

Fitness flux and ubiquity of adaptive evolution

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Natural selection favors fitter variants in a population, but actual evolutionary processes may decrease fitness by mutations and genetic drift. How is the stochastic evolution of molecular biological systems shaped by natural selection? Here, we derive a theorem on the fitness flux in a population, defined as the selective effect of its genotype frequency changes. The fitness-flux theorem generalizes Fisher's fundamental theorem of natural selection to evolutionary processes including mutations, genetic drift, and time-dependent selection. It shows that a generic state of populations is adaptive evolution: there is a positive fitness flux resulting from a surplus of beneficial over deleterious changes. In particular, stationary nonequilibrium evolution processes are predicted to be adaptive. Under specific nonstationary conditions, notably during a decrease in population size, the average fitness flux can become negative. We show that these predictions are in accordance with experiments in bacteria and bacteriophages and with genomic data in *Drosophila*. Our analysis establishes fitness flux as a universal measure of adaptation in molecular evolution.

adaptive evolution | fitness landscapes | fluctuation theorems in statistical physics | fundamental theorem of natural selection

Adaptive processes have taken center stage in molecular evolutionary biology. Deep sequencing of populations opens unprecedented opportunities to trace the genomic basis of adaptation in population-genetic studies within and across species as well as in time series of evolution experiments. Various methods are used to infer natural selection from such data; however, their results lack a common gauge and are sometimes difficult to compare. This paper develops the concept of fitness flux in a population as a generic measure of adaptation applicable to molecular data. Whereas fitness characterizes the state of a population at a given point in time, fitness flux can be accumulated in a population's history over a period (a precise definition of this quantity will be given below). Fitness flux, not fitness, turns out to be the right variable to show that adaptive evolution is a generic state of natural populations. The notion of fitness flux is already implicitly contained in Fisher's fundamental theorem of natural selection (1), which states that any fitness difference within a population leads to adaptation in an evolution process governed by natural selection alone. The fitness flux of this deterministic process equals the (additive) fitness variance in the population. Hence, the flux is positive when adaptation occurs and zero otherwise.

Generalizing this picture to realistic processes of molecular evolution has been a long-standing problem (2–8). The solution presented here involves a number of important conceptual steps. First, molecular processes are always stochastic because of genetic drift and mutations, and we include these forces into a stochastic theory of fitness flux. Second, we extend the observation of this dynamics to the time scales of genomic data, describing populations by histories of genotype composition and demography that may extend beyond their coalescence time. Third, natural selection itself is treated as dynamic on these time scales. We generalize static fitness landscapes, a concept introduced by S. Wright (9), to explicitly time-dependent models of selection referred to as fitness seascapes. The time dependence of selection reflects the changing ecology of a species. It has complex and opposing effects on adaptation (10–15): rapid fluctuations enhance the stochasticity of the evolutionary process and impede

long-term adaptation (11), but persisting changes open windows of positive selection and are the very cause of adaptive evolution and fitness flux (13, 15). The production of fitness flux by adaptive processes is a nonequilibrium phenomenon and does not necessarily imply any increase in fitness. Surprisingly, this production obeys a general theorem, which provides a basis for quantifying adaptation in stochastic evolution. The theorem and its proof establish an important conceptual link between evolutionary genetics and stochastic nonequilibrium thermodynamics. A close analogy of the fitness-flux theorem to its deterministic counterpart, Fisher's theorem, emerges in the case of stationary evolution. The average fitness flux is positive in nonequilibrium stationary states, which are associated with adaptive evolution (15). The flux is zero at evolutionary equilibrium, where no adaptations occurs. The power of the fitness-flux theorem will be shown by a number of applications to evolution experiments in microbes and to cross-species genome comparison in flies.

