



Genetics of adaptation

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Edited by Hopi Hoekstra, Harvard University, Cambridge, MA; received January 27, 2022; accepted May 24, 2022

The rediscovery of Mendel's work showing that the heredity of phenotypes is controlled by discrete genes was followed by the reconciliation of Mendelian genetics with evolution by natural selection in the middle of the last century with the Modern Synthesis. In the past two decades, dramatic advances in genomic methods have facilitated the identification of the loci, genes, and even individual mutations that underlie phenotypic variants that are the putative targets of natural selection. Moreover, these methods have also changed how we can study adaptation by flipping the problem around, allowing us to first examine what loci show evidence of having been under selection, and then connecting these genetic variants to phenotypic variation. As a result, we now have an expanding list of actual genetic changes that underlie potentially adaptive phenotypic variation. Here, we synthesize how considering the effects of these adaptive loci in the context of cellular environments, genomes, organisms, and populations has provided new insights to the genetic architecture of adaptation.

adaptation | evolution | genetics | population genetics | evolutionary biochemistry

The rediscovery of Mendel's work in 1900 showed that heritable variation is controlled by discrete genes. Even before this time, there were calls to link the study of mechanisms of inheritance of adaptive traits with Darwinism as a "test of natural selection" (1). The rediscovery of Mendel's work provided a needed mechanism, and this knowledge then drove theoretical and empirical efforts to understand the genetics of adaptation. More recently, a renewed awareness of the fact that genes encode molecules that function in cellular environments led to a merging of biochemistry with evolutionary biology, bringing a new perspective to our understanding of the genetics of adaptation.

While historically most studies started from traits and moved toward identification of their molecular basis, the advent of genomic analysis means that both forward and reverse genetic approaches are now feasible (Box 1). These approaches have enabled the identification of loci—and in a growing number of cases, genes and even alleles—underlying phenotypes predicted or known to be adaptive. There has been some debate in the literature about whether and when it is useful to identify the genetic architecture and molecular basis of adaptation (2–4). We argue that this body of work has provided empirical data to test theoretical models and answer long-standing questions about the genetics of adaptation, while also providing a unique perspective that informs our fundamental understanding of genes and the proteins they encode. This is particularly highlighted by the power of combining forward

and reverse genetic approaches to inform our understanding of what traits are adaptive, since many may not be immediately obvious (Box 2).

In this review, we first briefly synthesize some of what we have learned about the loci (i.e., the genes and mutations) underlying potentially adaptive phenotypic variation. We then survey what we have learned from the recognition that genes and mutations do not act in isolation, but rather in the context of genomes, organisms, and populations, focusing on the overall genetic architecture of adaptation (i.e., number of loci, effect sizes, origins, genomic distributions, and pleiotropic effects). We finish by using temperature adaptation as a specific example to highlight what we can gain from moving beyond genetic architecture to considering that genes encode biologically active molecules, ruled by the laws of physics, which function in complex cellular environments. It is an exciting time in the field, but there remains much to learn, and we thus end with some of our own thoughts about interesting avenues and opportunities for future research.

What Are the Genes and Mutations that Underlie Adaptation?

There are a growing number of beautiful examples in which researchers have identified putatively adaptive phenotypic differences between populations or closely related species (Fig. 1) and then used forward genetic approaches to identify the underlying genes and mutations. Here we highlight several cases, but it is far from an exhaustive list, so we refer readers to Gephebase, a database of genotype-phenotype associations for phenotypic variation in natural and domesticated species (29). Perhaps due to the ease of identifying color differences, the vast majority of morphological phenotypes for which underlying genes have been identified are related to pigmentation (29), which can be a target of both natural and sexual selection (30). Genes and mutations underlying variation in pigmentation have been identified in invertebrates, vertebrates, and plants, including for example: wing color patterns in *Drosophila* (reviewed by

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Author contributions: K.B. and C.L.P. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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Published July 18, 2022.

Box 1. Forward and reverse genetic approaches to the study of adaptation

Two main approaches to identify genetic variants underlying adaptive phenotypic variation are the “forward genetics of adaptation” (“top-down” in the sense of ref. 5), where one first identifies adaptive phenotypes and then asks what genes underlie them, and the “reverse genetics of adaptation” (“bottom-up” in the sense of ref. 5), where one first identifies genes that show signatures of natural selection, and then uses gene perturbations to ask what phenotypes they cause.

