , ℓ^*) are related,

$$\ell^* = \frac{k_0}{\gamma^* - \gamma_0} + O(\log(\epsilon_0 f_0 / s_\gamma^*)). \quad (3)$$

This relation has a simple biological interpretation: across all evolutionary regimes, mutations and genetic drift push recognition sites close to the edge of functionality. In our biophysical model, this edge is around the half-binding point ΔG_{50} , given by an excess number of k_0 matches over random sequence (thick contours in Fig. 2). Equation (3) shows that a typical population of recognition sites settles on the upper branch of the recognition Hill function close to the half-binding point. This implies that γ^* and ℓ^* depend in opposite ways on the model parameters $2Nc_0$ and κ [Figs. 3(b) and 3(c)]. Hence, any increase in code length goes along with a decrease in coding density.

C. Driving-dependent selection and fitness

As shown in Fig. 3(d), the scaled selection coefficient for code changes, $2Ns_\gamma^*$, increases in a monotonic pattern from weak to strong driving: weakly driven sites evolve under weak to marginal selection ($2Ns_\gamma^* \lesssim 1$), strongly driven sites under strong selection ($2Ns_\gamma^* \gg 1$). In other words, stronger driving pushes recognition sites closer to the edge of functionality. In the approximately exponential recognition fitness landscape, this implies a proportionally increased fitness drop, $f_0 - F_r(\gamma^*, \ell^*) = s_\gamma^* / \epsilon_0$, which reflects the cost of driving caused by the time lag between recognition target changes and adaptive responses of recognition sites.

This selection pattern is captured by our regime-specific analytical approximations. In the weak-driving regime, maximizing the potential $\Psi(\gamma|\ell)$ yields a balance condition for coding density, $2Ns_\gamma^*(\ell) = [\gamma^*(\ell) - \gamma_0](1 + \kappa) / (\gamma_0(1 - \gamma_0))$ (Appendix B). This says again that individual code letters evolve under at most marginal selection, $2Ns_\gamma^* \lesssim 1$; the highest values $2Ns_\gamma^* \sim 1$ are reached in the regime of compact sites with high coding density ($2Nc_0 \sim 1$, $\gamma^* \sim 1$). In the equilibrium limit ($\kappa = 0$), this relation is accordance with general results for mutation-selection-drift-equilibria of quantitative traits [51–53]. With an appropriately rescaled effective population size, it holds even if clonal interference is the dominant noise in sequence evolution [54]. Marginal selection implies that the stationary-state coding density γ^* can get close to but cannot reach 1, which explains why functional recognition interfaces typically retain substantial sequence variation.

Strong driving introduces an important qualitative shift of the evolutionary pattern. For $\kappa \gtrsim \tilde{\kappa}(2Nc_0)$, maintaining recognition requires substantial selection on individual code letters. In this regime, deleterious mutations are suppressed and the evolutionary process consists of recognition target changes and adaptive code changes [Figs. 2(d) and 9]. Again by maximizing $\Psi(\gamma|\ell)$, we obtain the strong-driving balance condition for coding density, $2Ns_\gamma^*(\ell) = [\gamma^*(\ell) - \gamma_0]\kappa / (\gamma_0(1 - \gamma^*(\ell)))$ (Appendix B). Selection strength and adaptative rate are now tuned to driving, which enables recognition sites to maintain a stationary functional state at different driving rates.

Importantly, the weak- and strong-driving balance conditions are highly universal: the scaled selection coefficient $2Ns_\gamma^*(\ell)$ does not depend on the fitness amplitude f_0 or the effective population size N .

D. Molecular complexity and evolutionary information

To characterize evolved recognition sites in information-theoretic terms, we evaluate the Kulback-Leibler distance between conditional code distributions under selection and at neutrality, $H(\ell) \equiv H(Q_{\gamma|\ell} | Q_{\gamma|\ell}^0)$ (Appendix B). In saddle-point approximation, this distance takes the form $H(\ell) = S(\gamma^*(\ell), \ell) - S(\gamma_0, \ell)$, where $S(\gamma, \ell)$ is the entropy (log density) of sequence states at given code length and coding density. Thus, $H^* \equiv H(\ell^*)$ measures the evolutionary information, or entropy decrease, generated by natural selection on functional site sequences in the stationary state.

How is information related to molecular complexity? As shown in Fig. 3(e), these quantities depend in opposite ways on the model parameters: long and fuzzy sites not only distribute evolutionary information onto more sequence positions but also evolve a reduced total information content. In such sites, the combinatorics of distributing $\gamma\ell$ matches onto a site of total length ℓ generates additional sequence entropy. Our analysis quantifies the notion of fuzziness by an information theory measure. Below, we discuss an important consequence: reduced information facilitates the evolution of altered or additional functions.

VI. RATCHET EVOLUTION OF CODE LENGTH

What kind of evolutionary mechanism generates the evolution of longer recognition codes? Consider first the long-term fitness effects of length changes: longer codes improve recognition but come with a cost of complexity. This tradeoff can be displayed by an effective fitness landscape for code length, $\bar{F}(\ell) = \bar{F}_r(\ell) + F_c(\ell)$, where the recognition component $\bar{F}_r(\ell)$ is defined by averaging $F(\gamma, \ell)$ over the conditional stationary distribution $Q(\gamma|\ell)$ [Fig. 4(a); Appendix C]. This component shows a diminishing-return nonlinearity; its balance with a linear component $F_c(\ell)$ leads to a κ -dependent maximum of the total fitness $F(\ell)$.

Importantly, however, code length changes do not happen alone, but they are always coupled to changes of the coding density. Moreover, given the separation of mutational timescales ($\nu \ll \mu$), the evolution of coding density settles to its stationary state before the next update of code length happens. These characteristics generate a fundamental asymmetry: code length extensions recruit new functional sequence units from random sequence, compression steps cut into existing functional sequence. Hence, extensions take place under net positive selection for recognition, compressions include a net constraint from locked-in target matches. These effects are described by average selection coefficients for extensions, $s_+(\ell)$, and for compressions, $s_-(\ell)$ (see Appendix C). Figure 4(b) shows these selection coefficients as arrows on the backbone of the evolutionary potential $\Psi(\ell)$; Fig. 4(c) displays their dependence on the code length. This reveals an asymmetry that defines an evolutionary ratchet operating by selection: $s_+(\ell)$ and $s_-(\ell + 1)$ do not sum up to 0, and both

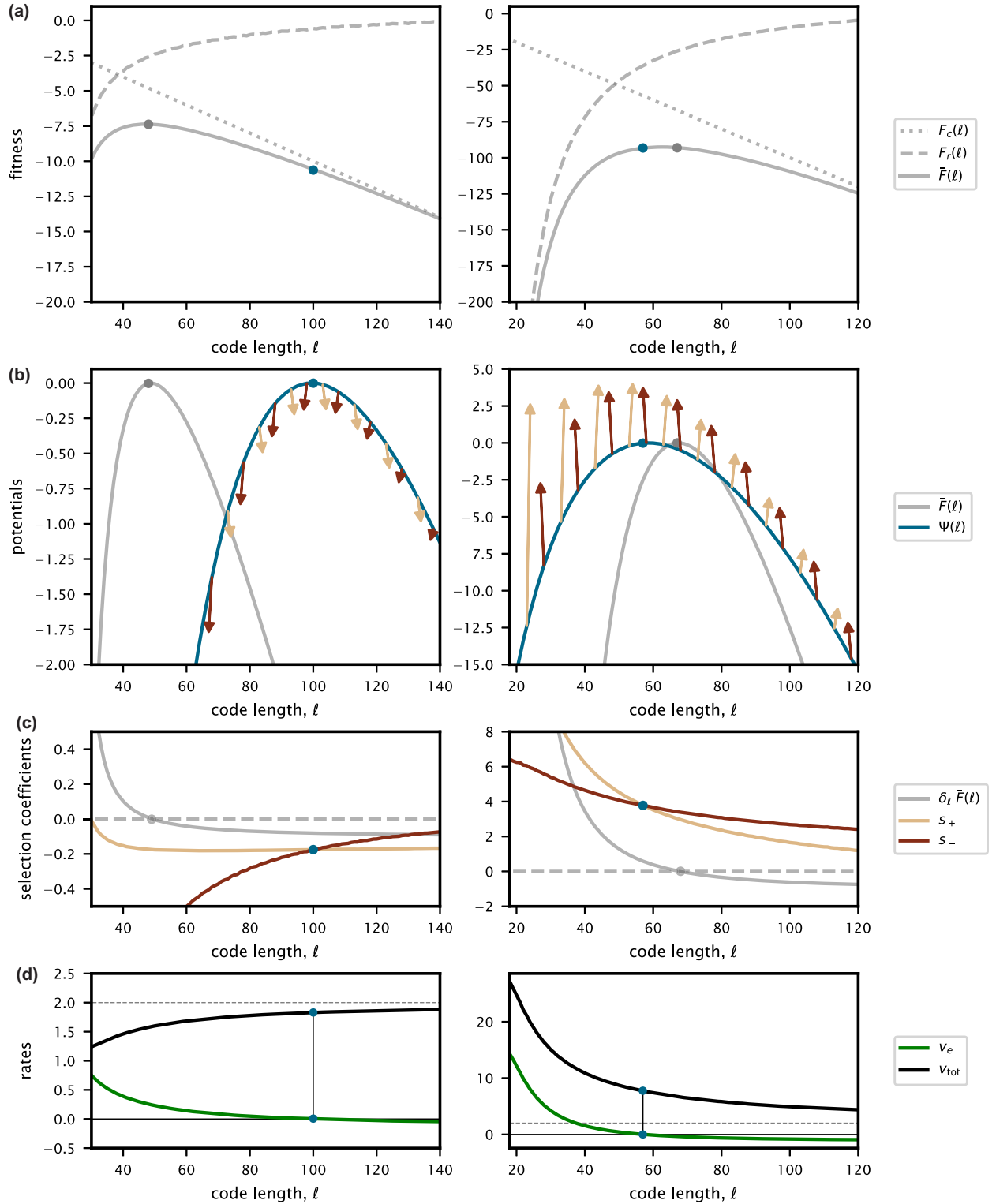


FIG. 4. Ratchet Evolution. Left: constraint ratchet at low driving and low cost ($\kappa = 0.1, 2Nc_0 = 0.1$). Right: Adaptive ratchet at strong driving and unit cost ($\kappa = 10, 2Nc_0 = 1$). (a) Effective fitness landscape for code length, $\bar{F}(\ell)$ (solid), recognition component, $\bar{F}_r(\ell)$ (dashed, plotted relative to the maximum recognition fitness), and complexity cost, $F_c(\ell)$ (dotted). (b) Evolutionary potential for code length, $\Psi(\ell)$. Effective selection coefficients for length extensions, $s_+(\ell)$ (beige), and length compressions, $s_-(\ell)$ (brown), are drawn as arrows on the backbone of the evolutionary potential. The code length ℓ^* given by the maximum of $\Psi(\ell)$ (blue dots) differs from the maximum point of the fitness landscape (gray dots). The fitness landscape $\bar{F}_r(\ell)$ is shown for comparison; $\bar{F}_r(\ell)$ and $\Psi(\ell)$ are plotted relative to their maximum points. (c) Effective selection coefficients, $s_+(\ell)$ (beige) and $s_-(\ell)$ (brown), compared to the slope of the effective fitness landscape, $\bar{F}(\ell + 1) - \bar{F}(\ell)$ (gray). (d) Total rate of length substitutions, v_{tot} (black), and elongation rate, $v_e(\ell)$ (green), compared to the neutral rate 2ν (gray dashed). Other evolutionary parameters: $\gamma_0 = 1/4, k_0 = 10, \epsilon_0 = 0.5, 2Nf_0 = 1000, \nu/\mu = 0.01, N = 10^5$.

selection coefficients differ from the increment in the effective fitness landscape $\bar{F}_r(\ell)$ [gray lines in Fig. 4(c)]. This establishes another main result of the paper: complexity evolves in a ratchet that clicks by different rules for forward and backward moves.

The asymmetry of selection has a drastic consequence: the evolution of code length cannot be described by any fitness landscape that depends only on ℓ . This would require $s_+(\ell) = -s_-(\ell + 1) = F(\ell + 1) - F(\ell)$; the arrows in Fig. 4(b) would collapse onto their backbone curve.

Next, to capture the dynamical effects of ratchet evolution, we evaluate the total substitution rate for code length, $v_{\text{tot}}(\ell) = \bar{v}_+(\ell) + \bar{v}_-(\ell)$, and the net elongation rate, $v_e(\ell) = \bar{v}_+(\ell) - \bar{v}_-(\ell)$. Depending on the complexity cost parameter c_0 and the driving rate κ , we find two different modes of ratchet evolution.

