© 2022. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2022) 225, jeb243411. doi:10.1242/jeb.243411

COMMENTARY

Epigenomics as a paradigm to understand the nuances of phenotypes

Cornelia Fanter[1], Carla Madelaire[1], Diane P. Genereux[2], Frank van Breukelen[1], Danielle Levesque[3] and Allyson Hindle[1,*]

ABSTRACT

Quantifying the relative importance of genomic and epigenomic modulators of phenotype is a focal challenge in comparative physiology, but progress is constrained by availability of data and analytic methods. Previous studies have linked physiological features to coding DNA sequence, regulatory DNA sequence, and epigenetic state, but few have disentangled their relative contributions or unambiguously distinguished causative effects ('drivers') from correlations. Progress has been limited by several factors, including the classical approach of treating continuous and fluid phenotypes as discrete and static across time and environment, and difficulty in considering the full diversity of mechanisms that can modulate phenotype, such as gene accessibility, transcription, mRNA processing and translation. We argue that attention to phenotype nuance, progressing to association with epigenetic marks and then causal analyses of the epigenetic mechanism, will enable clearer evaluation of the evolutionary path. This would underlie an essential paradigm shift, and power the search for links between genomic and epigenomic features and physiology. Here, we review the growing knowledge base of gene-regulatory mechanisms and describe their links to phenotype, proposing strategies to address widely recognized challenges.

KEY WORDS: Epigenetics, Gene regulation, Phenotype, Physiology

Introduction

Next-generation sequencing has become commonplace over the past decade, and extensive genomic and epigenetic data are increasingly available for non-model species (Zoonomia Consortium, 2020). Emergence of these resources has made it easier to explore the multitude of factors that potentially shape gene expression, but the nuances of both phenotype and epigenetics mean connecting genome to phenome has remained challenging. A few studies have identified single-gene sequence changes that drive phenotype changes (Hiller et al., 2012; Romney et al., 2018; reviewed in Smith et al., 2020), but many other phenotypes are multifactorial, resulting from complex interactions among genomic, epigenomic, epitranscriptomic and proteomic mechanisms.

[1]School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV 89154, USA. [2]Vertebrate Genome Biology, Broad Institute, Cambridge, MA 02142, USA. [3]School of Biology and Ecology, University of Maine, Orono, ME 04469, USA.

*Author for correspondence (allyson.hindle@unlv.edu)

C.F., 0000-0002-4591-1866; C.M., 0000-0002-4399-715X; D.P.G., 0000-0001-5770-0989; F.v.B., 0000-0001-9653-0273; D.L., 0000-0003-0132-8094; A.H., 0000-0002-1983-9573

In this Commentary, we propose a comparative physiology-centric paradigm to discover epigenomic states that shape organismal phenotypes. This approach leverages, rather than simplifies the complexity of phenotypes and their dependence on environmental conditions will reveal otherwise inaccessible connections. It acknowledges that identifying epigenomic states correlated to specific phenotypes is an essential step towards distinguishing the subset with (potentially complex) mechanistic connections to phenotype and that discovering underlying epigenetic states is, in turn, essential for identifying selective pressures that could underlie evolution of novel physiological phenotypes. Even findings only part of the way along this series of steps may furnish information crucial for specific research goals. For example, identifying epigenetic states correlated to specific physiological phenotypes may be sufficient for developing diagnostics, and identification of driver states can inform predictive models even while relevant selective pressures remain unknown.

We discuss practical considerations for implementing our proposed research paradigm. We describe challenges in documenting genetic and environmentally induced phenotypic variation, and for identifying gene×environment interactions and relevant epigenetic states through available data analysis packages. In doing so, we highlight how deeper exploration of complex phenotypes can advance our understanding of the epigenetic underpinnings of physiological responses and help to prioritize populations, developmental time points, and environmental and behavioral states for further study.

The move from categorial to continuous phenotyping

Assigning complex physiological phenotypes to discrete categories offers appealing simplicity (Fig. 1A). Classical physiology often took this approach, defining species according to their ability to achieve extremes (Irschick and Higham, 2016; Kooyman, 1966; Schmidt-Nielsen et al., 1956). Although this focus on extreme physiology yielded many formative insights, the field has now embraced the more nuanced perspective that most phenotypes are continuous, potentially changeable through time and often shaped by multiple molecular mechanisms that are themselves fluid and complex (Rosenblum et al., 2010). This shift is closely paired to a greater appreciation that phenotype states are contingent on ecological context (Lane et al., 2019; Winterová and Gvoždík, 2021). For example, the magnitude of seasonal acclimation in three newt species was not consistent for specific individuals across years, suggesting an important role for environmental modulation, and underscoring the importance of considering individual variation when attempting to discern species-level phenomena (Winterová and Gvoždík, 2021). Microbiome variation among individuals can also affect phenotypic variation in a common environment (Kolodny and Schulenburg, 2020). Often-unmeasured sources of

