

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

Special Issue: *The Year in Evolutionary Biology*

REVIEW

Genetic constraints on adaptation: a theoretical primer for the genomics era

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Genetic constraints are features of inheritance systems that slow or prohibit adaptation. Several population genetic mechanisms of constraint have received sustained attention within the field since they were first articulated in the early 20th century. This attention is now reflected in a rich, and still growing, theoretical literature on the genetic limits to adaptive change. In turn, empirical research on constraints has seen a rapid expansion over the last two decades in response to changing interests of evolutionary biologists, along with new technologies, expanding data sets, and creative analytical approaches that blend mathematical modeling with genomics. Indeed, one of the most notable and exciting features of recent progress in genetic constraints is the close connection between theoretical and empirical research. In this review, we discuss five major population genetic contexts of genetic constraint: genetic dominance, pleiotropy, fitness trade-offs between types of individuals of a population, sign epistasis, and genetic linkage between loci. For each, we outline historical antecedents of the theory, specific contexts where constraints manifest, and their quantitative consequences for adaptation. From each of these theoretical foundations, we discuss recent empirical approaches for identifying and characterizing genetic constraints, each grounded and motivated by this theory, and outline promising areas for future work.

Keywords: dominance; epistasis; pleiotropy; trade-off; population genetics

Introduction

Interest in genetic constraints extends back to the earliest days of evolutionary genetics research, with several prominent constraints recognized by founding figures in evolutionary theory. Fisher, for example, provided the first compelling analysis of pleiotropy as a limiting factor in the emergence of adaptive genetic variation (Ref. 1, pp. 34–35, and Ref. 2). Haldane noted the constraints to adaptation from genetic dominance, and the inefficiency of natural selection when beneficial mutations are recessive.³ Wright emphasized the importance of epistatic gene interactions during adaptation, and the challenge of optimizing fitness when epistasis is strong.⁴ Each showed how constraints arise from intrinsic properties of genetic transmission or trait expression, inhibiting the tendency of natural selection to increase fitness over time.

Research on genetic constraints has since expanded profoundly, and themes of constraint now

occupy prominent positions in some of the most active areas of evolutionary research. The modern study of evolutionary genetic constraints has two particularly notable features. First, constraints have broad evolutionary implications. They are thought to be central to the maintenance of genetic variation,^{5,6} the evolution of separate sexes,⁷ and the evolutionary dynamics of genomes.^{8,9} Constraints also carry implications for ageing,¹⁰ extinction,¹¹ and species invasions.¹² A second feature of this research is its heavy reliance on mathematical models. Empirical tests of genetic constraints are motivated by, and interpreted in light of, formal theory. The widespread application of genomics to most areas of biology has further tightened the interplay between theory and empirical research on genetic constraints.

This review has two main aims. First, we seek a theoretical synthesis between the major forms of evolutionary genetic constraint. With this in mind,

we present a population genetic primer for five classes of constraint: (1) genetic dominance, (2) pleiotropy, (3) fitness trade-offs between individuals of a population, (4) sign epistasis, and (5) genetic linkage between loci. In seeking conceptual unification, we develop analogs of Fisher's fundamental theorem of natural selection (Ref. 1, pp. 34–35, and Refs. 13 and 14) to quantify short- and long-term consequences of genetic constraints for adaptation. We acknowledge that the literature on genetic constraints is massive, with individual constraints often subjects of solo reviews^{15–17} or discussed as composite factors affecting the evolution of quantitative traits.^{18,19} By presenting comparable metrics of adaptation, we hope to provide a common currency for evaluating individual constraints, and a theoretical platform for empirical studies of constraint.

Our second goal is to highlight recent empirical progress, promising approaches, and challenges in validating mechanisms of evolutionary genetic constraint. The recent proliferation of experimental evolution studies and population genomics inference methods has greatly expanded our ability to infer the molecular genetic basis of adaptation (e.g., Refs. 20 and 21). Nevertheless, genetic constraints often leave weak genomic signals, or apply to species where experimental evolution is intractable. Studies of genetic constraints therefore face challenges that go beyond the usual struggles in deciphering the genetic basis of fitness (e.g., Ref. 22, p. 23, and Ref. 23). Knowledge of the theory helps to clarify these challenges.

Quantifying genetic constraints

Studies of the evolutionary genetics of adaptation rely heavily upon two distinct modeling traditions—each best suited for a different time scale of evolutionary divergence. The first tradition focuses on short-term responses to selection from standing genetic variation, typically across one or a few generations. This tradition includes Fisher's fundamental theorem of natural selection,^{1,13} and prominent models from evolutionary quantitative genetics (e.g., Refs. 14, 24, and 25). The second tradition—largely developed for contexts of molecular evolution—focuses on long-term dynamics of adaptation, proceeding through the fixation of new, beneficial mutations. This tradition stems from theory developed by Kimura and Ohta,²⁶ Maynard Smith,²⁷ Gillespie,²⁸ and others (reviewed in Refs. 29

and 30). We apply both modeling traditions—as outlined below—to quantify impacts of genetic constraints on the dynamics of adaptation.

Short-term rates of adaptation. A population's short-term potential for adaptation depends on the variance in fitness among members of the population, and the extent to which this fitness variance is heritable. Under standard, Mendelian inheritance, Fisher¹ showed that natural selection increases population mean fitness at a rate that is proportional to the additive genetic variance for fitness (V_A). This is the basis of Fisher's fundamental theorem of natural selection:

$$\Delta_{NS} \bar{w} = \frac{V_A}{\bar{w}} \quad (1)$$

(contra Fisher, we use the discrete time version; Ref. 1, pp. 34–35, and Refs. 13 and 14), where $\bar{w}$ refers to mean fitness, and Δ_{NS} denotes change, per generation, due to natural selection. The expression characterizes the change in mean fitness owing to effects of natural selection on allele frequency changes, and it neglects other factors that mediate population fitness (e.g., changes in environment; see Refs. 13, 31, and 32). Fisher's Fundamental Theorem is notable for its elegance and utility. It places a spotlight on population metrics that matter most for short-term adaptation (selection, genetic variation, and inheritance), and provides context for exploring evolutionary genetic factors that promote or constrain adaptation. Fisher's Fundamental Theorem suggests a straightforward way to quantify genetic constraints: each constraint may be understood by its impact on the evolution of mean fitness. Absolute genetic constraints arise when there is no additive genetic variance for fitness ($V_A = 0$), and quantitative constraints arise from factors that reduce V_A (e.g., Ref. 11). In the spirit of this approach, we develop simple expressions, similar to Eq. (1), for change in mean fitness under each scenario of genetic constraint (see Appendix 1, online only, for further details).

Long-term rates of adaptation. To characterize long-term rates of adaptation, we use an analog of Fisher's Fundamental Theorem based on the fixation of new, beneficial mutations. We assume that the population is initially fixed for an ancestral genotype that mutates to alleles favored by selection. The fitness effects of beneficial mutations are assumed

to be small, and selection is strong relative to the mutation rate (i.e., $u \ll 1/N_e \ll s \ll 1$, where u is the mutation rate, N_e is the effective population size, and s is the fitness benefit of the substitution; see Ref. 30). Under these conditions, the waiting time until fixation of the next beneficial substitution is roughly independent of the fitness effect of the allele that eventually fixes (e.g., because sojourn times are negligible relative to the intervals between substitutions; Ref. 27). The rate of increase in mean fitness is:

$$\Delta_{NM} \bar{w} = P \cdot E[s | \text{fix}] \quad (2)$$

(e.g., Refs. 33 and 34), where $\Delta_{NM} \bar{w}$ denotes the expected rate of increase in mean fitness from the next beneficial substitution, $E[s | \text{fix}]$ is the expected increase in fitness caused by the next beneficial substitution, and P is the probability, per generation, that a beneficial mutation arises that ultimately fixes within the population. Whereas, Fisher's Fundamental Theorem is purely deterministic, Eq. (2) includes stochastic effects of mutation and drift, which are captured in the parameter P , and the fitness effect of the next fixed mutation.

Dominance

... a single recessive mutation has an infinitely small chance of spreading, however favourable it is, simply because selection cannot begin to operate in its favour till two recessive genes are present in the same zygote. But before this happens "blind accident and blundering mischance" will have extinguished the gene (Haldane 1932; ref. 35, p. 201)

Dominance interactions between alleles influence the contribution of each locus to additive genetic variance for fitness, and the efficiency with which natural selection promotes adaptation (e.g., Refs. 3, 5, and 35). Haldane's work was particularly prominent among early theories for the impact of dominance on adaptation,^{3,35–37} and highlighted the central role of dominance in the spread of beneficial mutations. Haldane's insights on dominance were foundational to 20th century (pre-molecular) evolutionary interpretations of conspicuous adaptations for mimicry, industrial melanism, and insecticide resistance.^{38,39} They later inspired prominent models of dominance and constraint in genome evolution (e.g., Refs. 39–41). Below, we outline the core of this theory, along with major empirical insights on dominance as an evolutionary constraint.