Forward genetics of adaptation. Once adaptive phenotypes under selection (6, 7) have been identified, a variety of approaches can be employed to understand the underlying genetic architecture, such as QTL mapping, recombinant inbred line (RIL) mapping, and GWAS (8). QTL and RIL approaches are based on controlled crosses, while GWAS can be used more widely (e.g., in species that are not easy to cross or rear in the laboratory, or with long generation times). These approaches identify intervals and candidate genes, but ultimate proof of causality requires molecular follow-up, high-resolution genetic studies, and allele replacements with technologies like CRISPR/Cas9 (9), in which phenotypic effects of particular alleles are assessed. The major advantage of the forward genetic approach is that it does not assume prior knowledge of candidate genes, and it can identify genes that would not a priori have been considered as candidates. Beyond the technical limitations of detecting loci of small effect (2, 10–12), a major disadvantage of forward genetic approaches is that we are biased toward phenotypes that are easily observed to differ between populations, and we might miss traits that are less obvious but relevant for adaptation (Box 2).

Reverse genetics of adaptation. The most widely used starting point for a reverse genetic approach, often referred to as a “genome scan,” is to sample individuals from distinct populations and perform whole-genome sequencing, reduced-representation sequencing, or transcriptome sequencing. Then, statistical methods are applied to identify regions of the genome with signatures of divergent selection, and/or associations between genotypes and environmental factors (13, 14). A major advantage of a “genome-scan informed” reverse genetic approach is that it can be done on virtually any species or population. Moreover, as this approach is agnostic to phenotype, in principle it allows an unbiased view of the genetic basis of adaptation, allowing identification of adaptive traits that may not manifest as obvious phenotypic differences between populations (Box 2). An important disadvantage of the reverse genetic approach is that technical biases and other factors—such as demography, gene flow, and recombination—can influence inferences of selection (13–17). Furthermore, interpretation of lists of candidate genes, even if they were to represent a “complete” view of adaptive signals in the genome, is nevertheless biased by our own interpretations and tendency to focus on genes for which a clear hypothesis can be made (13). Thus, it is critical that these types of analyses are coupled with functional follow-up studies to understand the signatures observed.

Combining forward and reverse genetic approaches. The above approaches are complementary ways of reaching our ultimate goal in the genetics of adaptation, namely to know fitness effects of specific phenotypes, the underlying genetic architecture, and the selective forces acting on traits. The problem with both approaches is that each only fills in part of the puzzle (5), thus, we gain the most power combining them. Nevertheless, despite the call of Stinchcombe and Hoekstra (18) to do so in 2008, combining these approaches remains limited to relatively few systems (see refs. 19–21 for some examples). We therefore renew this call!

ref. 31), mimetic butterflies (32–35), and peppered moths (35, 36); body color patterns in stick insects (37) and the harlequin ladybird (38); coat color in rock pocket mice (39) and *Peromyscus* (40–42); seasonal coloration in snowshoe hares (43); hair color in humans (44); throat color variation in warblers (45); color patterns in cichlids (46); loss of pigment in cavefish (47); and flower color in *Mimulus* (48–50), *Petunia* (51), *Phlox* (52), *lochroma* (53), and *Helianthus* (54). Genes that underlie other ecologically relevant traits have also been identified, including skeletal traits relevant to predation and foraging in sticklebacks (55–58), beak size and shape in Darwin’s finches (59, 60), and flowering time in *Arabidopsis thaliana* and its relatives (reviewed by ref. 61). As reviewed in this issue, there are also a growing number of studies that have identified genes underlying behavioral evolution (62).

Several key insights emerged directly from the identification of genes and mutations that underlie these traits that would not have otherwise been clear. First, several of these studies highlighted the importance of ancestral standing variation as a source of adaptive alleles in the repeated evolution of the same trait (*Standing Variation*

vs. De Novo Mutation?). Second, this growing dataset has also shown that both coding mutations and *cis*-regulatory changes contribute to phenotypic evolution (*Synergistic vs. Antagonistic Pleiotropy?*). Third, the many cases of independent evolution of similar phenotypes have shown that even though many genes can in principle cause the same phenotype (reviewed by refs. 63 and 64), the same genes tend to get reused, suggesting constraints make some routes more likely than others (*How Can Incorporating Knowledge of Biochemistry and Biophysics Inform Our Understanding of the Genetics of Adaptation?*).

What Is the Genetic Architecture of Adaptation?