Journal of Experimental Biology (2022) 225, jeb243411. doi:10.1242/jeb.243411

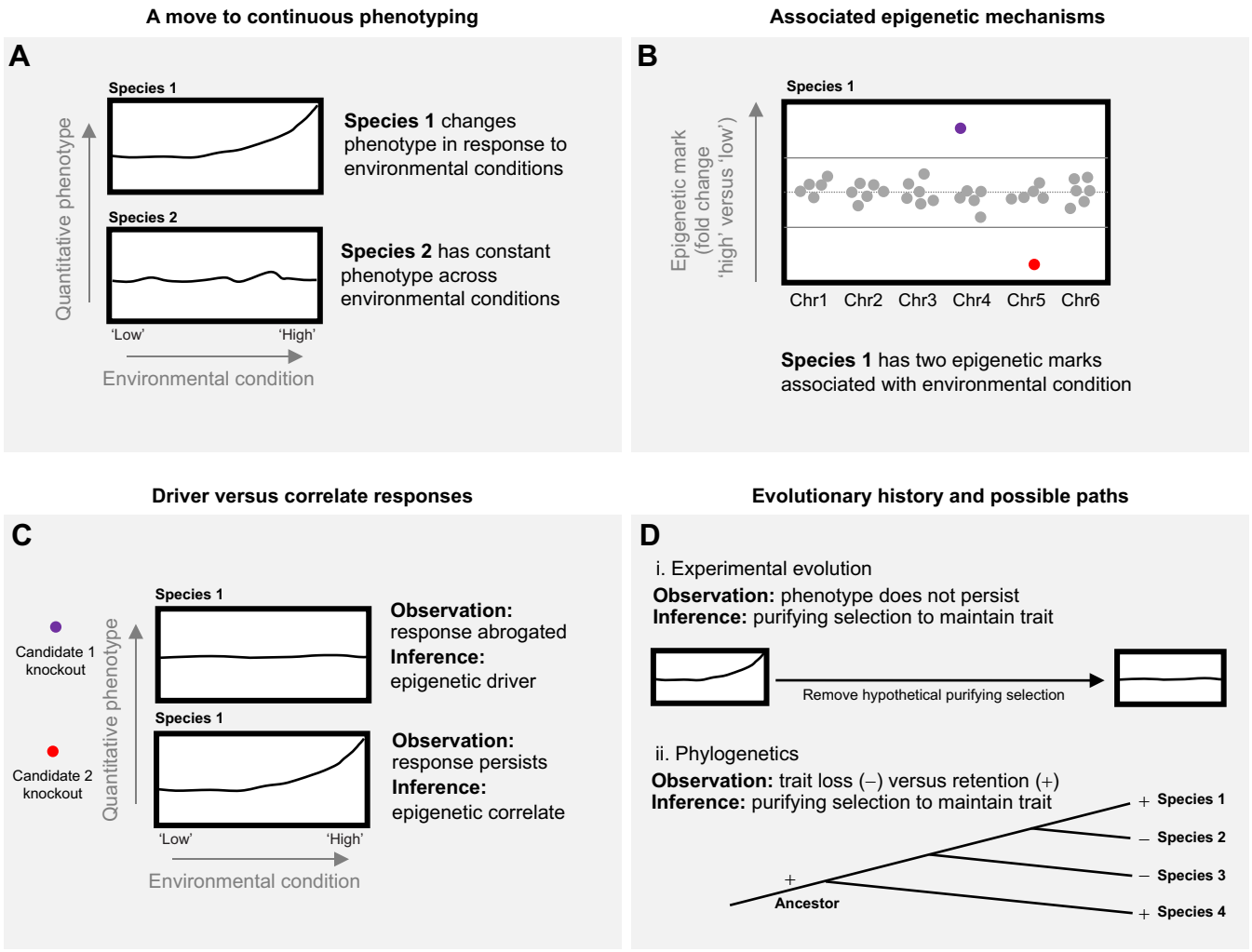

Fig. 1. A comparative physiology-centric paradigm to discover epigenomic states that shape organismal phenotype. (A) The ongoing shift from collection of categorical to continuous phenotypes is essential for clarifying both the scale and scope of physiological response to environment conditions. (B) Comparing epigenomic marks between individuals under disparate environmental conditions, an approach modeled on genome-wide association studies, will reveal state changes associated with physiological responses to environmental conditions. Chr, chromosome. (C) Experimental testing is essential to distinguish epigenetic changes that drive phenotype from those that are merely correlated. (D) Insights into the evolutionary origins of an epigenetic response are potentially accessible through: (i) experimental evolution to identify purifying selective pressures essential for maintenance of a given phenotype; (ii) phylogenomic analyses that use evolutionary history to identifying traits that have emerged convergently in species that inhabit similar environments. Which, if either, approach is feasible will depend largely on the feasibility of manipulative studies, generation time and other species-specific considerations.

variation create both challenges for study reproducibility and opportunities for more comprehensive measurement of variance (reviewed in Voelkl et al., 2020).

Phenotypic plasticity arises when the environment affects the manifestation of a trait encoded by a given genotype via changes in gene expression (as reviewed in Kilvitis et al., 2017). In this Commentary, we define any alteration that does not affect genomic sequence directly as an epigenetic mechanism (Waddington, 1956). Epigenetic processes often occur on shorter timescales than genetic changes and can therefore play an important role in modulating phenotype in rapid response to environmental pressures (Burggren, 2016; Kilvitis et al., 2017).

Distinguishing between genetic and environmental contributions to phenotypic variation within and between species can be experimentally difficult. We argue that this challenge can be addressed without resorting to simplifying phenotypes. Using a common garden framework, which enables measurement of phenotypic variation that persists even when individuals are subjected to identical conditions (de Villemereuil et al., 2016), researchers can quantify reaction norms, the range of phenotype that can arise from a single genotype. Variation observed in a common environment cannot derive from differences in immediate environmental impacts and so enables the separation of genetic and epigenetic components of variation (i.e. standing genetic diversity) within or between species. As natural selection can act only in the presence of heritable variation (which is primarily genetic, although see recent reviews on transgenerational epigenetic inheritance, e.g. Burggren, 2015; Lind and Spagopoulou, 2018), any phenotypic variation observed under identical conditions can hint at the capacity of a species to evolve. Recent studies confirm the power of a common garden approach for distinguishing environmental from genomic contributors to functional and physiological phenotypes in a wide range of species. In low-bush blueberry, for example, this approach revealed a strong role for microenvironmental variation over genetic variation in the timing of leafing-out (McDonough MacKenzie et al., 2018). In contrast, the same approach suggested a primary role for genetic variation among subspecies in white-crowned sparrows' responses to photoperiod

(Ramenofsky et al., 2017) and in oak species' response to freezing (Cavender-Bares and Ramírez-Valiente, 2017). Insights into how genotypes manifest across environments may also be gained from a modified common garden experimental approach, by replicating the same experiments across different environments (reviewed in de Villemereuil et al., 2016).

Choosing environmental conditions to assay

Recent advances give increasing attention to the impact of environmental factors in shaping traits. Depending on the trait of interest the impacts of an environmental stressor can differ vastly if the exposure is acute or chronic (reviewed in Schulte et al., 2011), or if it occurs in a particularly sensitive stage of development (e.g. Levy et al., 2015). The spatial scale of environmental variation is also an important consideration for determining conditions that affect phenotype. For example, most species do not experience temperature at the level of weather stations, so consideration of microclimates is important (reviewed in Sears et al., 2011). Similarly, focus on environmental means can obscure important effects of variability (Dillon and Woods, 2016; Dowd et al., 2015). Therefore, when selecting habitats for a comparative study or designing common garden experiments in controlled environments, it is useful to have advanced knowledge of the relative importance of the timing, scale and variability of environmental conditions. Similarly to early studies in comparative physiology and other fields, the easiest approach often is to oversimplify at first, beginning with differences that manifest under extremes of environment, then progress towards discovery of finer-grain relationships between a continuous phenotype and the environment. Here, we review recent work in the field to summarize a workflow that moves stepwise from discovering epigenetic states that are correlated to a trait of interest to interrogating their possible mechanistic connections.

Associating epigenetic features to phenotype states

Once the range of physiological phenotypes has been recorded across relevant environmental conditions, the search for epigenetic modulators of phenotype becomes largely analogous to the search for relevant genetic variation and genome-wide association studies (GWAS) become a useful approach for generating lists of trait-correlated candidate genetic variants to screen for causal impacts in follow-up assays (Fig. 1B). In their earliest form, GWAS required pairing whole-genome sequences with binary phenotypes for each individual, then searching for genomic variants whose frequencies differed significantly between 'case' and 'control' groups.

This second step – comparison of states across phenotype classes – has more recently been adapted to account for quantitative and continuous phenotypes (Schmid and Bennewitz, 2017; Simons et al., 2018). Computationally, 'case' and 'control' groups are easily expanded into more than two categories, such as ancestry groups to understand human disease biomarkers (e.g. Sun et al., 2021), with important considerations for statistical power across multi-group analyses and controlling for false discovery rate in multiple tests (Brzyski et al., 2017). Continuous phenotypes require linear regression analysis to associate a quantified trait metric with genomic data, which can be computationally intensive in large datasets (Haller et al., 2015; Wu et al., 2021). Analytical approaches also exist to evaluate GWAS data against multiple inter-related phenotypes (Bradbury et al., 2007; Sha et al., 2018; Wang and Zhang, 2021).

Comparison of states across phenotype classes can also present challenges for epigenomic studies. Although a single genome can be taken to represent an individual organism across any set of conditions, epigenomic information associated with an organismal phenotype that is responsive to the environment cannot be defined by any one data set. Researchers seeking to identity epigenomic correlates of complex, continuous physiological phenotypes therefore must identify a set of discrete environment conditions to use as an initial proxy for an entire range, decide which tissue types to assay, and fund collection of the potentially large number of datasets required to characterize epigenomic states over a wide range of conditions.

Epigenome-wide association studies (EWAS; Lappalainen and Greally, 2017; not to be confused with 'environment-wide association studies', a common tool in disease epidemiology) partially address these challenges. EWAS, closely modeled on traditional GWAS, typically implements pairwise comparisons of genome-wide DNA methylation data from individuals subjected to 'baseline' as compared to altered states. Initial applications of EWAS typically simplified data collection by focusing on a single tissue type. Despite the potential information loss inherent to discretizing or even binarizing continuous phenotypes and limiting analyses to how just a subset of tissues may respond to a given environmental stimulus, EWAS have been successful in uncovering DNA methylation changes associated with organismal responses to diverse environmental conditions (reviewed for animals in Hu and Barrett, 2017; Navarro-Martín et al., 2020), ranging from hypoxia at altitude (Nanduri et al., 2017) to ocean acidification (Putnam et al., 2016).

