Common, consequential, and actively maintained: a neo-balance view of genetic variation

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Abstract

A fundamental and still unfinished task of evolutionary biology is to understand the nature of genetic variation and the evolutionary forces that shape it. The traditional approach to this problem is through retrospective inference from patterns in contemporary, static DNA sequence data. The prevailing view that emerged from these studies and from theoretical research is that segregating genetic polymorphism is either deleterious or effectively neutral and thus governed primarily by purifying natural selection and stochastic forces such as genetic drift or draft. In this Perspective, we review emerging evidence that challenges this paradigm. In particular, evolution-in-action studies that directly measure selection at individual loci throughout the evolutionary process have revealed hundreds to thousands of loci with common polymorphism exhibiting strong fitness effects and suggest that these polymorphisms must be maintained through some form of balancing selection. These data call for a new framework for understanding population genetic variation, which we term neo-balance. We discuss the theoretical and practical implications of this new paradigm and outline an empirical agenda to incorporate these observations into the study of biological variation and evolution.

Main Text

During the eighteenth and nineteenth centuries, plant and animal breeders demonstrated that practically all traits evolve toward the desired values when subjected to artificial selection. This observation was famously influential for Darwin and is the topic of the very first chapter of the *Origin of Species*. While the abundant genetic variation implied by this responsiveness has since been confirmed to be ubiquitous across life, the question of which evolutionary forces generate and maintain it remains stubbornly unresolved[1,2].

The currently dominant framework in evolutionary genetics is rooted in the 'classical' model, originally championed by H. J. Muller[3]. This model holds that at any given functional locus there exists a single optimal, wild-type allele and that all other variants represent deleterious departures from this optimum. Under this view, natural selection acts primarily as a diversity-

purging process — most commonly eliminating the persistent input of deleterious mutations via purifying selection, and more rarely driving the rapid fixation of unconditionally advantageous ones.

The discovery of an unexpected abundance of molecular variation in the 1960s led to an expansion of the classical model to allow for a class of neutral or nearly neutral polymorphisms. Specifically, Kimura and others proposed the neutral theory of molecular evolution, which posits that most variants segregating within species are inconsequential to fitness[4,5]. This theory was further refined by Ohta, who postulated an additional abundant class of mutations with fitness effects that are slightly deleterious in an absolute sense but still sufficiently small relative to the power of genetic drift to render them effectively neutral[6]. The evolutionary fates of both neutral and nearly neutral mutations are governed primarily by drift rather than selection, and it was presumed that molecular divergence between species was largely explained by these mutations. Subsequently, however, tests of selection at individual loci and across genomes found positive selection to be responsible for an unexpectedly large fraction of functional divergence between species[7–13]. These findings, together with theory on selection at linked sites led to further modifications of the classical framework that emphasized the impacts of positive and purifying selection on linked neutral and nearly neutral polymorphisms[14–18]. Ultimately, the classical model and its subsequent theoretical refinements constitute what has become known as the neo-classical framework[1], the field's current standard model. Its core premise is that at any given locus there exists a single optimal allele, which represents the unconditionally favored state that purifying selection drives the population toward via the elimination of mutational departures (Table 1).

The key alternative to the neo-classical framework is balance theory, the intellectual foundations of which were established by Dobzhansky and colleagues during the mid-twentieth century[19,20]. Balance theory did not dispute, and can in fact accommodate, key features of the neo-classical model: the central importance of purifying selection, the prevalence of deleterious mutations, the existence of common neutral variation, and the action of positive selection. However, it challenges the notion that a single optimal wild-type allele exists at each locus. Instead, balance theory proposes that at a substantial number of loci there exist alternative alleles that are beneficial in some contexts and deleterious in others. Natural selection then acts to preserve these alternative alleles, in effect actively maintaining consequential functional diversity within species. The specific mechanisms that maintain diversity include spatially and/or temporally variable selection, the many possible forms of negative frequency dependence, and other diversity-preserving mechanisms such as storage effects, heterozygote advantage (absolute or marginal), and beneficial reversals of dominance[1,21–28]. Despite their mechanistic differences, these balancing processes share a common outcome: they slow or even prevent the fixation of any single functional allele. Critically, and in direct contrast to the neo-classical framework, balance theory predicts that many common polymorphisms in natural populations should (i) have

large absolute fitness effects, (ii) be actively maintained by natural selection, and (iii) tend to shift from strongly advantageous to strongly deleterious depending on context and thereby serve as the substrate for adaptation to local conditions and speciation[1,21] (Table 1; Fig. 1).