A list of genes and mutations that are known to underlie adaptive traits only provides a small window into adaptation. Genes and mutations do not function in isolation, but rather in the context of genomes, organisms, and populations. Here, we summarize how forward and reverse genetic studies have provided empirical data that test and

Box 2. The hidden side of adaptation: Type A traits

In evolutionary biology we are justifiably drawn to traits that distinguish populations or species. This has led to many beautiful examples of genes underlying potentially adaptive traits (see text). Recently, work combining genome scans with functional follow-up to study adaptation to whole-genome duplication in *Arabidopsis arenosa* (22–24) showed that traits that do not differ in obvious ways among ancestral and derived populations may nevertheless be important in adaptation (25). For example, genome scans suggested that genome-wide recombination rate might have been under selection in a derived polyploid lineage (22, 26, 27), but it was later found that ancestral diploids and derived tetraploids do not significantly differ in recombination rate (24). However, newly generated polyploids show a substantial increase in recombination rate associated with meiotic instability (24), suggesting that there was indeed adaptation in recombination rate in the polyploids, but the end result was to return the tetraploid to the diploid level. Thus, trait maintenance can leave signatures of selection in the genome, without yielding trait differences between derived and ancestral populations. We previously referred to this kind of “restorative” evolution as a “type A” pattern (28). This example further highlights how functional follow-up can inform genome scan data. That evolution can act on trait maintenance is also clear from the protein evolution literature, where there are numerous examples of adjustments needed to maintain critical enzyme functions at normal levels in novel conditions (see text).

shape theoretical predictions about the number and effect size of loci that contribute to adaptation, the origin of adaptive alleles, the distribution of those alleles in the genome, and whether alleles have pleiotropic effects on phenotypic evolution and adaptation.

Many Loci of Small Effect vs. Few Loci of Large Effect? Many of the examples discussed in the previous section are focused on loci that have a relatively large effect on phenotypes. Such loci are consistent with the proposal made shortly after the rediscovery of Mendelian genetics that loci of large effect might allow complex adaptations to evolve in a single leap (65, 66). However, this was in direct contrast to Darwin’s view of evolution as a slow and

gradual process (67). Thus, there was much debate about whether evolution proceeds in small steps, via many changes of infinitesimally small effect, or whether evolution proceeds in leaps via a few loci with large effect. This debate largely ended when R. A. Fisher reconciled Darwinian gradualism with Mendelian genetics in the infinitesimal model of adaptation (68). Despite a key correction to the model by Motoo Kimura (69), who predicted that loci of intermediate effect would be most likely to contribute to adaptation, Fisher’s micromutational view dominated thinking for much of the last century (65, 66). However, the rise of quantitative trait loci (QTL) mapping studies around the end of the last century revealed that phenotypic differences between populations or closely related species could

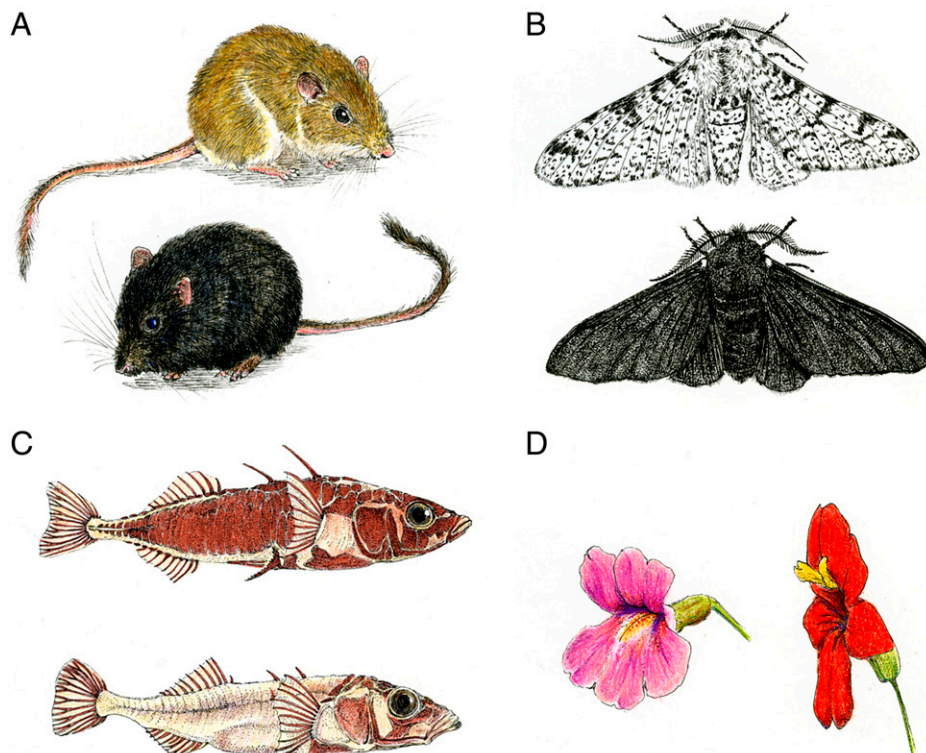


Fig. 1. Examples of systems used to study the genetics of adaptation. (A) Pocket mice (*Chaetodipus intermedius*); (B) peppered moths (*Biston betularia*); (C) threespine sticklebacks (*Gasterosteus aculeatus*), with red marking bony structures; (D) monkeyflowers (*Mimulus lewisii* and *Mimulus cardinalis*; note: these species are now in the genus *Erythranthe*). See text for references. Drawings by K.B.