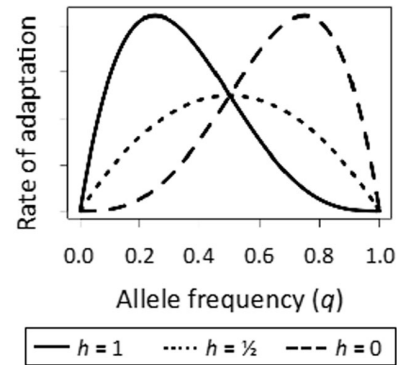


Figure 1. Dominance and the short-term rate of adaptation. The rate of adaptation, based on Eq. (3) is a function of the frequency of the B allele. There are three scenarios of dominance shown: (i) no dominance ($h = 1/2$), (ii) complete dominance of B ($h = 1$), and (iii) complete recessivity of B ($h = 0$).

Dominance and short-term rates of adaptation

The effect of dominance on short-term adaptation can be illustrated by revisiting Fisher's fundamental theorem of natural selection for a single, diploid locus with two alleles: an A allele at frequency p , and B allele at frequency q . Relative fitnesses of the three genotypes are $w_{AA} = 1$, $w_{AB} = 1 + sh$, and $w_{BB} = 1 + s$, where s is the homozygous benefit of the B allele ($s > 0$ assuming that B is beneficial), and h is the dominance coefficient of B relative to A (under directional selection, $0 < h < 1$; $h < 1/2$ corresponds to partial recessivity; $h > 1/2$ to partial dominance; $h = 1/2$ to codominance). Following standard theory (i.e., discrete generations, random mating, small s , Ref. 42, pp. 90–93), the deterministic rate of change in mean fitness across a generation is:

$$\Delta_{NS} \bar{w} \approx \frac{s^2 q (1-q) [1 + (q-p)(1-2h)]^2}{2\bar{w}}. \quad (3)$$

As before, the rate of adaptation is proportional to the additive genetic variance for fitness (V_A in this case is the numerator in Eq. (3)). Dominance mediates the rate of adaptation through its effect on V_A . Equation (3) implies that V_A decreases and constraints arise when $(q-p)(1-2h) < 0$. In out-bred populations, adaptation is constrained relative to the additive case ($h = 1/2$) if, on average, recessive alleles are rare and dominant alleles are common (Fig. 1). This is because the effects of rare, recessive alleles are masked within the heterozygous individuals in which they typically occur, which poses a challenge (highlighted in Haldane's quote) of

selecting for rare beneficial alleles and selecting against recessive ones.

Dominance and long-term rates of adaptation

The extent to which dominance constrains long-term adaptation hinges upon a much broader question in evolutionary genetics: to what extent is adaptation limited by the availability of beneficial mutations? Constraints from dominance are alleviated when adaptation is fuelled by standing genetic variation,^{39,43} or when the size of an adapting population is so large that the appearance of beneficial mutations that can establish is virtually instantaneous.^{27,44} However, in populations that initially lack beneficial genetic variation, the average dominance coefficient among new beneficial mutations can profoundly affect the rate at which new mutations arise and fix within a population.

In a population initially lacking an allele favored by selection, the average time for the allele to arise that can establish is (see Ref. 45):

$$T = \frac{1}{1 - e^{-4N_e u sh}} \approx \frac{1}{4N_e u sh}, \quad (4)$$

where u is the mutation rate to the beneficial allele, and $2sh$ is the fixation probability of each copy of the mutant (assuming $h > 0$; $4N_e u sh < 1$). From Eq. (2) (where $P = 1/T$), the rate of adaptation from new mutations is: $\Delta \bar{w}_{NM} = 4N_e u h s^2$, which increases with the dominance coefficient of the beneficial mutation; partial recessivity among beneficial mutations constrains adaptation.

Haldane's Sieve and the genetic basis of adaptive phenotypes

Dominant beneficial mutations are more likely to become fixed than recessive ones. To the extent that adaptation relies on new mutations, the preferential fixation of dominant alleles—a phenomenon known as “Haldane's Sieve”^{39,46}—generates clear theoretical predictions for the dominance of substitutions that underlie adaptive phenotypes. Specifically, the distribution of dominance among beneficial substitutions (those fixed by selection) should be upwardly biased relative to the distribution of dominance among new, beneficial mutations. This intuition may be formalized by considering a set of beneficial mutations, each with fitness effect of s , and variable dominance coefficients. Using $2sh$ to represent the fixation probability of a beneficial

mutation with dominance coefficient of h , the average dominance among fixed mutations is:

$$\bar{h}_{fix} \approx \bar{h}_b + \frac{\sigma_b^2}{\bar{h}_b}, \quad (5)$$

where $\bar{h}_b$ and σ_b^2 represent the mean and variance of dominance coefficients among beneficial mutations.⁴⁷ Since $\sigma_b^2/\bar{h}_b$ must be positive, the average dominance of fixed mutations will be higher than the dominance of random beneficial mutations.

Signals of constraint due to genetic dominance

The rate of adaptation and its genetic basis critically depend on the distribution of dominance among beneficial mutations. Yet direct inference of dominance among random beneficial mutations is logistically prohibitive. Visible mutations and those arising in mutation-accumulation experiments are overwhelmingly deleterious, and largely recessive in expression ($h \sim 0.25$, on average; Refs. 5 and 48). On the other hand, few experimental systems allow for direct estimation of dominance among beneficial mutations; data on beneficial alleles are sparse.⁴¹ Consequently, most evidence for the dominance of beneficial alleles is indirect, and typically based on analyses of genome evolution.

Haldane's Sieve and plant mating systems.

Species' mating systems affect the manifestation of Haldane's sieve, and as such, different genetic architectures of adaptation in species with different mating systems provide indirect evidence for constraint due to dominance (e.g., Refs. 47, 49, and 50). Charlesworth (Refs. 47 and 51) recognized that Haldane's sieve (Eq. (6)) breaks down in species undergoing self-fertilization (e.g., many flowering plants), with adaptive substitutions in highly selfing species reflecting the true distribution of dominance among beneficial mutations. On this point, Ronfort and Glémin⁴⁹ leveraged available genomic data on the number, location, and dominance effects of loci underlying plant domestication phenotypes, and observed that the distribution of dominance for fixed quantitative trait loci was upwardly biased in outcrossing species compared to selfers. Their results suggest that a large fraction of beneficial mutations is recessive. Such alleles are effectively unavailable for adaptation in obligately outcrossing species.

Ploidy and the rate of adaptation. The masking of expression due to dominance is primarily an issue under diploid or polyploidy inheritance. Haploid expression eliminates constraints from dominance, and facilitates the spread of rare beneficial mutations. If beneficial mutations are typically recessive or partially recessive, haploid populations should adapt more rapidly than diploids (see Refs. 52 and 53). Experimental evolution studies using yeast—where evolving lineages may be haploid or diploid—appear to validate this prediction.^{54,55}

Comparative genomic studies of chromosomes with different ploidy levels provide another approach for inferring dominance among beneficial mutations (reviewed in Refs. 8 and 56). Sex-linked mutations are haploid in one sex (e.g., males in species with X chromosomes), visible to selection irrespective of dominance, and more likely to fix than equivalent mutations on the autosomes.⁴⁰ Consequently, elevated rates of adaptive substitution for X-linked compared to autosomal genes may imply that beneficial mutations are, on average, recessive or partially recessive,^{40,41} though the inference is contingent on assumptions about the nature of adaptation itself.^{8,39,57,58} Elevated patterns of X-linked (or Z-linked) divergence have been reported in several species, and in some of these cases, these “faster-X effects” appear to be driven by positive selection,⁸ suggesting that beneficial mutations are often partially recessive.

Pleiotropy

... with more dimensions, there are more ways for a random mutation to “go wrong.” (Orr 1998; ref. 59, p. 939)

Well-adapted individuals must do many things right, from defending against natural enemies to competing for resources and reproductive opportunities. Performance in any one of these fitness components invariably depends on the expression of many traits; notable examples include the diverse array of mechanical and chemical traits that plants use to defend against insect herbivores,⁶⁰ and the multivariate sexual signals used by males to attract mates.^{61,62} However, when multiple traits jointly impact fitness, adaptation is complicated by pleiotropic genetic variation, in which individual variants affect the expression of more than one trait, including traits expressed at different life stages.⁶³ Current data suggest that most mutations

are indeed pleiotropic, affecting multiple traits or fitness components.^{5,6,64} This pleiotropy can give rise to genetic and evolutionary nonindependence between different traits, genetic trade-offs between different fitness components (e.g., Refs. 63 and 65), and reduced capacity for adaptation.

Pleiotropy and short-term rates of adaptation

Quantitative genetics theory provides a straightforward way to quantify effects of pleiotropy, manifested in genetic correlations (see Ref. 66), on the short-term rate of adaptation. In a population where fitness depends on the expression of n quantitative traits, the rate of change in mean fitness is:

$$\Delta \bar{w}_{NS} \approx \frac{\boldsymbol{\beta}^T \mathbf{G} \boldsymbol{\beta}}{\bar{w}} = \frac{\boldsymbol{\beta}^T \mathbf{D} \boldsymbol{\beta}}{\bar{w}} + \frac{\sum_i \sum_{j \neq i} G_{ij} \beta_i \beta_j}{\bar{w}} \quad (6)$$

(see, e.g., Refs. 67 and 68), where $\mathbf{G}$ is the standardized additive genetic variance–covariance matrix for the traits, $\boldsymbol{\beta}$ is a column vector of “selection gradients” that defines the direction and strength of directional selection on each trait, and $\mathbf{D}$ is a vector of the diagonal elements of $\mathbf{G}$; the superscript T denotes vector transposition. Equation (6) applies under standard quantitative genetics assumptions, and assumes that nonlinear selection is weak; for generalizations of the model that allow for strong stabilizing or disruptive selection, see Refs. 67 and 68). Elements of $\mathbf{G}$ and $\boldsymbol{\beta}$ include: β_i , the selection gradient of the i th trait; G_{ii} , the genetic variance of the i th trait; and G_{ij} , the genetic covariance between traits i and j .

