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The Epigenome and Beyond: How Does Non-genetic Inheritance Change Our View of Evolution?

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Synopsis Evidence from across the tree of life suggests that epigenetic inheritance is more common than previously thought. If epigenetic inheritance is indeed as common as the data suggest, this finding has potentially important implications for evolutionary theory and our understanding of how evolution and adaptation progress. However, we currently lack an understanding of how common various epigenetic inheritance types are, and how they impact phenotypes. In this perspective, we review the open questions that need to be addressed to fully integrate epigenetic inheritance into evolutionary theory and to develop reliable predictive models for phenotypic evolution. We posit that addressing these challenges will require the collaboration of biologists from different disciplines and a focus on the exploration of data and phenomena without preconceived limits on potential mechanisms or outcomes.

Introduction

Heritability is at the core of our understanding of both adaptation and evolution. Most analyses of heritability assume that changes in the DNA sequence are the foundation of heritable changes in phenotype. However, given our improved understanding of the importance of epigenetics, it is possible that heritable changes in phenotypes not dictated by DNA sequence also have significant effects on both adaptation and evolution. Given known connections between epigenetic variation and environmental variation, it is important to determine the extent to which the environment can impact and perturb epigenetic pathways both within individual organisms and in subsequent generations. Transgenerational epigenetic effects are particularly important because they are most likely to impact responses to natural selection and long term evolutionary change. Furthermore, the ways that epigenetic pathways are employed and regulated differs among organisms, but we have little knowledge of this variation across the diversity of life. Addressing these questions will have a significant impact on our understanding of heritability, evolution, and adaptation and

might lead to improved, more predictable models of phenotypic evolution.

Epigenetics defined

For the purpose of this article, we broadly define epigenetics as heritable changes in phenotype in the absence of changes in primary DNA sequence and in the absence of the initial trigger for those changes. It is important for the trigger to be absent in subsequent generations to ensure that the phenotypic change is inherited rather than being reestablished each generation *de novo*. Molecular mechanisms often considered as the basis of epigenetic inheritance include cytosine methylation, various histone modifications, and chromatin structure, as well as certain types of small RNAs (Felsenfeld 2014). Although specific biochemical modifications such as DNA methylation and histone modification are known to be associated with epigenetic changes in gene expression (Giaimo et al. 2019; Gökbüget and Blleloch 2019; Ninova et al. 2019; Sheikh et al. 2019), we are agnostic with respect to the mechanism. Epialleles are distinct epigenetic variants at the same locus leading

to different phenotypes. Epigenetic changes are distinctive because they are heritable, labile, and potentially influenced by the environment (Costa and Dean 2019; Kelly et al. 2020; Matuleviciute et al. 2021). Because of these qualities, epigenetic changes generate patterns of inheritance that are not necessarily amenable to traditional Mendelian genetic analysis and thus will require distinctive strategies that incorporate qualities such as stochasticity and heritable responses to environmental conditions.

Epigenetics and evolution

Transgenerational transmission of variation in information is the basis of evolutionary change, yet existing conceptualizations consider only direct inheritance of variation in DNA. If whole organism phenotypes are composed of a mosaic of characters that are inherited through distinct mechanisms, evolutionary theory will have to determine how to simultaneously include different processes to predict change. Attempts have been made to incorporate non-genetic inheritance mechanisms into evolutionary models. For example, Klironomos et al. (2013) model how epigenetic changes can interact with genetic changes and impact adaptive evolution. Day and Bonduriansky (2011) and Bonduriansky et al. (2012) model several modes of non-genetic inheritance and find interesting impacts on evolutionary trajectories, including the possibility of transmission of acquired characters under certain circumstances. It has been attempted to estimate the contribution of epigenetic factors to adaptive evolution (e.g., see Skinner et al. 2014), but we currently do not know how important these alternative inheritance mechanisms are for evolutionary processes. Without an understanding of the frequency at which non-genetic, and in particular epigenetic, inheritance occurs as well as the stability of the non-genetic information, clear predictions about potential evolutionary impacts will be elusive. A mechanistic understanding of how these indirect pathways of inheritance function will dictate how we determine their impact on evolutionary processes. The inclusion of a new focus on both biotic and abiotic environmental factors will require a change in the overall scale at which we are currently analyzing these phenomena. This question will require the integration of approaches and expertise from a number of biological disciplines in order to truly assess the full importance and functionality of these extra-chromosomal pathways and mechanisms of inheritance.