be due to relatively few loci of moderate to large effect (70–75). These empirical findings propelled Allen Orr to revisit Fisher's geometric model of adaptation in a landmark theoretical paper in which he predicted that the distribution of phenotypic effect sizes of adaptive loci should be exponential, with many loci of small effect and a few loci of large effect, and that large-effect loci should be favored, especially when a population is far from the optimum (76).

Orr's theoretical work reassured empiricists that identifying loci, or even individual mutations, with detectable effects on adaptation was actually a feasible goal. Indeed, as highlighted above, there are now numerous examples of (mostly) large-effect loci underlying putatively adaptive traits in natural populations. However, it is unclear whether these iconic examples are representative. To date, the only direct tests of Orr's prediction used data on the percent variance explained by QTL that contribute to ecologically relevant traits. Although these QTL mapping studies are biased toward the identification of large and moderate effect loci (2, 10–12), metaanalyses of empirical QTL data revealed a general architecture of a few loci of large effect with many loci of small to intermediate effect in plants (77, 78) and sticklebacks (79). These data are consistent with Orr's prediction, suggesting that many traits evolving in nature are likely to have a polygenic architecture and hint that loci with large phenotypic effects might be the exception rather than the rule. Importantly, most of these studies are focused on the genetic basis of phenotypes, which are often assumed to be adaptive, but in reality we are measuring effect sizes of genetic variants on phenotypes and not on fitness (5). Thus, elucidating the genetic architecture of adaptation itself remains a major challenge for the future.

Standing Variation vs. De Novo Mutation? One key component of Orr's model is that adaptation is based on the fixation of de novo mutations, rather than mutations already present in the founding population. Indeed, until recently, most models had not considered adaptation from standing genetic variation. However, quantitative genetic studies show that most populations possess abundant genetic variation that enable a rapid response to selection (80). Recent theoretical work also suggests that adaptation from standing genetic variation is more rapid than adaptation from de novo mutation, and standing genetic variants (particularly of small effect) have a higher probability of fixation under a wide range of conditions (81, 82). Theory, therefore, suggests that adaptation via standing genetic variation might be particularly important in cases of rapid adaptation (83).

The relative roles of adaptation via standing variation versus de novo mutation can be examined with three different approaches (83). First, adaptation from standing variation and de novo mutations leave different population genetic signatures in genome scans (82, 84–87). Second, identifying the alleles that underlie adaptation allows us to test whether those alleles are still present in the ancestral population. For example, identification of the allelic variants underlying lactase persistence in human populations showed that some variants arose de novo, while others spread via selection on standing variation (88). Third,

determining the phylogenetic history of adaptive alleles can reveal whether they arose before a current population adapted to a particular environment. For example, haplotypes at two loci associated with variation in beak shape and size in Darwin's finches are ~1 million y old, predating very recent selection on these traits (59, 60). Similar evidence for selection on old genetic variants in rapid adaptation is accumulating in many plant and animal systems (89).

Despite this growing evidence for a role of selection on standing genetic variation in adaptation, there are also examples of de novo mutation contributing to recent phenotypic evolution. Strikingly, independent de novo mutations of an enhancer at the *Pitx1* gene underlies the repeated evolution of pelvic loss in stickleback populations (90). This locus is in a fragile site in the genome, suggesting that loci with high mutation rates might overcome the problem of waiting for new mutations (91). Although great progress has been made in particular case studies (only some of which are highlighted here), the relative contributions of de novo mutation and standing genetic variation to adaptation in natural populations, and under which particular conditions each might play a role, remains an open question.

Distributed vs. Clustered Genetic Architecture? When organisms are adapting to multiple different aspects of a local environment (e.g., predators, prey, parasites, abiotic factors), mechanisms that facilitate coinherence of adaptive phenotypes and prevent the formation of unfit combinations of genotypes might be favored, particularly when organisms experience divergent selection in the face of gene flow (92, 93). One mechanism of trait coinherence is pleiotropy, when a single allele affects multiple phenotypes, and another is tight linkage of multiple adaptive alleles, which could be caused by chromosomal rearrangements, such as inversions or fusions (94, 95) or gene transposition (96). This theory predicts a clustered genetic architecture for adaptation, in which multiple adaptive traits map to the same region of the genome (97). Indeed, clustering of QTL has been reported in both plants and animals (79, 98–105). Similarly, genome scans often (but do not always) find so-called "genomic islands," which are large regions (containing multiple genes) of high genetic differentiation between populations, although determining whether these islands result from divergent selection on multiple loci is challenging (13–17). It is clear, however, that clusters of QTL, complex phenotypic polymorphisms, and genomic islands are often associated with chromosomal inversions or chromosomal fusions (106–108). It will be particularly interesting to test whether such genomic clusters act as "supergenes," with large effects not only on phenotypes but on fitness, as was done, for example, in coastal versus inland populations of *Mimulus* (109).