The final expression in Eq. (6) partitions the rate of adaptation into two subcomponents. The first ($\boldsymbol{\beta}^T \mathbf{D} \boldsymbol{\beta} / \bar{w}$) describes the effects of selection and genetic variation for each trait, individually, on the rate of adaptation; it is always positive provided the traits vary and at least one is under directional selection. The second term (with the double-summation) captures the effects of genetic correlations on adaptation; it may be positive or negative, depending on the sign and magnitude of elements in the summation. Genetic correlations constrain adaptation when the second term is negative, and promote adaptation when it is positive. Intuitively, constraints are likely to emerge when: (1) traits that positively covary are selected in opposite directions ($\beta_i \beta_j < 0$, $G_{ij} > 0$, leading to $G_{ij} \beta_i \beta_j < 0$), and (2)

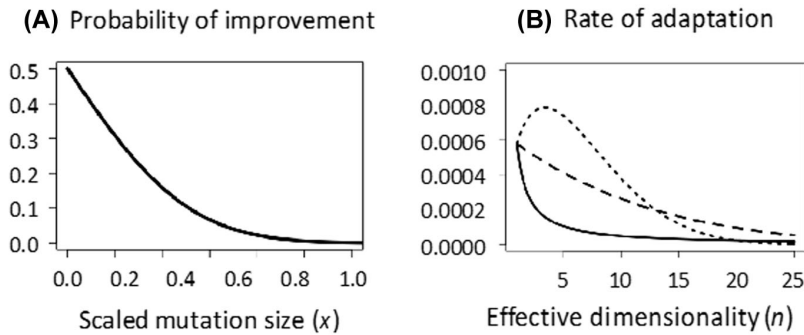


Figure 2. Pleiotropy and adaptation in Fisher's geometric model. (A) The probability that a random mutation with scaled effect size of x is favorable. The curve is based on Eq. (7); the scaled mutation size, x , is a function of on the “complexity” or effective dimensionality of the organism (n), the absolute size of the mutational effect (r), and the population's distance from the optimal phenotype (z). (B) The rate of adaptation in Fisher's geometric model. The three curves are based on equations presented in Appendix 2 (online only), with parameters: $Nu = 1$, $\omega = 0.5$, $z = 0.5$, $a = 0.05$.

traits that negatively covary are selected in the same direction ($\beta_i\beta_j > 0$, $G_{ij} < 0$, leading to $G_{ij}\beta_i\beta_j < 0$).

Pleiotropy and long-term rates of adaptation

In *The Genetic Theory of Natural Selection*, Fisher (Ref. 1, pp. 38–41) raised the question: what are the “statistical requirements” for adaptation of a high-dimensional organism to a complex environment? Fisher showed that the probability that a random mutation improved fitness of its carriers hinges upon three factors: (1) the degree of maladaptation of the population (represented by z : the disparity between the optimal phenotype for the environment and the phenotype expressed by members of the population); (2) the total phenotypic effect size of the mutation (represented by r); and (3) the number of independent trait dimensions under selection (represented by n orthogonal traits, where the effective n may be much smaller than the actual number of traits; see Refs. 69 and 70). For large n , the probability that a random mutation with effect size of r is beneficial is:

$$\Pr(s > 0) \approx 1 - \Phi[r\sqrt{n}/2z] \quad (7)$$

(Ref. 1, p. 40), where $\Phi[x]$ is the cumulative distribution function for the standard normal distribution. Although Fisher provided no derivation, Eq. (7) has since been derived using geometric and probabilistic arguments (see Refs. 59, 71–74). Equation (7) reveals that the probability of improvement is a declining function of $x = r\sqrt{n}/2z$, which represents the “effective mutation size” on Fisher's scale (for discussion, see Ref. 59). The effective mutation

size increases with both the complexity of the organism (with n) and the ratio of the absolute mutation size versus the distance to the optimum (r/z). Effectively large mutations are unlikely to be favorable because they disrupt high-dimensional phenotypes; “small” mutations are much less disruptive, and have a high probability of being beneficial (Fig. 2A).

Fisher's model has since been extended to characterize the distribution of fitness effects among mutations, and the dynamics of adaptation. Each of these applications is contingent on assumptions about ploidy, phenotypic orientations among random mutations, and the functional relationship between z and fitness. For simplicity, assume that the population is haploid (but see Refs. 48, 63, 65, and 75), mutations are randomly oriented in phenotypic space, and fitness is a Gaussian function of the form: $w(z) = \exp[-\omega z^2]$, where ω is a positive constant that defines the rate of fitness decline with the distance from the optimum. Assuming high dimensionality ($n > \sim 10$) and small mutant fitness effects ($|s| < 1$), the distribution of fitness effects among mutations with magnitude r is roughly normal, with a mean and variance (respectively) of (see Refs. 76 and 77):

$$\bar{s} = -\omega r^2 \quad (8a)$$

and

$$\sigma^2 = \frac{(2\omega z r)^2}{n} \quad (8b)$$

Note that the effective size of a mutation in Fisher's scale (x ; see above) is a simple function of the mean

and variance: $x = -\bar{s}/\sigma$. This makes intuitive sense: the probability that a random mutation is beneficial must decline when the average mutational fitness effect ($\bar{s}$) is strongly deleterious relative to the dispersion of s around the mean. Building off of Eqs. (8a) and (8b), the long-term rate of adaptation is given by Eq. (2), with expressions for P and $E(s | \text{fix})$ provided in Appendix 2 (online only).

The effect of pleiotropy on the rate of adaptation depends on the scaling of mutation size (r) with dimensionality (n). Following Wang *et al.*,⁶⁴ let $r = an^b$, where a is a positive constant, and b is a scaling coefficient that determines the relation between r and n . Consider three idealized scaling scenarios: (1) independence between r and n ($b = 0$); (2) positive scaling between r and n , with mean effect size per dimension independent of n ($b = 0.5$); and (3) positive scaling between r and n , with effect size per trait increasing with n ($b > 0.5$). Wang *et al.*⁶⁴ showed that the rate of adaptation declines with n when $b \leq 0.5$ (cases 1 and 2), leading to an unequivocal cost of complexity. However, when $b > 0.5$, the rate of adaptation is maximized at intermediate n (Fig. 2B), suggesting that modest pleiotropy may promote adaptation. Alternative frameworks of adaptation under pleiotropy paint a less rosy picture, with pleiotropy unequivocally decreasing the potential for adaptation (see Ref. 78).

Signals of pleiotropic constraint

The impact of pleiotropic genetic correlations on short-term adaptation can be empirically evaluated whenever estimates of $\mathbf{G}$ and ideally $\boldsymbol{\beta}$ are available for the same population,^{67,79,80} or trait values are tracked across multiple generations.^{81–83} Short-term constraints arise when there is misalignment between $\boldsymbol{\beta}$ and the major axis of variability in $\mathbf{G}$.^{18,84} Meta-analyses of published estimates of $\boldsymbol{\beta}$ and $\mathbf{G}$ suggest that short-term constraints of pleiotropy are far from ubiquitous, with pleiotropy about as likely to enhance as to constrain adaptation,⁶⁷ though such studies are of course limited by the subset of traits that happen to be measured. In cases where information is available for $\mathbf{G}$, but not for selection, it is possible to identify hypothetical orientations of selection that will elicit a weak or inconsistent evolutionary response (i.e., when aligned with nearly null subspaces, Ref. 85); though selection estimates are required to prove the realization of such constraints.

Studies focusing on long-term prospects for adaptation often focus on the pleiotropic effects of mutations on trait sets. Some of the largest scale studies are based on phenotyping of gene-knockout strains in model organisms, or whole-genome transcriptional profiles (a multivariate phenotype) of mutation accumulation lines (MA lines; see discussions in Refs. 85 and 86). Gene deletion data suggest that pleiotropy is restricted, with most genes affecting a small subset of examined traits (Refs. 64 and 87, but see Ref. 88). On the other hand, recent MA studies indicate that pleiotropy may be widespread, particularly when high-dimensional phenotypes are explored.^{89,90} Resolving how these studies relate to adaptive change, however, remains a challenge, as data from gene knockout and MA experiments are often difficult to directly link to major parameters of models of adaptation, where fitness is the phenotype of interest. Nevertheless, recent applications in genomics and experimental evolution have helped to clarify two key parameters of Fisher's geometric model: the scaling coefficient, b , and the effective dimensionality, n , which together influence the so-called “costs of complexity.” These are discussed below.