Theory of Fitness Flux

Population Histories and Fitness Flux. We start by introducing the notion of fitness flux and its relationship to fitness. Consider first the microevolution of a population containing a resident and a mutant genotype. The genotype evolution of the population can be described by a series of observations $\mathbf{x} = (x_0, x_1, \dots, x_n)$ of mutant frequencies at successive times (t_0, t_1, \dots, t_n) , which we refer to as the history of the population. Natural selection governs this process by a difference in reproductive rate of the mutant against the resident genotype, s , which may depend on frequencies and time. Here, we consider selection coefficients $s(x, t)$ with persistence times of many generations that affect the evolution of genotype frequencies and can be measured, for example, by growth-competition experiments in a microbial population. Measurements of population histories are assumed to be sufficiently dense so that the changes in selection as well as frequency changes ($\Delta x_i = x_{i+1} - x_i$) in each time interval ($\Delta t_i = t_{i+1} - t_i$) are small. The fitness flux can then be defined as a measure of adaptation at a given point of the population history: $\phi(t_i) = s(x_i, t_i)\Delta x_i/\Delta t_i$ is the product of the selection coefficient and the rate of frequency change. The cumulative fitness flux (Eq. 1)

$$\Phi(\mathbf{x}) = \sum_{i=0}^{n-1} \phi(t_i)\Delta t_i = \sum_{i=0}^{n-1} s(x_i, t_i)\Delta x_i \quad [1]$$

measures the total adaptation of the entire population history (13). For a history containing a substitution process (i.e., a transition from initial frequency $x_0 = 0$ to final frequency $x_n = 1$) under constant selection, the flux Φ equals the selection coefficient s of the new genotype against the old genotype. This picture is easily extended to sequences of length L with 4^L

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positive (i.e., the cumulative flux Φ increases), and there is adaptation: Fisher's populations move uphill on fitness landscapes. However, the theorem is valid only under the restrictive assumption that evolution is deterministic and dominated by natural selection, which excludes mutations and genetic drift. Hence, Fisher's theorem applies only to microevolution of established alleles in the limit of large populations under strong selection, a regime that is violated by low-frequency alleles in any finite population.

To describe realistic processes of molecular evolution over longer evolutionary times, we must include mutations and genetic drift in our scenario. These stochastic evolutionary forces compete with natural selection and invalidate Fisher's theorem. The fixation of slightly deleterious mutations and mutation load are well-known examples for such effects. Thus, the fitness flux of an individual population can become negative: stochastically evolving populations can move downhill on fitness landscapes (27). Of course, stochastic evolutionary theory no longer addresses single populations but ensembles of evolving populations with time-dependent distributions $P(x, t)$ of genotype or allele frequencies. The interpretation of such distributions is strictly probabilistic, because genotype space and allele-frequency space for multiple genomic loci are very high dimensional and can never be sampled by the time course of a single population or by an actual ensemble of populations. Simple observables in populations and their histories, however, can be sampled and compared with predictions of the probabilistic theory. This is the case for the theorem to be established below, which is an identity involving the cumulative fitness flux.

Evolutionary Equilibria and Fitness. We first show how fitness and fitness flux can be evaluated in stochastic population ensembles. The evolution of the frequency distribution $P(x, t)$ is determined by the conditional probabilities $G(x', t', x, t)$ of the transition from initial frequencies x at time t to frequencies x' at a later time t' . We define evolutionary equilibrium as a stationary (time-independent) distribution $P_{\text{eq}}(x)$, which satisfies the so-called detailed balance condition $G(x', t', x, t)P_{\text{eq}}(x) = G(x, t', x', t)P_{\text{eq}}(x')$ for arbitrary times and frequencies. Detailed balance says that in equilibrium, the probability of any evolutionary transition equals the probability of the reverse transition. This definition is well-known in statistical physics, but it is more restrictive than the definitions in much of the population-genetics literature, where any stationary state is called equilibrium. For simplicity, we assume that the mutation-drift process in the absence of selection has an (approximate) equilibrium $P_0(x)$. This is the case if the mutation rates are independent of time and satisfy mild additional conditions (*Methods*). We do not require that the actual process has reached this equilibrium. Given a neutral equilibrium, the full process in an arbitrary time-independent fitness landscape $F(x)$ also has an equilibrium, and its frequency distribution is of remarkably simple form (Eq. 2),

$$P_{\text{eq}}(x) = P_0(x)e^{NF(x)} \quad [2]$$

(*Methods*). Here, N is the effective population size, and the additive constant of the fitness landscape is given by normalization of the distribution P_{eq} . Kimura's (27) U-shaped distributions for a single two-allele locus at neutrality and under directional selection are classic examples of evolutionary equilibria and the fitness relation (Eq. 2). Various forms of this relation have been used to infer scaled fitness landscapes $NF(x)$ from histograms of the distributions $P_0(x)$ and $P_{\text{eq}}(x)$ obtained from genomic data (28, 29) (for a recent review, see ref. 15). The fitness relation has an obvious information-theoretic interpretation (30): the function $NF(x)$ is the relative log-likelihood of the distributions $P_{\text{eq}}(x)$ and $P_0(x)$, and its expectation value