Many Linked Mutations vs. a Single Pleiotropic Mutation? When multitrait QTL clusters are identified, it is usually unknown whether they are due to pleiotropic effects of single mutations or tight linkage between multiple causative mutations. It is difficult to distinguish these two scenarios when QTL clusters are found in regions of low recombination, such as inversions. However, a handful of studies have carefully dissected the molecular basis of a genomic cluster. In some cases, there is evidence for

pleiotropic effects of a single mutation (110–113); in others, there is evidence that linked mutations cause multiple phenotypes to map to the same location (42, 58, 114–116). A recent study shows evidence for both linked and pleiotropic mutations within a single locus (117). Disentangling the relative roles of pleiotropy and linkage is challenging, but identifying the phenotypes associated with a specific allele can help us determine whether selection is acting directly on a particular trait or on a correlated trait, and allows us to test whether pleiotropy promotes or constrains adaptation, a topic to which we now turn.

Synergistic vs. Antagonistic Pleiotropy? Although the sections above suggest that pleiotropy can play a beneficial role in adaptation, pleiotropy has classically been considered to constrain adaptation because the likelihood that an allele or a mutation has beneficial effects when it affects many traits is low; such antagonistic pleiotropy imposes a “cost of complexity” (68, 118, 119). This idea of pleiotropy as a constraint on adaptation led to the hypotheses that morphological evolution might predominantly involve changes in *cis*-regulatory elements of developmental regulatory genes because such mutations are less likely to have pleiotropic effects than coding changes (120). Indeed, many of the examples listed earlier provide evidence that mutations in *cis*-regulatory elements do contribute to morphological evolution. A metaanalysis also shows that the evolution of morphological traits between species is most often due to *cis*-regulatory mutations (121). However, there are also examples of *cis*-regulatory mutations with pleiotropic effects (113, 122–124), as well as numerous examples of coding mutations that contribute to trait evolution (29, 121). Moreover, the finding that there are mutations with pleiotropic effects on phenotypic variation in the wild (110–113, 117) suggest that pleiotropy is not always a crippling constraint. Thus, when considering the role of pleiotropy in evolution, it is crucial to consider whether individual mutations (and not genes) have pleiotropic effects on phenotypes (121).

Although both theory and empirical data have suggested that pleiotropy is universal—that is, “all loci affect all traits” (125–128)—knockout studies in model systems as well as QTL studies have provided limited evidence of pleiotropy at the gene level (129–131), though there has been vigorous debate about whether these methods are suitable to detect pleiotropy (126, 132, 133). Nonetheless, loci that are pleiotropic generally exhibit synergistic pleiotropy in which an increase in the number of traits affected by a mutation is correlated with an increase in the per-trait effect size (129, 130). These authors suggest that mutations with synergistic pleiotropy can overcome the cost of complexity by facilitating large steps toward a fitness optimum and also predict that intermediate levels of pleiotropy should be favored. This prediction is challenging to test because pleiotropy is extremely difficult to measure (126). However, two recent studies have supported the prediction that adaptive loci have intermediate levels of synergistic pleiotropy (134, 135). Such synergistic pleiotropic alleles might be particularly prevalent in cases of adaptation from ancient standing genetic variation where the alleles have already been tested by selection (83).

Pleiotropic mutations might also be favored when populations are far from the optimum (136), similar to large-effect mutations (76). Further tests of the relative roles of synergistic and antagonistic pleiotropy in promoting or constraining adaptation are clearly required, although quite challenging to do.

How Can Incorporating Knowledge of Biochemistry and Biophysics Inform Our Understanding of the Genetics of Adaptation?

Another important realization in the 20th century was that genes encode proteins (or functional RNAs) that operate in complex cellular contexts and are subject to biophysical constraints (137–139). For largely historical reasons, biochemistry and evolutionary biology mostly developed in isolation throughout the 20th century, but more recently, the two fields have been merging into what has been called “evolutionary biochemistry” (139).