Effective complexity. Dimensionality or “complexity” in Fisher's geometric model (n) does not necessarily equate to the total number of traits of the organism, but rather, to an effective number of dimensions, each potentially comprised of composite trait sets. An organism's effective n —a function of the spectrum of mutant effects and the multivariate pattern of selection across traits—is likely to be much smaller than an organism's actual number of traits.⁷⁰ Two applications of Fisher's geometric model provide means to infer effective dimensionality. First, data on the distribution of mutant fitness effects can be used to indirectly infer effective n , under assumptions of multivariate normality of mutational effects across the contributing traits.⁷⁰ Applications of this approach have yielded low estimates of n —typically on the order of $n < 10$ (reviewed in Ref. 91). The second approach uses fitness data from experimental evolution to infer n , and is based on theoretical predictions of the genetic load for fitness at mutation–selection–drift equilibrium.^{92,93} Applications of the second approach yield much higher estimates of n ($n > 10$ in two viruses; Ref. 92). It is unclear which

approach provides the more relevant measure of organismal complexity.

Is there a cost of complexity? Whether there is a “cost of complexity”³³ depends on effective complexity (n), and the scaling of mutations with dimensionality (i.e., the b parameter; see above). Using gene knockout data from yeast, Wang *et al.*⁶⁴ found that b was significantly greater than 0.5 (their estimate: $b \sim 0.6$) and large enough to potentially eliminate a cost of complexity, provided n was modest-to-small. Although the true range of effective n remains an open question,^{85–88} the low n estimates, mentioned above, suggest that the cost of complexity may be small. Nevertheless, further work is clearly needed to reconcile different estimates of n , and resolve the plausible values of n and b across a larger range of taxa.

Fitness trade-offs between types of individuals

If ... a breeder observed that some of his pigeons ... varied into pale blue; could he by long-continued selection make a breed, in which the males alone should be of this tint, whilst the females remained unchanged? I will here only say, that this, though perhaps not impossible, would be extremely difficult; for the natural result of breeding from the pale-blue males would be to change his whole stock, including both sexes, into this tint. (Darwin 1871; ref. 94, pp. 284–285).

Different members of a population are sometimes subject to wildly divergent patterns of natural selection. Yet evolutionary divergence between these subsets of the population may be constrained by shared patterns of inheritance. Consider the classic example of sexual dimorphism.⁷ Each sex inherits nearly identical genetic material, and most genetic variation similarly affects phenotypes expressed by both sexes (see Refs. 95 and 96). Although selection favors different phenotypes in each sex, shared inheritance limits evolutionary divergence between them,⁷ and generates fitness trade-offs at loci affecting divergently selected traits (i.e., “sexual antagonism,” Ref. 71). Analogous trade-offs can also arise from disruptive selection between distinct habitats in a species’ range,⁹⁷ morphs with different reproductive strategies,⁹⁸ and castes of social insect species.⁹⁹

Fitness trade-offs are conceptually similar to pleiotropic trade-offs between traits (see above, plus Refs. 100 and 101), yet with a key distinction: *fit-*

Table 1. Relative fitness in a trade-off model between two environments of selection^a

Genotype:	AA	AB	BB
Fitness, environment 1:	1	$1 - s_1/2$	$1 - s_1$
Fitness, environment 2:	$1 - s_2$	$1 - s_2/2$	1
Frequency at birth ^b :	p^2	$2pq$	q^2

^aSelection coefficients fall within the range, $0 < s_1, s_2 < 1$, so that AA individuals are favored in environment 1 and BB individuals are favored in environment 2.

^bHardy–Weinberg genotype frequencies are approximate for models of sex-specific selection. In reality, heterozygosity is inflated by sex differences in selection, though the effect is mild when selection coefficients are small (i.e., $0 < s_1, s_2 < 1$; Ref. 42, pp. 97–98, Ref. 190). Although we use the approximations in deriving Eqs. (9a–9c), our predictions about F_{ST} make no such assumptions (see Appendix 1, online only).

ness trade-offs partially or completely decouple the typically tight association between additive genetic variance for fitness and the rate of adaptation (as in Fisher’s fundamental theorem; Eq. (1)). In contrast to scenarios of pleiotropy between traits expressed by the same individual (see above), selection does not necessarily maximize fitness of any single type of individual in the population, and at equilibrium, fitness trade-offs allow for the maintenance of additive genetic variation for fitness. Loci exhibiting fitness trade-offs contribute much less to adaptation than they do to additive genetic variance for fitness, leading to sluggish rates of adaptation, and persistence of genetic variants that harm a large fraction of the population.

Fitness trade-offs and rates of adaptation

Classical population genetic models of fitness trade-offs focus on two distinct contexts of selection: trade-offs between habitats,^{102–104} and trade-offs between sexes.^{105,106} Although these theoretical traditions have largely developed independently of one another, unification between them is achievable under the following simplifying assumptions, which we adopt below (and see Appendix 1, online only). We consider the simplest model of a single, deterministically evolving diploid locus with two alleles—each having a codominant (i.e., additive) effect on fitness in each environment or sex (Table 1). We assume that the fitness effects of genetic variation are small to modest (i.e., $0 < s_1, s_2 < 1/2$, in Table 1), and that generations are discrete, with a life cycle of: (1) birth, (2) sex- or habitat-specific selection, (3) random mating

(postselection), (4) reproduction, and (5) death of the adults. Under habitat selection, our model conforms to the Levene model with full migration.¹⁰² Under sex-specific selection, we use a random-mating approximation, which works well for the specified parameter range (see Table 1 footnote; and for the many extensions of this basic model, Refs. 106–112).

Under sex-specific selection,¹⁰⁶ or habitat-specific selection with strong density regulation of adults (i.e., “soft” selection; Refs. 102 and 107), there are three meaningful metrics of adaptation: (1) the mean fitness of individuals from environment 1 ($\bar{w}_1 = 1 - qs_1$); (2) the mean fitness of individuals from environment 2 ($\bar{w}_2 = 1 - ps_1$); and (3) the weighted geometric mean fitness, $\bar{w}_G = (\bar{w}_1)^C (\bar{w}_2)^{1-C}$, where C is the fraction of breeding adults from environment 1 ($C = 1/2$ in the sex-specific selection model). Under the stated assumptions, the rate of change of each fitness metric is:

$$\Delta_{NS} \bar{w}_G = \frac{s_1 s_2 \bar{w}_G (\hat{p} - p)^2}{(\bar{w}_1 \bar{w}_2)^2} \sqrt{V_1 V_2}, \quad (9a)$$

$$\Delta_{NS} \bar{w}_1 = \frac{s_2 (\hat{p} - p)}{\bar{w}_1 \bar{w}_2} V_1, \quad (9b)$$

$$\Delta_{NS} \bar{w}_2 = \frac{s_1 (p - \hat{p})}{\bar{w}_1 \bar{w}_2} V_2, \quad (9c)$$

where $\hat{p} = [Cs_1 - (1 - C)s_2(1 - s_1)]/s_1 s_2$, and V_1 and V_2 represent the additive genetic variance for fitness in environments 1 and 2, respectively. The model has three possible equilibria, including $p = 0$ and $p = 1$. The third equilibrium applies when $0 < \hat{p} < 1$, corresponding to balancing selection and the maintenance of polymorphism. Otherwise, selection favors fixation of either B (when $\hat{p} < 0$) or A (when $\hat{p} > 1$). Additional equilibrium points are possible under forms of dominance that are not considered here (see Refs. 105 and 106).

Inspection of Eqs. (9a) and (9c) reveals that trade-offs decouple adaptation from the additive genetic variance for fitness, leading to three key departures from Fisher’s fundamental theorem (Eq. (1)). First, in a nonequilibrium population ($p \neq 0$; $p \neq 1$; $p \neq \hat{p}$), where V_1 and V_2 are both positive, mean fitness always declines in one environment and increases in the other (and thus, $\Delta_{NS} \bar{w}_1 \cdot \Delta_{NS} \bar{w}_2 < 0$). Second, the rate of adaptation to each environment is

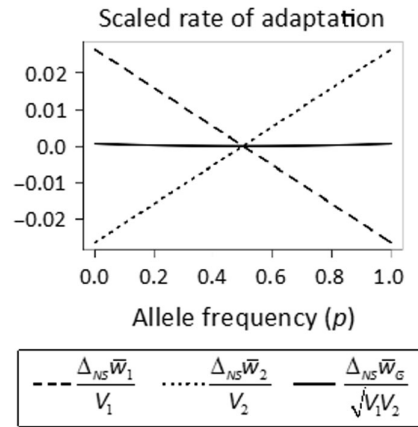


Figure 3. Rates of adaptation under a symmetrical fitness trade-off ($s_1 = s_2 = 0.05$; $C = 1/2$). Scaled rates of change are plotted for each of the three metrics of fitness described in the text. Scaling is relative to the additive genetic variance for fitness (see the expressions below the figure), and results are based on Eqs. (9a–9c). Given this scaling, genetic constraints are reflected in the reduction of each metric relative to one ($\Delta_{NS} \bar{w}_i / V_i = 1$ corresponds to no constraint; $\Delta_{NS} \bar{w}_i / V_i = 0$ corresponds to absolute constraint; $\Delta_{NS} \bar{w}_i / V_i < 0$ corresponds to a reduction in adaptation over time). Note that the y -axis is far below one, which shows that the rate of adaptation, using any of these fitness metrics, is much less than the additive genetic variance for fitness.

substantially hindered by the trade-off, by at least a factor proportional to C . In the two-sex case ($C = 1/2$), for example, if selection is weak in males relative to females (e.g., s_1/s_2 approaches zero, where s_1 and s_2 are male and female selection coefficients), then the rate of female adaptation is roughly half the additive genetic variance of female fitness. Selection is essentially sex limited, with female-beneficial alleles inherited by both sexes, but only responding to selection in one.