A. Weak driving, constraint ratchets

In the weak-driving regime ($\kappa \lesssim \tilde{\kappa}(2Nc_0)$), we find $v_{\text{tot}} < 2\nu$, indicating a net constraint on code length evolution. At small ℓ , length extensions are approximately neutral because new positions initially sample random sequence, whereas compressions are under constraint in functional sequence units. These asymmetric rates generate a net elongation rate $v_e(\ell) \lesssim \nu$ [Figs. 4(c) and 4(d), left]. Hence, an initially compact recognition site ratchets up slowly to a cost-dependent stationary code length ℓ^* . This length overshoots the point of maximal fitness defined by the landscape $\bar{F}(\ell)$ [Fig. 4(c)], showing again that selection is not given by gradients of this landscape, Appendix C). Importantly, because elongation in this regime relies on nearly neutral extensions, constraint ratchets produce complex recognition sites only at very low complexity cost, $2Nc_0 \ll 1$ but become ineffective at higher cost [Fig. 3(a)]. On larger scales of functional units, comparable ratchet mechanisms have been invoked to explain the complexity of RNA editing [7], cellular machines [8,10], and protein domains [11,13–15]. In contrast to the adaptive ratchet described below, this mechanism operates near equilibrium and relies on nearly neutral forward steps rather than strong directional selection.

B. Strong driving, adaptive ratchets

In the strong-driving regime [$\kappa \gtrsim \tilde{\kappa}(2Nc_0)$], all length changes become adaptive, i.e., take place with positive selection coefficients $s_+(\ell)$ and $s_-(\ell)$. In other words, length extension and compression steps increase recognition, responding to preceding target mutations. This generates fast ratchets with total click rates $v_{\text{tot}}(\ell)$ and small- ℓ elongation rates $v_e(\ell)$ well above their neutral values [Figs. 4(c) and 4(d), right]. At stationarity, beneficial extensions balance against beneficial compressions. The balance condition for the code length now reads $v_{++}(\ell) = v_{--}(\ell)$, where both length extensions (adding a match) and compressions (removing a mismatch) are beneficial (Appendix C, Fig. 10). It determines a universal click rate

$$\frac{v_{\text{tot}}^*}{2\nu} = 2Nc_\gamma^* = \frac{C(\gamma^* - \gamma_0)}{\gamma_0(1 - \gamma^*)} \kappa, \quad (4)$$

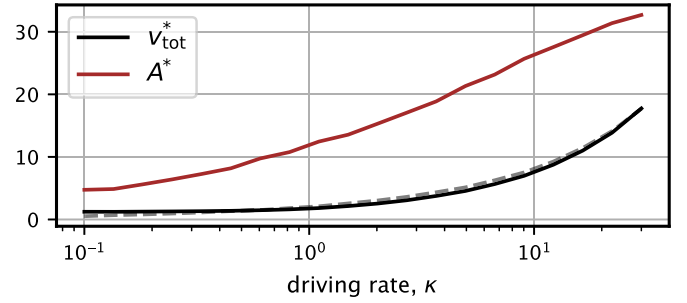


FIG. 5. Adaptive Tinkering. Average click rate (total rate of length substitutions) of adaptive ratchets in the stationary state as a function of the driving rate, $v_{\text{tot}}^*(\kappa)$; numerical solution (black solid) and analytical approximation by Eq. (4) (black dashed). Number of adaptive paths, $A^*(\kappa)$, as given by Eq. (5) (red). Other evolutionary parameters: $2Nc_0 = 1$, $\gamma_0 = 1/4$, $k_0 = 10$, $\epsilon_0 = 0.5$, $2Nf_0 = 1000$, $\nu/\mu = 0.01$, $N = 10^5$.

in the analytical approximation for the stationary state, where C is a constant of order 1 (Fig. 5, Appendix C). This relation shows that adaptive ratchets are powered by recurrent target mutations: the faster the target evolves, the faster the ratchet clicks. In contrast to constraint ratchets, adaptive ratchets generate molecular complexity also in the face of substantial costs ($\kappa \gtrsim 1$, $2Nc_0 \sim 1$). The ratchet dynamics of recognition sites has important consequences for the evolution of recognition functions, to which we now turn.

C. Complexity-dependent speed of adaptation

The most prominent effect of a longer code is an increased supply of selectable variation. We measure this effect by an effective number of mutational paths for adaptive evolution of recognition starting from a given site genotype, where each path is weighted by its contribution to the speed of adaptation. As detailed in Appendix C, this number equals a suitably normalized expectation value of the population variance of the recognition trait, $A = \langle \text{Var}(\Delta G/k_B T) \rangle / (2\mu N \epsilon_0^2)$. Sites with larger A adapt their code faster at a given fitness gradient $\partial F / \partial(\Delta G)$; equivalently, such sites require less selection for recognition to maintain a given steady-state recognition R (Appendix C) at a given driving rate κ . Therefore, long and fuzzy sites can maintain function up to larger driving rates ($\kappa_{\text{max}} \sim 2NAf_0$).

The effective number of adaptive paths is determined solely by the genetic architecture of the recognition trait; the actual speed of adaptation, or fitness flux, also depends on the global parameters μ , N , ϵ_0 , and on the fitness amplitude f_0 [49]. In the minimal model, the number of adaptive paths is related in a simple way to coding density and code length. When code changes are under strong selection, we find stationary-state values

$$A^* = \frac{\gamma_0}{1 - \gamma_0} (1 - \gamma^*) \ell^* \quad (5)$$

(Appendix C). Thus, by increasing ℓ^* and decreasing γ^* , adaptive ratchets increase the number of accessible beneficial mutations and accelerate their response to moving targets. In the strong-driving regime, A^* is a strongly increasing function

of the driving rate (Fig. 5). In other words, the recognition system adapts its own adaptability to moving targets.

D. Adaptive tinkering and functional alterations

Around the strong-driving stationary-state average ℓ^* , the ratchet dynamics of the code length becomes diffusive ($|v_e| \ll v_{\text{tot}}$), as in near-neutral evolution, but the diffusion rate is adaptively enhanced [$v_{\text{tot}} \gg 2\nu$; Figs. 4(d) and 5]. These characteristics define a ratchet regime of adaptive tinkering at large code length. The range of this regime is given by the width of the potential peak $\Psi(\ell)$, which is defined as the r.m.s. variation $\Delta\ell$ around ℓ^* and can be computed from the curvature of the evolutionary potential. We find a broad regime of adaptive tinkering, $\Delta\ell \sim \ell^*$ with a proportionality factor of order 1 (Appendix C). Rapid diffusion and broad length distributions are specific effects of adaptive ratchets. For evolution in a single-valued fitness landscape $F(\ell) = F_r(\ell) - c_0\ell$, the diffusion rate at the peak is bounded by the neutral rate, $v_{\text{tot}}^* \leq 2\nu$. Moreover, if F_r is a power of ℓ , length fluctuations around the peak scale as $\Delta\ell \sim (\ell^*)^{1/2}$ (Appendix C).

Together, the turnover of recognition code includes point mutations at rates $u_+^* \sim \kappa\mu$ and length changes at rates $v_{\text{tot}}^* \sim \kappa\nu$, both of which are tuned by the seascape of target changes. Thus, by adaptive tinkering, recognition sites rapidly scan sequence space while their function is maintained. This seascape-driven process is much faster than tinkering by near-neutral changes to which only later selection for new functions latches on. It enhances the probability of evolving functional refinements (for example, more specific target binding by epistatic interactions between sequence units) and of adopting new functions (examples are overlapping binding sites in bacteria [55,56]).

Evolutionary paths of recognition sites can also end with loss of function, which becomes frequent when site evolution cannot keep up with a fast-moving target. Typical paths show loss of function (decrease of ΔG) followed by collapse of the site sequence (driven by the cost of complexity), as shown in Appendix C, Fig. 11. We conclude that adaptive ratchets evolve a transient gain of molecular complexity, which can be locked in by additional functions or is exposed to pruning.

VII. COMPLEXITY OF IMMUNE RECOGNITION

The following example shows how selection on complexity can act in the adaptive immune system, a rapidly evolving recognition system of high global complexity [57]. In the presence of an antigen, immune B cells produce neutralizing antibodies that bind to specific target sites on its surface (called antigenic epitope sites) [58]. In a primary infection, a part of the responding B cells is stored as immune memory to protect against future infections by the same pathogen (another part evolves high affinity to their cognate epitope by affinity maturation, a rapid evolutionary process under selection for recognition [16,59–61]). Fast-evolving pathogens, however, are moving targets: they change epitope sequences by accumulation of mutations, which can lead to eventual escape from immune recognition and protection. This process, called antigenic drift, is frequently observed in RNA viruses, including human influenza, norovirus, and

SARS-CoV-2 [62–65]. Here we develop a minimal biophysical model for the immune recognition of an evolving antigen and its consequences for host fitness.

A natural measure for the molecular complexity of immune responses to a given antigen is the effective number of epitopes targeted by neutralizing antibodies. For an antigen with a total of g epitopes, we record the epitope-specific antiserum potencies, $\mathbf{Z} = (Z_1, \dots, Z_g)$, which measure the relative probabilities that each epitope is bound by antibodies [66]. We then define the immune recognition complexity g_r in terms of a Shannon entropy, $\log g_r \equiv \langle S(\mathbf{z}) \rangle$, where $\mathbf{z} = \mathbf{Z} / \sum_{\alpha=1}^g Z_{\alpha}$ is the potency distribution of a given host and angular brackets denote averages over the host population. This function counts epitopes weighed by the strength of their cognate immune response. Thus, immune recognition complexity is closely related to the established notion of immunodominance [67,68]—low complexity implies high immunodominance and vice versa—and is known to vary substantially between viral pathogens and between human hosts [69–71].

The complexity of immune recognition is a coevolutionary trait: it depends on pathogen factors, such as the number of accessible epitopes, and on global parameters of the host immune repertoire, including the number of B cell lineages and the length of the antibody-epitope binding motif [66]; more details are given in Appendix D. Therefore, hosts can change the effective number of targeted epitopes by long-term evolution of their immune systems. For example, humans and mice differ substantially in the number of B cell lineages and consistently in the immunodominance of B cell immune responses [72,73].

A. Model for recognition function

Infections by an antigen with g accessible epitopes induce the production of an antiserum with epitope-specific potencies $\mathbf{Z}$. We define the effective target affinities $\log \mathbf{Z} \equiv (\Delta G_1, \dots, \Delta G_g) / (k_B T)$ as the biophysical recognition phenotype for the immune memory response of this system; this phenotype characterizes the interaction of a specific host and a specific pathogen strain. The affinities govern the recognition of each epitope, $R_{\alpha} = H(\Delta G_{\alpha} - \Delta G_{50})$, where ΔG_{50} denotes a threshold antibody level required for clinical protection against reinfection [74,75]. The form of the single-epitope recognition function is analogous to Eq. (1) in our model for gene regulation. The full protection function takes the form $R = 1 - (1 - R_1) \times \dots \times (1 - R_g)$, assuming an exposed individual is protected as long as the antigen is recognized at any of its epitopes.

This form of the recognition and protection functions is well supported by empirical findings. First, clinical studies show that human immune protection correlates well with overall antibody titers in serum, independently of the epitope distribution of the response [74,75]. Second, functional binding of a single epitope (monoclonal response) is sufficient to confer full protection to the host [70]; consistently, a single epitope mutation can cause loss of protection [76].

How does the immune recognition in a secondary infection depend on antigenic drift of the pathogen? A minimal molecular model of this process is sketched in Fig. 6(a). A

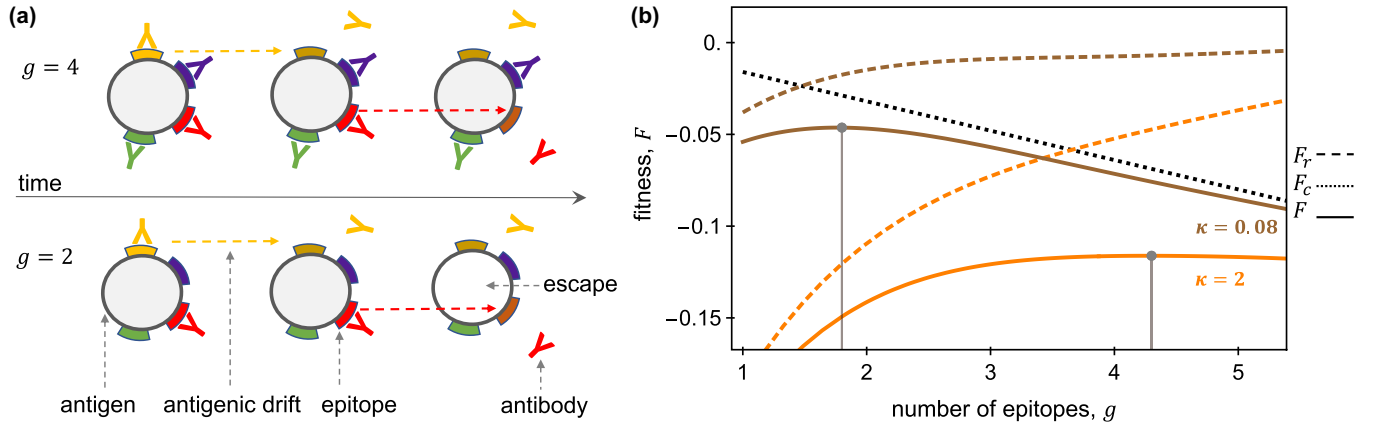


FIG. 6. Complexity-dependent recognition and fitness of the adaptive immune system. (a) Immune recognition and escape evolution (schematic). Antibodies neutralize pathogens by binding to specific target sites (epitopes). The number of initially bound epitopes, g , measures the complexity of the immune response. Antigenic evolution of the pathogen (dashed arrows) changes the target sequences and reduces recognition of each epitope, eventually leading to escape from immune recognition. These dynamics is shown for $g = 2$ and $g = 4$. (b) Complexity-dependent fitness landscape, $F(g)$ (solid), recognition component, $F_r(g)$ (dashed), and complexity cost, $F_c(g)$ (dotted black). Stationary-state values g^* are marked by gray dots; $F_r(g)$ is plotted relative to the maximum recognition fitness and in units of the infection cost parameter f_0 . This landscape is shown for slow- and fast-evolving pathogens with effective antigenic mutation rates $\kappa = 2$ (orange) and $\kappa = 0.08$ (brown). To be compared with Fig. 4(a). Evolutionary parameters: $\epsilon_0 = 2.5$, $\Delta G_i = \Delta G_{50} + 2\epsilon_0 k_B T$, $c_0/f_0 = 0.016$.

primary infection generates immune memory with g equal high-affinity components $\Delta G_\alpha = \Delta G_0 > G_{50}$ ($\alpha = 1, \dots, g$) against the infecting strain, resulting in a recognition complexity $g_r = g$ (Appendix D). Subsequent antigenic drift occurs by epitope mutations with effect $\Delta \Delta G_\alpha / (k_B T) = \epsilon_0$ and point substitution rate ρ , generating stochastically reduced affinities ΔG_α against evolved strains. Thus, antigen evolution degrades the expected molecular recognition $R(t)$ for recurrent exposures at a time t after the previous infection, leading eventually to loss of recognition and reinfection.

