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Opinion

Understanding 'Non-genetic' Inheritance: Insights from Molecular-Evolutionary Crosstalk

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Understanding the evolutionary and ecological roles of 'non-genetic' inheritance (NGI) is daunting due to the complexity and diversity of epigenetic mechanisms. We draw on insights from molecular and evolutionary biology perspectives to identify three general features of 'non-genetic' inheritance systems: (i) they are functionally interdependent with, rather than separate from, DNA sequence; (ii) precise mechanisms vary phylogenetically and operationally; and (iii) epigenetic elements are probabilistic, interactive regulatory factors and not deterministic 'epialleles' with defined genomic locations and effects. We discuss each of these features and offer recommendations for future empirical and theoretical research that implements a unifying inherited gene regulation (IGR) approach to studies of 'non-genetic' inheritance.

Highlights

'Non-genetic' inheritance (NGI) involves a wide range of epigenetic, cytoplasmic, and other mechanisms. The term inherited gene regulation (IGR) provides a unifying concept for the diverse heritable factors that may alter offspring gene expression.

It is crucial to be aware of three features of NGI systems: they are functionally dependent with, rather than separate from, DNA sequence information; the precise mechanisms are highly diverse within and across taxa; and they act probabilistically rather than as 'epialleles' with defined genomic locations and effects.

These often-overlooked properties point to promising empirical and theoretical research avenues in evolution and ecology. A group of molecular and evolutionary biologists offers an integrated perspective on this emergent research area.

Inheritance beyond DNA Poses Key Questions for Evolution and Ecology

Biologists are currently engaged in a lively conversation about whether it is necessary to expand our view of biological inheritance to include 'non-genetic' factors [1–3]. In particular, molecular epigenetic mechanisms (such as DNA methylation, histone modifications, and small noncoding RNAs) have been interpreted as additional 'streams' of phenotypic information distinct from DNA sequence transmission [4–8]. In comparison with DNA sequence variation, which is transmitted with great fidelity across numerous generations, these factors have complex and potentially unpredictable dynamics: they may arise stochastically or be induced by specific environmental conditions, and they may persist from one to several generations (reviewed in [9–16]). Researchers in many fields are now confronting an unexpected question: must we fundamentally revise our understanding of inheritance to incorporate these new insights?

For evolutionary biologists, the phenotypic impact of inherited 'non-genetic' factors and their potential contribution to adaptation and diversification are pressing issues. Although many questions remain, mounting evidence indicates that these transgenerational mechanisms may substantially influence phenotypic outcomes in a wide range of organisms (reviewed in [17–22]). Induced inherited effects may be negative: for instance, parent individuals with a nutrient-poor, high-fat, or high-sugar diet may transmit altered DNA methylation states to offspring that result in metabolic or developmental disorders [23–25]. Alternatively, stressful parental conditions may induce gametically transmitted changes that promote adaptive phenotypes in offspring encountering similar stresses [26–32]. Because (unlike allele frequency change) such induced, inherited effects could cause adaptive adjustments in many individuals in a population (and their descendants) after only one generation, they are of particular interest to ecologists and evolutionary biologists as a potential mechanism for rapid adaptation to environmental changes [33,34].

In addition to phenotypic effects on individual organisms, 'non-genetic' factors may influence the adaptive potential of populations [6,35–37]. Recent studies of wild populations have shown the

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role of epigenetic variation in several aspects of population dynamics relevant to local divergence [38–42], invasion potential [43,44], migration propensity [45], developmental morph determination [46,47], and host–parasite interactions [48–50]. Note that field studies are challenged to determine the precise source of epigenetic variation (i.e., direct environmental induction, inheritance from previously induced generations, or locally selected 'epialleles' [51]), and to exclude the possibility that epigenetic differences among populations are simply downstream consequences of genetic differences [52,53]. Theoretical models indicate that 'non-genetic' factors, despite their transient nature, may substantially change selection gradients and heritabilities, and hence alter evolutionary trajectories [7–9,54,55].

Toward a More Precise View of 'Non-genetic' Inheritance

Although much progress has been made in understanding the ecological and evolutionary role of 'non-genetic' inheritance (NGI) [37,52,56], further advances may be hampered by three common simplifications about these molecular systems (for mechanistic reviews [13,57–59]). First, the very term 'non-genetic' is inaccurate: inherited epigenetic factors and DNA sequence are not distinct but functionally interdependent [52,53]. Second, processes such as DNA methylation are not uniform mechanisms, but operate in a multiplicity of ways depending on both species and mode of induction (e.g., [60,61]). Third, most epigenetic variants are not deterministic 'epialleles' with defined genomic locations and effects, but probabilistic, interactive regulatory factors [62].