Why now?

While epigenetics began as a study of oddities in a few model organisms, we now have numerous well-studied examples of epigenetic inheritance in a variety of species, and there are many examples of unexplained forms of heritability across the tree of life that need to be explored further (Felsenfeld 2014). Technological innovations such as next-generation sequencing methods and genome-wide epigenome assays have enabled researchers to explore epigenetic phenomena at an unprecedented scale and depth (Park 2008; Meaburn and Schulz 2012; Robertson and Richards 2015; Arora and Tollefsbol 2021). In addition, CRISPR/dCas9 (Clustered Regularly Interspaced Short Palindromic Repeats/dead Cas9)-based methods for epigenome editing have made it possible to test specific hypotheses in novel ways (Rots and Jeltsch 2018; Schoelz and Riddle 2020). For example, we are now able to add or remove DNA or histone modifications at targets of interest using dCas9 fusions to chromatin modifiers. Through site-specific genome editing using CRISPR/Cas9, insulator elements, small RNA sources, or small RNA targets can be removed or added to generate and study new epialleles. These approaches will allow us to further dissect the connections between epigenetic marks, chromatin, and nuclear processes, such as the suppression of recombination by cytosine methylation documented in *Arabidopsis* (Yelina et al. 2015). We are now at a point where we can begin to determine the extent to which the heritability of large numbers of traits in multiple species is dependent on known epigenetic mechanisms, such as DNA and histone modification. Thus, it is possible to obtain reliable information about the extent of epigenetic variation in known pathways within individuals, between individuals and populations, as well as between species. Knowledge of the level and type of epigenetic variation is the first step in understanding the impact of this variability on phenotypes.

While DNA and histone modifications are an attractive target of study, analyses of epigenetic phenomena should not be restricted to known mechanisms. It is tempting to simply apply known technologies such as bisulfite or chromatin immunoprecipitation (ChIP) sequencing on a much larger scale, but such a focus on well-understood mechanisms carries the risk of missing important aspects of epigenetic inheritance. There is ample evidence from yeast, for instance, that prions can mediate heritable changes that impact host fitness in the absence of changes in the DNA sequence or in chromatin

modifications (Tuite 2016; Chakravarty and Jarosz 2018). It is also worth noting that until very recently, little was known about the importance of small RNAs, a class of molecules that now sit at the center of our understanding of epigenetic regulation (Weick and Miska 2014; Martinez and Köhler 2017; Skvortsova et al. 2018; Weiser and Kim 2019). Therefore, it would be beneficial to take a broader and more integrated view, remaining open to the existence of additional mechanisms mediating epigenetic inheritance—a point well illustrated by a recent preprint on bioRxiv that documents the inheritance of cellular memory through retrotransposon capsids (Moore et al. 2020). Furthermore, this approach can even be extended to behavioral or cultural evolution, where information is transferred also between generations without the involvement of changes in the DNA sequence. Of particular interest would be behaviors that clearly impact selection, such as those linked to niche construction as described by Clark et al. (2020). Overall, the goal should be not just to find more examples of what we know, but also to uncover phenomena that may involve novel mechanisms.