B. Model for host fitness

Fitness effects of infections accumulate during a host's infection history. This process includes recurrent infections by evolving pathogens, such as influenza, and is modulated by the efficiency of immune responses. To capture these joint effects, we introduce a minimal, two-component model for host fitness $F(g) = F_r(g) + F_c(g)$, which is analogous to Eq. [2] in our evolutionary model for gene regulation. First, a recognition-dependent term $F_r(g) = f_0 \langle R \rangle$ is generated by the fitness cost of infections. Here we evaluate the stationary-state population average of the protection function, $\langle R \rangle$, describing a population of adult individuals subject to multiple reinfections by evolving pathogens. As before, the fitness function $F_r(g)$ is blind to the molecular details of the recognition machinery; epitope complexity, g , carries a fitness benefit only if it generates an increased average immune protection. Second, a fitness cost of complexity, $F_c(g)$, can arise from multiple factors, including the size of the naive immune repertoire: larger recognition complexity g requires a larger number of naive B cell lineages [66], generating a larger burden of repertoire generation, maintenance, and off-target recognition (Appendix D). For simplicity, we summarize these costs into a linear fitness term, $F_c(g) = -c_0 g$.

C. Selection for complex immune responses by antigenic drift

We now evaluate the steady-state population average of the host fitness $F(g)$ as a function of two key parameters: the number of targeted epitopes, g , and the effective speed of antigenic evolution, $\kappa = \rho \tau$ [Fig. 6(b)]. Here τ denotes the average time between host-pathogen encounters. We find a link between immune recognition complexity and target mutation rate remarkably similar to Fig. 4(a): multi-epitope immune responses play out a fitness benefit $F_r(g)$ specifically against fast-evolving antigens ($\kappa \gtrsim 1$). Again, the recognition fitness component shows a diminishing-return nonlinearity, leading to a κ -dependent maximum of the total fitness $F(g)$. The underlying dynamical mechanism is similar as well: immune responses targeting complex epitope states adapt more efficiently to moving antigenic targets. A related effect has recently been discussed in Ref. [27]: antigenic drift can favor generalist over specialist immune responses against multiple antigens. For even faster target evolution ($\kappa \gg 1$), defense by immune memory becomes inefficient, in accordance with the results of Ref. [17].

In summary, we have shown that fast antigenic drift can induce selection for complex adaptive immune responses, in line with our general picture of complexity in dynamical recognition systems. This finding is consistent with observations: several fast-evolving RNA viruses, including influenza [25,77], norovirus [64], and SARS-Cov-2 [78–80], are subject to multi-epitope responses, although successful targeting of a single epitope is sufficient for neutralization [70]. However, the minimal model is insufficient to describe the full dynamics of immune recognition complexity. These processes depend on the long-term co-evolution of host immune systems and multiple viral pathogens [81], which is beyond the scope of this paper. Other features of immune recognition not contained in the minimal model include a feedback of recognition onto the speed of target evolution. If epitope mutations come

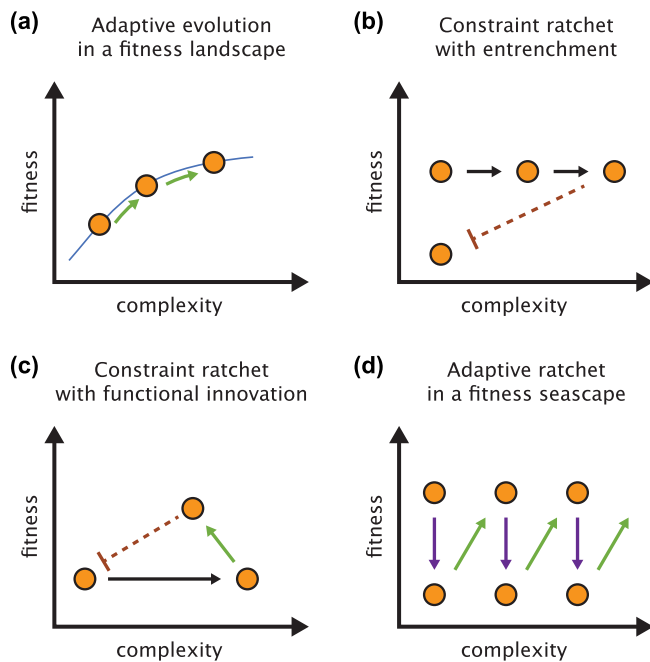


FIG. 7. Evolution of molecular complexity. The schematic distinguishes four modes of evolution: (a) Adaptive evolution in a static fitness landscape. Complexity-increasing changes carry a direct fitness benefit (green arrows), e.g., in a complex environment. (b) Constraint ratchet, entrenchment. Complexity increases by neutral evolution (black arrows); the complex state gets locked in by the buildup of negative selection against the reverse dynamics (red dashed line). (c) Neutral evolution inducing functional innovation. Complexity increases without a fitness benefit (black arrows); complex states are stabilized by the subsequent adaptive evolution of new functions (green arrow), which suppresses subsequent reduction of complexity (red dashed line). (d) Adaptive ratchet. Complexity increases by adaptive evolution (green arrows) in response to a moving target; the externally driven target dynamics degrades fitness (purple arrows).

with a collateral cost to the pathogen (for example, through their effect on protein stability), a complex immune response can slow down or halt antigenic drift; this effect has recently been reported for measles [71].

VIII. DISCUSSION

Organisms live in dynamic environments and evolve changing functions in them. When do these dynamics generate complexity? Here, we characterize two evolutionary pathways to molecular complexity for recognition sites in DNA or proteins. In contrast to the classical picture of Darwinian evolution in a static fitness landscape [Fig. 7(a)], these pathways involve ratchet evolution under time-dependent selection, where opposite steps of code extension and compression do not have opposite selection coefficients [Figs. 7(b)–7(d)].

Ratchet modes of recognition site evolution can be distinguished by the underlying selective forces. A classical evolutionary ratchet operates by constraint: site extensions are near-neutral, selection for the recognition function builds up in the extended state and suppresses subsequent site compressions [Figs. 7(b) and 7(c)]. Comparable evolutionary

mechanisms have previously been discussed for protein and whole-genome evolution. Proteins can evolve by neutral gene duplication to more complex multimeric states that become evolutionarily stable by entrenchment: conditionally neutral processes, such as partial loss-of-function evolution in each daughter copy, make both copies dependent on each other and generate negative selection against loss of complexity [11,13–15] [Fig. 7(b)]. On larger genomic scales, it has been argued that the decreased effective population sizes of multicellular organisms induces an initially neutral increase of sequence complexity. The more complex genome state provides a substrate for the subsequent adaptive evolution of new functions, selection on which stabilizes this state [9] [Fig. 7(c)].

A neutral path to higher complexity can be realized under two conditions: the cost of complexity is small and the primary function is well-adapted. In this mode, negative selection against loss of complexity builds up as a collateral of stabilizing selection on the primary function in the complex state [Figs. 7(b) and 7(c)]. For example, in Lynch and Conery’s model of macroevolution, the genomes of higher organisms grow neutrally because reduced effective population sizes reduce the cost of complexity; later, functions can latch on and eventually be conserved by selective constraint [9].

For recognition sites in a strongly time-dependent environment, we find a new mode of molecular evolution that we call an adaptive ratchet. A moving target of recognition generates a fitness seascape: external changes drive the system away from its fitness peak and are balanced by continuous adaptive evolution. All extension and compression steps of the recognition site are driven by asymmetric, positive selection on recognition of the moving target [Fig. 7(d)]. Starting from a low-complexity initial state, we show that stronger selection for extension generates a fast and robust evolution of molecular complexity that can overcome a substantial cost of complexity. In this mode, complexity evolves as a collateral to positive selection on the primary function. The rate of adaptive ratchets is set by external driving forces, here recognition target changes, not by genetic drift. Hence, unlike classical arguments of complexity evolution, adaptive ratchets do not describe the refinement of functions in a static environment but the maintenance of functions in the face of a fitness seascape.

The adaptive ratchet mechanism identified here may operate in recognition systems exposed to persistent external driving. In prokaryotic adaptive immunity, CRISPR-Cas arrays expand through spacer acquisition in response to viral evolution, increasing both recognition diversity and architectural complexity [82,83]. In vertebrate immunity, affinity maturation and clonal diversification occur under sustained antigenic change [26]. Modular DNA-binding proteins such as C₂H₂ zinc finger arrays similarly exhibit variable numbers of repeat units, suggesting that architectural expansion can enhance adaptive flexibility under selection [84].

All evolutionary ratchets build on a separation of mutational timescales between functional changes at fixed complexity and complexity changes. This is a natural assumption for recognition systems, because alterations in the architecture of molecular recognition interfaces are subject to stronger conformational and selective constraints than point mutations that tune binding affinities. We have

shown that the separation of timescales generates a fundamental asymmetry: code length extensions take place into random sequence, compression steps cut into functional sequence, causing asymmetric selection for reverse evolutionary steps. Therefore, even without any explicit fitness benefit, complexity can evolve as a collateral of selection for function.

In a ratchet mode, recognition sites evolve a substantially larger code length than the minimum length, or algorithmic complexity, required for function (Figs. 2 and 3). Importantly, the increase of code length for a conserved function is not tied to an increase in evolutionary information (i.e., an entropy drop compared to neutral sequence). Rather, it is coupled to a decrease of coding density; that is, individual letters carry a reduced information about the target. We have shown that this suboptimal use of genomic real estate increases the number of mutational paths available for adaptation of recognition, generating an evolutionary advantage in dynamic environments. Complex and fuzzy sites in a fitness seascape evolve in a regime of adaptive tinkering, which is characterized by an increased number of adaptive paths and by rapid parsing of site sequence states (Fig. 5). Adaptive tinkering facilitates the adaptation to moving targets and increases the propensity for functional refinement or alteration, for example by tuning epistatic interactions within the recognition site or by recruiting additional targets for cooperative binding. We can summarize these dynamics as an evolutionary feedback loop: moving targets, by increasing the molecular complexity of their cognate recognition machinery, improve their own recognition.

How universal are evolutionary ratchets? We can expect this mechanism to operate in a broad range of molecular networks with multiple parallel subunits and a reservoir of potential units. Examples include recognition sites with multiple sequence matches, repertoires of immune response with multiple antibody lineages, and on larger scales, regulatory or metabolic networks with multiple genes contributing to the same function. Such networks allow ratchet modes of evolution to kick in: by preferential attachment of fit and deletion of unfit subunits, changes in network architecture can couple to selection for network function. Specifically, the adaptive ratchet model then predicts that external driving—here by moving recognition targets, evolving antigens, or time-dependent metabolic substrates—induces the evolution of complex networks with redundant subunits.

New high-throughput methods are currently used to map sequence and phenotypes of molecular interactions at an unprecedented depth. Prominent examples include transcription factor interactions in microbes [22,85,86] and receptor-antigen interactions in human immune repertoires [87–89]. This opens the perspective of new experimental screens to map correlations between external driving, network architecture, and molecular complexity. Experiments with exposure to moving targets can probe constraint or adaptive ratchet evolution. Such experiments can also become a powerful tool for synthetic evolution, to elicit molecular complexity of recognition and its potential for evolutionary innovation.

All code used for numeric computations and to generate figures can be found in a GitHub repository [90].

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All authors performed model development, analytical calculations, and numerical simulations. T.R. focused on the general model, R.M.T. on the application to immune systems. All authors contributed to writing the paper.