A twofold reduction in the rate of adaptation is the best-case scenario. As opposing selection becomes symmetric between environments, the disparity between fitness variance and the rate of adaptation increases. As highlighted in Figure 3, when $C = 1/2$ and selection is symmetrical ($s_1 \sim s_2$), the rate of adaptation to each environment is orders of magnitude lower than the additive genetic variance for fitness. At polymorphic equilibrium ($0 < p = \hat{p} < 1$), adaptation grinds to a halt, despite substantial fitness variance in both environments ($V_1 V_2 > 0$). Although natural selection invariably increases the population’s weighted geometric mean fitness (see

Refs. 7, 97, 104, and 113), the pace of improvement in this fitness metric is severely dampened by the trade-off (i.e., solid line in Fig. 3).

Signals of fitness trade-offs

Fitness trade-offs between sexes or habitats may contribute to a wide variety of patterns in genome structure and evolution, including the emergence of nonrecombining sex chromosomes,^{114,115} nonrandom genomic distributions of sex-biased genes,^{116–118} inversions and “genomic islands” of local and sex-specific adaptation,^{119–121} and chromosomal fusions.^{9,122} Nevertheless, many patterns predicted by trade-off theory can potentially arise in contexts where trade-offs are absent.^{123–125} Thus, consistency between empirical observations and predictions of trade-off theory—without direct corroborating evidence—is merely suggestive of a role for trade-offs in genome evolution.

Direct tests for genetic trade-offs must overcome steep logistical challenges. Part of the problem is that we seek to dissect the genetic basis of fitness itself. Given the strong possibility that fitness variance is dominated by either rare alleles of large effect, or common alleles of small effect, prospects for identifying the loci underlying fitness trade-offs appear grim, and reminiscent of Lewontin’s famous quip: “What we can measure is by definition uninteresting and what we are interested in is by definition unmeasurable”.²² This problem is particularly acute for trade-offs associated with male–female differences, or other scenarios where selection is likely to be weak relative to gene flow (cf. local adaptation under high gene flow; Ref. 126). Below, we discuss two recent approaches for identifying genes underlying fitness trade-offs, focusing on applications to identify sexually antagonistic genes as a case studies of how progress can be made on what is likely one of the more empirically challenging trade-off cases.

Sexually antagonistic alleles and association studies. Genome-wide association studies (GWAS) are well suited for detecting causal loci with intermediate-frequency alleles of large effect. As a result, the potential to identify loci mediating fitness trade-offs increases considerably when contributing alleles are common, and maintained by balancing selection (e.g., when $C = \frac{1}{2}$, $s_1 = s_2$). Two recent studies have identified candidate sexually antagonistic loci of this nature. First, a GWAS of sex-specific fitness in a population of collared flycatchers iden-

tified a common single nucleotide polymorphism (minor allele frequency of ~ 0.4) with estimated sexually antagonistic effect on lifetime reproductive success.¹²⁷ Second, a study on Atlantic salmon identified a single gene with two common alleles, accounting for roughly 40% of the variance in age of maturity—a key life-history trait that is thought to be under opposing selection between the sexes.¹²⁸ These studies highlight the promise of association studies in well-monitored vertebrate populations, where fitness metrics are obtainable, and sexually antagonistic loci may be directly identified through GWAS. Nevertheless, the number of candidate loci detected so far is surprisingly low, and a reflection more of the inherent biases of GWAS (e.g., toward common large-effect alleles), than a complete picture of spectrum of loci exhibiting fitness trade-offs.

Detecting sexually antagonistic loci from between-sex F_{ST} . In populations where direct fitness measurements are not feasible, estimates of allele frequency differences between differentially selected subsets of the population can potentially identify major loci underlying fitness trade-offs. In the case of sexual antagonism, theory predicts that sex differences in selection should lead to sex-biased transmission rates of differentially selected alleles: female-beneficial alleles are preferentially transmitted from mothers to offspring, whereas male-benefit alleles are preferentially transmitted through fathers.^{129,130} If individuals of each sex are sampled and sequenced *after* selection has driven allele frequency differences between them, loci with the greatest frequency differences between sexes should represent the best candidates for sexually antagonistic loci.

Two issues of practicality limit the identification of sexually antagonistic loci via allele frequency differences between sexes. First, allele frequency differences are affected by several factors in addition to sex-specific selection (discussed in Ref. 131). These factors include: (1) other forms of differential selection between the sexes, (2) sex-biased genetic inheritance (e.g., X-linkage), (3) sex-biased demography, (4) genetic drift, and (5) effects of sampling of genotypes from each sex. Moreover, allele frequency differences between adults of each sex reflect only the viability component of selection and neglect genetic effects on other fitness components. Second, common metrics of

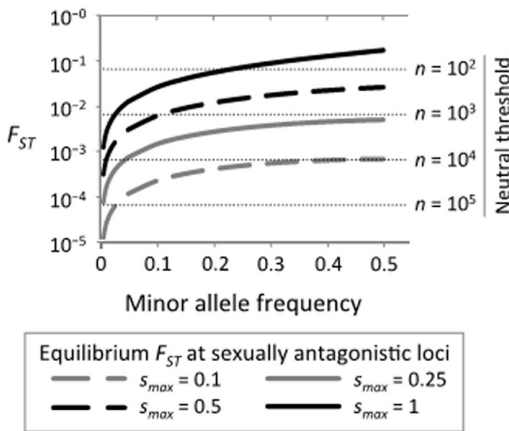


Figure 4. Challenges of detecting sexually antagonistic loci using between-sex F_{ST} . The horizontal lines show the upper 99% confidence intervals of F_{ST} for neutral loci, each as a function of the number of haploid genomes sequenced in the experiment (n , with an equal number of female and male genomes sequenced). These neutral thresholds were calculated by simulating genotypes by independent binomial sampling of female and male allele frequencies. The theoretical curves show equilibrium expectations for F_{ST} under the sexually antagonistic selection model (Table 1), with $s_{\max} = \max(s_1, s_2)$; each of these curves is exact and applicable under strong or weak selection (e.g., exact equations for allele frequency change from Appendix 1, online only). The plots illustrate that sexually antagonistic selection must be very strong or the number of genomes sequenced very large to reliably detect candidate sexually antagonistic loci beyond the neutral threshold.

divergence, such as F_{ST} , should have low statistical power unless the number of sequenced individuals is very large. In the case of sexual antagonism, we can use the diploid model from above (Table 1) to quantify equilibrium F_{ST} under sexual antagonism relative to the F_{ST} distribution among neutral loci (Fig. 4). Under sexual antagonism (by itself), the upper bound for F_{ST} is quite low ($\max(F_{ST}) \sim 0.17$ in the additive model from Table 1), and approaches zero unless selection coefficients are large. Either unreasonable strong selection ($\max(s_1, s_2) > 0.5$) or extremely large samples of sequenced adults ($n > 10^4$) are needed for individual sexually antagonistic loci to have any chance of standing out against the genomic backdrop of neutrally evolving loci (as indicated by the horizontal lines of Fig. 4).

Acknowledging these challenges, Cheng and Kirkpatrick¹³¹ were able to circumvent several by evaluating genome-wide patterns of between-sex

F_{ST} , as a function of each gene's pattern of sex-biased expression (measured as mRNA abundance). They reasoned that many issues of sampling should similarly impact most genes in the genome; gene subclasses with enriched F_{ST} , relative to genome-wide patterns, should be enriched for sexually antagonistic loci. Their analysis focused on autosomes, where allele frequencies at birth are expected to be equal between the sexes (this is not necessarily the case for X-linked loci, e.g., Ref. 132, pp. 45–47, 278–281). Moreover, by examining patterns of F_{ST} across thousands of genes, they could overcome power limitations for individual loci. They showed that F_{ST} in modern human samples is highest among genes with intermediate sex-biases in transcription, suggesting that sexually dimorphic expression may not necessarily resolve fitness trade-offs between the sexes.

Sign epistasis

The problem of evolution as I see it is that of a mechanism by which the species may continually find its way from lower to higher peaks . . . In order that this may occur, there must be some trial and error mechanism on a grand scale by which the species may explore the regions surrounding the small portion of the field which it occupies. (Wright 1932; ref. 4, pp. 358–359)

We have assumed up to this point that adaptation largely proceeds by fixation of alleles that individually increase fitness. But what if adaptation requires genetic combinations that are individually harmful, yet collectively beneficial? This form of genetic interaction—called “sign epistasis” (i.e., mutations have positive fitness effects in some genetic backgrounds and negative effects in others; Ref. 15)—poses a problem for evolution. Beneficial genetic combinations respond poorly to selection unless they are inherited as single hereditary units. In sexually reproducing populations, where recombination constantly breaks them apart, beneficial genetic combinations may fail to spread, even when they provide a substantial improvement over more common genotypes in the population. This raises two questions. First, how often does adaptation rely on beneficial genetic combinations? And second, how much is this expected to dampen the rate of adaptation? The first question is undoubtedly the more difficult to answer, and we will return to it later. The second, as a theoretical question, is more tractable,

and indeed, has been a central topic in evolutionary theory for the better part of a century.