To illustrate the insights that can be gained by considering the biochemical and biophysical properties of adaptive genetic changes along with the evolutionary genetic patterns described in the first part of this review, we highlight work on adaptation to temperature as an example, although other environmental factors can have similar effects. Temperature directly affects the biochemical and biophysical properties (i.e., structure and/or function) of nearly every biomolecule in a cell (including proteins, mRNA, tRNA, ribosomes, membranes), albeit to varying degrees (137–139). Most proteins function best in a specific stability range, and virtually any amino acid change can alter stability and flexibility to at least some degree (138, 139). At more extreme high temperatures, proteins can unfold partly or completely. The importance of unfolding as another potentially evolutionarily relevant force is highlighted by work in yeast, where it was shown that even trace amounts of a functionally irrelevant unfolded protein (YFP) in cells can elicit a costly unfolded-protein stress response (140). But how does this relate to adaptation?

Examples of Temperature-Associated Protein Adaptation. Here we discuss several examples showing that biochemical or biophysical properties of proteins can be altered during adaptive evolution. These examples also provide clear evidence of trade-offs, where an allele is beneficial in one environment, but not another.

Phosphoglucose isomerase. Phosphoglucose isomerase (Pgi) is an enzyme critical for gluconeogenesis and glycolysis and therefore the activity of muscles. Distinct Pgi alleles show temperature-associated clines in many insect species, and alternate alleles affect fitness-associated traits (e.g., growth, survival, mobility, and reproduction) in different temperatures (141–145). In characterizing the enzyme variants encoded by different alleles, evidence emerged for a trade-off between the kinetic efficiency of the enzyme and the thermostability of the protein, providing a biochemical explanation for why different alleles might be adaptive in different environments (141, 145).

Malate dehydrogenase. Malate dehydrogenase (MDH) is an enzyme essential in the citric acid cycle and several other critical metabolic reactions. In honey bees, distinct MDH alleles independently show latitudinal clines on four

continents (146). One allele (“M”) is more common at higher latitudes in all four clines and confers higher fitness in cool environments and lower fitness in warm environments. The protein encoded by the M allele is more heat-sensitive than the variants encoded by alleles from lower latitudes, but is more flexible and thus retains higher activity in the cold (146). In mussels, MDH alleles from a heat-tolerant and a cold-tolerant species differ by only two amino acids, one of which is sufficient to increase substrate affinity and turnover at low temperatures in the cold-adapted variant (147). In a striking example of cross-kingdom convergence, MDH also shows evidence of local adaptation in *A. thaliana*, two *Viola* species, and *Lathyrus japonicus*, where MDH alleles from cold-adapted and warm-adapted populations encode variants that differ in their activity and thermal lability, consistent with habitat of origin (148–150).

Tubulin. Tubulin proteins polymerize to form microtubules, which are essential to cell function. The dynamics of this process are extremely sensitive to cold; unsurprisingly, tubulin subunits commonly show signs of having evolved otherwise rare substitutions in cold-adapted organisms: A cold-tolerant alga has a mutation in a tubulin gene that causes an amino acid change predicted to increase resistance to destabilization (151). Antarctic fish tubulins have mutations thought to promote microtubule polymerization and suppress depolymerization in the cold. While their microtubules are therefore cold-stable, the trade-off is that they have slower polymerization and depolymerization dynamics (152). A cold-adapted ciliate and glacier ice worms also carry similar mutations that increase hydrophobicity of internal amino acids (and thus protein stability) and stabilize lateral contacts between protofilaments (153, 154). In ice-crawlers, an insect that lives on glaciers, a gene encoding a tubulin subunit shows evidence of having been under selection on two continents (155). This last example nicely highlights how understanding the biophysics of tubulin in relation to temperature provides insights that can explain what might otherwise be a puzzling gene to find in a genome scan for habitat adaptation.

Other types of changes. The examples above highlight the potential importance of selection on protein stability and flexibility during adaptation to temperature, but there is also evidence that other biophysical properties can be important. For example, selection on superoxide dismutase (SOD1) in long-lived great apes seems to have targeted amino acid changes that help prevent aggregation (156). Evidence from myoglobin in deep-diving cetaceans hints that stability-increasing mutations can be selected not because they alter protein function, but because they slow protein turnover and thus increase concentrations of active protein (157).