$N\langle F \rangle = \sum_x NF(x)P_{\text{eq}}(x)$ equals the relative (Kullback–Leibler) entropy of these distributions, $N\langle F \rangle = H(P_{\text{eq}}|P_0)$.

Reverse Histories and Fitness Flux. Equilibrium may be the exception rather than the rule in the evolution of biological systems under natural selection. A generic process evolves from arbitrary initial conditions in an arbitrary (time-dependent or nongradient) fitness seascape, generating a frequency distribution $P(x, t)$ that may be far from equilibrium. This distribution can still be compared with the neutral equilibrium distribution $P_0(x)$ by the relative log likelihood $\mathcal{H}(x, t) = \log[P(x, t)/P_0(x)]$, which, however, no longer equals the scaled fitness $NF(x)$ as in equilibrium. We now derive a nonequilibrium identity similar to Eq. 2 for the probability distribution $\mathcal{P}(\mathbf{x})$ of population histories in a given time interval (t_0, t_n) . We define for each history \mathbf{x} a reverse history $\mathbf{x}^T = (x_0^T, \dots, x_n^T)$. Starting from the point $x_0^T = x_n$ at time t_0 , the history \mathbf{x}^T evolves through the population states of \mathbf{x} in reverse order, and each transition has the opposite fitness effect as the corresponding transition in the original history (Fig. 1). Using methods originally developed in nonequilibrium thermodynamics (31–34), we can show that the probability of the reverse history is given by (Eq. 3)

$$\mathcal{P}(\mathbf{x}^T) = \mathcal{P}(\mathbf{x})e^{-N\Phi(\mathbf{x}) + \Delta\mathcal{H}(\mathbf{x})}, \quad [3]$$

where $\Phi(\mathbf{x})$ is the cumulative fitness flux and $\Delta\mathcal{H}(\mathbf{x}) = \mathcal{H}(x_n, t_n) - \mathcal{H}(x_0, t_0)$ is the difference in relative log likelihood between the initial and final point of the original (forward) history (*Methods*).

Fitness-Flux Theorem. We can now state the theorem: an evolutionary process with mutations, genetic drift, and selection given by an arbitrary fitness seascape satisfies the identity (Eq. 4)

$$\langle e^{-N\Phi + \Delta\mathcal{H}} \rangle = 1. \quad [4]$$

The angular brackets denote an average over the probability distribution of population histories, $\langle f \rangle = \sum_{\mathbf{x}} f(\mathbf{x})\mathcal{P}(\mathbf{x})$, in a given time interval (t_0, t) . Using Eq. 3, we recognize this history average as the sum $\sum_{\mathbf{x}^T} \mathcal{P}(\mathbf{x}^T)$, which equals unity by normalization of the probability distribution of reverse histories. The identity (Eq. 4) is a genuine nonequilibrium relation: it is valid for arbitrary initial conditions $P(x, t_0)$ and arbitrary time-dependent selections. It belongs to a set of relations known as fluctuation theorems, which have played an important role in the nonequilibrium statistical physics of mesoscopic systems over the past decade (31–34). As an immediate consequence of Eq. 4, the fitness flux in any fitness seascape has a lower bound,

$$N\langle \Phi \rangle \geq \Delta H, \quad [5]$$

where $\Delta H = \langle \Delta\mathcal{H} \rangle = H(P(t)|P_0) - H(P(t_0)|P_0)$ is the relative entropy difference between initial and final frequency distribution. If the underlying assumption of existence of a neutral equilibrium is dropped, relations analogous to Eq. 4 and the inequality 5 still hold for the total nonequilibrium flux, which is the sum of fitness flux and neutral mutation flux (*SI Text*).