Theories of epistatic adaptation

Sustained interest in epistatic interactions and evolution owes much to the influence of Sewall Wright, who heavily emphasized epistasis in his adaptive landscape metaphor.^{4,133,134} The adaptive landscape describes the mapping of fitness from the field of possible genotype combinations: fitness “peaks” in the landscape represent genetic combinations with high fitness, and fitness “valleys” represent maladaptive genetic combinations. During his lifetime, Wright consistently emphasized the challenge of adaptation in the face of sign epistasis, which he assumed was rampant, and the difficulty of transitioning between lower and higher fitness peaks separated by valleys.¹³³ This perceived challenge was a major undercurrent to Wright’s view of population subdivision as the primary facilitator of adaptation via peak shifts (i.e., his “shifting balance” theory; Refs. 4 and 135).

Although Wright’s “shifting balance” view of evolution is not as highly regarded as it once was,¹³⁴ issues pertaining to sign epistasis and peak shifts nevertheless continue to spur new theory, including recent applications inspired by cancer evolution and adaptation in large populations (e.g., Refs. 15, 136, and 137). Below, we highlight some of the major findings of these theories, and later discuss empirical approaches to test for epistatic constraints on adaptation. For simplicity, we center the theoretical discussion around Crow and Kimura’s¹³⁸ influential analysis of sign epistasis between a pair of fitness-interacting haploid loci. Theory for the diploid case, which has been developed elsewhere, exhibits similar evolutionary dynamics (e.g., Refs. 15, 139, and 140, pp. 322–330).

Sign epistasis and short-term rates of adaptation

Crow and Kimura’s¹³⁸ model considers evolution of a pair of loci, each with two alleles (*A* and *a* at the first locus; *B* and *b* at the second). The system of four genotypes includes two corresponding to fitness peak genotypes (*AB* and *ab*), and two fitness valley genotypes (*Ab* and *aB*; Table 2). As Crow and Kimura point out, the evolutionary dynamics of the two-locus system depend upon three factors: (1) the initial patterns of polymorphism in the population, (2) the strength of selection for “peak” genotypes

Table 2. Relative fitness, per genotype, in a haploid model of sign epistasis^a

Genotype:	<i>ab</i>	<i>Ab</i>	<i>aB</i>	<i>AB</i>
Fitness:	1	$1 - t$	$1 - t$	$1 + s$
Frequency at birth ^b :	x_1	x_2	x_3	x_4

^aFitness parameters are biologically constrained as follows $0 < t$, $s < 1$, though note that many of the approximations given below assume that $t, s \ll 1$.

^bUnder weak selection and loose linkage, we can roughly approximate the genotype frequencies at linkage equilibrium: $x_1 = (1 - x)(1 - y)$, $x_2 = x(1 - y)$, $x_3 = y(1 - x)$, $x_4 = xy$, where x is the frequency of *A* and y is the frequency of *B*. The approach follows Crow and Kimura (Ref. 138, p. 444). Approximations accounting for linkage disequilibrium are more complicated, but tractable when selection is modest relative to recombination (see Ref. 191).

relative to “valley” genotypes, and (3) the degree of physical linkage between loci. A few simple calculations illustrate how these factors combine to influence adaptation.

Assuming weak selection and loose linkage (so that the loci are roughly at linkage equilibrium; Table 2), the short-term rate of adaptation is approximately:

$$\Delta_{NS}\bar{w} \approx \frac{x(1-x)[y(s+2t)-t]^2 + y(1-y)[x(s+2t)-t]^2}{\bar{w}}, \quad (10)$$

where x is the frequency of *A* and y is the frequency of *B*. A set of critical allele frequencies, at $x^* = y^* = t/(s+2t)$, determine the threshold between selection for *ab* versus *AB*. When frequencies of *A* and *B* alleles are both below the threshold, the superior *AB* genotype is too rare to spread, and *ab* eventually becomes fixed. The population will then remain “stuck” on the lower peak of the fitness landscape. When frequencies of *A* and *B* exceed the threshold, the *AB* genotype will spread and eventually fix.

The dynamics of adaptation under loose linkage are illustrated in Figure 5, which shows the additive and total genetic variance for fitness (V_A and V_T , where V_A is the numerator of Eq. (10)) as a function of the frequencies of the *A* and *B* alleles. Although the total fitness variance of the population is positive when either locus is polymorphic, V_A and the rate of adaptation trend toward zero when the frequencies of *A* and *B* approach the threshold, $x^* = y^* = t/(s+2t)$. In this case, the rate of adaptation toward

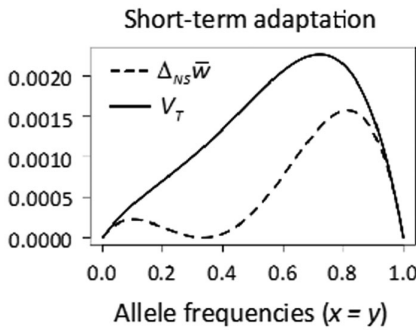


Figure 5. Sign epistasis between a pair of unlinked loci, and the short-term rate of adaptation. The dashed line shows the rate of adaptation as a function of the allele frequencies of *A* and *B*, which are assumed to be equal. The curve is based on Eq. (10), with parameters $s = t = 0.05$. Note that at the frequency threshold, $x = y = t/(s + 2t) = 0.25$, there is no additive genetic variance for fitness, and mean fitness does not evolve. The solid curve shows the total genetic variance for fitness (V_T), given by the following function:

$$V_T = s^2xy(1 - xy) + t^2[x(1 - y) + y(1 - x)][1 - x(1 - y) - y(1 - x)] + 2stxy[x(1 - y) + y(1 - x)].$$

either peak is slow, since nearly all fitness variance in the population is epistatic (Fig. 5). The picture improves considerably when *A* and *B* reach high frequencies, yet getting there is a problem if both alleles are initially rare.

Tight linkage between *A* and *B* alleles significantly improves matters. In this case, selection always favors the spread of the optimal genotype, *AB*, as long as the fitness benefit of *AB* is large relative to the rate of recombination between the loci (*AB* is favored when $s > r/(1 - r)$, where s is the fitness benefit of the *AB* genotype and r is the rate of recombination between the loci, per meiosis; Refs. 138 and 140, pp. 322–330). “Peak shifts” are favored if transitions to the new peak provide a large enough fitness gain relative to the rate at which recombination breaks up coadapted gene combinations. Or as Crow and Kimura¹³⁸ put it: “In Wright’s metaphor, the effect of linkage is to raise the valley between the two adaptive peaks and with extremely close linkage to provide a direct bridge.”

Sign epistasis and long-term rates of adaptation

Over the long run, stochastic processes of mutation and genetic drift can further facilitate peak shifts, though the question is, how fast? Specifically, in a population that is initially fixed for the

suboptimal peak genotype (*ab*), how much time is expected to pass before the population transitions to the higher peak? For the case of loose linkage, drift plays the key role in mediating peak shifts, which are most permissible in small populations (see Refs. 137 and 141). The waiting time to a peak shift increases roughly exponentially with population size (N), and thus, the rate of adaptation becomes a declining function of N .

Under complete linkage between interacting loci, peak shifts potentially occur by two distinct population genetic pathways: (1) the drift-induced pathway (as under loose linkage), and (2) a simultaneous invasion pathway, where an *AB* double-mutant chromosome spreads and eventually fixes. To illustrate the dynamics, we follow Weinreich and Chao’s¹⁵ analysis of peak shift transitions, applied to the haploid model from Table 2 (for a more comprehensive analysis, see Ref. 137). Under strong selection and weak mutation ($u \ll 1/N \ll s$, $t \ll 1$, where u is the mutation rate, per locus), the mean waiting time for pathway (1) is:

$$T_1 \approx \frac{e^{2Nt} - 1}{4Nut} + \frac{1 - e^{-2N(t+s)}}{2Nu(t+s)}. \quad (11a)$$

The mean time for pathway (2) is:

$$T_1 \approx \frac{t(1 - e^{-2Ns})}{4Nu^2s}. \quad (11b)$$

The overall mean waiting time, $T = 1/(1/T_1 + 1/T_2)$, depends primarily on the smaller of T_1 and T_2 (see Ref. 142). As shown in Figure 6(A), the drift-induced pathway dominates in small populations, and the simultaneous substitution pathway dominates in larger ones. The population size that maximizes the waiting time for the peak shift, and minimizes the rate of adaptation, corresponds approximately to the transition point between these two regimes. In this case, constraint arising from epistasis is amplified in populations of intermediate size (Fig. 6B).