DATA AVAILABILITY

No data were created or analyzed in this study.

APPENDIX A: EVOLUTIONARY MODEL

1. Recognition trait and function

In a biophysical model of recognition, a quantitative trait characterizing a recognition site is its binding affinity to the recognition target, ΔG . Here we measure this quantity with the zero point given by its expectation value in random sequence; the results do not depend on this choice. The recognition function, defined as the equilibrium occupancy of a binding site with affinity ΔG at physiological temperature T is then given by

$$R = \frac{1}{1 + \exp[(\Delta G_{50} - \Delta G(\gamma, \ell))/k_B T]}, \quad (\text{A1})$$

where ΔG_{50} denotes the functionality threshold (half-binding point). In our minimal model, the reduced binding affinity takes the simple form

$$\frac{\Delta G(\gamma, \ell)}{k_B T} = (\gamma - \gamma_0)\ell\epsilon_0, \quad (\text{A2})$$

which depends on the site length ℓ , the fraction of target-matching sequence units in the site, $\gamma = k/\ell$, the average fraction of matches in random sequence, γ_0 , and the reduced affinity gain per match, $\Delta\Delta G/k_B T = \epsilon_0$.

For a given background sequence $\mathbf{a} = (a_1, \dots, a_L)$, the functionality threshold is given by the condition that the Boltzmann factor of the functional site equals the sum of Boltzmann factors for spurious binding at all background sequence positions,

$$N \exp\left[\frac{\Delta G_{50}(\mathbf{a})}{k_B T}\right] = Z_b(\mathbf{a}) \quad (\text{A3})$$

with

$$Z_b(\mathbf{a}) = \sum_{r=1}^{L-\ell+1} \exp\left[\frac{\Delta G(a_r, \dots, a_{r+\ell-1}) - \Delta G_0}{k_B T}\right]. \quad (\text{A4})$$

Here N is the expected number of TF molecules diffusing along the DNA backbone, and ΔG_0 is the affinity of the diffusive molecular state. A typical transcription factor in prokaryotes has a 1D diffusion density $\rho = N/L \ll 1$, which is a finite fraction of the total TF density in the cell [91].

The expectation value of the functionality threshold in a random ensemble of background sequences is given by the quenched average

$$\frac{\Delta G_{50}}{k_B T} = -\log N + \sum_{\mathbf{a}} P_0(\mathbf{a}) \log Z_b(\mathbf{a}), \quad (\text{A5})$$

where $P_0(\mathbf{a})$ denotes the probability of genotype $\mathbf{a}$ in the background ensemble. Following Ref. [39], we evaluate Eq. (A5) in the so-called annealed approximation, averaging over Z_b instead of $\log Z_b$. In this approximation, the average factorizes into the contributions of individual sequence positions,

$$\begin{aligned} \frac{\Delta G_{50}}{k_B T} &= -\log N + \log \sum_{\mathbf{a}} P_0(\mathbf{a}) Z_b(\mathbf{a}) \\ &= -\log N + \log \left[L \prod_{i=1}^{\ell} \sum_a p_0(a) e^{\epsilon_i(a)} \right], \end{aligned} \quad (\text{A6})$$

where $p_0(a)$ denotes the probability of nucleotide a and $\epsilon_i(a)$ its affinity contribution at position i of the site. In the minimal model with our convention on the zero point of affinity, matches occur with probability $p_{0,+} = \gamma_0$ and have $\epsilon_+ = (1 - \gamma_0)\epsilon_0$, mismatches occur with probability $p_{0,-} = 1 - \gamma_0$ and have $\epsilon_- = -\gamma_0\epsilon_0$. We obtain

$$\frac{\Delta G_{50}}{k_B T} = -\log \rho + \ell \log [\gamma_0 e^{(1-\gamma_0)\epsilon_0} + (1 - \gamma_0) e^{-\gamma_0\epsilon_0}]. \quad (\text{A7})$$

The first term depends on the density of TF molecules but is independent of ℓ ; the second term accounts for the sequence heterogeneity of the random ensemble. As discussed in the main text, TF binding sites have a minimum functionality threshold $\Delta G_{50}^{\min}$, corresponding to a minimum number $k_0 = \Delta G_{50}^{\min} / (\epsilon_0 k_B T)$ of excess matches over random sequence and a minimum length $\ell_{\min} = \Delta G_{50}^{\min} / (\epsilon_0 (1 - \gamma_0) k_B T)$. Thus, we can rewrite the functionality threshold in the form

$$\frac{\Delta G_{50}}{k_B T} = \epsilon_0 k_0 + (\ell - \ell_{\min}) \frac{\Delta \Delta g_{50}}{k_B T} \quad (\text{A8})$$

with a threshold shift per unit length,

$$\frac{\Delta \Delta g_{50}}{k_B T} = \log [\gamma_0 e^{(1-\gamma_0)\epsilon_0} + (1 - \gamma_0) e^{-\gamma_0\epsilon_0}]. \quad (\text{A9})$$

By Eq. (A2), the half-binding point corresponds to a code density

$$\begin{aligned} \gamma_{50} &= \gamma_0 + \frac{1}{\epsilon_0 \ell} \frac{\Delta G_{50}}{k_B T} \\ &= \gamma_0 + \frac{k_0}{\ell} + \frac{(\ell - \ell_{\min})}{\epsilon_0 \ell} \frac{\Delta \Delta g_{50}}{k_B T}. \end{aligned} \quad (\text{A10})$$

In our numerical analysis shown in Figs. 3–5 and 7–11, we use a recognition function (A1) with the full, length-dependent form (A8)–(A10) of the recognition threshold. The length-dependent term remains subleading over the range of relevant coding lengths, as shown in Fig. 8. Therefore, we use the length-independent form $\Delta G_{50} / (k_B T) \approx \epsilon_0 k_0$, corresponding to $\gamma_{50} \approx \gamma_0 + k_0 / \ell$ in the analytical approximations, as in Eq. (A18).

2. Sequence evolution rates

In the minimal model, the sequence evolution of recognition involves the three classes of changes shown in Fig. 1. (i) Point mutations of the recognition sequence at constant code length occur at a rate μ per unit of sequence [Fig. 1(a)].

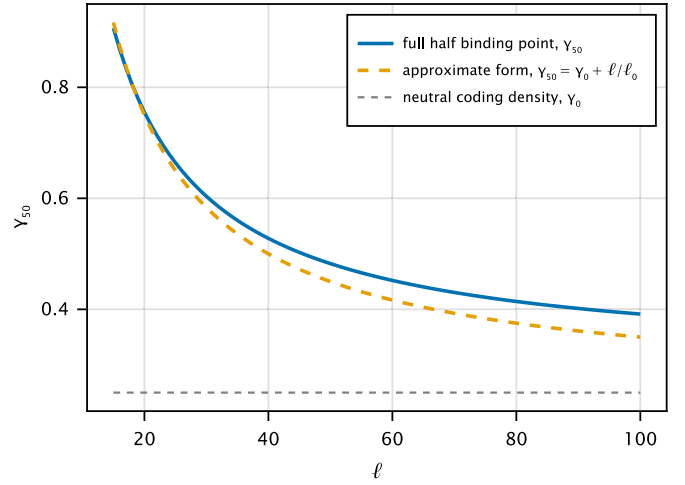


FIG. 8. Length dependence of the recognition threshold. The half-binding coding density γ_{50} of the minimal recognition model is shown in the full form (A10) (blue) and in the approximation used for analytical estimates, $\gamma_{50} = \gamma_0 + k_0 / \ell$ (orange). The neutral coding density γ_0 (gray) is shown for comparison. Parameters as in Fig. 3.

These changes have point mutation rates

$$\mu_+(\gamma) = \mu(1 - \gamma) \frac{\gamma_0}{1 - \gamma_0}, \quad \mu_-(\gamma) = \mu\gamma, \quad (\text{A11})$$

where the mutation rates are weighted such they only reflect mutations that lead to changes in coding density. These mutations have selection coefficients

$$s_+(\gamma, \ell) = s_\gamma \left(\gamma + \frac{1}{2\ell}, \ell \right), \quad s_-(\gamma, \ell) = -s_\gamma \left(\gamma - \frac{1}{2\ell}, \ell \right). \quad (\text{A12})$$

depending on the direction of selection (beneficial/deleterious, indicated by the index $\pm$). Here

$$s_\gamma(\gamma, \ell) = F \left(\gamma + \frac{1}{2\ell}, \ell \right) - F \left(\gamma - \frac{1}{2\ell}, \ell \right) \quad (\text{A13})$$

is the fitness increment in the landscape (1) in midpoint discretisation. Equations (A11) and (A12) determine the substitution rates

$$\begin{aligned} u_+(\gamma, \ell) &= \mu_+(\gamma) g(s_+(\gamma, \ell)), \\ u_-(\gamma, \ell) &= \mu_-(\gamma) g(s_-(\gamma, \ell)) \end{aligned} \quad (\text{A14})$$

in the low-mutation regime ($\mu N \ell \lesssim 1$). Here $g(s) = N\pi(s)$ is Haldane's factor in a population of effective size N [92],

$$g(s) = \frac{2Ns}{1 - \exp(-2Ns)}, \quad (\text{A15})$$

which is proportional to the fixation probability of an individual mutation, $\pi(s)$. With an appropriately changed effective population size, these substitution rates apply even if the global sequence evolution takes place under clonal interference [54,93]. (ii) Recognition site extensions and compressions add or remove one unit of sequence [Figs. 1(b) and 1(c)] and occur at a rate $\nu \ll \mu$. These changes have mutation

rates

$$\begin{aligned} v_{++}(\gamma) &= v\gamma_0, \\ v_{+-}(\gamma) &= v(1 - \gamma_0), \\ v_{-+}(\gamma) &= v(1 - \gamma), \\ v_{--}(\gamma) &= v\gamma \end{aligned} \quad (\text{A16})$$

and selection coefficients

$$\begin{aligned} s_{++}(\gamma, \ell) &= F\left(\frac{\gamma + 1/\ell}{1 + 1/\ell}, \ell + 1\right) - F(\gamma, \ell), \\ s_{+-}(\gamma, \ell) &= F\left(\frac{\gamma}{1 + 1/\ell}, \ell + 1\right) - F(\gamma, \ell), \\ s_{-+}(\gamma, \ell) &= F\left(\frac{\gamma}{1 - 1/\ell}, \ell - 1\right) - F(\gamma, \ell), \\ s_{--}(\gamma, \ell) &= F\left(\frac{\gamma - 1/\ell}{1 + 1/\ell}, \ell - 1\right) - F(\gamma, \ell). \end{aligned} \quad (\text{A17})$$

For the subsequent analytical treatment, we use the exponential approximation of the target binding probability (A1), (A2) in the regime of functional sites,

$$\begin{aligned} R(\gamma, \ell) &\simeq 1 - \exp\left[\frac{\Delta G_{50}}{k_B T} - (\gamma - \gamma_0)\ell\epsilon_0\right] \\ &\approx 1 - \exp[\epsilon_0 k_0 - \epsilon_0 \ell(\gamma - \gamma_0)]. \end{aligned} \quad (\text{A18})$$

In the last expression, we also use the length-independent form $\Delta G_{50}/(k_B T) \approx \epsilon_0 k_0$, which provides a good approximation to the full form (A8) throughout the range of site lengths relevant for this paper (Fig. 8).