Applications of the Theorem

Population histories and fitness fluxes are increasingly accessible to experimental observation (17–21). As an example, consider a recent experiment describing the adaptation of a bacterial population to antibiotic stress (17). This process involves five amino acid substitutions in a specific protein (i.e., $2^5 = 32$ different genotypes and $5! = 120$ different population histories differing in the order of these substitutions). The selection coefficient of each substitution in each genetic background has been measured, and the cumulative fitness flux of a population history is simply the sum of the selection coefficients of its substitutions in

Numerical simulations of equilibrium populations shown in Fig. 2A illustrate the identical statistics of $N\Phi$ and ΔH for individual histories and the resulting ensemble distributions. By Eq. 3, the identity $N\Phi = \Delta H$ implies that the probability of any history equals that of its reverse history, $\mathcal{P}(\mathbf{x}) = \mathcal{P}(\mathbf{x}^T)$, which expresses detailed balance of the equilibrium ensemble: beneficial substitutions of selection coefficient $s > 0$ occur at the same rate as deleterious substitutions of selection coefficient $-s$. Hence, the average fitness flux $\langle \phi \rangle$ vanishes, and the average fitness $\langle F \rangle$ remains constant. Evolutionary equilibrium has been realized, for example, in an experiment with bacteriophages (35). Under stationary experimental conditions, the phage populations have been found to relax to constant fitness values that are independent of the previous history of the population and increase with population size, consistent with the behavior of the ensemble average $\langle F \rangle$ predicted by Eq. 2. This self-averaging of individual populations suggests that the phage genome has a sufficient number of independently evolving loci.

For the approach to equilibrium, the inequality 5 implies that the free fitness $N\langle F \rangle - H$ always increases monotonically with time and is maximal at equilibrium (4, 36), which is an evolutionary analog of Boltzmann's H theorem. Consistent with this scenario, the recent analysis of a long-term experiment of bacterial evolution under constant conditions has found an increase of fitness and a decrease of fitness flux ϕ over time (21).

Nonequilibrium Stationary States. In a generic population ensemble, the maximum principles of equilibrium (4, 36) and the detailed balance condition $N\Phi = \Delta H$ for individual histories are violated. For any stationary nonequilibrium ensemble, the inequality 5 with $\Delta H = 0$ predicts that the average fitness flux $\langle \phi \rangle = d\langle \Phi \rangle/dt$ is a positive constant: populations steadily accumulate a surplus of beneficial over deleterious changes. Thus, adaptation takes place, although the average fitness $\langle F \rangle$ remains constant. For most individual populations, the cumulative flux over a sufficiently long time interval Δt is close to the ensemble average, $\Phi \approx \langle \Phi \rangle = \langle \phi \rangle \Delta t > 0$. The full theorem (Eq. 4) shows that selection is overcome by genetic drift and $N\Phi < \Delta H$ in an exponentially small subset of populations. Muller's ratchet, the scenario of degradation by an excess of deleterious substitutions, is, thus, very unlikely as a stationary process but requires, for example, a dwindling population size.

Stationary nonequilibrium evolution can be caused by time-dependent selection (15). Here, we consider a minimal stochastic fitness seascape defining independently fluctuating selection coefficients at individual genomic loci. This process generates an ensemble of populations with joint histories of selection and genotype, which is shown in the numerical simulations of Fig. 2B. In accordance with the fitness-flux theorem, the cumulative flux $\langle \Phi \rangle$ in the stationary state increases at a constant positive rate $\langle \phi \rangle$. Typical populations adapt to changing selection pressures by a surplus of beneficial over deleterious substitutions. Recent population-genetic studies of *Drosophila* genomes have shown evidence for adaptive evolution at a genome-wide level (37, 13, 38). Positive fitness flux (with values $N\Phi$ of order 10 per genomic substitution) (13) has been inferred from joint estimates of rate and average selection coefficient of point mutations, supporting the conclusion that a substantial fraction of the observed substitutions is adaptive at substantial levels of selection.

Demographic Nonequilibrium. The fitness-flux theorem also captures nonequilibrium processes generated by the demographic history of a population (*Methods*). A simple example is a population bottleneck with a decline transition from equilibrium at an initial population size to an (approximate) equilibrium at a lower population size, which is followed by a recovery transition to equilibrium at the initial population size. This is exactly the protocol of the bacteriophage experiment discussed above (35).