Extending EWAS from methylation to other epigenetic marks

EWAS have been primarily applied to identify phenotype-associated changes in DNA methylation states (see above); however, they are readily extensible to any epigenetic mark or combination of marks that can be assayed across individuals exposed to a range of environment conditions. For a review of epigenetic markers and relevant detection methods, see Cazaly et al. (2019) and Table S1. Both chromatin immunoprecipitation and DNA sequencing (ChIP-seq) and assay of transposase-accessible chromatin (ATAC-seq) provide strategies for identifying genomic regions for which epigenetic states differ across two or more environmental conditions, especially when it is not clear initially which epigenetic mark to study. ChIP-seq identifies genomic sequences bound to and likely regulated by known transcription factors. This approach requires antibodies to pull down crosslinked protein and DNA. Sequencing this DNA subset identifies genomic regions bound to regulatory factors. This approach can quantify gene regulation occurring by a broader set of epigenetic mechanisms (i.e. transcription factors), but its requirement for ChIP-seq validated antibodies presents limitations; while commercially available antibodies are increasingly validated for ChIP assays, like most antibodies, epitope binding is only predicted and not validated for non-model species.

ATAC-seq, like ChiP-seq, can identify genomic regions that may be transcriptionally active in a given tissue, while escaping the requirement for species-specific antibodies. ATAC-seq identifies regions of open chromatin by applying a transposase that inserts a primer-binding site, which is targeted for amplification during library preparation. Regions whose open/closed states differ across phenotype states and conditions can be followed up with targeted approaches that assay specific classes of epigenomic marks and directly assess potential functional impacts (e.g. Bysani et al., 2019). Moreover, because it does not require use of antibodies or other species-specific resources, ATAC-seq is readily extensible to

diverse species, including those that are exceptionally small for which available cell count is limited (Kissane et al., 2021).

Extending from single- to multi-tissue comparisons

Although responses of individual organs can reveal key molecular features of complex physiological phenotypes, collection and integration of data across tissue types (Husby, 2020; Torson et al., 2020) can provide deeper, organism-level inferences. A growing set of databases (e.g. GTEx, TiGER) offer regulatory information from multiple organs of a variety of model organisms. Multi-tissue proteomic and transcriptomic screens in hibernating ground squirrels indicate both tissue-specific molecular function (e.g. supporting ATP availability in skeletal muscle to fuel shivering during rewarming) and broader themes (e.g. suppression of wound healing and immune function during hibernation, seasonal fasting) that help reveal the molecular underpinnings of the hibernator phenotype (Grabek et al., 2015). In the African savannah butterfly, seasonal transcription responses have a systemic, whole-body component, even though the greatest changes are associated with specific tissues (Oostra et al., 2018).

Distinguishing cause from correlation

Although some epigenetic features associated with a phenotype may be causal, others can be merely incidental to new environmental conditions (Rey et al., 2020; van Oers et al., 2020). Hypothesis-neutral '-seq' approaches, designed to identify associations with epigenetic marks, cannot distinguish between them to establish cause and effect (Hu and Barrett, 2017; Torson et al., 2020; van Oers et al., 2020). Similarly, levels of mRNA or protein do not reveal whether expression is causal to, or simply correlated to, phenotypic responses (Torson et al., 2020). Moreover, as transcriptomic data do not correlate well with the proteome (Edfors et al., 2016; Gygi et al., 1999; Nie et al., 2007), it is clear that different levels of regulatory mechanisms of gene expression can control the manifestation of phenotype. Many recent studies have shown that DNA methylation and histone/chromatin modifications correlate with physiological adjustments and fitness (Rey et al., 2020; Sheldon et al., 2020). Such studies identify candidate genes of interest but cannot discern causality.

Mechanisms that may underlie correlation without causation

Three-dimensional genomic architecture is one of several mechanisms that can produce potential correlations of epigenetic state to phenotype in the absence of causative relationships. Genomic architecture is an essential component of epigenetic regulation as it determines not just chromosomal accessibility for transcriptional machinery, but also binding efficiency of transcription factors and access to enhancer regions (Fig. 2A). Spatial proximity of genome regions resulting from three-dimensional architecture can create correlated epigenetic states, with genome regions that are proximate tending to have similar DNase sensitivity, methylation, histone modifications and gene expression levels, even when the proximate regions are on different chromosomes (Khrameeva et al., 2012). Correlation of expression among nearby genes is even more obvious at smaller spatial scales. Open chromatin positions are key locations for regulatory elements that drive epigenetic modification of target gene expression (Klemm et al., 2019), but chromatin accessibility in these regions may also increase the expression of adjacent genes. Environmental perturbations may also affect gene expression through several mechanisms in parallel. For example, hypoxia is a potent environmental signal that affects metabolism by stimulating glycolysis for ATP generation, resulting in the accumulation of lactate. Cellular metabolites are well-known histone modulators, and lactate is no exception. Lactate inhibits histone deacetylase activity (Latham et al., 2012), but also directly modifies histone lysine residues by lactylation (Zhang et al., 2019). Zhang et al. (2019) reported a correlation between H3K lactylation and increased expression of *Arg1* and *Vegfa* in M1 macrophages. However, hypoxia-inducible factor (HIF) signaling may also be a driver for increased *Arg1* and *Vegfa* expression, operating under similar environmental conditions. The authors provide evidence for H3K18 lactylation as the epigenetic cause of phenotypic change in macrophage polarization by demonstrating both a timing difference between induction of histone lactylation versus HIF accumulation (only the former coincided with *Arg1* and *Vegfa* expression changes), as well as a lack of detectable HIF binding to the promoters of these genes of interest (Zhang et al., 2019).

Testing for causal relationships

Distinguishing epigenetic causation from correlation requires experimental designs that identify epigenetic states essential to a given phenotype (Fig. 1C). The choice of approach is dependent on the tools available for the species of interest. Species with extensive molecular toolkits are easily manipulated genetically; those that lack well-established genomic resources may still be amenable to experimental manipulation. In some cases, established model organisms can serve as useful alternatives to direct study in the species of interest. To date, studies in models such as mouse, flies and some fishes have identified causal epigenetic mechanisms that respond to environmental cues and shape physiological phenotypes (e.g. Romney et al., 2018; Turecki and Meaney, 2016; Xu et al., 2014).

Extensive investigation of epigenetic components of human disease has been powered by the availability of sophisticated tools for identifying causative epigenetic events. For instance, type 2 diabetes is under epigenetic influence and some of the underlying mechanisms have been elegantly demonstrated in rats (Bansal and Simmons, 2018). Experiments using the intrauterine growth restriction model (IUGR) revealed that expression of the pancreatic homeobox domain 1 (*Pdx1*) is permanently reduced in adult beta cells. The molecular processes are complex. mSin3A-HDAC binding affects multiple processes associated with acetylation and methylation in the fetus and neonate. Accumulation of resultant H3K9 dimethylation marks eventually leads to recruitment of DNA methyltransferase 3A, resulting in the permanent reduction in *Pdx1* expression into adulthood. Elucidating this mechanism was possible only via very thorough sampling in a rodent model and using a well-established model like IUGR. Given the successive processes of deacetylation, reduced trimethylation by H3K4m, and recruitment of H3K9 to promote dimethylation, sampling of fewer time points would have failed to capture the mechanism, highlighting potential challenges for implementing similar studies in species that lack well-developed experimental systems.