Heritable epigenetic changes are particularly difficult to assess for the same reasons that they are interesting: they are both transmissible and unstable. For this reason, analysis of instances in which DNA sequence is largely invariant, will be invaluable to examine. For example, in plants, it is relatively easy to generate doubled haploids that are homozygous for all loci (Hooghvorst and Nogués 2020; Jacquier et al. 2020). Clonal populations of plants and fungi also make it possible to examine the intersection of the environmental and epigenetic changes over time and space (Taylor et al. 2015; Ahn et al. 2017). For vertebrates, pedigreed populations under long-term study or isogenic lineages such as those of the mangrove rivulus fish *Kryptolebias marmoratus* might offer similar opportunities (Fellous et al. 2018). Due to their limited genetic variation, these types of populations are ideally suited to identify heritable epigenetic changes.

An additional source of data to identify new heritable epigenetic changes might be found in genome-wide association studies (GWASs). In these studies, there is still a large amount of missing heritability across the myriad GWAS performed for both plant and animal traits (Brachi et al. 2011; Young 2019). This unexplained variance could be due to the relative instability of traits that are driven by epigenetic mechanisms. Focusing on the heritability of these GWAS-tested traits also could provide a resource for identifying novel epigenetic mechanisms of

inheritance (Cortijo et al. 2014; Koch 2014; Schmitz 2014; Aller et al. 2018). Because many genetic variants identified as associated with a phenotype do not have an obvious function (i.e., they do not change a protein sequence or affect a known transcription factor binding site, etc.), it is possible that they are linked to stable epialleles, and thus, the association with the phenotype of interest is not due to genetic variation, but due to a linked epigenetic modification. Due to their relative stability, epialleles like the peloric epiallele of the *cycloidea* locus (*Lcyc*) in toadflax *Linaria vulgaris* (Cubas et al. 1999), the *mantled* epialleles Good Karma and Bad Karma in the oil palm *Elaeis guineensis* (Ong-Abdullah et al. 2015), or the Colorless non-ripening (*Cnr*) epiallele in the tomato *Solanum lycopersicum* (Manning et al. 2006) behave similarly to Mendelian alleles and were identified using traditional mapping approaches but turned out not to be due to DNA sequence variation. These examples highlight the possibility that GWAS candidates with no obvious sequence polymorphism might instead represent stable epialleles, as known cases likely represent only a fraction of the stable epialleles that occur in nature.

Unstable epialleles will be more difficult to detect, but it is also likely that the plant and animal breeding communities have encountered them as instances of unexplained phenotypic variation. Based on past experience, potentially fruitful phenotypes on which to initially focus would be flower or fruit morphology and color in various plants. Studies of variation in these traits led to the discovery of paramutation, co-suppression, transposable elements (TEs), and more (McClintock 1950; Brink 1956; Napoli et al. 1990; Hollick 2017). The pathways that influence these phenotypes are now well understood (Albert et al. 2014; Karlova et al. 2014; Spencer and Kim 2018; Woźniak and Sicard 2018), and breeders have been selecting for variation, some of which is unstable, for centuries. Many plant breeders have saved “odd ears” from corn plants, or seeds of strains showing unusual phenotypes and/or inheritance patterns, for later study. Sadly, “later” often means never, and many researchers will remember times in their career when they came across an unexplained oddity that would have been interesting to study but that was put aside due to practical considerations such as the chances of getting funding for these studies, the potential for success, ease of study, and the likelihood of success. Tapping into this often ignored type of variation, while risky, has the potential to reveal new insights into epigenetic mechanisms and their role in generating phenotypic diversity.

New technologies enable experiments designed to test the relationship between epigenetics and evolution

Over the last 10 years, methods development and technical innovations made a plethora of long-standing research questions amenable to study. One of these questions is the evolutionary significance of phenotypic variation under epigenetic control. To address this question, the development of low-cost, high-throughput sequencing methods has been essential. Combining these next-generation sequencing approaches with standard assays used to interrogate the epigenome and epitranscriptome has produced genome-wide profiles from diverse sets of epigenetic/epitranscriptomic marks in numerous cell types in many species (Ji et al. 2015; Zhong 2016; Lu et al. 2021). These methods allow us to assay numerous heritable histone, DNA, and RNA modifications, and higher-level chromatin structure can be profiled by a variety of methods that assay accessibility to nucleases (DNase-seq), transposases [ATAC (Assay for Transposase-Accessible Chromatin)-seq], and other processes [FAIRE (Formaldehyde-Assisted Isolation of Regulatory Elements)-seq, salt extraction, etc.]. These methods have delivered a view of the epigenome in unprecedented detail and complexity.