Parallelism, predictability, and pleiotropy. One striking feature of the examples highlighted above is that they show extensive parallels across taxa, even across kingdoms. One possible explanation is that a subset of proteins may be functionally constrained to have features that make them particularly prone to temperature-dysfunction. Likely, genes encoding proteins involved in temperature-sensitive processes—like enzyme kinetics, phase-separation, aggregation, and polymerization—will be overrepresented in temperature

adaptation in a wide range of taxa, leading to a degree of repeatability. As mentioned in *Synergistic vs. Antagonistic Pleiotropy?*, another question of importance in evolutionary genetics is whether a locus, a gene, or a mutation is pleiotropic. Defining pleiotropy is challenging for core cellular proteins, as it ultimately depends how we define a trait. If we consider the trait to be a molecular phenotype (like a single enzymatic reaction or the polymerization of microtubules), the proteins described above are not really pleiotropic because they are each responsible for a specific enzymatic reaction or cellular process. On the other hand, these proteins are critical for cellular functions and survival and could affect many macroscopic traits across the entire organism. Thus, at the organismal level, allelic variants in these proteins have pleiotropic effects. This highlights that we really need to consider what exactly we mean when we discuss whether or not traits are in fact pleiotropic.

Many Loci of Small Effect or Few Loci of Large Effect? As discussed above, an important question in evolutionary biology is whether adaptation proceeds via many loci of small effect or few of large effect. This question can also be applied to protein evolution. As described below for temperature adaptation, the answer seems to be “a bit of both.” As also noted above, mutations that cause amino acid changes in proteins are rarely truly neutral, as nearly all will affect protein flexibility or thermostability (138). Given this, what kinds of marks does temperature adaptation of proteins leave in the genome, and on what time scales?

Evidence for genome-wide effects. Certain amino acids can stabilize or destabilize proteins, leading to the prediction that organisms should have amino acid profiles that reflect their optimal growth temperatures. Indeed, in prokaryotes, there is an extremely good correlation between proteome-wide amino acid profiles and optimal growth temperature (158). In eukaryotes, the correlations are weaker, yet there are some global trends: Endothermic vertebrates generally have a more “thermophilic” amino acid profile (as defined from both biophysical predictions and empirical data) than ectothermic vertebrates (159). Average proteome thermostability correlates broadly with optimal temperatures across a wide range of organisms, including eukaryotes (160). At a finer evolutionary scale, there are amino acid composition differences between heat-adapted and cold-adapted tube worm species that suggest greater protein stability in the heat-adapted species (161). Similarly, mussel species that span nearly a 60 °C temperature range show differences in predicted protein flexibility that correlate with habitat temperature (162). The examples above highlight that, at least over medium to long evolutionary timescales, selection for protein stability, flexibility, or other biophysical properties is relevant, strong enough to out-compete drift, and polygenic enough to leave genome-wide signatures.

Short-term patterns. The previous paragraph highlights that thermal adaptation leaves genome-wide signatures over long evolutionary time, but what patterns do we see as populations adapt to a new habitat on a much shorter evolutionary time-scale? Are examples like Pgi or MDH just the most obvious of a long list of small-effect players, or are they at the front line of temperature adaptation, with genome-wide patterns appearing as part of a long-term

polygenic “fine-tuning”? The data thus far hint at the latter: modeling of protein stabilities in *Escherichia coli*, yeast, and *Caenorhabditis elegans* suggests cells “live on the edge of proteostasis catastrophe,” yet protein stabilities range widely in cells. The authors thus speculate that during temperature-triggered cell dysfunction, the problem is not the “proteome average,” but the subset of proteins that forms a long especially unstable tail of the distribution (163). A more recent study specifically tested whether declining cell viability with rising temperature is due to global protein denaturation or the loss of particular key players. At temperatures where cells begin to lose viability, only a subset of proteins are denatured or aggregated (164). We might thus expect that a subset of especially sensitive proteins would be among the first to be under selection during temperature adaptation, while the rest of the genome adjusts amino acid composition more slowly.

Genomic patterns observed to date are consistent with the idea that temperature-sensitive proteins are indeed quicker to adjust, with the rest of the genome following over time. For example, in thermotolerant invasive mussel species, there is a genome-wide signal of increased thermostability of the proteome, but it is strongest in a subset of proteins highly expressed in warmer temperatures (162). Cold-adapted (derived) tube-worm species show a lower tendency for heat-adaptive amino acid compositions than heat-adapted species, but changes in some proteins are more pronounced, suggesting a subset of proteins is particularly under pressure to evolve during thermal adaptation (161). Genome scans, genome-wide association studies (GWAS), and QTL mapping studies also support the idea that effects of temperature adaptation on the proteome are large enough to be detected, but adaptation is multigenic, with candidate genes encompassing broad ranges of functional classes of genes (165–167). Here, however, it is important to note that in these studies, temperature may not be the only selective force.