The fact that balancing selection can maintain some polymorphisms is not in dispute. For example, there is widespread agreement that the sickle-cell hemoglobin (HbS) polymorphism at the human β-globin locus (*HBB*), which causes sickle cell anemia in homozygotes but confers protection against malaria in heterozygotes, is maintained in malaria-endemic regions by heterozygote advantage[29]. Furthermore, classic studies of variation at the *G6PD* and major histocompatibility complex (MHC) loci in humans have provided evidence that diversity at these loci is also maintained by balancing mechanisms[30,31]. In addition, research by E. B. Ford and colleagues in the mid-twentieth century showed that the direction and intensity of selection can fluctuate over just a few generations in wild moth populations[32]. More recently, an analytical investigation by Charlesworth (2015) indicated that additive variance in fitness components in *Drosophila* is too large to be explained by the opposing forces of mutation and purifying selection alone, suggesting that balancing selection must contribute to its maintenance[2]. Yet, these canonical examples and indirect lines of evidence alone do not systematically answer the broader question of whether balancing selection is a dominant force maintaining consequential functional polymorphism genome-wide, both in our own species and in species across the tree of life.

In this Perspective, we first describe how the primary approach to studying genetic variation — inference-based methods applied to static snapshots of DNA sequences — has limited power to detect balanced polymorphism, leading to broad acceptance of the neo-classical framework. Next, we review how recent technological and conceptual advances have enabled a new approach: evolution-in-action studies in which selection on individual genetic variants is measured directly. We discuss how applying this longitudinal approach across diverse taxa is revealing abundant, large-effect, common polymorphisms that are likely maintained by balancing selection. These new data challenge the neo-classical paradigm and carry far-reaching implications for both fundamental and applied issues in biological research.

Table 1. Distinguishing premises and predictions of the neo-classical and balance models.

	Neo-classical	**Balance**
Core premise	Single, optimal wild-type allele at each functional locus	No single optimal allele; alternative alleles are beneficial in some contexts and deleterious in others
Primary role of selection	Diversity-purging	Diversity-maintaining
Common polymorphisms	Effectively neutral	Predominantly neutral with a subset exhibiting large, context-dependent fitness effects

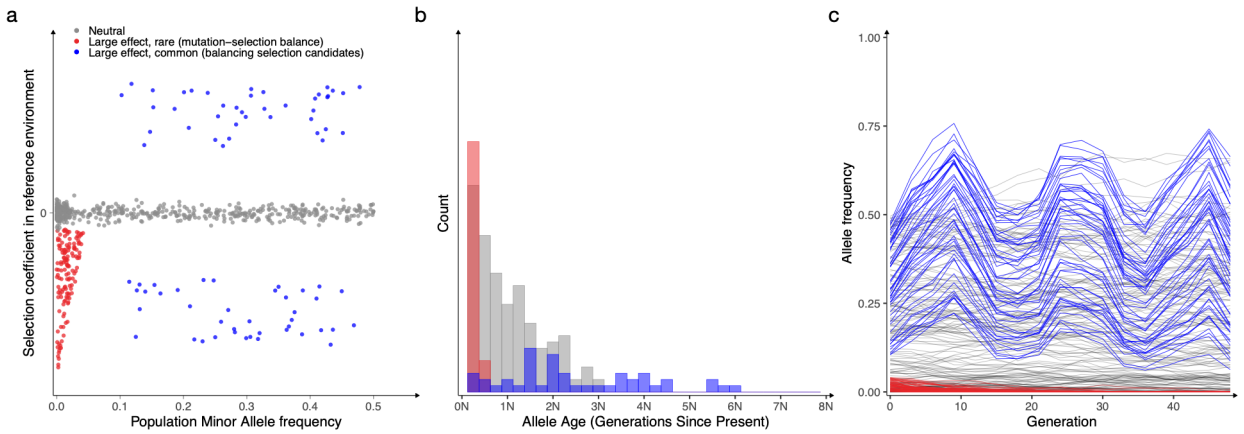

Figure 1 | Characteristics of representative polymorphisms subject to distinct evolutionary forces. (a) Expected distribution of polymorphisms as a function of their selection coefficient in a single reference environment and their population allele frequency. Polymorphisms subject to drift are grey, those subject to purifying selection are red, and those putatively balanced owing to context-dependent effects are blue. (b) Expected distribution of ages for each class of alleles depicted and colored as in (a). (c) Expected temporal trajectories for each class of alleles depicted and colored as in (a).

The search for balancing selection in static genetic and genomic data

Extensive evolutionary genetics research throughout the mid- and late twentieth century aimed to resolve the debate between the classical and balance models, but the results were fundamentally equivocal[1]. The advent of DNA sequencing technology seemed to offer a path forward[33]. Researchers could for the first time quantify patterns of variation at single-nucleotide resolution in contemporary sequence data and, in conjunction with theoretical expectations under different evolutionary scenarios, infer the underlying processes that gave rise to those patterns[34]. This molecular population genomics line of inquiry has been exceptionally productive and, to a large extent, consistent with the neo-classical model, establishing the key roles of purifying selection and random genetic drift[13,16,35–39]. Moreover, in a seeming blow to balance theory, whereas genome-wide scans for positive selection revealed that positive selection is common across taxa[7–11], similar scans for balancing selection have been much less fruitful[40,41]. This led many to conclude that the process has at most a minimal impact on the observed patterns of variation within species[21,40,41].