Many of these methods are applicable across a wide range of species and thus, the data available for scientists to estimate the extent of variation among epigenome features within and between organisms as well as within and between populations and species have increased exponentially. With the recent foray of epigenome profiling methods into the scale of single cells (Luo et al. 2020; Armand et al. 2021; Carter and Zhao 2021), we anticipate that the amount of data available will increase by orders of magnitude in the near future. This increase in capacity will open up additional areas of inquiry, but will also increase the challenges associated with handling the increased data complexity. Thus, computational methods and tools to efficiently and appropriately handle these data will become increasingly important. While computational modeling *per se* is not new, the methods to process highly dimensional data and model the complex interactions between genetics, epigenetics, and environment have become available only recently (e.g., see English et al. 2015; Roessler et al. 2018; Boyce et al. 2020; Sandholtz et al. 2020). Tools to integrate these data types and to partition phenotypic variance components are still being developed and refined. In addition, the integration of these tools into evolutionary models will require further development of computational

methods in multi-disciplinary collaborations between evolutionary biologists, geneticists, molecular biologists, and computational biologists.

Targeted manipulation of features within the epigenome that are now available will make it possible to directly test hypotheses (Holtzman and Gersbach 2018). zinc finger (ZNF) nucleases, transcription activator-like effector nucleases (TALEN), and CRISPR/dCas9 systems can be used to specifically target regions in the genome of model systems for epigenetic manipulation (Waryah et al. 2018; Agbleke et al. 2020; Schoelz and Riddle 2020). With these methods, it is possible to experimentally assess the phenotypic effects of specific modifications and epigenetic changes. Follow-up experiments utilizing artificial selection then can test directly if this epigenetic information and the associated phenotypes can be acted on by selection and serve as the basis for evolutionary change and adaptation. In particular, the CRISPR/dCas9-based approaches likely will be transferable from model systems into other species, thus allowing for hypotheses regarding the epigenetics–evolution relationship to be tested across different branches of the tree of life. These approaches present exciting new possibilities, as we might explore if we can purposefully employ epigenome engineering to drive evolution, at least in the short term.

Challenges and opportunities

Our understanding of epigenetic mechanisms of inheritance is incomplete. Our understanding of even the most well-studied phenomena is largely correlative, often anecdotal, and restricted to a relatively small number of model organisms. We know very little about the impact that these epigenetic mechanisms have on most organisms, although there is certainly evidence that there is a great deal of variation with respect to specific mechanisms among species (Höck and Meister 2008; Rodrigues and Zilberman 2015; Zhang et al. 2015; Bewick et al. 2016; Drinnenberg et al. 2016; Zilberman 2017; Muller et al. 2019). DNA methylation, for instance, is essential for proper epigenetic regulation in many species but is nearly absent in many other species (e.g., *Drosophila* and nematodes). We know very little about how selection operates on these pathways/mechanisms and how changes in these pathways mediate changes in responses to selection on the phenotypes they affect (Drinnenberg et al. 2019; Mbichi et al. 2020; Mulholland et al. 2020). Epigenetic variation in social insects promises to be a fruitful area for investigation. For example, the

extensive phenotypic differences seen between queen and worker bees of the same genotype are epigenetic in nature, highlighting a biological system that functions based on epigenetic control of caste phenotypes. Expanding epigenetic studies into additional branches of the tree of life has the potential to reveal rules governing epigenetic inheritance and the impact that epigenetically controlled phenotypic variation may have on evolutionary trajectories.