An overall picture emerges that adjustment of particular temperature-sensitive proteins is important for temperature adaptation in the short to medium-term, followed by highly polygenic fine-tuning in the longer term. This pattern is in keeping with the idea that adaptation often involves a distribution of effect sizes, with a few loci of large effect, and many of small to medium effect, and that the distance from the optimum as well as lineage age matter as to how many of each are found (76, 168, 169). The same seems to be true at the level of amino acids within proteins, where long-term fine-tuning appears to manifest as an overall shift in amino acid composition, while earlier adjustments likely occur through fewer larger-effect mutations (as in some of the specific examples noted above). Thus, the genes that are under selection in response to thermal challenges may be unpredictable in terms of known functions in thermal responses, but may be predictable in terms of biophysical properties.

Is Epistasis Important in Protein Evolution? An important topic in evolutionary biology is epistasis, the interactions among gene products or mutations (170). When considering protein evolution, epistasis is also very relevant, both at the inter- and intraprotein levels. A change in one

protein that affects its properties can place pressure on partner proteins to evolve in response to maintain functional interactions, which can lead to coevolution of “modules” of genes encoding interacting proteins (171, 172). Evolutionary biochemistry has also shown that within-gene epistasis is rampant, which has important consequences for whether or not particular mutations are beneficial, deleterious, or neutral. There is strong empirical evidence that folding, flexibility, or stability-altering mutations that do not themselves alter protein function can provide a context that allows, or disallows, the evolution of functional mutations elsewhere in a protein (173–176). These effects are not necessarily subtle; in extreme cases, a single point change can range from highly beneficial to deadly, depending on what other changes are present elsewhere in the protein (e.g., refs. 177 and 178). Interestingly, directed evolution experiments suggest that larger proteins, which tend to be more stable, are also more “evolvable,” since their stability can offset destabilizing effects of functional changes (179). The nonindependence of changes within the same protein-coding sequence has important implications for how different alleles of a gene can evolve. Thinking of protein evolution as an “adaptive walk” through fitness landscapes, epistatic interactions can dramatically change the accessibility of particular paths (138, 171, 180).

Looking to the Future

It is an interesting time for the continued extension of Mendelian genetics to evolutionary biology. We have come far in identifying genes that underlie adaptive or putatively adaptive traits and in understanding the genetic architecture of adaptive evolution. New advances in sequencing technology opened expansive views across genomes and allow implementation of reverse genetics in adaptation. But, as with all exciting times, much remains to be learned. Here we list a few ideas of forward avenues.

Functional Studies. Functional follow-up of phenotypic and fitness effects of loci, alleles, and mutations identified in genome scans or mapping studies, particularly for polygenic traits, is crucial. Such studies enable a mechanistic understanding of possible biophysical or developmental constraints that lead to the use of particular mutations or genes in adaptation, the contributions of pleiotropy and epistasis, and how the effects of particular alleles on phenotypes are translated into effects on fitness. Combining forward and reverse genetic studies, as well as directly mapping the genetic basis of phenotypes and fitness in the wild (e.g., refs. 181 and 182) will also be fruitful ways to connect genotype, phenotype, and fitness (5). Such studies will allow us to translate the genetic architecture of adaptation into a “functional architecture” of adaptation.

Polygenic Adaptation. Although many of the examples provided in this review involve loci with relatively large effects on phenotypes, it is clear that most traits have a polygenic architecture. Thus, both theoretical and empirical work is needed to better understand the signatures of polygenic adaptation in the genome and to identify the mechanistic basis of polygenic traits (169).

Beyond SNPs in Genome Scans. To date, most genome scans have only considered single nucleotide polymorphisms, but it is increasingly clear that structural variation, like gene duplications, transposable elements, or inversions can play a role in adaptation (183). Many new methods, including those that provide phased data, have been developed to capture structural variants (e.g., ref. 184), and it will be exciting to incorporate these more fully into empirical and theoretical studies of adaptation.

Interdisciplinary Collaboration. Obtaining a holistic understanding of adaptation requires the integration of genetic, cellular, molecular, developmental, biochemical, and biophysical studies in the laboratory, with studies of selection in the field and a “feeling for the organism” in the wild. This is well beyond the expertise of any one person or

research group. We therefore highlight the continued need for increased communication and collaboration across traditionally disparate fields, which will allow us to understand adaptive evolution from the identity of genetic variants to their effects on cells, organisms, populations, and ecosystems. We need look no further than the fusion of Mendelian genetics with Darwinian evolution to remind us of the power of such cross-fertilization!

Data Availability. There are no data underlying this work.

ACKNOWLEDGMENTS. We thank the editors for inviting us to contribute to this Special Feature collection. We also note that there is a vast amount of excellent work done on the genetics of adaptation that we cannot cover, and we apologize to those authors whose work is thus not featured. Finally, we thank Arnaud Martin and two anonymous reviewers for their very helpful and constructive feedback on the manuscript.

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