However, the limitations of an inferential approach to detecting balancing selection are notable[34,42]. The primary signature of balancing selection in static data is the persistence of polymorphisms at intermediate frequency for longer than expected under neutrality (Fig. 1b)[43]. This can be a very long time indeed — for instance, in humans an allele must be at least ~1 million years old before we can be confident that it is older than expected under neutrality[44]. Such ancient alleles can in principle be detected as trans-species polymorphisms and/or through

elevations in local nucleotide diversity around the balanced locus[43,45–48]. While this signature has revealed and validated classic instances of long-term balancing selection[43,48–51], it inherently misses instances of balancing selection that began more recently[42,44]. Furthermore, this inferential approach is insufficiently powered for species with relatively high rates of recombination (e.g., *Drosophila*), because even when sufficient time has elapsed since the onset of selection, the genomic signature of true trans-species polymorphism is winnowed to such a small region that it becomes impossible to identify[34,42,49,52]. These power limitations were recently quantified via simulation by Brandt and colleagues (2026), who demonstrated that even under favorable conditions — strong, symmetric selection with allele frequencies near 50% — more than two-thirds of instances of long-term balancing selection remain undetected by modern methods, and the true missed fraction is likely considerably higher[42].

Another signature of balancing selection in static sequencing data is based on a prediction originally made by Lewontin and Krakauer (1973): selection should produce anomalous patterns of allele frequency variation across loci, relative to the neutral patterns of variation dictated by genetic drift and population structure[53]. Specifically, loci under balancing selection in which favored genotypes are shared across populations (e.g., due to heterozygote advantage) are expected to show allele frequencies that are less differentiated between populations than the genome-wide average, because they are refractory to genetic drift. Alternatively, if balancing selection is driven by spatial variation in selective pressures, such that different genotypes are favored in different populations, selected loci might show greater allele frequency differentiation than neutral loci. Thus, the allele frequencies of balanced polymorphisms could be either more or less similar between populations than expected under neutrality, making these tests difficult to interpret in practice. Nevertheless, genome-wide scans for 'outlier' loci in a range of taxa have detected polymorphisms with signatures of local adaptation to specific environmental regimes[54–56]. Yet, again, it is challenging to discern whether observed allele frequency variation arises as a result of spatial structure induced by differing phenotypic optima for traits under stabilizing selection[55,57], or in fact represents the maintenance of large-effect polymorphism at intermediate equilibrium via some form of context or frequency dependence[58,59].

Despite the acknowledged lack of power, the dearth of empirical evidence for balancing selection led to the view that balancing selection likely plays at most a minor role in shaping patterns of diversity within species[21,40]. Because this view aligned with the long-standing theoretical expectation that the presence of a large number of balanced alleles would lead to an unsustainable genetic load on populations[60–62], the neo-classical framework has become the *de facto* standard model in population and quantitative genetics research[63]. Consequently, most current approaches in comparative and quantitative genomics assume that practically all high-frequency polymorphisms are effectively neutral, and that existing large-effect functional variants are deleterious and maintained at low population frequency due to mutation–selection balance[13,37–39].

Longitudinal sampling across taxa reveals large-effect, common polymorphism

The past decade has seen a rise in longitudinal studies of genomic variation, a trend driven by two key factors: (i) the growing appreciation that evolution can be quantified 'in action' on timescales ranging from weeks to hundreds of years, and (ii) the increasing affordability and accuracy of generating high-throughput genomic data from both contemporary and historical specimens[64,65]. The first of these factors was in large part facilitated by decades of eco-evolutionary research that tracked phenotypic and fitness variation across generations[66]. Such studies have been invaluable in demonstrating that evolution can proceed rapidly and predictably in response to fluctuating ecological change[67–69]. Yet, what has been missing are the genome-wide polymorphism data required to test whether balancing mechanisms are operating broadly across the genome, at what fraction of loci, and with what strength.

Here, our specific focus is on genomic evolution-in-action studies conducted across multiple generations and in the natural context in which the focal species lives and evolves. The frequencies of genome-wide polymorphisms (most often single nucleotide polymorphisms, or 'SNPs') observed throughout the time series can be used to directly measure the fitness effects of these variants (see Box 1). Thus, this approach allows for the direct observation of the evolutionary forces acting on variation, circumventing the key limitations of the inferential approaches described above. Our main question is whether the data that have emerged from these direct measurements indicate the existence of polymorphisms that both segregate at common frequency (e.g., >5% population frequency) and exhibit selection coefficients large enough (e.g., ~10%) that direct selection should have driven their fixation within hundreds of generations, even in populations of modest size[70]. Further, we are particularly interested in the identification of polymorphisms with context-dependent effects on fitness, a prerequisite for maintenance via balancing mechanisms (Fig. 1c). We note that the studies we highlight here do not represent an exhaustive review of longitudinal studies of genomic variation, but rather aim to exemplify, using a diverse array of taxa, the general features of genomic variation that are emerging from this approach.