By Eq. 2, the population decline leads to a loss in scaled fitness, $\Delta_1 H = \Delta_1 \langle NF \rangle < 0$, which is exactly compensated by the gain during recovery, $\Delta_2 H = \Delta_2 \langle NF \rangle = -\Delta_1 H$. The cumulative fitness flux $\langle \Phi_1 \rangle$ of the decline transition is allowed to become negative but is more than offset by the flux $\langle \Phi_2 \rangle$ of the recovery transition, such that the total flux $\langle \Phi_1 + \Phi_2 \rangle$ becomes positive. These predictions are confirmed by numerical simulations as shown in Fig. 2C. They imply that the positivity of fitness flux is not limited to stationary evolution at constant population size. Any population whose size changes periodically or fluctuates stochastically around some average will, over sufficiently long periods of time, acquire a positive cumulative flux.

Strong-Selection Limit and Fisher's Theorem. Laboratory evolution experiments often involve very high selection pressures. In this regime, the probability of fitness-lowering frequency transitions becomes very small according to Eq. 3, and only evolutionary histories with monotonically increasing fitness flux are accessible to the system. This reduction of histories has been observed in the bacterial evolution experiment of ref. 17. The evolutionary process over longer time intervals remains stochastic, because every new mutation appears by chance in an individual and its fate at small-population frequencies is always governed by genetic drift. The remainder of its substitution process, however, becomes deterministic and follows the single, most probable evolutionary history. The cumulative flux of the deterministic history grows at a rate equal to the fitness variance in the population. In this limit, the stochastic theory of fitness flux contains Fisher's theorem (*Methods*).

Conclusions

Here, we have established fitness flux as a measure of adaptation in molecular evolution under time-dependent selection and population size, mutations, and genetic drift. The fitness-flux theorem lays the statistical foundation of this measure and makes testable predictions in diverse contexts of experiment and genomics. The concept of fitness flux and the theorem can be formulated for the evolution of genotypes, as we have done here, but also for the evolution of complex molecular phenotypes.

The applications of the fitness-flux theorem discussed in this paper show that an increase of Φ is an almost universal evolutionary principle of biological systems. Positive contributions to the fitness flux arising from adaptive genotype changes accumulate over evolutionary periods of time. Negative contributions are limited to time intervals with a systematic loss of adaptation ($\Delta H < 0$), which cannot occur continuously in viable populations. In this sense, fitness flux is a more fundamental characteristic of evolution than fitness, for which no comparable growth law holds.

Ever since Fisher speculated about a connection between the fundamental theorem and the second law of thermodynamics (1), conceptual links between biological evolution and statistical thermodynamics have been discussed (39, 40). Here, we have established a generic connection away from equilibrium, which links molecular evolution and thermodynamics as stochastic processes driven by time-dependent forces. The fitness-flux theorem shows that this dynamics follows common statistical principles in both fields.

Methods

Evolution Equation and Equilibrium Distributions. In a space of k different genotypes or genomic alleles, we describe the evolution of a population by time-dependent frequencies $\mathbf{x}(t) = (x^1, \dots, x^k)(t)$ with the normalization $\sum_{\alpha=1}^k x^\alpha(t) = 1$ and, the evolution of an ensemble of populations by a time-dependent frequency distribution $P(\mathbf{x}, t)$ with the normalization $\sum_{\mathbf{x}} P(\mathbf{x}, t) = 1$. For populations of large effective size N , it is convenient (but not necessary) to approximate frequencies by continuum variables and to describe their evolution by a diffusion equation (Eq. 6) (41),

$$\frac{\partial}{\partial t} P(x, t) = \sum_{\alpha, \beta=1}^{k-1} \frac{\partial}{\partial x^\alpha} \left[\frac{1}{N} \frac{\partial}{\partial x^\beta} g^{\alpha\beta}(x) - v^\alpha(x, t) \right] P(x, t). \quad [6]$$