Another challenge currently limiting our understanding of epigenetic inheritance is that epigenetic information is dynamic. Time is an essential component of epigenetic regulation because the causes of epigenetic changes are manifest both in individual organisms as they develop and in their progeny. But we know very little about the dynamics of these epigenetic changes, either spatially or temporally. There are, for instance, multiple epigenomes/epitranscriptomes even within an individual, with different cell types/tissues having distinct epigenomes and epitranscriptomes (Bernstein et al. 2010; He et al. 2011; Brown and Celniker 2015; Tam and Ho 2020; Carter and Zhao 2021; Luo et al. 2021). In addition, patterns of epigenetic modification change as tissues differentiate or experience different environmental conditions (e.g., see Garrett-Bakelman et al. 2019). Thus, we need to carefully consider at what scale (e.g., cells and tissue) and at what time we need to sample to understand the inheritance of the epigenome between parents and offspring. It is also important to recognize the role of stochastic changes in epigenetic states, which can occur due to the imperfect inheritance and/or maintenance of epigenetic information. In many cases, these changes in epigenetic information are likely to be ascribed to Gene $\times$ Environment effects impacting the epigenetic information, and in many cases, they may well be. However, it is also possible that some degree of stochasticity is an intrinsic, and perhaps even selectively advantageous, feature of an epigenetically regulated regulatory pathway. Furthermore, data from for example aging studies suggest that the level of stochasticity is not constant over an individual's lifespan (Sen et al. 2016). Thus, any model for these phenomena should incorporate variation in stochasticity.

Interestingly, the best and most ubiquitous examples of stable epigenetic inheritance are linked to the silencing of TEs, which make up a substantial proportion of most eukaryotic genomes (Choi and Lee 2020; Sundaram and Wysocka 2020; Ritter and Niederhuth 2021). The bulk of these examples involve selection in favor of silencing of TEs rather than regulation of gene expression (Morgan et al.

1999; Blumenstiel 2011; Song and Schaack 2018; Deniz et al. 2019; Ondičová et al. 2020; Liu et al. 2021). For example, late-flowering *FWA* epialleles in *Arabidopsis* are linked to the loss of cytosine methylation at remnants of a SINE (short interspersed nuclear element) (Kinoshita et al. 2007). Thus, if one is interested in epialleles that affect specific phenotypes, there is a substantial signal-to-noise problem, as a great deal of epigenetic variation is a consequence of ongoing efforts by the host to regulate parasitic DNA, rather than to regulate genes. It will therefore be important to recognize distinctions between selectively neutral epigenetic variation arising from transposon silencing and epigenetic variation that has a direct effect on fitness.

To the extent that epigenetic differs from genetic inheritance, we will need to transform how we approach predictive modeling of phenotypes. For each trait, robust models will have to include contributions of both epigenetic and genetic factors that underlie that phenotype, but also will need to include environmental factors as well as the interactions among these main effects. Because both the epigenotype and the environment are dynamic, temporal components may be required to account for the fact that a significant portion of phenotypes change over time. Currently, these types of complex models are not available. One way to collect the data necessary for developing these models might be the reaction norm approach taken by Kronholm et al. (2016), who estimated phenotypic variance in *Neurospora crassa* in response to various conditions in select epigenetic pathway mutants. While challenging, the existing barriers to developing comprehensive models provide important areas of inquiry in order to drive this important research direction forward, with machine learning being one particularly promising avenue.

Finally, it is important to note that epigenetic variation not linked to known chromatin modifications is likely to have been ignored. Given the well-known epialleles that are linked to cytosine methylation, researchers with evidence of alleles with non-Mendelian behaviors know to investigate the cytosine methylation status. If that fails to reveal evidence of a causal mechanism, select histone modifications might be checked, as antibodies and kits for ChIP are widely available at this point. However, if this approach also fails to reveal a clear cause for the non-Mendelian inheritance, the investigation likely will stop, if it even reached this point, and the investigation will be seen as a dead end. Based on these limitations, it may well be that re-examination of data from a wide variety of

experiments in fields as diverse as plant and animal breeding, ecology, and medicine could yield valuable insights into hidden sources of epigenetic variation.