Multivoltine insects

Multivoltine invertebrates, which experience multiple generations per year, are among the most widely used systems for quantifying evolution in action. For example, Lynch and colleagues (2024) conducted a ten-year population-genomic survey (~35 generations) of the aquatic crustacean *Daphnia pulex*, generating allele frequency data from nearly 1,000 isolates of an unmanipulated pond population. Across the time series, common variation appeared quasi-neutral, with mean selection coefficients near zero. However, selection varied substantially through time (Fig. 2a), and within any given year individual genomic sites exhibited net selection coefficients ranging over 10–20%[71]. In a similar system, Pfenninger and Foucault (2022) monitored a wild population of the midge *Chironomus riparius* across three consecutive years (~30 generations) within a highly dynamic stream environment. They identified on the order of

10,000 SNPs with frequency shifts that exceeded neutral expectations, the average selection coefficient of which was approximately 30%. Crucially, the standard deviation of calculated selection coefficients was much larger than the mean (0.68 vs. 0.30), indicating substantial changes in magnitude, and frequent reversals in the direction, of selection throughout the observation window[72].

Our own research, as well as that of others, has focused on diverse populations of the model vinegar fly, *Drosophila melanogaster*, inhabiting temperate environments. Through wild population sampling, as well as sampling from replicated, semi-natural mesocosms, we have repeatedly identified several hundred loci harboring SNPs with allele frequency shifts that fluctuate in concert with seasonal transitions (i.e., alleles favored during spring and summer are selected against during fall and winter). The observed magnitude of allele frequency shifts indicates strong selection, with selection coefficients often exceeding 10% per locus and per generation (Fig. 2b)[73–76]. The identified SNPs are widespread across the genome and, critically, the same SNPs have been independently identified across sampling years and locales, supporting their functional relevance and suggesting that the genomic basis of adaptation itself is predictable[74,76]. While there is evidence for a potential causal role of large, cosmopolitan inversions in patterns of seasonal adaptation[74,77,78], most selected variants are unlinked to inversions. Recent studies that have increased the resolution of temporal sampling have revealed an unexpectedly complex selective landscape in the system: deterministic shifts in allele frequency can occur on monthly and even weekly (roughly single-generation) timescales, and the SNPs identified on these finer temporal scales can be distinct from those responding to more gradual environmental shifts[75,76]. Finally, complementary lab-based and manipulative studies have elucidated a role for fundamental life-history trade-offs and beneficial dominance reversals in the long-term maintenance of this functional variation in this system[76,79].

Plants

Evidence for large-effect, common polymorphisms revealed by longitudinal sampling also extends to plants. For example, Kelly (2022) analyzed a 23-year time series of allele frequency data in the yellow monkeyflower *Mimulus guttatus*, finding that several thousand SNPs fluctuated in concert, alternating between two regimes — one associated with early investment in vegetative tissue and delayed flowering, the other with rapid progression to flowering (Fig. 2c)[80]. More recently, in a large-scale outdoor evolution experiment with *Arabidopsis thaliana*, Wu and colleagues (2026) found coordinated patterns of allele frequency movement within and across field-based common-garden sites, consistent with synchronized selection imposed by temporally shifting environmental conditions. Selection coefficients at selected loci were again large, on the order of 50% at candidate functional genes, with convincing evidence of context-dependent fitness effects: alleles originating from warmer regions of the species' range showed upward trajectories in warmer experimental sites and downward trajectories in colder sites[81].

Long-lived vertebrates

Longitudinal genomic data are sparser for vertebrate species, for which longer generation times reduce the feasibility of direct, multi-generational sampling (Box 1). Nonetheless, long-term monitoring programs, such as those for species of conservation concern, provide valuable datasets for the evaluation of fitness-relevant variation through time. For example, Cosgrove and colleagues (2025) co-analyzed multi-generational pedigree and genomic data from the Florida Scrub-Jay, finding that loci harboring fitness-relevant variation tend to exhibit sex-dependent effects and antagonistic pleiotropy, both of which likely contribute to the long-term maintenance of the variation[82].

In humans, advances in the retrieval of ancient DNA from excavated specimens allow longitudinal data to be generated by reconstructing allele frequency trajectories across historical time. For example, Irving-Pease and colleagues (2024) showed that while an analysis of genomic data from present-day individuals alone yielded no significant evidence of selection at trait-associated loci, incorporation of genotype data from ancient genomes identified 21 loci with signatures of directional selection[83]. Strikingly, many of the loci subject to selection did not sweep to fixation as expected for advantageous mutations under the neo-classical framework, but rather plateaued in their trajectories, potentially consistent with the initiation of balancing mechanisms. Akbari and colleagues (2026) leveraged an impressively large ancient DNA dataset generated from 15,836 West Eurasians to detect selection at 347 putatively independent loci, an order of magnitude more than previous scans had detected. The authors inferred directional selection at many of these loci, yet the temporal trajectories at over half of them indicated selection coefficients that were not constant through time, with some variants increasing in frequency by several percent before declining by a similar amount over just 4,000 years (Fig. 2d)[84]. Still, a key challenge for studies that rely on allele frequency estimates from a single unmanipulated population is accounting for unobserved population structure, which may generate spurious signatures of selection; this challenge can be addressed using complementary experimental and analytical approaches (Box 1).