We can then relate the selection coefficients (A17) to the selection coefficient for point mutations of the recognition sequence in Eq. (A13),

$$\begin{aligned} s_{++}(\gamma, \ell) &= c_{++} s_\gamma(\gamma, \ell) - c_0, \\ s_{+-}(\gamma, \ell) &= c_{+-} s_\gamma(\gamma, \ell) - c_0, \\ s_{-+}(\gamma, \ell) &= c_{-+} s_\gamma(\gamma, \ell) + c_0, \\ s_{--}(\gamma, \ell) &= c_{--} s_\gamma(\gamma, \ell) + c_0, \end{aligned} \quad (\text{A19})$$

where c_0 denotes the complexity cost parameter in the fitness landscape Eq. (1) and

$$\begin{aligned} c_{++} &= \frac{1 - \exp[-\epsilon_0(1 - \gamma_0)]}{2 \sinh(\epsilon_0/2)}, \\ c_{+-} &= \frac{1 - \exp[\epsilon_0 \gamma_0]}{2 \sinh(\epsilon_0/2)}, \\ c_{-+} &= \frac{1 - \exp[-\epsilon_0 \gamma_0]}{2 \sinh(\epsilon_0/2)}, \\ c_{--} &= \frac{1 - \exp[\epsilon_0(1 - \gamma_0)]}{2 \sinh(\epsilon_0/2)}. \end{aligned} \quad (\text{A20})$$

Here we compute one selection coefficient explicitly,

$$\begin{aligned} s_{++}(\gamma, \ell) &= F\left(\frac{\gamma + 1/\ell}{1 + 1/\ell}, \ell + 1\right) - F(\gamma, \ell) \\ &= f_0 \left(\exp[\epsilon_0 k_0 - \ell(\gamma - \gamma_0)] - \exp\left[\epsilon_0 k_0 - (\ell + 1)\left(\frac{\gamma + 1/\ell}{1 + 1/\ell} - \gamma_0\right)\right] \right) - c_0 \\ &= f_0 (\exp[\epsilon_0 k_0 - \epsilon_0 \ell(\gamma - \gamma_0)] - \exp[\epsilon_0 k_0 - \epsilon_0 \ell(\gamma - \gamma_0) - \epsilon_0 + \epsilon_0 \gamma_0]) - c_0 \\ &= f_0 \exp[\epsilon_0 k_0 - \epsilon_0 \ell(\gamma - \gamma_0)] (1 - \exp[-\epsilon_0(1 - \gamma_0)]) - c_0 \\ &= f_0 (\exp[\epsilon/2] - \exp[-\epsilon/2]) \exp[\epsilon_0 k_0 - \epsilon_0 \ell(\gamma - \gamma_0)] c_{++} - c_0 \\ &= f_0 (\exp[\epsilon_0 k_0 - \epsilon_0 \ell(\gamma - 1/2\ell - \gamma_0)] - \exp[\epsilon_0 k_0 - \epsilon_0 \ell(\gamma + 1/2\ell - \gamma_0)]) c_{++} - c_0 \\ &= c_{++} s_\gamma(\gamma, \ell) - c_0; \end{aligned} \quad (\text{A21})$$

the other coefficients are obtained in the same way. These selection coefficients depend on the direction of change (extension/compression, first index $\pm$) and the direction of selection (beneficial/deleterious, second index $\pm$). The approximate expressions in terms of the fitness increment s_γ are used for the analytical closure discussed below. The exponential prefactors, which are of order one, reflect the shift in the half-binding point γ_{50} induced by a length change and the switch to the midpoint discretization in Eq. (A13). Equations (A16) and (A17) determine the total substitution rates of extensions and compressions,

$$\begin{aligned} v_+(\gamma, \ell) &= v_{++}(\gamma) g(s_{++}(\gamma, \ell)) + v_{+-}(\gamma) g(s_{+-}(\gamma, \ell)), \\ v_-(\gamma, \ell) &= v_{-+}(\gamma) g(s_{-+}(\gamma, \ell)) + v_{--}(\gamma) g(s_{--}(\gamma, \ell)), \end{aligned} \quad (\text{A22})$$

(iii) Recognition target changes occur at a rate $\rho = \kappa \mu$ per unit of sequence, independently of the recognition function [Fig. 1(d)]. Hence, beneficial and deleterious target changes have rates

$$\rho_+(\gamma) = \kappa \mu (1 - \gamma) \frac{\gamma_0}{1 - \gamma_0}, \quad \rho_-(\gamma) = \kappa \mu \gamma, \quad (\text{A23})$$

which have the same combinatorial factors as the point mutations (A11) and are independent of the fitness landscape $F(\gamma, \ell)$.

Individual evolutionary trajectories $(k, \ell)(t)$ generated by the substitution dynamics with rates $u_\pm(\gamma, \ell)$, $v_\pm(\gamma, \ell)$, and $\rho_\pm(\gamma)$ are shown in Figs. 2(b) and 2(d) and in Appendix C, Fig. 11.

APPENDIX B: STATIONARY-STATE ANALYSIS

1. Evolutionary potentials and stationary distribution

Given the separation of mutational timescales for coding density and code length ($\nu \ll \mu$), the stationary distribution factorizes,

$$Q(\gamma, \ell) = Q(\gamma|\ell) Q(\ell). \quad (\text{B1})$$

The conditional distribution of the coding density takes the form

$$Q(\gamma|\ell) = \exp[\Psi(\gamma|\ell)] \quad (\text{B2})$$

with a potential function given by detailed balance,

$$\Psi(\gamma|\ell) = \sum_{k'=0}^{\gamma\ell} \log \frac{u_+(k'/\ell, \ell) + \rho_+(k'/\ell, \ell)}{u_-((k'+1)/\ell, \ell) + \rho_-((k'+1)/\ell, \ell)} - \log Z_\gamma(\ell). \quad (\text{B3})$$

Here $Z_\gamma(\ell)$ is a normalization factor and the point substitution rates $u_\pm, \rho_\pm$ are given by Eqs. (A14) and (A23). This distribution defines the conditional average of an observable $x(\gamma, \ell)$,

$$\bar{x}(\ell) \equiv \sum_\gamma x(\gamma, \ell) Q(\gamma|\ell). \quad (\text{B4})$$

Because $Q(\gamma|\ell)$ is approximately Gaussian (Fig. 9), such averages can be accurately evaluated by steepest descent, $\bar{x}(\ell) \approx x(\gamma^*(\ell), \ell)$, where $\gamma^*(\ell) = \arg \max_\gamma Q(\gamma|\ell)$ denotes the conditional saddle-point coding density. In particular, this procedure yields an effective fitness landscape for code length,

$$\bar{F}(\ell) = \bar{F}_r(\ell) + F_c(\ell) = f_0 - \frac{1}{\epsilon_0} s_\gamma^*(\ell) - c_0 \ell, \quad (\text{B5})$$

where $s_\gamma^*(\ell) \equiv s(\gamma^*(\ell), \ell)$ is the selection coefficient at the saddle-point coding density. Here we use an exponential approximation of the Hill recognition function $R(\gamma, \ell)$ in the regime of functional sites, which implies $s_\gamma = \epsilon_0 \partial F_r / \partial (\Delta G / k_B T) = \epsilon_0 (f_0 - F_r)$. The marginal distribution of code length,

$$Q(\ell) = \exp[\Psi(\ell)], \quad (\text{B6})$$

is again determined by detailed balance,

$$\Psi(\ell) = \sum_{\ell'=\ell_{\min}+1}^{\ell} \log \frac{\bar{v}_+(\ell' - 1)}{\bar{v}_-(\ell')} - \log Z_\ell, \quad (\text{B7})$$

where Z_ℓ is a normalization factor, $\ell_{\min}$ is the minimal length of functional sites and $\bar{v}_\pm$ are the average extension and deletion rates given by Eqs. (A22) and (B4). From the full distribution $Q(\gamma, \ell)$, we define the corresponding distribution for functional sites,

$$Q_f(\gamma, \ell) = \frac{1}{Z_f} R(\gamma, \ell) \exp[\Psi(\gamma, \ell)]. \quad (\text{B8})$$

This distribution weighs sequence states by the recognition (target binding) probability, $R(\gamma, \ell)$. Examples of the distribution of functional sites $Q_f(\gamma, \ell)$ are plotted in Figs. 2(a) and 2(c); the evolutionary potential $\Psi(\ell)$ is shown in Fig. 4(b).

The evolutionary potential $\Psi(\gamma, \ell)$ simplifies at low and high target mutation rates, which allows for simple analytical

estimates of the global maximum point (γ^*, ℓ^*) and gives insight in the evolutionary determinants of site complexity.

2. Weak-driving regime

For $\kappa \lesssim 1$, the evolutionary potential for coding density takes the form

$$\Psi(\gamma|\ell) \simeq S(\gamma, \ell) + \frac{2N}{(1+\kappa)} F_r(\gamma, \ell) - \log Z_\gamma(\ell), \quad (\text{B9})$$

where

$$S(\gamma, \ell) = \log \left[\binom{\ell}{\gamma\ell} \gamma_0^{\gamma\ell} (1-\gamma_0)^{(1-\gamma)\ell} \right] \quad (\text{B10})$$

$$= \text{const} - \frac{\ell(\gamma - \gamma_0)^2}{2\gamma_0(1-\gamma_0)} + O((\gamma - \gamma_0)^3) \quad (\text{B11})$$

is the entropy, defined as the log number of sequence states, at a given recognition density. This entropy serves as a steepest-descent approximation for the Kulback-Leibler distance between the code density distribution $Q_{\gamma|\ell}$ and its neutral counterpart $Q_{\gamma|\ell}^0$,

$$H(Q_{\gamma|\ell}|Q_{\gamma|\ell}^0) \simeq S(\gamma^*(\ell), \ell) - S(\gamma_0, \ell) \simeq \frac{\ell(\gamma^*(\ell) - \gamma_0)^2}{2\gamma_0(1-\gamma_0)}, \quad (\text{B12})$$

which measures the information content of a regulatory motif of length ℓ . By Sanov's theorem, the expected number of off-target sites in a random sequence of length L is given by $L \exp[-H(Q_{\gamma|\ell}|Q_{\gamma|\ell}^0)]$, which sets a lower bound on the information content [39,41].

At equilibrium ($\kappa = 0$), the linear combination of S and F in (B9) reduces to the well-known free fitness that generating the Boltzmann-Gibbs distribution of the substitution process (A14) [40,94]. Remarkably, the extension to driven processes involves a simple rescaling of the effective population size by a factor $(1+\kappa)$. This can be derived a weak-selection approximation, which is appropriate close to equilibrium. In this regime, we expand the rates $u_\pm(\gamma, \ell)$ and $\rho_\pm(\gamma)$ to first order in the selection coefficient $s_\gamma(\gamma, \ell)$. We can then write the stochastic dynamics of the mean coding density γ as a diffusion process [54,95],

$$\dot{\gamma} = \frac{\Delta(\gamma, \ell)}{2N} \left((1+\kappa) \frac{\partial S(\gamma, \ell)}{\partial \gamma} + 2N \frac{\partial F(\gamma, \ell)}{\partial \gamma} \right) + \zeta(t). \quad (\text{B13})$$

The stochastic force $\zeta(t)$ has mean $\langle \zeta(t) \rangle = 0$ and covariance

$$\langle \zeta(t) \zeta(t') \rangle = \frac{\Delta(\gamma, \ell)}{2N} \delta(t - t'), \quad (\text{B14})$$

which is related to the diversity of the coding density in a population of size N ,

$$\begin{aligned} \Delta(\gamma, \ell) &= \frac{2N}{\ell} [\mu_+(\gamma, \ell) + \mu_-(\gamma, \ell)] \\ &= \frac{2N\mu}{\ell} \left[\gamma + \frac{\gamma_0}{1-\gamma_0} (1-\gamma) \right]. \end{aligned} \quad (\text{B15})$$

The neutral process includes recognition site and target mutations, leaving the neutral distribution $Q_0(\gamma|\ell) = \exp[S(\gamma, \ell)]$

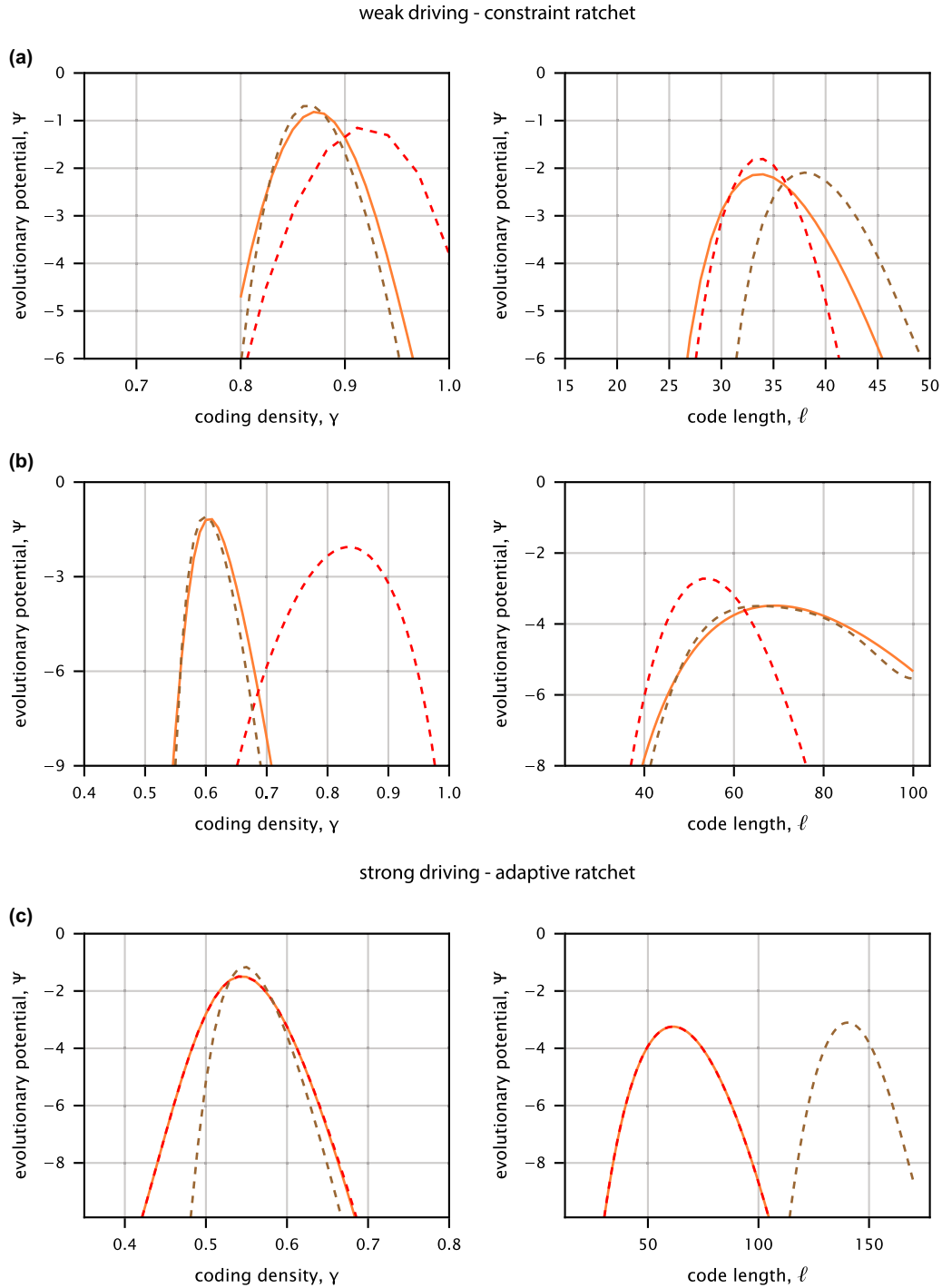


FIG. 9. Approximate evolutionary potentials. Left: Evolutionary potential for coding density at the expected stationary-state code length, $\Psi(\gamma|\ell^*)$. Right: Evolutionary potential for code length, $\Psi(\ell)$. Full potentials (orange) are compared to the corresponding weak-driving (brown) and strong-driving (red) approximations. Evolutionary regimes: (a) weak-driving regime, $2Nc_0 = 1$, $\kappa = 0.1$; (b) weak-driving regime at low cost, $2Nc_0 = 0.2$, $\kappa = 0.1$; (c) strong-driving regime, $2Nc_0 = 1$, $\kappa = 15$. Other parameters as in Fig. 2.

unaffected by the driving. In contrast, the population diversity Δ includes only site mutations. This diversity governs the response to selection in Eq. (B13), while target mutations are independent of the fitness landscape $F(\gamma, \ell)$.