Moving forward

One key problem will be recognizing patterns of inheritance that are distinct from those that result from only genetic variation that follows the rules of Mendelian inheritance. These new inheritance patterns likely will be considerably more stochastic and contingent, making them difficult to detect as well as difficult to incorporate into existing models. Traditionally, cases of epigenetic inheritance have been identified initially due to deviations from expected Mendelian ratios in simple experimental crosses, an approach that continues to be a promising first step. It might also be possible to investigate candidate phenotypes and partition phenotypic variation due to genetics, epigenetics, and environment using quantitative genetics approaches. Machine learning can be used to reveal patterns that do not match normal genetic inheritance patterns in more complex situations. Machine learning might also be able to help us develop new inheritance models that take into account the contribution of stochastic and contingent inheritance patterns, thus allowing us to better model phenotypic variance, its inheritance, and evolution. Ultimately, new tools and open minds will be needed to recognize unusual inheritance patterns.

In order to determine the scale and magnitude of how these mechanisms affect trait inheritance across scales and populations, the proposed work will involve interactions between evolutionary biologists, geneticists, molecular biologists, ecologists, computational biologists, and systems biologists working across a variety of phyla.

Integration: what are the roles for each biological field in this research question

- (1) Computational biologists will be needed because the approaches that are being widely adopted and developed as well as those that are needed for addressing this question tend to be data-intensive and produce numerous large-scale datasets.
- (2) Transmission geneticists are very good at finding new epigenetic phenomena, while population geneticists are very good at finding variation in allele (and epiallele) frequencies. In order to understand the overall impact of epigenetic variation, both in populations and over time, it will be important for these specialists to work

closely with both ecologists and evolutionary biologists.

- (3) Molecular biologists will be needed because the methodologies and approaches that need to be developed will require molecular genetic techniques and expertise. Thus, molecular biologists will continue to be at the forefront in providing the methodological framework to drive our understanding of this important research question and to uncover novel mechanisms of epigenetic inheritance.
- (4) Ecologists will reveal the bidirectional connections between phenotype and the environment. Environmental conditions have the potential to drive changes in extra-genetic inheritance, while simultaneously acting as the background in which resulting phenotypic variation is tested by selection.
- (5) Evolutionary biologists will be required to understand how epigenetic inheritance determines the long-term change of populations and species and to develop predictive frameworks that incorporate novel pathways of inheritance into models of transgenerational change and response to environmental challenges.
- (6) Organismal biologists will be needed to bring phenotypes with potentially unusual transmission behaviors to the attention of transmission geneticists and to provide an understanding of how phenotypes showing epigenetic inheritance impact the organism.

Conclusion

Exploring the diversity of epigenetic inheritance could reveal novel mechanisms that explain examples of transmission that cannot be understood by primary sequence-based mechanisms. Understanding how different pathways determine the inheritance of each part of a larger phenotypic output could lead to independent manipulation of components. For instance, the regulation of disease resistance genes in plants involves a complex relationship between genetic and epigenetic mechanisms (Deng et al. 2017). Epigenetic modes of inheritance could be especially important for understanding social and cultural modes of inheritance that cannot be explained under current molecular mechanisms of inheritance. Complex social traits including collective behavior, empathic learning, and social network structure that exist only in groups of many individuals are likely to be influenced by epigenetic processes. An inheritance that operates outside of DNA sequence transmission opens the potential for

horizontal and vertical pathways of transmission both within and among groups and species, which would fundamentally shift the rules of evolutionary change. Ultimately, uncovering new mechanisms by which biological inheritance takes place would transform our understanding of how organisms respond to environmental changes and the consequences of selection, both artificial and natural. The types of studies we encourage are inherently risky and will require a certain amount of risk-tolerance from researchers as well as funders, but we believe they are worth pursuing because they have the potential to transform our understanding of phenotypic evolution.

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Conflict of interest

The authors declare no conflict of interest.

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