The matrix $g^{\alpha\beta}(x)$ characterizes genetic drift, and the evolution rates $v^\alpha(x, t) = m^\alpha(x) + \sum_{\beta} g^{\alpha\beta}(x) s_\beta(x, t)$ contain the contributions of mutations and time-dependent selection with coefficients $s_\beta(x, t)$ (for details, see *SI Text*). A stationary neutral process with mutation rates μ_α^β satisfying the detailed balance conditions $\mu_\alpha^\beta/\mu_\beta^\alpha = p_0^\beta/p_0^\alpha$ can be shown to have an asymptotic equilibrium distribution $P_0(x)$ in the regime $\mu N L \ll 1$ for genotypes or $\mu N \ll 1$ for allele frequencies in linkage equilibrium (*SI Text*). Under the same conditions, the corresponding process in any time-independent fitness landscape $F(x)$ has an equilibrium distribution $P_{\text{eq}}(x)$ of the form (Eq. 2), as shown by inspection of the diffusion equation (Eq. 6).

Derivation of the Fitness-Flux Theorem. Consider the ensemble of population histories $\mathbf{x} = (x_0, \dots, x_n)$ with a sufficiently dense set of observation times $t_i = t_0 + i\delta$ ($i = 0, \dots, n$). For a generic diffusion process of the form (Eq. 6), this ensemble is characterized by a probability distribution $\mathcal{P}(\mathbf{x})$ of histories, which determines the frequency distribution $P(x_n, t_n) = \sum_{x_0, \dots, x_{n-1}} \mathcal{P}(\mathbf{x})$ and has the normalization $\sum_{\mathbf{x}} \mathcal{P}(\mathbf{x}) = 1$. Using standard methods of statistical mechanics, we compute the conditional probability of a history for a given initial state, $G(\mathbf{x}) = \mathcal{P}(\mathbf{x})/P(x_0, t_0)$, and its neutral counterpart $G_0(\mathbf{x})$. These enjoy the relation $G(\mathbf{x}) = G_0(\mathbf{x}) \exp[N\Phi(\mathbf{x}) + \frac{N\delta}{4} \sum_{i=0}^{n-1} [v^2(x_i, t_i) - m^2(x_i, t_i)]]$ with scalar products v^2 and m^2 defined in *SI Text*. We now consider the reverse history $\mathbf{x}^T = (x_n^T, \dots, x_0^T)$, which is given by $x_i^T = x_{n-i}$ ($i = 0, \dots, n$), and, by definition, evolves in the time-reversed fitness landscape $F^T(x, t) = F(x, t_n - t + t_0)$ (Fig. 1). Using the

method of refs 32–34, we compare the conditional probabilities of original and reverse history (Eq. 7),

$$\frac{G^T(\mathbf{x}^T)}{G(\mathbf{x})} = \frac{G_0^T(\mathbf{x}^T)}{G_0(\mathbf{x})} e^{-N\Phi(\mathbf{x})} = \frac{P_0(x_n)}{P_0(x_0)} e^{-N\Phi(\mathbf{x})}. \quad [7]$$

Inserting the definition of the relative log likelihood, $P(x, t) = P_0(x) e^{\eta(x, t)}$, then yields Eq. 3, and the theorem (Eq. 4) follows by summation over all histories. For a time-dependent population size parameterized in terms of a reference size, $N(t) = \zeta(t)N_0$, and the scaled fitness flux defined as $N_0\Phi(\mathbf{x}) = \sum_{i=0}^{n-1} N(t_i)\Delta x_i s(x_i, t_i)$, the identity (Eq. 4) remains valid in very good approximation for low mutation rates. This is shown by an inhomogeneous rescaling of time by a factor $1/\zeta(t)$ in the evolution equation (Eq. 6). A more detailed derivation of the theorem is given in *SI Text*.

Fisher's Theorem. In the strong-selection limit of the evolutionary process (Eq. 6), the evolution of a polymorphic population is dominated (except for small frequencies of $x < 1/Ns$) by its most probable history, which follows the deterministic evolution equation ($dx_i^\alpha/dt = \sum_{\beta} g^{\alpha\beta}(x_i(t)) s_\beta(x_i(t), t)$). Hence, its fitness flux Φ_* increases at a rate $\dot{\Phi}_*(t) = d\Phi_*/dt = (dx_*/dt) s(x_*(t), t) = [s(x_*(t), t)]^2$, which is the fitness variance in the population (*SI Text*). Fisher's theorem is the projection of this identity from genotype frequencies to gene-allele frequencies.

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