Signals of epistatic constraints

Sign epistasis between adaptive mutations may ultimately lead to two types of constraint. First, there may be no accessible pathway to an adaptive genotype if intermediate genotypes between the ancestral and a derived, adaptive state are deleterious. In such cases, transitions to higher fitness states must proceed through drift-induced peak shifts, or

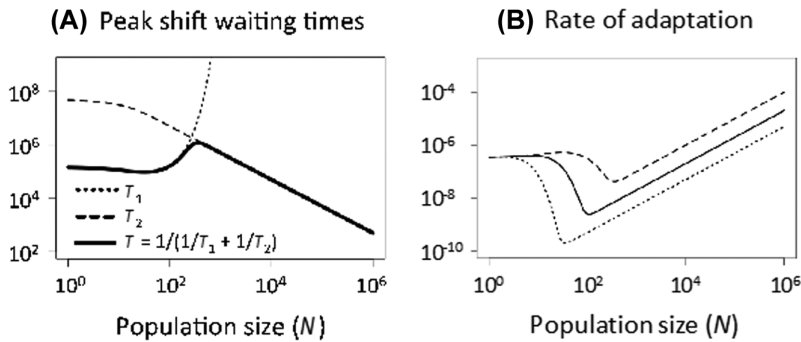


Figure 6. Peak shifts and long-term rates of adaptation under sign epistasis and tight linkage. (A) The mean waiting time for a peak shift under the drift-induced pathway (T_1), the simultaneous invasion pathway (T_2), and the overall time (T). Results are based on Eqs. (11a) and (11b), using parameters: $u = 10^{-5}$, $t = 0.01$, $s = 0.05$. Panel A closely follows Figure 2 of Weinreich and Chao,¹⁵ which was our inspiration. (B) The rate of adaptation of a pair of tightly linked, epistatically beneficial mutations. Results are based on Eq. (2), substituting $T = 1/P$, and T based on Eqs. (11a) and (11b), with the parameters $s = 0.05$, $u = 10^{-5}$.

through changes in environment that alter the fitness landscape and generate new routes to evolutionary improvement. Second, in cases where there are permissible population genetic pathways to a complex adaptive genotype (i.e., pathways that do not involve deleterious intermediates), sign epistasis can potentially reduce the number of accessible routes to the adaptive state. Given sufficient time and available genetic variation, adaptation may eventually occur, yet the rate of the evolutionary transition is constrained by the reduction of the number of viable population genetic paths to the adaptive state. Two general approaches have been taken to test these possibilities.

The prospective approach. The most conceptually straightforward approach for evaluating constraint from sign epistasis is to directly measure the individual and interactive fitness effects of random mutations. Direct estimation of individual mutant fitness effects is itself a challenge (e.g., Ref. 143), yet experimental effort for higher order mutant combinations quickly eclipses effort for single-mutant effects. For example, the number of pairwise interactions between n point mutations is $n(n-1)/2$ —an intractably large number of assays for even modest n . Nevertheless, studies that have managed to assay single and interactive effects of mutations in a single gene show that sign epistasis may be fairly common (reviewed in Ref. 144). For example, Olson *et al.*¹⁴⁵ found that most function-reducing mutations of the GB1 protein can actually improve function when combined with other mutations of the gene.

The retrospective approach. Another approach for evaluating evolutionary consequences of sign epistasis is to map fitness along the set of possible population genetic pathways between a known ancestral and a derived, adaptive genotypic state. The approach is retrospective because the evolutionary end state has usually been identified because it has been successfully reached. Although such studies do not tell us about impassable constraints that populations fail to overcome, they are valuable in what they can tell us about evolutionary accessible routes toward complex adaptations. We note two prominent examples of this approach. First, Weinreich *et al.*¹⁴⁶ used experimental populations of *Escherichia coli* to characterize fitness along the set of possible pathways leading to a 5-substitution allele causing antibiotic resistance. They found that only 18 of the $5! = 120$ possible evolutionary pathways were accessible by natural selection (in only 15% of pathways were each of the ordered substitutions favored by natural selection), and only a fraction of these viable routes was evolutionarily likely. Second, Ortlund *et al.*¹⁴⁷ used “ancestral gene reconstruction” to directly evaluate the effects of ancestral genotypes on the evolution of hormone receptor function. They demonstrated that neutral intermediate steps are sometimes required before later substitutions are favored by natural selection.

Genetic linkage

For, unless advantageous mutations occur so seldom that each has had time to become predominant before the next

appears, they can only come to be simultaneously in the same gamete by means of recombination. (Fisher 1930; ref. 1, p. 104)

Tight physical linkage between loci facilitates adaptation in some contexts of epistatic selection,¹³⁸ and the evolution of coadapted gene complexes (e.g., mimicry supergenes, Refs. 46 and 148; haplotypes conferring sex-specific adaptation, Refs. 112, 120, and 149; and locally adapted inversions, Ref. 150). The sign epistasis scenario, presented above, provides a particularly compelling example of linkage promoting adaptation that would otherwise be unlikely if the interacting loci segregated independently of one another. Yet in many other contexts of selection, tight linkage substantially constrains adaptation, by hindering the fixation of beneficial mutations, or prohibiting the removal of deleterious ones.^{151–153} Below, we outline constraints arising from tight linkage, leaning heavily upon an expansive theoretical literature concerning fitness benefits and costs associated with sexual versus asexual reproduction.^{154–157} In line with this theoretical tradition, we contrast the short- and long-term dynamics of adaptation under complete linkage versus adaptation under free recombination. While we do not explicitly consider the evolutionary origin or maintenance of sex and recombination, the major constraints that we outline play a central role in the evolution of sex (see Ref. 155).

Genetic linkage and short-term constraints

Linkage constrains adaptation by hindering the rate at which unconditionally beneficial mutations at different loci can be combined onto the same chromosome, and subsequently fixed within a population. In sexually reproducing populations, recombination can rapidly yield chromosomes bearing multilocus beneficial genotypes. However, the production of beneficial genotypes may be slow in the absence of recombination, as in asexual populations and genetic systems of closely linked loci. A simple, two-locus model of adaptation illustrates this effect of tight linkage on the short-run dynamics of adaptation.

Consider a haploid genetic system with two bi-allelic loci and genotypes ab , Ab , aB , and AB (Table 3), with alleles A and B being the beneficial alleles. We assume that their beneficial fitness effects are small ($1 \gg s > 0$), and epistasis between beneficial alleles is either weak or absent

Table 3. Relative fitness, per genotype, in a haploid genetic system with two beneficial alleles and weak epistasis between them^a

Genotype:	ab	Ab	aB	AB
Fitness:	1	$1 + s$	$1 + s$	$(1 + s)^2 + \varepsilon$
Frequency at birth:	x_1	x_2	x_3	x_4

^aAlleles A and B are beneficial, with $0 < s \ll 1$, and weakly epistatic: $|\varepsilon|$ on the order of s^2 or smaller. Epistasis between beneficial alleles is reinforcing (positive) when $\varepsilon > 0$; epistasis is antagonistic (negative) when $\varepsilon < 0$; $\varepsilon = 0$ corresponds to the case of no epistasis (i.e., multiplicative fitness) between loci.

(i.e., $|\varepsilon|$ on the order of s^2 or smaller, where ε represents the degree of epistasis; Table 3). Linkage disequilibrium—the statistical association between the beneficial alleles—is defined as the difference between observed and random expectations for the haplotype frequencies: $D = x_4 - xy$, where $x = x_2 + x_4$ is the population frequency of the A allele, and $y = x_3 + x_4$ is the frequency of the B allele. With complete linkage between the loci (no recombination), the change in mean fitness due to selection is given by:

$$\Delta_{NS} \bar{w} = \frac{s^2 [x(1 - x) + y(1 - y)]}{\bar{w}} + \frac{2s^2 D}{\bar{w}} + O(s^3, s\varepsilon), \tag{12}$$

where $\bar{w} = (1 + sx)(1 + sy) + xy\varepsilon + D(s^2 + \varepsilon)$. When epistasis and selection at each locus are both weak (as assumed here), the first two terms of Eq. (12) provide a reasonable approximation of the rate of adaptation in the absence of recombination between the loci. The first term of Eq. (12) describes the rate of adaptation in the absence of linkage disequilibrium, whereas the second describes the modifying effect of linkage disequilibrium (D) on the rate of adaptation under tight linkage. It is clear from the second term that linkage increases the rate of adaptation when AB chromosomes are enriched in the population ($D > 0$), and constrains adaptation when AB chromosomes are deficient ($D < 0$). The question is: which form of D —positive or negative—are we most likely to see in nature?

Two evolutionary factors—epistasis, and genetic drift interacting with selection—generate predictable patterns in D over time, with direct implications for linkage constraints (Ref. 26, Chapters 6

and 7, and Refs. 155 and 158). First, in the absence of genetic drift, epistasis predicts the direction of linkage disequilibrium, with positive epistasis between beneficial alleles leading to positive D ($\varepsilon > 0$ and $D > 0$), and negative epistasis leading to negative D ($\varepsilon > 0$ and $D < 0$). Tight linkage should therefore constrain adaptation when beneficial alleles at different loci exhibit negative epistasis between them (equivalent to reinforcing, or “synergistic,” epistasis between deleterious mutations; Ref. 159). Theory predicts that negative epistasis should be common,¹⁶⁰ with supporting empirical evidence from beneficial mutations in *E. coli* and an RNA virus,¹⁶⁰ deleterious mutations in the Hsp90 gene in yeast,¹⁶¹ and deleterious mutations in the human genome.¹⁶²

Second, interactions between selection and genetic drift will also lead to negative linkage disequilibrium between beneficial mutations ($D < 0$, corresponding to a deficit of double-beneficial genotypes; see Ref. 155). The effect arises because drift generates random statistical associations between loci, but the rate at which any arising disequilibrium dissipates will differ between positive and negative linkage disequilibrium. Selection will rapidly fix combinations of beneficial mutations with positive D and erode the disequilibrium in short order. In contrast, beneficial combinations with negative D are more likely to persist as polymorphisms. Selection is simply less effective at removing or fixing the combinations of beneficial and deleterious alleles, which leads to a negative D , on average, among all segregating beneficial alleles. This phenomenon (referred to as the “Hill-Robertson Effect” after Ref. 163, see also Ref. 153) amplifies genetic constraints associated with tight linkage.