Attempts to identify causative epigenetic mechanisms in one species and then extrapolate to another must be implemented with caution. In addition to the challenges of determining whether species-specific responses are confounded by genome annotation incompleteness or the challenges of identifying true, cross-species 1:1 orthologs of multi-copy genes, epigenetic modifications and their impacts can be clade specific, or even species specific. For example, Fellous and Shama (2019) evaluated epigenetic

Journal of Experimental Biology (2022) 225, jeb243411. doi:10.1242/jeb.243411

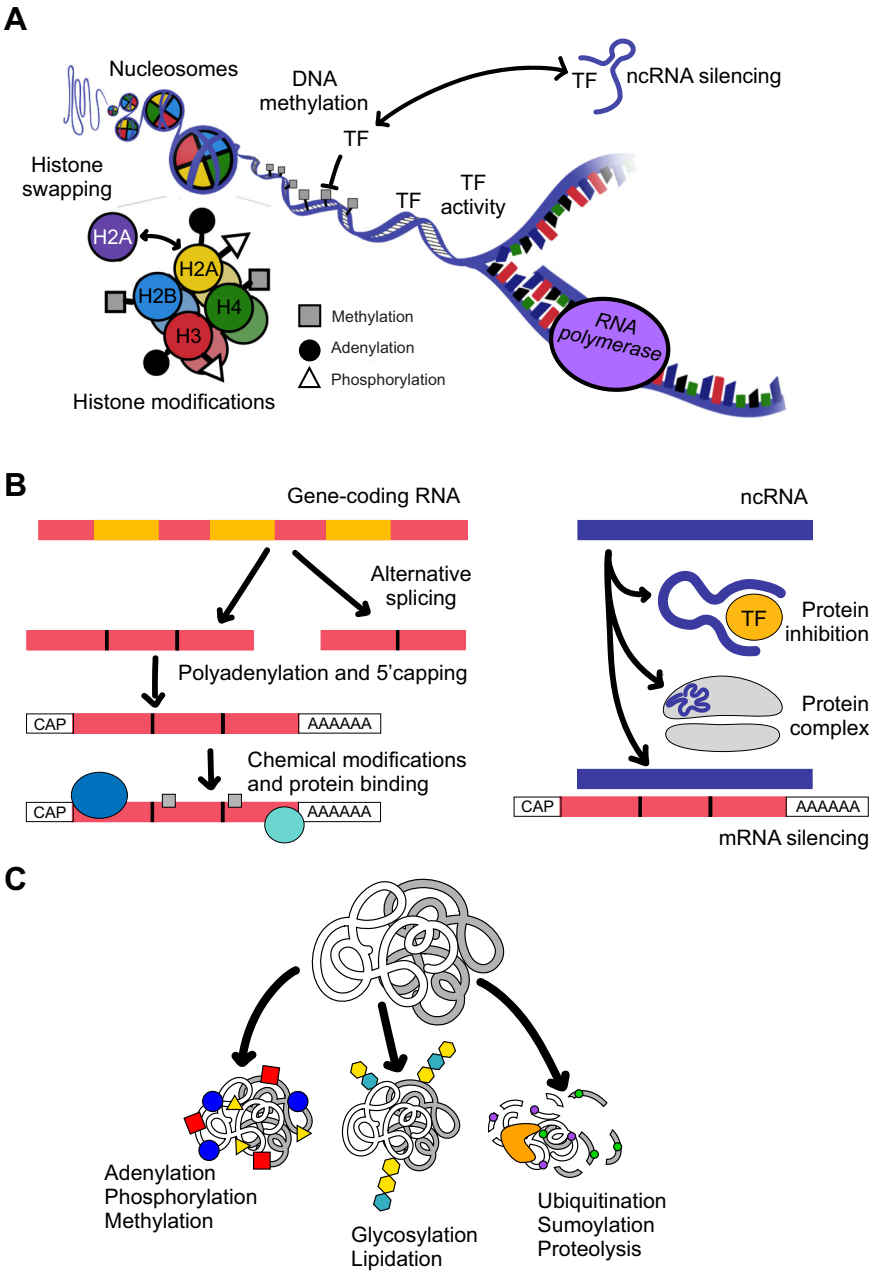

Fig. 2. Overview of epigenomic mechanisms that can impact gene expression. (A) Regulating gene expression by targeting transcription: regulatory factors that control genomic accessibility and gene product production, including DNA methylation, histone modifications, histone variants and the regulation of transcription factor (TF) binding. These regulatory mechanisms can inhibit or enhance transcription initiation. (B) The fate of RNA regulates gene expression: regulatory mechanisms also include any RNA modifications that alter function, including alternative splicing, polyadenylation and 5′ capping, export from the nucleus (not depicted), chemical modifications and protein binding. These mechanisms can inhibit or enhance transcription initiation via protein binding or they can inhibit or enhance translation. (C) Post-translational modifications impact gene expression: regulatory mechanisms also include any protein modifications that alter function, including adenylation, glycosylation, lipidation, methylation, phosphorylation, sumoylation and ubiquitination. These modifications may result in proteolysis. Other modifications not depicted here include amidation, hydroxylation and ubiquitylation.

pathways in sticklebacks and identified teleost-specific epigenetic states.

In species that so far lack well-developed experimental systems, statistical approaches that draw information from a range of epigenetic data types may be useful. For example, the Decorate differential epigenetic correlation test exploits cluster analyses and gene location information to better leverage sets of DNA methylation, ATAC-seq, and histone modification ChIP-seq (Hoffman et al., 2020) data and integrate information across them (Cazaly et al., 2019).

Despite constraints arising from limited genomics resources for ‘non-model’ organisms, manipulating gene function can enable assessment of causative links between gene and phenotype on a gene-by-gene basis (e.g. Marutani et al., 2021 performed adeno-associated virus short hairpin RNA gene knockdown in adult 13-lined ground squirrels), as well as via wide-scale RNA interference genetic screening (e.g. Kampmann et al., 2015 in mammalian cells). Genome-wide analyses of high-altitude Tibetan horses found that mutations in *EPAS1* (which encodes HIF-2α) cause an increase in HIF-2α protein abundance when mutagenesis was performed in a human alveolar epithelial cell line (Liu et al., 2019). These sequence-level changes experimentally resulted in protein overexpression, which in turn increased the capacity of alveolar cells for HIF signaling. Gain- and loss-of-function studies such as these allow researchers to directly pinpoint genes with functional ties to phenotype. Epigenetic pathways themselves can also be experimentally modified (activated or repressed) to provide evidence of epigenetic modulators of physiology. In the blind cavefish, *Astyanax mexicanus*, for example, eye-specific genes are methylated during development, repressing their transcription and leading to eye degeneration (Gore et al., 2018). Eye regression is developmentally preceded by decreased expression of eye-specific crystallins (Gore et al., 2018). The hypothesized mechanism of epigenetic control for eye degeneration is DNA methyltransferase

Dnmt3bb.1; this gene maps to an eye-specific quantitative trait locus in the cavefish genome (McGaugh et al., 2014) and gene expression is 1.5-fold elevated in the blind cave morphs compared to surface morphs (Gore et al., 2018). *Dnmt3bb.1* null mutant zebrafish develop enlarged eyes and retinal hyperplasia, providing functional evidence for a mechanistic link between epigenetic transcriptional repression leading to eye degeneration (Gore et al., 2018).

Loss-of-function experimentation requires careful attention to developmental timing, such as the impact of silencing genes during development versus in adulthood. Work in model systems has often revealed only poor correlations between phenotypic outcomes with these two approaches (Kok et al., 2015; Rossi et al., 2015). At least partially, this is a likely outcome from removing or disabling an entire gene sequence, which may contain regulatory elements acting elsewhere in the genome, versus RNA interference approaches, which act between transcription and translation (Fig. 2B). Importantly, these intricacies are much less likely to be understood in non-model organisms, leading to potentially confounding factors in experiments seeking to manipulate gene function to evaluate epigenetic mechanisms.

Linking epigenetic change to evolution

Determining the evolutionary history of a response becomes especially interesting once a given genetic or epigenetic state has been determined to be not only associated with but directly causal to a physiological phenotype (Fig. 1D). This has been recently reviewed (e.g. Ashe et al., 2021; Stajic and Jansen, 2021) and remains especially important for comparative physiology, which relies heavily on evolutionary context. For epigenetic mechanisms determined to be directly causal to phenotype, phylogenetic and evolutionary analyses could determine whether the trait emerged as a direct result of the presence or relaxation of a selective pressure. If epigenetic states are themselves heritable (Ashe et al., 2021; Stajic and Jansen, 2021), it is important to consider how to distinguish whether such heritable states implicate adaptation.