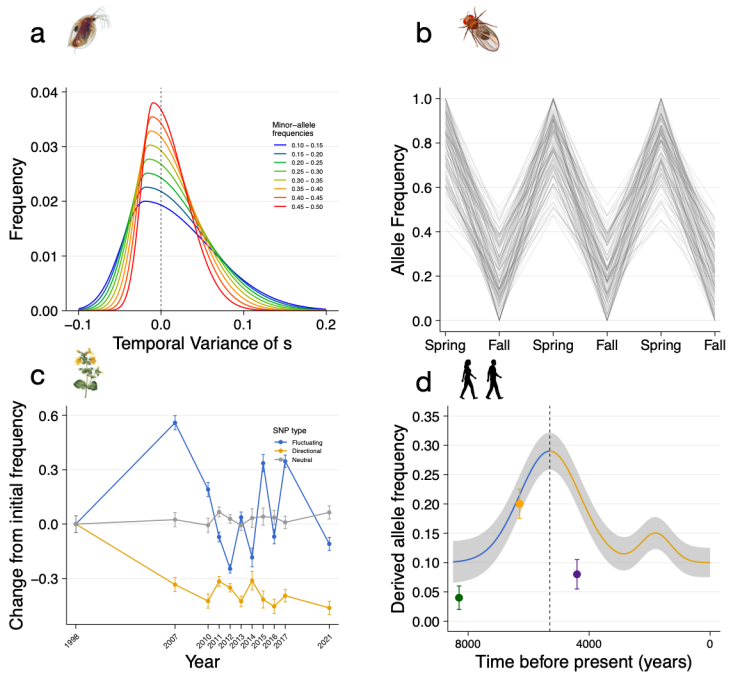

Figure 2 | Fluctuating selection revealed by longitudinal sampling across taxa. (a) Distributions of the site-specific estimates of temporal variances in selection coefficients (after removing the contribution from sampling error) across allele frequency bins (colors), identified in a 10-year population-genomic survey of the microcrustacean *Daphnia pulex*. Panel modified from ref. [71]. (b) Trajectories of SNPs with evidence of selection across three consecutive spring-to-fall transitions in a wild population of *Drosophila melanogaster*. Allele frequencies are polarized so that spring frequencies are higher than fall frequencies. Panel modified from ref. [73]. (c) Example trajectories of SNPs testing positive for fluctuating (blue) or directional (orange) change, together with a neutral SNP (grey), identified in a 23-year population-genetic survey of a natural population of the yellow monkeyflower *Mimulus guttatus*. Error bars indicate ± one standard error. Panel modified from ref. [80]. (d) Frequency trajectory of the derived allele for a SNP in a major risk gene for hemochromatosis (HFE C282Y) in human populations. The shaded region represents the 95% CIs around the estimated trajectory, while the circles denote frequencies in Western hunter-gatherers (green, $n = 131$), early European farmers (orange, $n = 452$) and steppe pastoralists (purple, $n = 293$) (bars around circles indicate 95% CIs). Segments of the time series are colored blue or red according to whether they fall before or after the manually selected peak frequency, respectively. Panel modified from ref. [84].

Box 1. Methodology and considerations for longitudinal analysis of genomic variation

Timescales: The appropriate observational timescale for quantifying evolution-in-action varies across study systems and is largely a function of the focal species' life history. For short-lived organisms, such as annual plants and multivoltine insects, DNA can be collected from living individuals sampled months to years apart, while for longer-lived species, such as humans, researchers may need to rely on the retrieval of ancient DNA from excavated or preserved specimens. While anomalous (or extreme) environmental change can drive evolution over one or a few generations, longer time-series data (e.g., spanning tens to hundreds of generations) may capture specific environmental regimes that reveal the functional relevance of variation that appears neutral on shorter timescales (i.e., is conditionally neutral)[85]. Similarly, increasing the resolution of sampling may reveal dynamics that remain hidden in coarser data, such as changes in the magnitude of selection or fluctuations in its direction[71,76,86]. In practice, phenotypic data on fitness-relevant traits may provide useful preliminary evidence in any given system to justify the initiation of genomic sampling, while also informing the appropriate sampling frequency.