Maximizing the potential $\Psi(\gamma|\ell)$ determines the conditional expectation value in saddle-point approximation, $\gamma^*(\ell)$. This point satisfies a balance condition between selection, site mutations and the associated genetic drift, and target

mutations. At equilibrium, this reduces to the well-known detailed balance between deleterious and beneficial substitutions [Fig. 2(b)]. By Eqs. (B9) and (B11), the weak-driving balance condition relates selection and coding density,

$$2Ns_\gamma^*(\ell) = \frac{(1 + \kappa)}{\gamma_0(1 - \gamma_0)}(\gamma^*(\ell) - \gamma_0). \quad (\text{B16})$$

The evolutionary potential for length takes the weak-driving form

$$\Psi(\ell) \simeq \frac{\epsilon_0 k_0}{2} 2N\bar{F}_r(\ell) + 2NF_c(\ell) - \log Z_\ell, \quad (\text{B17})$$

where $k_0 = \Delta G_{50}/(\epsilon_0 k_B T)$ and the recognition component, $\bar{F}_r(\ell)$, is determined by Eq. (B5). To derive this form, we use again a weak-selection approximation and expand the substitution rates for extensions and compressions, $v_\pm(\gamma, \ell)$, to first order in the selection coefficients s_γ and c_0 . From Eqs. (A16)–(A22), we obtain

$$v_+(\gamma, \ell) = v(1 - Nc_0) + O(s_\gamma^2, s_\gamma c_0, c_0^2), \quad (\text{B18})$$

$$v_-(\gamma, \ell) = v(1 - (\gamma - \gamma_0)Ns_\gamma(\gamma, \ell) + Nc_0) + O(s_\gamma^2, s_\gamma c_0, c_0^2). \quad (\text{B19})$$

We can now compute the potential increment in the steepest-descent approximation for γ ,

$$\begin{aligned} \Psi(\ell) - \Psi(\ell - 1) &= \log \frac{v_+(\gamma^*(\ell - 1), \ell - 1)}{v_-(\gamma^*(\ell), \ell)} \\ &= [\gamma^*(\ell) - \gamma_0]Ns_\gamma^*(\ell) - 2Nc_0 + O(s_\gamma^2, s_\gamma c_0, c_0^2) \\ &= \frac{\epsilon_0 k_0}{2} [2N\bar{F}_r(\ell) - 2N\bar{F}_r(\ell - 1)] \\ &\quad + 2N[F_c(\ell) - F_c(\ell - 1)]. \end{aligned} \quad (\text{B20})$$

The last approximation uses that selection is proportional to the recognition load, $s_\gamma = \epsilon_0(f_0 - F_r)$, and its expectation value is approximately inversely proportional to the site length, $s_\gamma^*(\ell) \sim k_0/\ell$, by Eqs. (2) and (B16). Equation (B17) then follows by summation of the increment up to a given value of ℓ . Maximizing $\Psi(\ell)$ determines the balance condition for code length,

$$k_0 \frac{\partial}{\partial \ell} s_\gamma^*(\ell) = -c_0. \quad (\text{B21})$$

Together with Eq. (B16) and the leading-order relation between coding density and length, Eq. (2), we obtain an analytical closure for the global maximum point (γ^*, ℓ^*) in the weak-driving regime,

$$\frac{\ell^*}{k_0} = \frac{1}{\gamma^* - \gamma_0} \simeq \sqrt{\frac{1 + \kappa}{\gamma_0(1 - \gamma_0)2Nc_0}}. \quad (\text{B22})$$

Compared to the effective fitness $\bar{F}(\ell)$, as given by Eq. (B5), the potential $\Psi(\ell)$ upweighs the recognition component, which implies that the stationary-state code length ℓ^* overshoots the maximum of $\bar{F}(\ell)$ in the weak-driving regime [Figs. 4(a) and 4(b)].

In Fig. 9 we compare the full potentials $\Psi(\gamma|\ell)$ and $\Psi(\ell)$ with their the weak-driving approximations. This approximation produces the correct scaling of the equilibrium point with the cost parameter c_0 . In particular, the condition that evolutionary equilibrium leads to near-maximal coding density, $\gamma^*(\kappa=0) \approx 1$, sets a constraint roughly equal to the selection for coding density, $2Nc_0 \approx 2Ns_\gamma^*(\kappa=0) \approx 1$. This informs our choice of the cost parameter for the crossover to strong driving.

3. Strong-driving regime

For $\kappa \gtrsim 1$, selection on code changes becomes strong ($s^*(\ell) \gg 1$), causing deleterious point mutations to freeze-out. The resulting balance condition for the coding density γ^* involves beneficial site mutations and target mutations [Fig. 2(d)],

$$u_+(\gamma^*, \ell) + \rho_+(\gamma^*) = \rho_-(\gamma^*). \quad (\text{B23})$$

To first order, we obtain

$$\gamma_0(1 - \gamma^*(\ell))(2Ns_\gamma^*(\ell) + \kappa) = \gamma^*(\ell)(1 - \gamma_0)\kappa. \quad (\text{B24})$$

Like its weak-selection counterpart, Eq. (B16), this condition relates selection and coding density,

$$2Ns_\gamma^*(\ell) = \frac{\gamma^*(\ell) - \gamma_0}{\gamma_0(1 - \gamma^*(\ell))} \kappa. \quad (\text{B25})$$

Similarly, strong selection causes deleterious extensions and compressions to freeze-out ($2Ns_{+-} \ll 1$, $2Ns_{--} \ll 1$), and the balance condition for code length becomes

$$\gamma_0 s_{++}^* = (1 - \gamma^*)s_{-+}^*. \quad (\text{B26})$$

Inserting the approximations (A17) and solving for s_γ^* , we obtain

$$s_\gamma^* = \frac{1 - (\gamma^* - \gamma_0)}{\gamma_0 c_{++} - (1 - \gamma^*)c_{-+}} c_0, \quad (\text{B27})$$

which is the strong-selection counterpart of Eq. (B21). The analytical closure in the strong-driving regime given by Eqs. (B25) and (B27),

$$\begin{aligned} 0 &= \frac{2Nc_0}{\kappa} \gamma_0(1 + \gamma_0 - \gamma^*)(1 - \gamma^*) \\ &\quad + (\gamma^* - \gamma_0)(c_{++}\gamma_0 - c_{-+}(1 - \gamma^*)), \end{aligned} \quad (\text{B28})$$

determines the expectation values (γ^*, s^*) in saddle-point approximation; the expected code length, ℓ^* , is then obtained from Eq. (2). The full potentials $\Psi(\gamma|\ell)$ and $\Psi(\ell)$ and their strong-driving approximations are shown in Fig. 9.

APPENDIX C: DYNAMICAL ANALYSIS

1. Ratchet evolution

The ratchet dynamics derived in this paper is characterized by a breakdown of detailed balance for evolution under selection. Specifically, the minimal model for recognition sites generates net selection coefficients for code length changes,

$$2Ns_\pm(\ell) = g^{-1}\left(\frac{\bar{v}_\pm(\ell)}{v}\right), \quad (\text{C1})$$

where v is the basic mutation rate of extensions and compressions, $\bar{v}_\pm(\ell)$ are the corresponding substitution rates defined by Eqs. (A22) and (B4). The function g^{-1} is the inverse of the Haldane function g defined in Eq. (A15). Ratchet evolution of code length is defined by a decoupling of selection for reverse changes,

$$s_+(\ell) \neq s_-(\ell + 1). \quad (\text{C2})$$

This inequality says that the substitution rates $\bar{v}_\pm(\ell)$ cannot be generated from the neutral rate v and selection by any fitness landscape $F(\ell)$, which would imply $s_+(\ell) =$

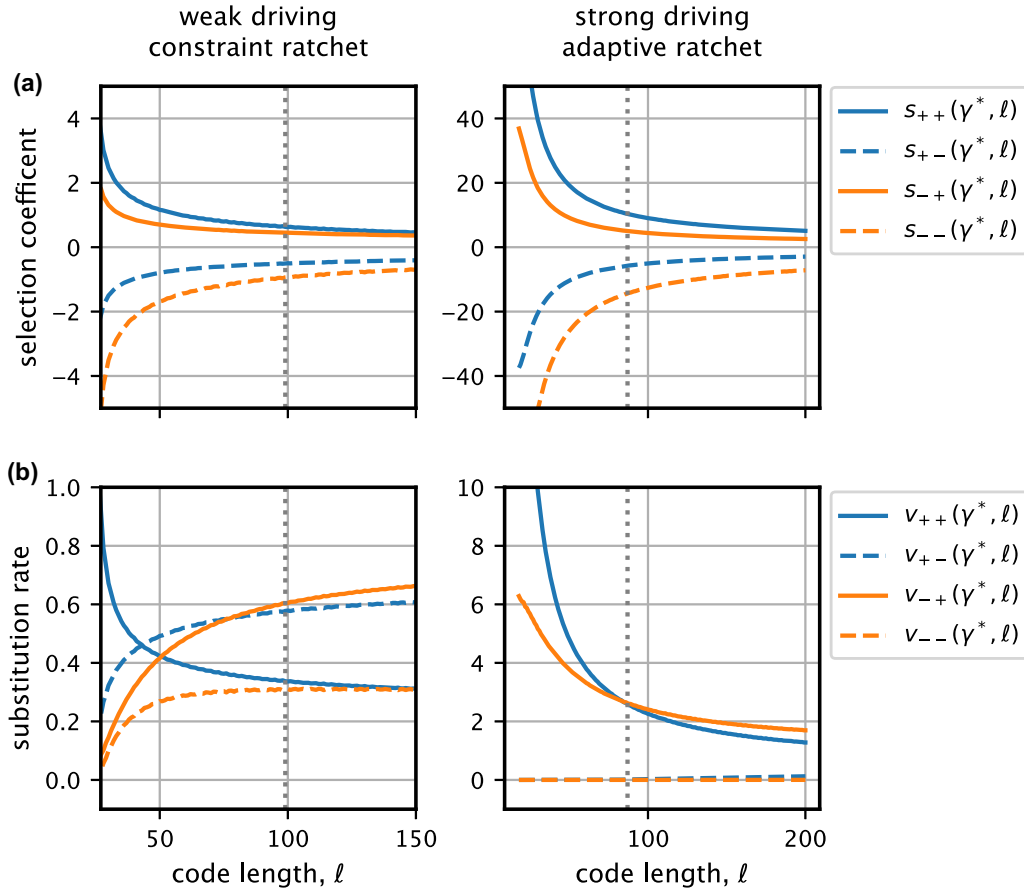


FIG. 10. Selection coefficients and substitution rates for code extensions and compressions. (a) Selection coefficients for two classes of length extensions (blue) and length compressions (orange): mutations generating matches (solid) and mismatches (dashed). (b) Corresponding substitution rates. Left: constraint ratchet regime ($\kappa = 0.1$, $2Nc_0 = 0.1$). Right: adaptive ratchet regime ($\kappa = 10$, $2Nc_0 = 1$). Other parameters as in Fig. 2.