Confirming adaptation

'Adaptation' is a term widely used in comparative biology. It can have many meanings, most tied either to phenotypes that differ between species, or encompassing the evolutionary history of physiological specializations. Here, we focus on evolutionary aspects, defining physiological specializations as bona fide 'adaptations' only if several types of evidence are available: (1) presence of evolutionary selection directly for the trait of interest; (2) organismal fitness benefit; and (3) genetic or epigenetic component mechanisms. Challenges in collecting datasets sufficient to assess all three criteria remains a problem. Epigenetic changes that impact phenotype can induce responses ranging from favorable, to neutral or even maladaptive with respect to fitness (Ghalambor et al., 2007); we consider only those that increase fitness to be adaptive. For example, blind cavefish morphs, which live in very low-light environments, lack neural tissue that is present and supports vision in conspecifics living at the surface. Fish lacking relevant neural tissue receive a demonstrable fitness benefit in low-light environments (Moran et al., 2014) fulfilling criteria for defining loss of this tissue as a relevant adaptation.

In assessing the epigenetic state that underlies a phenotype as a possible adaptation, it is essential to acknowledge that not all associated genetic or epigenetic differences are necessarily under direct selective pressure. Indeed, the assumption that a given state is independent of a directly relevant selection pressure is an invaluable null hypothesis, helpful for distinguishing adaptations from exaptations. Exaptations emerge when existing traits, including those that arose through positive selection adaptations, assume an additional, novel function where none had previously existed. The new function afforded by an exaptation may benefit the organism, but is not itself a direct result of the initial selective pressure.

Light generation in fireflies is a classic example of a trait that, while now associated with high fitness, may not have arisen as an adaptation per se. Fireflies possess luciferin, which acts as a substrate for oxidation and associated release of light energy. This molecule likely evolved as an antioxidant defense system in the firefly respiratory tracheal system (Dubuisson et al., 2004). Only later was the light-generating capacity arising from substrate oxidation co-opted into its recognized roles in predator avoidance, foraging advantage and intra-specific communication.

Numerous gains and losses of the capacity for light generation are evident in the cladogenesis of fireflies; a greater tendency towards loss suggests ongoing purifying selection as relevant for maintenance of luminescence (Martin et al., 2017). This highlights another feature of exaptation: the observation that purifying selection on a given function is currently contributing to its maintenance does not, itself, indicate a role for that selective pressure in the evolutionary origins of that function.

Coevolution can obfuscate epigenetic processes. In the 1940s and 1950s, Waddington popularized the terms 'epigenetics' and 'canalization', arguing that most mutations will either be neutral or deleterious. Canalization would predict an active process of suppressing the manifestation of nearly all phenotypes arising through novel mutations (Waddington, 1942). But what if a trait that is not itself adaptive, co-evolves with other, more adaptive traits? Could there be a time that neutral or deleterious phenotypes might emerge as collateral damage of selection on high-fitness innovations? Indeed, a famous Waddington experiment may involve such a trait. When *Drosophila melanogaster* pupae were exposed to heat shock, Waddington found expression of a 'crossveinless' wing phenotype (Waddington, 1942, 1953). After 20 generations of selection for the crossveinless phenotype, it appeared in >90% of flies even without heat shock. It is hard to imagine how lack of crossveins in the wings might allow a fly to better withstand heat shock. Indeed, we now know the crossveinless phenotype is the result of an alteration of a critical process in limb formation. Bone morphogenetic protein (BMP) signaling mechanisms along with an interaction from heat shock protein 90 (HSP90) underlie much of this regulation (e.g. Marcus, 2001; O'Connor et al., 2006). When animals experience sustained stress, HSP90 is diverted from its normal cellular roles. The crossveinless phenotype is now allowed to be expressed (see Siegal and Bergman, 2002 for review) and recent data suggest that it may decrease fitness (Nair and Dearden, 2016). Importantly, HSP90 has a clearly defined role in maintenance of gene silencing in the absence of stress (Wong and Houry, 2006). This means that during sustained stress, when HSP90 is diverted to increased chaperone roles, organisms may be 'rolling the dice' by unsilencing mutations that had been subject to canalization. While some of these unmasked mutations may lead to better survivorship, others might be totally unrelated to fitness.

Adaptive potential and response to environmental change

At the population level, both genetics and epigenetics define adaptive potential. Epigenetic processes can operate on much faster timescales compared to genetic differences whose persistence over generations is modulated by selective pressures. Specifically, populations may rapidly become epigenetically differentiated owing to microenvironment-induced modifications (Fig. 2C),

even if they remain genetically similar via ongoing gene flow (Rey et al., 2020), offering the potential for populations to persist even if relevant genetic variation is rare or absent from the population – so long as epigenetic responses support endurance in local conditions (Burggren, 2018). Common garden experiments are proving a powerful approach to identify species whose comparatively low capacity for rapid phenotypic change suggests that they will be severely imperiled by the unprecedented conditions of climate change (Kilvitis et al., 2017; van der Wiel and Bintanja, 2021). Already, common garden experiments have helped to distinguish between species whose phenological variation is principally genetic, suggesting that directional selection could be the primary substrate for response to climate change, from those whose phenology remains extensively malleable by microenvironment, suggesting capacity for rapid epigenetic response (De Kort et al., 2014; Liu and El-Kassaby, 2019; Xiankui and Chuankuan, 2018). Variants on the common garden framework specifically offer the opportunity to quantify 'epigenetic potential', defined by Kilvitis et al. (2017) as high phenotypic plasticity via environment-induced epigenetic modifications, may be poised to change much faster than natural selection would permit (Burggren, 2016). Even so, the capacity for rapid epigenetic response to climate change is not a panacea. If new conditions exceed the boundaries of species' evolved reaction norms, their responses will be random with respect to fitness, with potential for disastrous outcomes (Consuegra and Rodríguez López, 2016; McGuigan et al., 2021).

Finally, a cautionary note: researchers exploring the evolutionary outcomes of epigenetic responses to environmental change must take care to distinguish the fitness effects of immediate, individual-level epigenetic responses from the effects of potentially heritable epigenetic change (Burggren, 2015). For example, if members of a species possess the capacity for high phenotypic plasticity via environment-induced epigenetic modifications, and these changes generally support survival in novel conditions, then the capacity for epigenetic response can be said to be beneficial even if relevant epigenetic states are not inherited through the germline. Emerging examples do suggest that some environment-induced epigenetic changes can be transmitted across generations, which has been recently reviewed (Anastasiadi et al., 2021).

Conclusions

In many respects, integration across sub-disciplines will define the future of biology (Holford and Normark, 2021). Physiological phenotypes, in particular, result from many interacting genomic and epigenomic processes, and insights from all relevant fields will be essential to understand their mechanistic basis and evolutionary history. Although comparative physiology increasingly acknowledges the continuous nature of phenotype, establishing physiological mechanisms in epigenetic and evolutionary contexts requires intersections with computational approaches that can be data intensive and are often simplified to strip away the complexities of phenotypes. In epigenetic analyses this may be simplified into pairwise comparisons, and in evolutionary analyses, to trait gain or loss. Integration will be especially important across disciplines, as we move forward in understanding complex biological themes. Inter-disciplinary discussions between genome biologists, comparative physiologists and computational scientists will enable selection of species, experimental designs, sampling timepoints and data analysis that leverage the complexities of physiology.

Integration is also important across physiological scales. Our understanding of mechanisms underlying a phenotype obtained from the study of a single regulatory element might change dramatically when combined with other data. By integrating profiles at the genomic, epigenetic, epitranscriptomic and proteomic processes, we come closer to isolating the driving factors of physiology and phenotype. Following a bioinformatic approach of integrating diverse levels of molecular regulation, testing directional hypotheses with studies that target specific mechanisms will clearly demonstrate how phenotype is defined by the organism. This approach is more consistent with the view of physiology as an emergent property.

Competing interests

The authors declare no competing or financial interests.

Funding

This work was funded by the National Science Foundation (grants no. 1656063, no. 2022046, and no. 2022012).