Quantifying selection: The key measurement derived from longitudinal studies of genomic variation is the frequency of alleles segregating at polymorphic sites, most often in the form of single nucleotide polymorphisms (SNPs). These frequencies can in turn be used to reconstruct the underlying temporal trajectories of polymorphisms and, in effect, directly quantify the evolutionary forces acting upon them. Thus, this approach overcomes a key limitation of inference from static data — it does not hinge upon a genomic signature that may have decayed over time or that has yet to accrue. Instead, the fitness effects of segregating variation can be evaluated directly and within the ecological context in which it evolved and is maintained[64,87]. Evidence that selection has acted upon any particular SNP can be garnered from multiple signatures in time-series allele frequency data: parallel shifts across independent replicate populations, correlated frequency shifts across successive sampling intervals and/or across similar ecological/environmental stages, or a magnitude of frequency change that exceeds expectations under a model of genetic drift alone[64,88–90]. Critically, the frequency trajectories of selected SNPs allow direct estimation of their selection coefficients; SNPs whose selection coefficients fluctuate through time provide evidence of context-dependent balancing mechanisms (Fig. 1c).

Technical considerations: Disentangling technical artifacts and noise from selection is a key challenge in the analysis of time-series genomic data. For example, the identification of SNPs with extreme allele frequency shifts is susceptible to the 'winner's curse', whereby the effect sizes of candidate SNPs selected on the basis of extreme shifts are systematically overestimated. To mitigate this potential bias, candidate SNPs can be validated using various forms of out-of-sample prediction whereby selection coefficients are re-estimated from data that are independent of those analyzed in the original time series. This can be accomplished via comparison across independently evolving populations — for example, by evaluating genomic variation in isolated habitats (e.g., lakes)[71], or by using mesocosms that preclude migration among study populations evolving independently in response to a shared natural environment[75,76]. These designs further mitigate the challenge of disentangling the effects of selection from shifts in population structure (e.g., migration, admixture, or bottlenecks), which may give rise to spurious inferences of selection, particularly in the absence of a well-characterized demographic null model. Finally, quantifying linkage disequilibrium among identified SNPs is a critical step in determining the number of truly independent loci underpinning signatures of selection.

The neo-balance paradigm: implications and a new methodological agenda

The studies discussed above have indicated that the genomes of many species, from insects to plants to humans, contain on the order of hundreds to several thousand large-effect, common polymorphisms that shift in frequency by 1–10% over periods ranging from a few to a few hundred generations. The selection coefficients implied by the observed rates of change are large enough that unconditional directional selection would have driven these polymorphisms rapidly to fixation[70]. Instead, the persistence of these polymorphisms at intermediate frequencies likely reflects some form of context-dependent fitness effects, a hypothesis bolstered by their temporally fluctuating selection coefficients. Critically, this fluctuating behavior was only detectable through the direct measurement of allele frequencies as evolution proceeded in real time, and it would have remained largely hidden from inference using static data alone. These direct measurements of genomic variation further revealed that inferred rates of evolutionary change increase as sampling resolution increases[76], a trend that mirrors the classic observation by Gingerich (1983) that rates of morphological evolution inferred from the fossil record are inversely related to the interval over which they are measured[86]. These patterns are further supported by recent laboratory microbial-evolution studies that have quantified context dependence in the fitness effects of large-effect adaptive mutations[91,92].

Together, these observations represent a fundamental challenge to the neo-classical paradigm, *sensu stricto*. Yet, they do not discount the decades of previous work that have rigorously demonstrated the validity of numerous features of this framework: the importance of purifying selection, the prevalence of positive selection and selective sweeps, and the dramatic effects of genetic drift and linked selection on genome-wide patterns of variation[8,9,14,16,45,93–95]. Accordingly, in the context of the original debate with which we motivated this Perspective, what we advocate here is a revision of our standard model, one that accommodates the well-established forces recognized by the neo-classical model while also incorporating the previously underappreciated prevalence and impact of balancing selection — a revised framework we refer to as neo-balance. The implications of the neo-balance framework are widespread, and in the remainder of this Perspective we discuss several areas of biological research in which these findings are salient and potentially disruptive. We then outline the key outstanding questions regarding the common, large-effect polymorphisms identified by longitudinal approaches and describe a new methodological agenda to address them, an agenda that simultaneously takes advantage of current technology and embraces the varied contexts within which species live and evolve.