Genetic linkage and long-term constraints

Long-term dynamics of adaptation in absence of recombination depend on two chance events. First, new beneficial mutations must arise to allow for adaptive evolutionary change. Second, to eventually become fixed in the population, beneficial mutations must arise in otherwise high-quality genetic backgrounds. In sexually reproducing populations, recombination can allow beneficial alleles to potentially “escape” from an initially low-quality background. In asexual species, however, the lack of recombination renders escape impossible; the initial association between a beneficial mutation and

its genetic background becomes a primary determinant of its evolutionary fate. This poses a dilemma for the long-term rate of adaptation under tight linkage (Ref. 1, p. 122). A low mutation rate ensures that most genomes should carry few or no deleterious alleles, yet adaptation will then be strongly limited by the rarity of beneficial mutations. With high mutation rates, we have the opposite problem: beneficial mutations are abundant, yet their beneficial effects will typically be negated by high mutation loads elsewhere in the genome.

The balance between deleterious and beneficial mutations can be illustrated with a simple, haploid model of mutation and selection. In a sexually recombining population where beneficial alleles evolve independently of deleterious ones, the probability that a new beneficial mutation eventually fixes is roughly $\sim 2s_b$, where s_b is the fitness benefit of the allele (e.g., Ref. 36). The long-run rate of adaptation depends on the rate at which beneficial mutations arise in the population, the probability that each is fixed, and the average effect of mutations that do eventually fix. Taking each of these factors into account, the predicted long-run rate of adaptation in the presence of recombination is approximately:

$$\Delta_{NM} \bar{w}_{sex} = 2NU f_b E[s_b] \cdot E[s_b | est.], \quad (13)$$

where U is the genomic mutation rate, N is the population size, f_b is the fraction of mutations that are beneficial, $E[s_b]$ is the mean fitness effect of random beneficial mutations, and $E[s_b | est.]$ is the expected fitness effect of those that eventually establish in the population.

In an asexual population, the fixation probability of a beneficial mutation is complicated by the need to consider the initial genomic background that it arises in. If beneficial mutations are assumed to generally have smaller fitness effects than deleterious ones, then the fixation probability of a random beneficial allele is roughly $2s_b \cdot e^{-U(1-f_b)/s_H}$, where s_H is the harmonic mean fitness effect of deleterious mutations (the approximation draws from the theory of “background selection”; see Refs. 164–167). The rate of adaptation in the asexual population becomes:

$$\Delta_{NM} \bar{w}_{asex} = 2NU f_b e^{-U(1-f_b)/s_H} E[s_b] \cdot E[s_b | est.], \quad (14)$$

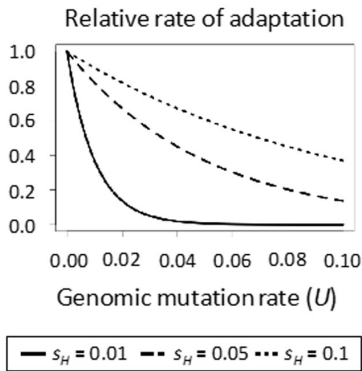


Figure 7. The relative rate of adaptation in an asexual relative to a sexual lineage. Theoretical predictions are based on Eqs. (13) and (14), and give the ratio of the long-term rate of adaptation under sexual versus asexual reproduction. All else being equal between sexual and asexual lineages, the ratio reduces to: $e^{-U(1-f_b)/s_H}$. Results assume that a small proportion of mutations are beneficial ($f_b = 10^{-5}$).

which is based on a similar result from Orr.¹⁶⁷ As shown in Figure 7, linkage greatly reduces the rate of adaptation when the average number of deleterious mutations per haploid genome is large (the mean number of mutations is $U/(1-f_b)/s_H$; see Ref. 42, p. 162, 554 and Ref. 167). Note that in the absence of recombination, the rate of adaptation is maximized at an intermediate rate of mutation: $\Delta_{NM}\bar{w}_{asex}$ is maximized when $U = s_H/(1-f_b) \approx s_H$ (see Ref. 167).

Extensions of the basic model have led to two refinements of the theory. First, large-effect beneficial mutations (i.e., where $s_b > s_H$) somewhat elevate the rate of adaptation by expanding the fixation probability of beneficial alleles, though this comes at a price of increasing the fixation rate of linked, deleterious alleles.^{152,168,169} Second, competition between different beneficial alleles that segregate simultaneously within the population (see Ref. 170), known as clonal interference, further dampens the rate of adaptation under linkage.¹⁶⁷ Deleterious mutations can also fix under drift even in the absence of any beneficial mutations (e.g., Refs. 151, 171, and 172).

Empirical evidence of constraints from tight linkage

Linkage constraints to adaptation, as outlined above, are more than theoretical possibilities—we now have considerable evidence that they are important in mediating the rate of adaptation in microbes, the population genetic dynamics of beneficial alleles,

and patterns of molecular evolutionary diversity and divergence in genomes. The strong empirical push to quantify constraints arising from linkage reflects sustained interest in the evolutionary consequences of recombination, and their potential implications for the evolution of sex. Below, we briefly touch upon approaches for inferring constraints to adaptation that arise from insufficient recombination, focusing on recent studies made possible by genome sequencing advances.

Experimental evolution and the dynamics of microbial adaptation.

Many microbial species are facultatively sexual, reproducing asexually under conditions of abundance, and sexually in contexts of crowding or starvation. One powerful way to examine the effect of sex and recombination on the rate of adaptation is to observe whether sexually reproducing lineages evolve more rapidly than asexually reproducing ones. Goddard *et al.*,¹⁷³ for example, experimentally evolved genetically engineered yeast strains that differed only in their mode of reproduction (with versus without recombination). They showed that the rate of adaptation to a new, harsh environment was boosted by sexual reproduction—in line with theoretical predictions. More recently, this approach was taken further by McDonald *et al.*,²⁰ who replicated the result (also in yeast), but this time tracked the evolutionary trajectories of beneficial and deleterious alleles as they spread within adapting sexual and asexual populations. They observed rampant interference between different loci in asexual populations, leading to a reduced efficiency in fixing beneficial mutations and eliminating deleterious ones. These constraints to adaptation were greatly mitigated in sexually recombining populations.

Molecular evolutionary inferences of selective constraints from linkage.

Some of the strongest evidence of linkage constraints comes from studies of recently diverged sex chromosomes in which there is a near-complete absence of recombination in one of the neo-sex chromosomes. Genes on the nonrecombining neo-Y disproportionately accumulate loss of function alleles and other deleterious mutations relative to their homologs on the recombining neo-X.¹⁷⁴ A second approach—contrasting population genetic signals of selection between genomic regions with low versus high recombination rates—provides further evidence of

linkage constraints in molecular evolutionary data. Several studies have found evidence of elevated and putatively deleterious protein divergence in low-recombining regions of the yeast^{175–177} and *Drosophila* genomes,^{178–181} but not in primates.¹⁸² Studies of positively selected divergence, particularly in *Drosophila*, similarly find evidence of elevated rates of adaptive substitution in genomic regions with high recombination rates.^{178,183–185} What remains unclear is whether these phenomena occur in a broad spectrum of species. Availability of genome-wide polymorphism and divergence data, along with fine-scale recombination rate estimates, will allow tests widely applied in model systems to be extended to a broader array of species.

Conclusions

Genetic constraints dampen the rate of adaptation by constricting genetic variation for fitness, reducing the fraction of mutations that are beneficial, and decreasing the efficacy with which natural selection is able to fix them. We have emphasized five general population genetic mechanisms that can generate constraints—dominance, pleiotropy, fitness trade-offs, sign epistasis, and tight linkage—each with fairly old conceptual origins in evolutionary theory. Yet owing to evolving interests within the field of evolutionary biology, along with technological advances in genomics, molecular biology, high-throughput phenotyping, and experimental evolution, some of the most compelling empirical examples of each form of constraint have been recent. Technological advances appear likely to drive further progress on the nature and evolutionary importance of genetic constraints, and future studies stand to benefit from ever-closer links between emerging data and (both old and new) theory.

Finally, although genetic constraints make adaptation more difficult—and rapid adaptation less likely—they do not necessarily prohibit it. Instead, time and population persistence are the ultimate arbiters of the impact of genetic constraints.¹¹ Constraints matter most in populations threatened by extinction, and where each additional limit to evolutionary capacity may serve as the proverbial straw that breaks the camel's back. Some forms of constraint, such as limited genetic variation, are exacerbated in threatened populations (e.g., Refs. 84 and 186), triggering an extinction vortex.¹⁸⁷ But what forms of genetic constraint, if any, are alle-

viated in threatened populations? Are some forms of constraint more likely to manifest in large, stable populations, where their impacts are comparatively benign? Although such questions have received some attention (e.g., in the context of fitness trade-offs: Refs. 100, 101, 188, and 189), their broader implications for genetic constraints remain unclear, particularly from an empirical perspective. Further attention to the ecology of genetic constraints therefore seems warranted, and may provide fertile areas for future exploration.

Acknowledgments

This research was funded by the Australian Research Council and the School of Biological Sciences at Monash University. We gratefully acknowledge the insightful comments and suggestions from three anonymous reviewers.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix 1: Discrete-time approximations for Fisher's Fundamental Theorem

Appendix 2: The rate of adaptation in Fisher's geometric model

Competing interests

The authors declare no competing interests.

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