References

Anastasiadi, D., Venney, C. J., Bernatchez, L. and Wellenreuther, M. (2021). Epigenetic inheritance and reproductive mode in plants and animals. *Trends Ecol. Evol.* **36**, 1124-1140. doi:10.1016/j.tree.2021.08.006

Ashe, A., Colot, V. and Oldroyd, B. P. (2021). *How Does Epigenetics Influence the Course of Evolution?*, Vol. 376, p. 20200111. The Royal Society.

Bansal, A. and Simmons, R. A. (2018). Epigenetics and developmental origins of diabetes: correlation or causation? *Am. J. Physiol. Endocrinol. Metab.* **315**, E15-E28. doi:10.1152/ajpendo.00424.2017

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y. and Buckler, E. S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* **23**, 2633-2635. doi:10.1093/bioinformatics/btm308

Brzyski, D., Peterson, C. B., Sobczyk, P., Candès, E. J., Bogdan, M. and Sabatti, C. (2017). Controlling the rate of GWAS false discoveries. *Genetics* **205**, 61-75. doi:10.1534/genetics.116.193987

Burggren, W. W. (2015). Dynamics of epigenetic phenomena: intergenerational and intragenerational phenotype 'washout'. *J. Exp. Biol.* **218**, 80-87. doi:10.1242/jeb.107318

Burggren, W. (2016). Epigenetic inheritance and its role in evolutionary biology: re-evaluation and new perspectives. *Biology* **5**, 24. doi:10.3390/biology5020024

Burggren, W. (2018). Developmental phenotypic plasticity helps bridge stochastic weather events associated with climate change. *J. Exp. Biol.* **221**, jeb161984. doi:10.1242/jeb.161984

Bysani, M., Agren, R., Davegårdh, C., Volkov, P., Rönn, T., Unneberg, P., Bacos, K. and Ling, C. (2019). ATAC-seq reveals alterations in open chromatin in pancreatic islets from subjects with type 2 diabetes. *Sci. Rep.* **9**, 1-12. doi:10.1038/s41598-019-44076-8

Cavender-Bares, J. and Ramírez-Valiente, J. A. (2017). Physiological evidence from common garden experiments for local adaptation and adaptive plasticity to climate in American live oaks (*Quercus* Section *Virentes*): implications for conservation under global change. In [Tree Physiology] *Oaks Physiological Ecology. Exploring the Functional Diversity of Genus Quercus L.* (ed. E. Gil-Pelegrín, J. Peguero-Pina and D. Sancho-Knapik D), vol 7. pp. 107-135. Springer. doi:10.1007/978-3-319-69099-5_4

Cazaly, E., Saad, J., Wang, W., Heckman, C., Ollikainen, M. and Tang, J. (2019). Making sense of the epigenome using data integration approaches. *Front. Pharmacol.* **10**, 126. doi:10.3389/fphar.2019.00126

Consuegra, S. and Rodríguez López, C. M. (2016). Epigenetic-induced alterations in sex-ratios in response to climate change: an epigenetic trap? *BioEssays* **38**, 950-958. doi:10.1002/bies.201600058

De Kort, H., Vandepitte, K., Bruun, H. H., Closset-Kopp, D., Honnay, O. and Mergeay, J. (2014). Landscape genomics and a common garden trial reveal adaptive differentiation to temperature across Europe in the tree species Alnus glutinosa. *Mol. Ecol.* **23**, 4709-4721. doi:10.1111/mec.12813

de Villemereuil, P., Gaggiotti, O. E., Mouterde, M. and Till-Bottraud, I. (2016). Common garden experiments in the genomic era: new perspectives and opportunities. *Heredity* **116**, 249-254. doi:10.1038/hdy.2015.93

Dillon, M. E. and Woods, H. A. (2016). Introduction to the symposium: beyond the mean: biological impacts of changing patterns of temperature variation. *Integr. Comp. Biol.* **56**, 11-13. doi:10.1093/icb/icw020

Dowd, W. W., King, F. A. and Denny, M. W. (2015). Thermal variation, thermal extremes and the physiological performance of individuals. *J. Exp. Biol.* **218**, 1956-1967. doi:10.1242/jeb.114926

Dubuisson, M., Marchand, C. and Rees, J.-F. (2004). Firefly luciferin as antioxidant and light emitter: the evolution of insect bioluminescence. *Luminescence* **19**, 339-344. doi:10.1002/bio.789

Edfors, F., Danielsson, F., Hallström, B. M., Käll, L., Lundberg, E., Pontén, F., Forsström, B. and Uhlén, M. (2016). Gene-specific correlation of RNA and protein levels in human cells and tissues. *Mol. Syst. Biol.* **12**, 883. doi:10.15252/msb.20167144

Fellous, A. and Shama, L. N. S. (2019). Genome survey of chromatin-modifying enzymes in threespine stickleback: a crucial epigenetic toolkit for Adaptation? *Front. Mar. Sci.* **6**, 721. doi:10.3389/fmars.2019.00721

Ghalambor, C. K., McKay, J. K., Carroll, S. P. and Reznick, D. N. (2007). Adaptive versus non–adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* **21**, 394-407. doi:10.1111/j.1365-2435.2007.01283.x

Gore, A. V., Tomins, K. A., Iben, J., Ma, L., Castranova, D., Davis, A. E., Parkhurst, A., Jeffery, W. R. and Weinstein, B. M. (2018). An epigenetic mechanism for cavefish eye degeneration. *Nat. Ecol. Evol.* **2**, 1155-1160. doi:10.1038/s41559-018-0569-4

Grabek, K. R., Martin, S. L. and Hindle, A. G. (2015). Proteomics approaches shed new light on hibernation physiology. *J. Comp. Physiol. B* **185**, 607-627. doi:10.1007/s00360-015-0905-9

Gygi, S. P., Rochon, Y., Franza, B. R. and Aebersold, R. (1999). Correlation between protein and mRNA abundance in yeast. *Mol. Cell. Biol.* **19**, 1720-1730. doi:10.1128/MCB.19.3.1720

Haller, T., Kals, M., Esko, T., Mägi, R. and Fischer, K. (2015). RegScan: a GWAS tool for quick estimation of allele effects on continuous traits and their combinations. *Brief. Bioinform.* **16**, 39-44. doi:10.1093/bib/bbt066

Hiller, M., Schaar, B. T., Indjeian, V. B., Kingsley, D. M., Hagey, L. R. and Bejerano, G. (2012). A "forward genomics" approach links genotype to phenotype using independent phenotypic losses among related species. *Cell Rep.* **2**, 817-823. doi:10.1016/j.celrep.2012.08.032

Hoffman, G. E., Bendl, J., Girdhar, K. and Roussos, P. (2020). decorate: differential epigenetic correlation test. *Bioinformatics* **36**, 2856-2861. doi:10.1093/bioinformatics/btaa067

Holford, M. and Normark, B. B. (2021). Integrating the life sciences to jumpstart the next decade of discovery. *Integr. Comp. Biol.* **61**, 1984-1990. doi:10.1093/icb/icab194

Hu, J. and Barrett, R. D. H. (2017). Epigenetics in natural animal populations. *J. Evol. Biol.* **30**, 1612-1632. doi:10.1111/jeb.13130

Husby, A. (2020). On the use of blood samples for measuring DNA methylation in ecological epigenetic studies. *Integr. Comp. Biol.* **60**, 1558-1566. doi:10.1093/icb/icaa123

Irschick, D. J. and Higham, T. E. (2016). *Animal Athletes: An Ecological and Evolutionary Approach*. Oxford University Press.