Theoretical population and quantitative genetics

The presence of large-effect variants at common frequency, as revealed by evolution-in-action studies, implies their long-term maintenance and raises the fundamental question of how such extensive genetic variation for fitness is maintained in natural populations[1]. It has previously been shown that the neo-classical explanation for this variation, namely that it represents a

balance between the input of deleterious mutations and their removal by purifying selection, is quantitatively insufficient to account for fitness variation in *Drosophila*[2]. Indeed, the longitudinal analyses reviewed here speak directly to this insufficiency, suggesting that temporally fluctuating selection may in fact be a prevalent and underappreciated balancing force[96]. This challenges two early theoretical predictions: (i) that temporal fluctuations could maintain polymorphism only under restrictive conditions due to the largely unsustainable genetic load induced by pervasive selection, and (ii) that phenotypic plasticity is the more likely means of persistence in variable environments[28,61,97,98]. However, recent theoretical research has shown how previously underexplored mechanisms, such as dominance reversals and storage effects, can alleviate such load and broaden the parameter space over which temporal fluctuations ultimately balance polymorphism in natural populations[24–26,99–102]. Alternatively, while the context-dependent fitness effects of these polymorphisms have been revealed via temporal fluctuations in the selective environment, it is also possible that their long-term maintenance is actually achieved via mechanisms other than temporally fluctuating selection alone (e.g., overdominance, frequency dependence, spatially variable selection). Resolving which of the many possible balancing mechanisms maintains these polymorphisms over long time periods is an exciting area of future research (see below).

Evolution-in-action studies might also help resolve additional puzzles in theoretical population and quantitative genetics that existing frameworks have long struggled to explain. For example, a long-standing issue in population genetics is a paradox first noted by Lewontin (1974): levels of genetic diversity vary far less across species than expected under neutral evolution given the enormous range of observed population sizes[1]. Proposed resolutions of this paradox have primarily invoked the neo-classical argument that purifying and positive selection are more effective in large populations[103]. The prevalence of fluctuating selection revealed by recent longitudinal sampling, however, suggests that this force may also play a contributing, if not dominant, role in this process. Specifically, selection fluctuating in intensity and direction can drive variance in allele frequencies at selected sites, as well as recurrent hitchhiking at linked neutral sites, in a manner that dramatically reduces overall genetic diversity[104,105].

The pervasiveness of fluctuating selection further provides exciting opportunities for new models in quantitative and population genetics. For example, models of polygenic adaptation have primarily focused on characterizing the architecture of adaptation under the scenario in which a quantitative trait is subject to long-term stabilizing selection around a constant optimum, followed by a gradual or abrupt shift toward a new optimum[106–109]. Such models have elegantly shown that the architecture of adaptation under such scenarios can range from selective sweeps, with large allele frequency shifts at one or a few loci, to a collective polygenic response in which subtle, coordinated frequency shifts occur at many loci throughout the genome[108–111]. Yet, the regime revealed by longitudinal studies — a continually fluctuating optimum that is tracked, at least in part, through shifts at large-effect, common polymorphisms — remains largely

unexplored, and understanding its underlying architecture may ultimately lend insight into how these large-effect alleles arise and are maintained in natural populations. In the context of demographic inference, existing models largely assume that variation in allele frequencies mostly reflects neutral demographic processes, an assumption already known to be violated by purifying selection and sweeps[93,94,112]. Pervasive fluctuating selection may provide an additional force confounding demographic signals, for example through its expected impact on effective population size[105]. Thus, new theoretical and inferential models are needed that accommodate a regime in which selection at many loci of individually large effect fluctuates simultaneously in direction and magnitude. Ultimately, these models should treat fluctuating selection not as a perturbation of directional adaptation, nor as neutral demographic noise, but as a primary and pervasive evolutionary force in its own right.

Genome-wide association studies

The common, large-effect variants revealed by longitudinal sampling shed new light on studies of quantitative genetic variation. For example, genome-wide association (GWA) studies across diverse species have consistently observed an inverse relationship between the effect size of mapped variants and their population allele frequency, a trend consistent with the neo-classical expectation that large-effect variants should be kept at low frequencies by purifying selection[113–119]. These observations, however, may also be compatible with the regime of balancing selection revealed by longitudinal studies. Specifically, while GWA studies estimate the marginal effect of a polymorphism on a pre-defined phenotypic trait, the fitness consequences of any given polymorphism are determined not by its effect on any single measured trait, but by its cumulative impact across the suite of traits that affect survival and reproduction[120,121]. The neo-balance framework can accommodate the relationship between effect size and frequency observed in GWA data if some key variants with individually modest effects on any single trait exert substantial aggregate fitness consequences via their cumulative pleiotropic effect across multiple fitness-relevant phenotypes[39,122–126]. This hypothesis is supported by the widespread sharing of association loci across seemingly distinct complex traits[127–129], as well as by recent efforts that have specifically mapped variation in components of fitness (e.g., life-history traits) and revealed the presence of large-effect polymorphisms[81,82,130]. Notably, recent theoretical work has also shown that the relationship between effect size and allele frequency of mapped variants could be a consequence of stabilizing selection acting on pleiotropic variants in a multidimensional phenotypic space[126]. Ultimately, and as we describe in more detail below, more studies are needed that fully integrate association mapping with longitudinal genomic and phenotypic data[83,84,131]. These data will enable systematic quantification of the relationship between a variant's frequency, its effects on individual phenotypic traits, and its consequences for fitness in natural populations.