$-s_-(\ell + 1) = F(\ell + 1) - F(\ell)$ and a detailed balance relation of the form $v_+(\ell)/v_-(\ell + 1) = g(s_+(\ell))/g(s_-(\ell + 1)) = \exp[2N(F(\ell + 1) - F(\ell))]$. In particular, the selection coefficients $s_{\pm}(\ell)$ decouple from the increment of the effective fitness landscape for code length, $\bar{F}(\ell + 1) - \bar{F}(\ell)$, as defined by Eq. (B5) [Fig. 4(c)]. This landscape maps the average long-term fitness effects of length changes but does not govern the ratchet dynamics. The selection coefficients $s_{\pm}(\ell)$ also decouple from the increment of the evolutionary potential, $\Psi(\ell + 1) - \Psi(\ell)$ [Fig. 4(b)]. In the stationary state of the ratchet, the substitution rates satisfy the detailed balance relation $\bar{v}_+(\ell)/\bar{v}_-(\ell + 1) = \exp[\Psi(\ell + 1) - \Psi(\ell)]$, but the link of these rates to the neutral rate ν by Haldane's formula is lost.

2. Adaptive tinkering

The ratchet substitution dynamics of extensions and compressions can be characterized by the total rate $v_{\text{tot}}(\ell) = \bar{v}_+(\ell) + \bar{v}_-(\ell)$, which sets the overall speed of code length evolution, and the net elongation rate, $v_e(\ell) = \bar{v}_+(\ell) - \bar{v}_-(\ell)$. Inspection of the potential $\Psi(\ell)$, Eq. (B7) with rates given by Eq. (A22), shows that the total rate takes the form $v_{\text{tot}}^*(\ell)/(2\nu) = 2NC(\ell)s_{\gamma}^*(\ell)$ with a slowly varying coefficient function $C(\ell) \sim 1$. In particular, we obtain an analytical estimate for the total ratchet rate in the stationary state, as given

by Eq. (3). This relation implies $v_{\text{tot}}^* < 2\nu$ in the weak-driving regime, where $2Ns_{\gamma} \sim 1$, and $v_{\text{tot}} > 2\nu$ for $\kappa \gg 1$, in accordance with the evaluation from the full rates.

In the strong-driving regime, the range of adaptive tinkering around ℓ^* can be defined by the r.m.s. code length fluctuations in the stationary state,

$$\Delta\ell = \left(\sum_{\ell} (\ell - \ell^*)^2 Q(\ell|\kappa) \right)^{1/2}. \quad (\text{C3})$$

We estimate $\Delta\ell$ from the curvature of the evolutionary potential for length,

$$\Delta\ell = \left(\frac{\partial^2 \Psi(\ell)}{\partial \ell^2} \Big|_{\ell^*} \right)^{-1/2} \sim \left(\frac{\partial \log[1 - \gamma^*(\ell)]}{\partial \ell} \Big|_{\ell^*} \right)^{-1/2} \sim \ell^*. \quad (\text{C4})$$

To derive this scaling, we Taylor-expand the potential $\Psi(\ell)$ around the point ℓ^* , using Eq. (B7) with rates given by Eq. (A22) in the strong-driving regime ($2Ns_{++}, 2Ns_{--} \ll 1$ and $v_{+-}, v_{-+} \approx 0$, as shown in Fig. 10). Then we use the leading-order scaling $\gamma^*(\ell) - \gamma_0 \sim \ell^{-1}$, consistent with Eq. (2). The analytical approximation of Eq. (C4) is in accordance with the tinkering range evaluated from the full distribution $Q(\ell)$ [Fig. 2(c)]. The ratchet generates

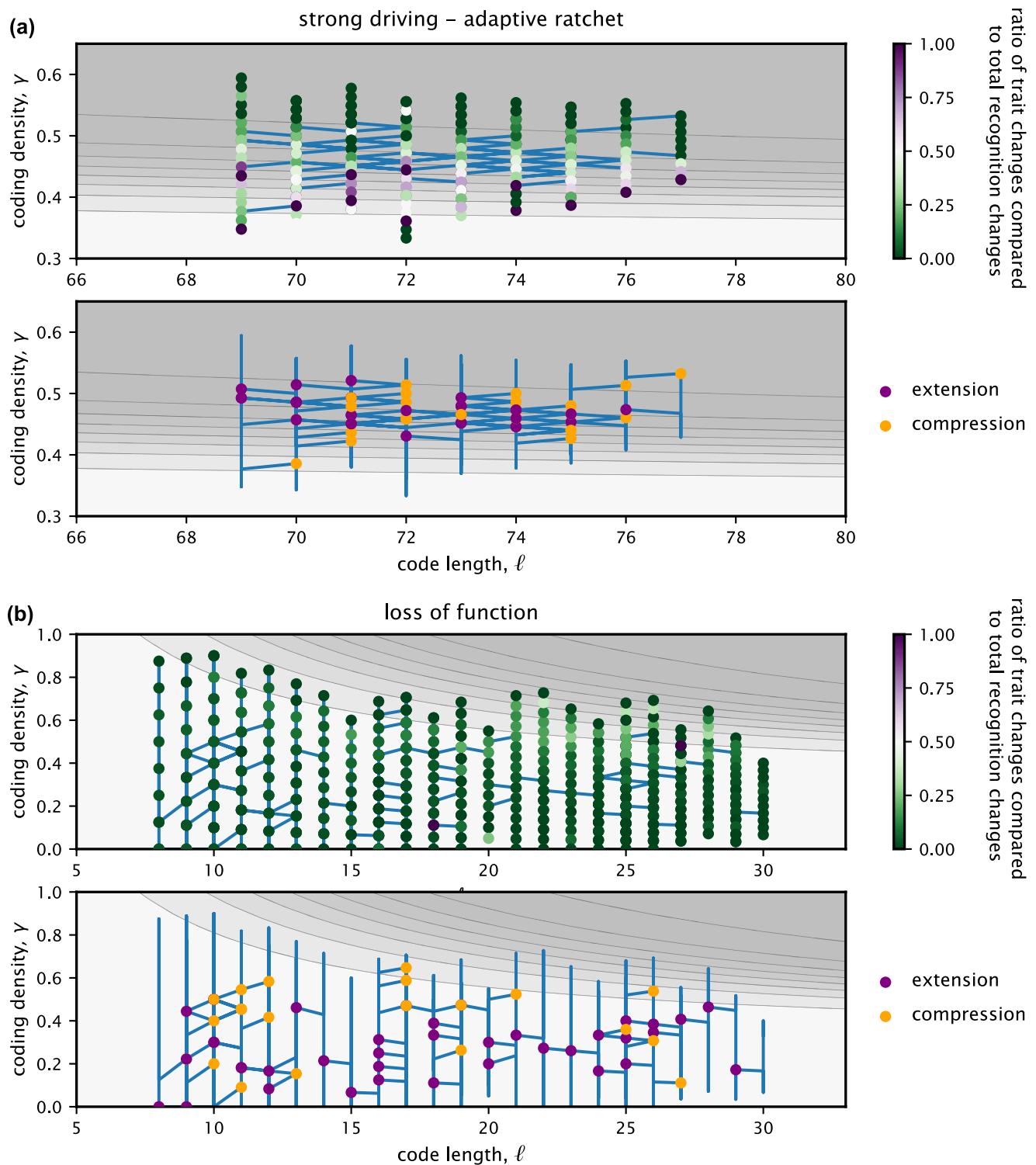


FIG. 11. Evolutionary trajectories at strong driving. Examples of trajectories obtained by numerical simulation (10^5 steps); intermediate states (γ, ℓ) are colored by the ratio of recognition site changes to total recognition changes, $u_{\text{tot}}/(u_{\text{tot}} + \rho_{\text{tot}})$ with $u_{\text{tot}} = u_+ + u_-$ and $\rho_{\text{tot}} = \rho_+ + \rho_-$ (top) or by the type of length change (bottom; compressions: orange; extensions: purple). (a) Trajectory of a functional site in the adaptive ratchet regime ($\kappa = 30$, $2Nc_0 = 1$, zoom of the path in Fig. 2(d), starting point $\ell = 100$, 10^5 warmup steps). This trajectory maintains a balance between target changes and subsequent adaptive code changes. (b) Trajectory leading to loss of function and subsequent collapse of code length ($\kappa = 100$, $2Nc_0 = 1$, starting point $\ell = 30$). Along this trajectory, adaptive code changes cannot keep up with target changes. Other evolutionary parameters as in Fig. 2.

anomalously broad length fluctuations compared to evolution in a univalued fitness peak of the form $F(\ell) = f_0 - c_r \ell^{-\alpha} - c_0 \ell$ ($\alpha > 0$). In that case, the stationary-state fluctuations scale differently, $\Delta \ell \sim (\ell^*)^{1/2}$, as can be seen by evaluating the Boltzmann-Gibbs distribution $Q_{\text{eq}}(\ell) \sim \exp[2NF(\ell)]$.

We note that the state of adaptive tinkering at high molecular complexity is transient because it is subject to an instability. At high driving rates, the coding density can drift towards the neutral value γ_0 and loose functionality; that is, adaptive recognition site mutations cannot keep up with the larger number of target mutations. Subsequent length decay of dysfunctional sites due to complexity cost $F_c(\ell)$ leads to a loss of the site. Figure 11 shows an example if an evolutionary trajectory ending with loss of the site.

3. Number of adaptive paths and fitness flux

The effective number of adaptive paths for a given quantitative trait, here ΔG , is defined as its suitably normalized heritable variation,

$$A(\gamma, \ell) = \frac{\text{Var}(\Delta G/k_B T)}{2\mu N \epsilon_0^2}, \quad (\text{C5})$$

where $2\mu N$ is the neutral sequence diversity per unit of length and $\epsilon_0^2 = \text{Var}(\Delta \Delta G/k_B T)$ is the variance of the mutational effect distribution. The number of adaptive paths is a measure of susceptibility to selection [96]. This can be seen by relating it to the change in mean population fitness by natural selection, $\phi_s \equiv \frac{d}{dt}|_{\text{sel}} E(F)$, in a trait-dependent fitness landscape $F(\Delta G)$. By Fisher's theorem, we obtain

$$\begin{aligned} \phi_s &= \text{Var}(F_r) \\ &= 2\mu N \epsilon_0^2 (F'_r(\Gamma))^2 A(\gamma, \ell) + O(F''_r(\Gamma) \text{Var} F_r), \end{aligned} \quad (\text{C6})$$

where $\Gamma = E(\Delta G/k_B T)$ denotes the population mean trait. The selective flux ϕ_s is a non-negative component of the fitness flux ϕ . In an evolutionary steady state, ϕ_s measures the amount of selective turnover required to maintain a time-independent average trait $\langle \Gamma \rangle$. The total fitness flux ϕ also includes the fitness change by evolution under mutations, selection, and genetic drift [49]. It vanishes in an evolutionary equilibrium state. In a driven stationary state, it has a time-independent average, $\langle \phi \rangle > 0$, that balances with the rate of fitness change by external fluctuations.

In a model with site-dependent trait effects ϵ_i ($i = 1, \dots, \ell$), we obtain an effective number of adaptive paths

$$A \simeq \begin{cases} \sum_{i=1}^{\ell} \frac{\epsilon_i^2}{\epsilon_0^2} \left(\frac{\gamma_0}{1-\gamma_0} (1-\gamma_i) + \gamma_i \right) & (\text{weak selection}) \\ \sum_{i=1}^{\ell} \frac{\epsilon_i^2}{\epsilon_0^2} \left(\frac{\gamma_0}{1-\gamma_0} (1-\gamma_i) \right) & (\text{strong selection}), \end{cases} \quad (\text{C7})$$

where $\gamma_i = 1$ for site positions with a target match and $\gamma_i = 0$ for positions with a mismatch. The weighting factors $\epsilon_i^2/\epsilon_0^2$ reflect the higher contributions of strong-effect mutations to the adaptive process; different regimes for transient adaptive dynamics have been discussed in Ref. [97]. In the weak-selection regime [$2N\epsilon_0 F'(\Gamma) \lesssim 1$], all positions contribute an expected sequence diversity $2N\mu$, generating the maximum trait diversity [95,96]. In the strong-driving regime [$2N\epsilon_0 F'(\Gamma) \gg 1$], the trait diversity narrows to positions with deleterious ma-

jority alleles ($\gamma_i = 0$). These positions contribute an expected trait diversity $2\mu N \frac{\gamma_0}{1-\gamma_0} \epsilon_i^2$, predominantly from mutations destined for fixation. Positions with beneficial majority alleles ($\gamma_i = 1$) freeze out; these positions have a much smaller trait diversity $\mu \epsilon_i^2 / [\epsilon_i F'(\Gamma)]$. Together, the number of adaptive paths A depends on the distribution of effects ϵ_i and the occupancy of beneficial and deleterious sequence states but decouples from the global parameters μ , N , and from the overall strength of selection, $F'(\Gamma)$.