Kampmann, M., Horlbeck, M. A., Chen, Y., Tsai, J. C., Bassik, M. C., Gilbert, L. A., Villalta, J. E., Kwon, S. C., Chang, H. and Kim, V. N. (2015). Next-generation libraries for robust RNA interference-based genome-wide screens. *Proc. Natl Acad. Sci. USA* **112**, E3384-E3391. doi:10.1073/pnas.1508821112

Khrameeva, E. E., Mironov, A. A., Fedonin, G. G., Khaitovich, P. and Gelfand, M. S. (2012). Spatial proximity and similarity of the epigenetic state of genome domains. *PLoS ONE* **7**, e33947. doi:10.1371/journal.pone.0033947

Kilvitis, H. J., Hanson, H., Schrey, A. W. and Martin, L. B. (2017). Epigenetic potential as a mechanism of phenotypic plasticity in vertebrate range expansions. *Integr. Comp. Biol.* **57**, 385-395. doi:10.1093/icb/icx082

Kissane, S., Dhandapani, V. and Orsini, L. (2021). Protocol for assay of transposase accessible chromatin sequencing in non-model species. *STAR Protoc.* **2**, 100341. doi:10.1016/j.xpro.2021.100341

Klemm, S. L., Shipony, Z. and Greenleaf, W. J. (2019). Chromatin accessibility and the regulatory epigenome. *Nat. Rev. Genet.* **20**, 207-220. doi:10.1038/s41576-018-0089-8

Kok, F. O., Shin, M., Ni, C.-W., Gupta, A., Grosse, A. S., van Impel, A., Kirchmaier, B. C., Peterson-Maduro, J., Kourkoulis, G., Male, I. et al. (2015). Reverse genetic screening reveals poor correlation between morpholino-induced and mutant phenotypes in zebrafish. *Dev. Cell* **32**, 97-108. doi:10.1016/j.devcel.2014.11.018

Kolodny, O. and Schulenburg, H. (2020). Microbiome-mediated plasticity directs host evolution along several distinct time scales. *Philos. Trans. R. Soc. B* **375**, 20190589. doi:10.1098/rstb.2019.0589

Kooyman, G. L. (1966). Maximum diving capacities of the Weddell seal, *Leptonychotes weddeili*. *Science* **151**, 1553-1554. doi:10.1126/science.151.3717.1553

Lane, J. E., Czenze, Z. J., Findlay-Robinson, R. and Bayne, E. (2019). Phenotypic plasticity and local adaptation in a wild hibernator evaluated through reciprocal translocation. *Am. Nat.* **194**, 516-528. doi:10.1086/702313

Lappalainen, T. and Greally, J. M. (2017). Associating cellular epigenetic models with human phenotypes. *Nat. Rev. Genet.* **18**, 441-451. doi:10.1038/nrg.2017.32

Latham, T., Mackay, L., Sproul, D., Karim, M., Culley, J., Harrison, D. J., Hayward, L., Langridge-Smith, P., Gilbert, N. and Ramsahoye, B. H. (2012). Lactate, a product of glycolytic metabolism, inhibits histone deacetylase activity and promotes changes in gene expression. *Nucleic Acids Res.* **40**, 4794-4803. doi:10.1093/nar/gks066

Levy, O., Buckley, L. B., Keitt, T. H., Smith, C. D., Boateng, K. O., Kumar, D. S. and Angilletta, M. J. (2015). Resolving the life cycle alters expected impacts of climate change. *Proc. R. Soc. B* **282**, 20150837. doi:10.1098/rspb.2015.0837

Lind, M. I. and Spagopoulou, F. (2018). *Evolutionary Consequences of Epigenetic Inheritance*, Vol. 121, pp. 205-209. Nature Publishing Group.

Liu, Y. and El-Kassaby, Y. A. (2019). Phenotypic plasticity of natural Populus trichocarpa populations in response to temporally environmental change in a common garden. *BMC Evol. Biol.* **19**, 231. doi:10.1186/s12862-019-1553-6

Liu, X., Zhang, Y., Li, Y., Pan, J., Wang, D., Chen, W., Zheng, Z., He, X., Zhao, Q., Pu, Y. et al. (2019). EPAS1 gain-of-function mutation contributes to high-altitude adaptation in Tibetan horses. *Mol. Biol. Evol.* **36**, 2591-2603. doi:10.1093/molbev/msz158

Marcus, J. M. (2001). The development and evolution of crossveins in insect wings. *J. Anat.* **199**, 211-216. doi:10.1046/j.1469-7580.2001.19910211.x

Martin, G. J., Branham, M. A., Whiting, M. F. and Bybee, S. M. (2017). Total evidence phylogeny and the evolution of adult bioluminescence in fireflies (Coleoptera: Lampyridae). *Mol. Phylogenet. Evol.* **107**, 564-575. doi:10.1016/j.ympev.2016.12.017

Marutani, E., Morita, M., Hirai, S., Kai, S., Grange, R. M. H., Miyazaki, Y., Nagashima, F., Traeger, L., Magliocca, A., Ida, T. et al. (2021). Sulfide catabolism ameliorates hypoxic brain injury. *Nat. Commun.* **12**, 1-19. doi:10.1038/s41467-021-23363-x

McDonough MacKenzie, C., Primack, R. B. and Miller-Rushing, A. J. (2018). Local environment, not local adaptation, drives leaf-out phenology in common gardens along an elevational gradient in Acadia National Park, Maine. *Am. J. Bot.* **105**, 986-995. doi:10.1002/ajb2.1108

McGaugh, S. E., Gross, J. B., Aken, B., Blin, M., Borowsky, R., Chalopin, D., Hinaux, H., Jeffery, W. R., Keene, A., Ma, L. et al. (2014). The cavefish genome reveals candidate genes for eye loss. *Nature Communications* **5**, 5307. doi:10.1038/ncomms6307

McGuigan, K., Hoffmann, A. A. and Sgrò, C. M. (2021). How is epigenetics predicted to contribute to climate change adaptation? What evidence do we need? *Philos. Trans. R. Soc. B* **376**, 20200119. doi:10.1098/rstb.2020.0119

Moran, D., Softley, R. and Warrant, E. J. (2014). Eyeless Mexican cavefish save energy by eliminating the circadian rhythm in metabolism. *PLoS ONE* **9**, e107877. doi:10.1371/journal.pone.0107877

Nair, A. and Dearden, P. (2016). A systems view of Waddington's genetic assimilation. *Int. J. Bioinform. Biol. Syst.* **1**, 10-17.

Nanduri, J., Semenza, G. L. and Prabhakar, N. R. (2017). Epigenetic changes by DNA methylation in chronic and intermittent hypoxia. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **313**, L1096-L1100. doi:10.1152/ajplung.00325.2017

Navarro-Martín, L., Martyniuk, C. J. and Mennigen, J. A. (2020). Comparative epigenetics in animal physiology: an emerging frontier. *Comp. Biochem. Physiol. D Genomics Proteomics* **36**, 100745. doi:10.1016/j.cbd.2020.100745

Nie, L., Wu, G., Culley, D. E., Scholten, J. C. M. and Zhang, W. (2007). Integrative analysis of transcriptomic and proteomic data: challenges, solutions and applications. *Crit. Rev. Biotechnol.* **27**, 63-75. doi:10.1080/07388550701334212

O'Connor, M. B., Umulis, D., Othmer, H. G. and Blair, S. S. (2006). Shaping BMP morphogen gradients in the Drosophila embryo and pupal wing. *Development* 133, 183-193. doi:10.1242/dev.02214.