Applied issues in global change biology and human disease

Beyond the implications for basic biological research, the results of evolution-in-action studies are pertinent to issues of societal concern. For example, the abundance of fitness-relevant variation revealed by longitudinal studies offers some hope regarding the capacity of species to respond in the current era of unprecedented environmental change. While numerous laboratory studies have quantified extensive standing variation with respect to specific global-change stressors, these experiments are limited in that they generally evaluate how species under isolated laboratory conditions respond to shifts in single abiotic stressors[132]. The multi-generational, longitudinal studies reviewed above have demonstrated how standing variation can facilitate persistence in the context of complex, rapidly fluctuating natural environments, a result complemented by numerous studies evaluating changes in genomic variation before and after extreme events[64,133,134]. However, it remains to be determined whether the variation that responds to contemporary natural environmental fluctuations will be the same as that which facilitates persistence in future, novel environments[135].

Results from evolution-in-action studies also provide a compelling evolutionary framework for understanding the incidence of non-communicable human disease. Recent empirical research has suggested that the prevalence of modern-day non-communicable diseases may in part be attributed to the mismatch between the environments in which risk-conferring alleles arose and the modern environments in which humans now live[136,137]. Longitudinal genomic data support the 'mismatch' hypothesis, suggesting that disease-risk variants may not always be deleterious mutations that have as yet escaped purifying selection, as expected under the neo-classical framework, but rather polymorphisms whose disease risk in modern environments is a by-product of their advantage in past, or unobserved contemporary, environments[136]. While the role of genetic drift in this process remains to be disentangled, the observed patterns underscore the necessity of studying human variation across contexts (see below). From a therapeutic standpoint, these data imply that drug targets derived from mapping conducted in a single population, environment, and point in evolutionary history may exhibit limited effectiveness across populations[138].

Outstanding questions and a new methodological agenda

Much remains to be reconciled in light of the potential strength and prevalence of balancing selection revealed by evolution-in-action studies. First, more finely resolved time-series data will be needed both to identify the full suite of large-effect, common polymorphisms segregating in natural populations and to fine-map the causal variants underlying selection. While longitudinal studies to date have identified on the order of several hundred to a few thousand large-effect common polymorphisms in a range of taxa, broadening the scope over which such studies are conducted (temporally, spatially, and taxonomically) could reveal an even greater number of such polymorphisms. This effort may ultimately better resolve the underlying architecture of these loci, such as whether they are spread evenly across the genome or predominantly within

tightly linked clusters (e.g., supergenes or inversions). Even if such variants remain relatively rare in comparison with the millions of variants segregating in many species, their aggregate effect on genome-wide patterns of variation could be substantial and thus warrants further investigation[104]. There is thus an urgent need for new theory both to explore the genome-wide impact of such polymorphism and to explain how populations persist despite the potential genetic load induced by this variation[62]. Similarly, it is unknown how such pervasive rapid adaptation can be reconciled with the high levels of phenotypic plasticity observed in ecologically relevant traits, plasticity that is often presumed to be the primary response to very rapid environmental change[139,140]. Determining theoretically the conditions under which adaptation dominates over plasticity and resolving empirically whether the same loci underpin rapid adaptation and control the plasticity of fitness-relevant traits, will be crucial in this regard.

Finally, functional characterization of candidate loci through high-throughput genome editing approaches will be required to establish the causal links between genotype, phenotype, and fitness that longitudinal allele frequency data alone cannot provide. This approach will be aided by efforts that integrate fine-mapping data with longitudinal genomic and phenotypic data in a manner that more directly links variant frequency, phenotypic effects, and fitness consequences across the range of contexts in which this variation arises and is maintained in nature[82–84,131]. Ultimately, these new data will allow us to discern the key features of these balanced loci: Are they alleles subject primarily to long-term balancing selection, or are they more often alleles that arose recently in evolutionary history? Are they generally pleiotropic, impacting suites of fitness-relevant traits in a coordinated manner? Are they specialized in their response to particular environmental conditions, or do they respond coherently to generic, coarse-grained shifts in ecological and demographic landscapes?

Importantly, this proposed empirical agenda is not as ambitious as it might once have seemed, because the technological infrastructure to pursue it is now available. High-resolution temporal genomic sampling is increasingly tractable across a wide range of taxa, and precision genome-editing tools such as CRISPR-Cas9 can now be deployed in both model and non-model organisms at a scale that makes systematic functional characterization of candidate loci feasible[141]. The convergence of these technological developments with the empirical foundation laid by the evolution-in-action studies reviewed here creates an unusually favorable moment to pursue a synthetic understanding of the nature, genetic architecture, and functional consequences of the variation that natural selection actively maintains. While this methodological agenda deviates from the approach that has historically characterized biological research — laboratory studies under carefully controlled, standardized conditions — our conviction is that embracing life's natural complexity will not generate a deluge of intractable findings. Rather, we believe it will reveal an emergent simplicity that elucidates the generic rules governing the generation and maintenance of life's diversity[142].

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