Here we focus on the expected number of adaptive paths in an ensemble of parallel-evolving populations, $A = \langle A \rangle$. In the strong-selection regime of the minimal model [$\epsilon_i = \epsilon_0$, $\langle \gamma_i \rangle = \gamma$], we obtain

$$A(\gamma, \ell) = \frac{\gamma_0}{1-\gamma_0} (1-\gamma) \ell. \quad (\text{C8})$$

Inserting the global stationary-state saddle point $(\gamma^*, \ell^*)(\kappa)$, this relation reduces to Eq. (4); the function $A^*(\kappa)$ is shown in Fig. 5. The number of adaptive paths can be compared to the expected adaptive speed of the recognition trait by point substitutions,

$$\langle \phi_G \rangle(\gamma, \ell) = \ell \epsilon_0 [u_+(\gamma, \ell) - u_-(\gamma, \ell)], \quad (\text{C9})$$

and the corresponding fitness flux,

$$\langle \phi \rangle(\gamma, \ell) = \ell [u_+(\gamma, \ell) - u_-(\gamma, \ell)] s_\gamma(\gamma, \ell); \quad (\text{C10})$$

the contribution of length changes to the adaptive fluxes is down by a factor $O(v/\mu)$. In the strong-driving regime, we obtain

$$\begin{aligned} \langle \phi_G \rangle(\gamma, \ell) &\simeq \ell \epsilon_0 u_+(\gamma, \ell) \\ &= 2\mu N \epsilon_0 s_\gamma(\gamma, \ell) A(\gamma, \ell) \end{aligned} \quad (\text{C11})$$

and

$$\begin{aligned} \langle \phi \rangle(\gamma, \ell) &\simeq \ell u_+(\gamma, \ell) s_\gamma(\gamma, \ell) \\ &= 2\mu N s_\gamma^2(\gamma, \ell) A(\gamma, \ell) \end{aligned} \quad (\text{C12})$$

using Eq. (A14). Thus, at a given value of s_γ , the expected fluxes $\langle \phi_G \rangle$ and $\langle \phi \rangle$ increase with A , while the selection s_γ required for a given value of $\langle \phi_G \rangle$ decreases with increasing A . In an evolutionary steady state, the adaptive fluxes match the effect of recognition target changes, as given by the strong-driving balance condition (B25). We obtain

$$\langle \phi_G^* \rangle(\ell) = \mu \kappa \ell \epsilon_0 (\gamma^*(\ell) - \gamma_0) \simeq \mu \kappa k_0 \epsilon_0, \quad (\text{C13})$$

and

$$\begin{aligned} \langle \phi^* \rangle(\ell) &= \mu \kappa \ell (\gamma^*(\ell) - \gamma_0) s_\gamma^*(\ell) \\ &= \frac{\mu \kappa^2 \ell}{2N} \frac{(\gamma^*(\ell) - \gamma_0)^2}{\gamma_0 (1 - \gamma^*(\ell))} \\ &\simeq \frac{\mu \kappa^2}{2N} \frac{k_0^2}{(1 - \gamma_0) A^*(\ell)}, \end{aligned} \quad (\text{C14})$$

where we use the leading-order scaling $\gamma^*(\ell) - \gamma_0 \sim k_0/\ell$, consistent with Eq. (2). According to these relations, the adaptive speed of the recognition trait becomes asymptotically independent of ℓ ; the fitness flux required to maintain the stationary state decreases with increasing number of adaptive paths.

APPENDIX D: IMMUNE RECOGNITION

1. Recognition complexity

The effective number of epitopes targeted by an adaptive immune response, g_r , depends on the total number of accessible epitopes, g , presented by the pathogen and on stochastic differences in immunogenicity, which are characteristics of the host's immune repertoire. For simplicity, we assume the pathogen has g independent, equally accessible, and statistically equivalent epitopes. In this case, the potencies $\mathbf{Z} = (Z_1, \dots, Z_g)$ of a given host are independent random variables drawn from a common distribution $Q(\mathbf{Z})$, which determines the population statistics of adaptive immune response to a given pathogen. Specifically, we compute the population-averaged log recognition complexity, $\log g_r \equiv \langle S(\mathbf{z}) \rangle$, where $S(\mathbf{z})$ denotes the Shannon entropy of the normalized potency distribution, $S(\mathbf{z}) = -\sum_{i=1}^g z_i \log z_i$ with $z_i = Z_i / \sum_{i=1}^g Z_i$.

A key feature of adaptive immune response is the density of B cell lineages with genetically different receptors, $\Omega(K)$, as a function of the binding constant K to a given epitope. The extremal-value statistics of this density of states determines two important repertoire parameters [66]: (i) the expectation value of strongest binding in a repertoire of L_0 lineages, $K^* = \langle \min K \rangle$, and the repertoire exponent $\beta^* \equiv (Kd/dK) \log \Omega(K)|_{K^*}$. This exponent is approximately determined by the repertoire size L_0 , the code length of the binding locus, ℓ , and the affinity variance in an ensemble of random sequences, $\sigma_{\log K,0}^2 \equiv \ell \epsilon_0^2$, where ϵ_0 denotes the affinity effect scale of point mutations in the receptor sequence. We obtain [66]

$$\beta^* \simeq \frac{\sqrt{2 \log L_0}}{\epsilon_0 \ell^{1/2}}. \quad (\text{D1})$$

At constant binding parameters ℓ and ϵ_0 , the repertoire exponent β^* increases with increasing repertoire size L_0 . At constant L_0 , the exponent decreases with increasing ℓ or ϵ_0 . Hence, by evolutionary changes of L_0 , ℓ , and ϵ_0 , host systems can independently vary the repertoire parameters K^* and β^* .

The repertoire exponent β^* determines the fluctuations of epitope-specific effective affinities $\log Z \equiv \Delta G/(k_b T)$, as given by the variance $\sigma_{\log Z}^2$. To compute this variance, we use three results of Ref. [66]: (i) Fluctuations in the maximum affinity have variance $\sigma_{\log K^*}^2 = \pi^2/(6\beta^{*2})$. (ii) In an exponential infection process, affinity changes induce correlated changes in B cell clone size, $N \sim (K^*)^{-\zeta/\beta^*}$ with $\zeta/\beta^* \approx 1$ for physiological parameters of human repertoires. (iii) Antiserum potencies are given by $Z \sim N/K^*$. Together, we obtain

$$\sigma_{\log Z}^2 = \sigma_{\log(N/K^*)}^2 \approx \frac{4\pi^2}{6} \frac{1}{\beta^{*2}}. \quad (\text{D2})$$

Next, we evaluate the recognition complexity by numerical simulations of stochastic lineage activation [66], generating an ensemble of potency distributions $\mathbf{z}$ in a repertoire with parameters L_0 , ℓ , and ϵ_0 . We obtain a remarkably simple scaling form,

$$\log g_r \simeq (\log g) \times \begin{cases} 1 & (\sigma_{\log Z}^2 \lesssim 1) \\ (\sigma_{\log Z}^2)^{-1} & (\sigma_{\log Z}^2 \gtrsim 1), \end{cases} \quad (\text{D3})$$

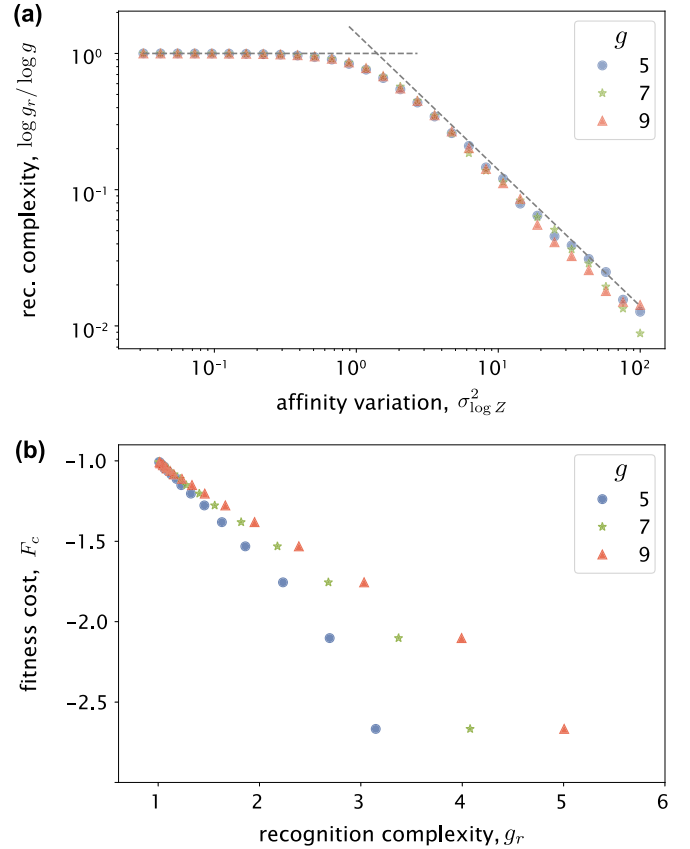


FIG. 12. Repertoire dependence and fitness cost of recognition complexity. (a) Scaled log recognition complexity, $\log g_r / \log g = \langle S(\mathbf{z}) \rangle$, plotted as a function of the target affinity variation $\sigma_{\log Z}^2$ together with the asymptotic forms given by Eq. (D3) (dashed lines). The population-averaged entropy is obtained numerically by sampling 1000 potency distributions $\mathbf{z}$ from the immune response distribution described in the text [66]. (b) Host fitness cost, $F_c(g_r)$. This function is derived from the fitness cost of repertoire size L_0 , as explained in the text. Repertoire parameters: $\beta^* = 1/\zeta = 2.5$; see Refs. [66,98–102].

as shown in Fig. 12(a). This form describes a cross-over from a high-complexity, low-immunodominance regime ($g_r \approx g$ for $\sigma_{\log Z}^2 \lesssim 1$) to a low-complexity, high-immunodominance regime set by immunogenicity fluctuations of the host repertoire ($g_r < g$ for $\sigma_{\log Z}^2 \gtrsim 1$).

The preceding analysis also establishes a generic fitness cost of recognition complexity, defining a cost component $F_c(g_r)$ in the fitness model of Eq. (2). Assuming a fitness cost of repertoire size L_0 , we can compute the resulting fitness landscape $F_c(g_r)$ by combining the repertoire relations (D1)–(D3). For example, a repertoire fitness cost of order $\log L_0$ (e.g., induced by off-target binding) produces the landscape $F_c(g_r)$ shown in Fig. 12(b), which describes a monotonically increasing, approximately linear cost over the relevant range of g_r (cf. Fig. 6). Other assumptions produce qualitatively similar landscapes, the details of which are beyond the scope of this paper. In the minimal fitness model discussed used in the main text, we use the linear approximation $F_c(g_r) = c_0 g_r$.

2. Recognition dynamics

Our minimal dynamics model has three characteristics: (i) Functional binding of antibodies to an antigenic epitope leads to neutralization of the antigen. The neutralization probability R is described by a Hill recognition function depending on the reduced binding affinity $\Delta G/(k_B T)$, as given by Eq. (A1) [74,75]. (ii) An infection at time t_0 targets g epitopes with initially equal affinities, $\Delta G_\alpha(t_0)/(k_B T) = \Delta G_i/(k_B T) = \Delta G_{50}/(k_B T) + 2\epsilon_0$ ($\alpha = 1, \dots, g$). This sets the entropy $S(z) = \log g$ and the recognition complexity $g_r = g$. (iii) Antigenic drift is a Poisson process that changes epitope sequences with a point substitution rate ρ ; each epitope mutation reduces the affinity by a constant amount, $\epsilon_0 = \Delta \Delta G/k_B T$. Thus, antigenic drift generates time-dependent affinities $\Delta G_\alpha(t)$ ($\alpha = 1, \dots, g$). (iv) Exposure of an individual to the evolving antigen is an independent Poisson process with rate τ^{-1} . For an exposure event at time t , we evaluate the recognition function

$$R(t) = 1 - \prod_{\alpha=1}^g \left(1 - \frac{1}{1 + \exp \left[\frac{1}{k_B T} (\Delta G_{50} - \Delta G_\alpha(t)) \right]} \right). \quad (\text{D4})$$

Neutralization by activation of immune memory occurs with probability $R(t)$, given that each epitope is an independent and sufficient channel for recognition of the antigen [76].

A full secondary infection occurs with probability $\Delta R(t) = 1 - R(t)$, resetting all affinities to ΔG_0 . Here we assume that significant updating of the immune response repertoire occurs only after full infections. Additionally, exposure events that occur between full infection events can boost serum antibody titers against the corresponding pathogen [103]. However, this does not affect our results, which rely on memory response rather than on serum antibodies.

We simulate long-term affinity trajectories $(\Delta G_1, \dots, \Delta G_g)(t)$ for the joint dynamics of antigenic drift and exposures. The effective speed of antigenic evolution, $\kappa \equiv \rho \tau$, is given by the expected number of mutations per epitope between subsequent exposures. We record the steady-state average antigenic fitness component $F_r(g, \kappa) = f_0 \langle \Delta R \rangle(g, \kappa)$; this quantity is proportional to the fitness cost of infections. Together with a complexity cost of the form $F_c(g) = -c_0 g$, we obtain a minimal host fitness function of the form (2).

The conditional fitness landscape $F(g|\kappa)$ determines the optimal epitope complexity for given antigenic speed, $g^*(\kappa)$. For low to moderate antigenic drift ($\kappa \lesssim 2$), $g^*(\kappa)$ increases with κ [Fig. 6(b)]. For higher values of κ , the number of epitopes needed for protection increases exponentially, inducing a prohibitive cost and a transition to $g^*(\kappa) = 0$. A negative correlation between the speed of antigenic evolution and the efficacy immune memory has also been mapped in multipathogen models [17].

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