Oostra, V., Saastamoinen, M., Zwaan, B. J. and Wheat, C. W. (2018). Strong phenotypic plasticity limits potential for evolutionary responses to climate change. *Nat. Commun.* **9**, 1-11. doi:10.1038/s41467-018-03384-9

Putnam, H. M., Davidson, J. M. and Gates, R. D. (2016). Ocean acidification influences host DNA methylation and phenotypic plasticity in environmentally susceptible corals. *Evol. Appl.* **9**, 1165-1178. doi:10.1111/eva.12408

Ramenofsky, M., Campion, A. W., Pérez, J. H., Krause, J. S. and Németh, Z. (2017). Behavioral and physiological traits of migrant and resident white-crowned sparrows: a common garden approach. *J. Exp. Biol.* **220**, 1330-1340. doi:10.1242/jeb.148171

Rey, O., Eizaguirre, C., Angers, B., Baltazar-Soares, M., Sagonas, K., Prunier, J. G. and Blanchet, S. (2020). Linking epigenetics and biological conservation: Towards a conservation epigenetics perspective. *Funct. Ecol.* **34**, 414-427. doi:10.1111/1365-2435.13429

Romney, A. L. T., Davis, E. M., Corona, M. M., Wagner, J. T. and Podrabsky, J. E. (2018). Temperature-dependent vitamin D signaling regulates developmental trajectory associated with diapause in an annual killifish. *Proc. Natl Acad. Sci. USA* **115**, 12763-12768. doi:10.1073/pnas.1804590115

Rosenblum, E. B., Römpler, H., Schöneberg, T. and Hoekstra, H. E. (2010). Molecular and functional basis of phenotypic convergence in white lizards at White Sands. *Proc. Natl Acad. Sci. USA* **107**, 2113-2117. doi:10.1073/pnas.0911042107

Rossi, A., Kontarakis, Z., Gerri, C., Nolte, H., Hölper, S., Krüger, M. and Stainier, D. Y. R. (2015). Genetic compensation induced by deleterious mutations but not gene knockdowns. *Nature* **524**, 230-233. doi:10.1038/nature14580

Schmid, M. and Bennewitz, J. (2017). Invited review: Genome-wide association analysis for quantitative traits in livestock–a selective review of statistical models and experimental designs. *Arch. Anim. Breed.* **60**, 335-346. doi:10.5194/aab-60-335-2017

Schmidt-Nielsen, K., Schmidt-Nielsen, B., Jarnum, S. A. and Houpt, T. R. (1956). Body temperature of the camel and its relation to water economy. *Am. J. Physiol. Legacy Content* **188**, 103-112. doi:10.1152/ajplegacy.1956.188.1.103

Schulte, P. M., Healy, T. M. and Fangue, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integr. Comp. Biol.* **51**, 691-702. doi:10.1093/icb/icr097

Sears, M. W., Raskin, E. and Angilletta, M. J., Jr. (2011). The world is not flat: defining relevant thermal landscapes in the context of climate change. *Integr. Comp. Biol.* **51**, 666-675. doi:10.1093/icb/icr111

Sha, Q., Wang, Z., Zhang, X. and Zhang, S. (2018). A clustering linear combination approach to jointly analyze multiple phenotypes for GWAS. *Bioinformatics* **35**, 1373-1379. doi:10.1093/bioinformatics/bty810

Sheldon, K. S., Padash, M., Carter, A. W. and Marshall, K. E. (2020). Different amplitudes of temperature fluctuation induce distinct transcriptomic and metabolomic responses in the dung beetle *Phanaeus vindex*. *J. Exp. Biol.* **223**, jeb233239. doi:10.1242/jeb.233239

Siegal, M. L. and Bergman, A. (2002). Waddington's canalization revisited: developmental stability and evolution. *Proc. Natl Acad. Sci. USA* **99**, 10528-10532. doi:10.1073/pnas.102303999

Simons, Y. B., Bullaughey, K., Hudson, R. R. and Sella, G. (2018). A population genetic interpretation of GWAS findings for human quantitative traits. *PLoS Biol.* **16**, e2002985. doi:10.1371/journal.pbio.2002985

Smith, S. D., Pennell, M. W., Dunn, C. W. and Edwards, S. V. (2020). Phylogenetics is the new genetics (for most of biodiversity). *Trends Ecol. Evol.* **35**, 415-425. doi:10.1016/j.tree.2020.01.005

Stajic, D. and Jansen, L. E. T. (2021). Empirical evidence for epigenetic inheritance driving evolutionary adaptation. *Philos. Trans. R. Soc. B* **376**, 20200121. doi:10.1098/rstb.2020.0121

Sun, Q., Graff, M., Rowland, B., Wen, J., Huang, L., Miller-Fleming, T. W., Haessler, J., Preuss, M. H., Chai, J.-F., Lee, M. P. et al. (2021). Analyses of biomarker traits in diverse UK biobank participants identify associations missed by European-centric analysis strategies. *J. Hum. Genet.* **67**, 87-93. doi:10.1038/s10038-021-00968-0

Torson, A. S., Dong, Y.-W. and Sinclair, B. J. (2020). Help, there are 'omics' in my comparative physiology! *J. Exp. Biol.* **223**, jeb191262. doi:10.1242/jeb.191262

Turecki, G. and Meaney, M. J. (2016). Effects of the social environment and stress on glucocorticoid receptor gene methylation: a systematic review. *Biol. Psychiatry* **79**, 87-96. doi:10.1016/j.biopsych.2014.11.022

van der Wiel, K. and Bintanja, R. (2021). Contribution of climatic changes in mean and variability to monthly temperature and precipitation extremes. *Commun. Earth Environ.* **2**, 1-11. doi:10.1038/s43247-020-00077-4

van Oers, K., Sepers, B., Sies, W., Gawehns, F., Verhoeven, K. J. F. and Laine, V. N. (2020). Epigenetics of animal personality: DNA methylation cannot explain the heritability of exploratory behavior in a songbird. *Integr. Comp. Biol.* **60**, 1517-1530. doi:10.1093/icb/icaa138

Voelkl, B., Altman, N. S., Forsman, A., Forstmeier, W., Gurevitch, J., Jaric, I., Karp, N. A., Kas, M. J., Schielzeth, H., Van de Casteele, T. et al. (2020). Reproducibility of animal research in light of biological variation. *Nat. Rev. Neurosci.* **21**, 384-393. doi:10.1038/s41583-020-0313-3

Waddington, C. H. (1942). Canalization of development and the inheritance of acquired characters. *Nature* **150**, 563-565. doi:10.1038/150563a0

Waddington, C. H. (1953). Genetic assimilation of an acquired character. *Evolution* **7**, 118-126. doi:10.1111/j.1558-5646.1953.tb00070.x

Waddington, C. H. (1956). Embryology, epigenetics and biogenetics. *Nature* **177**, 1241-1241. doi:10.1038/1771241a0

Wang, J. and Zhang, Z. (2021). GAPIT Version 3: boosting power and accuracy for genomic association and prediction. *Genomics Proteomics Bioinformatics*. doi:10.1016/j.gpb.2021.08.005

Winterová, B. and Gvoždík, L. (2021). Individual variation in seasonal acclimation by sympatric amphibians: a climate change perspective. *Funct. Ecol.* **35**, 117-126. doi:10.1111/1365-2435.13705

Wong, K. S. and Houry, W. A. (2006). Hsp90 at the crossroads of genetics and epigenetics. *Cell Res.* **16**, 742-749. doi:10.1038/sj.cr.7310090

Wu, P., Wang, B., Lubitz, S. A., Benjamin, E. J., Meigs, J. B. and Dupuis, J. (2021). Approximate conditional phenotype analysis based on genome wide association summary statistics. *Sci. Rep.* **11**, 2518. doi:10.1038/s41598-021-82000-1

Xiankui, Q. and Chuankuan, W. (2018). Acclimation and adaptation of leaf photosynthesis, respiration and phenology to climate change: a 30-year *Larix gmelinii* common-garden experiment. *For. Ecol. Manag.* **411**, 166-175. doi:10.1016/j.foreco.2018.01.024

Xu, S., Wilf, R., Menon, T., Panikker, P., Sarthi, J. and Elefant, F. (2014). Epigenetic control of learning and memory in Drosophila by Tip60 HAT action. *Genetics* **198**, 1571-1586. doi:10.1534/genetics.114.171660

Zhang, D., Tang, Z., Huang, H., Zhou, G., Cui, C., Weng, Y., Liu, W., Kim, S., Lee, S., Perez-Neut, M. et al. (2019). Metabolic regulation of gene expression by histone lactylation. *Nature* **574**, 575-580. doi:10.1038/s41586-019-1678-1

Zoonomia Consortium. (2020). A comparative genomics multitool for scientific discovery and conservation. *Nature* **587**, 240-245. doi:10.1038/s41586-020-2876-6