By translating a fine-grained literature on molecular mechanisms into broader properties, we provide a more precise basis for integrating 'non-genetic' modes of inheritance into evolutionary and ecological studies. The key shared features of these mechanisms can be clarified by the unifying concept of inherited gene regulation (IGR; Box 1). This term encapsulates the common effect of a wide range of transgenerational systems that can alter genome activity and hence gene expression in progeny (Figure 1), including genome-associated mechanisms such as DNA methylation as well as cytoplasmic cellular components and non-DNA-bound factors such as hormones (while ecological and cultural inheritance could possibly be included, we limit our scope to molecular systems). Although the precise regulatory impact of specific factors is not always known (and may be highly context dependent), the term IGR provides a mechanistically grounded framework to focus on heritable factors that alter gene expression and hence may be of ecological and evolutionary relevance (see Box 2 and Figure S1 in the supplemental information online for modeling implications).

Feature One: 'Non-genetic' and Genetic Aspects of Inheritance Are Inseparable

'Non-genetic' and genetic aspects of inheritance are often treated as separate streams of information, both conceptually and experimentally. Accordingly, statistical and modeling approaches

Box 1. Inherited Gene Regulation

Currently used terms such as 'epigenetic', 'non-genetic', and 'extra-genetic' bear a multiplicity of meanings in the literature (see Box 2, Figure 2 and Supplemental Table S1 in the supplemental information online for a detailed analysis of terms, their use, and their implications). Yet, the diverse molecular mechanisms involved in 'non-genetic' modes of inheritance share a common effect: they alter aspects of genome activity and affect progeny gene expression. To emphasize this essential property, Day and Bonduriansky [7] introduced the concept of 'inheritance of the gene interpretation machinery', which we modify as the more concise 'inherited gene regulation (IGR)'. Here, 'regulation' may be functional or adaptive, but (as exemplified by negative effects of 'non-genetic' inheritance) this is not necessarily the case. IGR accommodates genome-associated mechanisms as well as non-nuclear factors such as maternally provided RNA [63], proteins, hormones, or nutrients (all of which can be potent gene expression regulators [60]), and encompasses the broad concept of transgenerational plasticity [20,33]. Contextual specification through prefixes (e.g., gamete-mediated IGR, DNA methylation–mediated IGR, hormone-mediated IGR, or RNA-mediated IGR) permits descriptive precision while maintaining conceptual unity.

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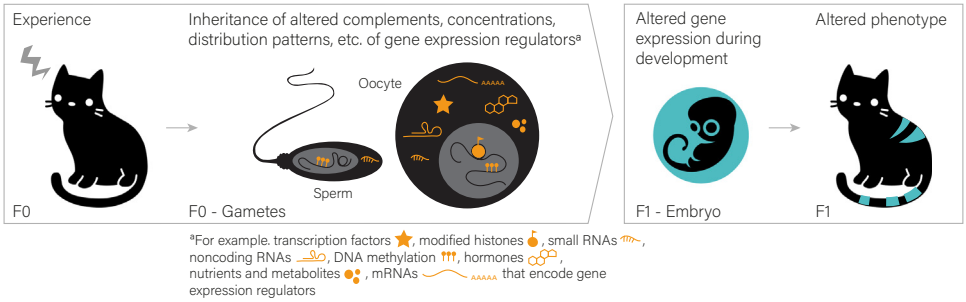

Figure 1. Inherited Gene Regulation (IGR). IGR encompasses all inherited factors that modify gene expression in offspring. This includes a wide range of molecular pathways, molecule types, and cellular compartments. *The use of cats is based on a conference remark comparing the 'dual' state of Schrödinger's cat with the dual nature of genetic/ 'non-genetic' inheritance.

often rely on the (linear) decomposition into 'genetic' and 'non-genetic' effects, and on the independent quantification of these effects (Box 2). Yet, gene sequence variants and heritable factors that regulate their activity are, in fact, deeply interwoven, suggesting that an interaction-based perspective may be more fruitful.

For reasons summarized in Figure 3A, IGR is never fully independent of DNA sequence. In the well-studied example of DNA methylation, marks are set, recognized, maintained, and erased by a large array of proteins that are encoded in the genome. Allelic variants of these genes can affect epigenetic induction and reversal dynamics [64], such that genetically distinct lines of the same species show different induced effects of parental conditions on offspring phenotype (e.g., [65–70]). In addition, DNA sequence at epigenetically targeted loci plays a role: whether or not a methylation mark can be set depends (among other factors) on the presence of CpG dinucleotides, and histone-modifying enzymes are frequently recruited by transcription factors that recognize specific DNA sequence motifs [71]. Finally, IGR mechanisms depend on the functional properties of the sequence surrounding the target locus [72]. In mammals, for instance, DNA methylation embedded in CpG-dense promoters contributes to gene silencing, whereas

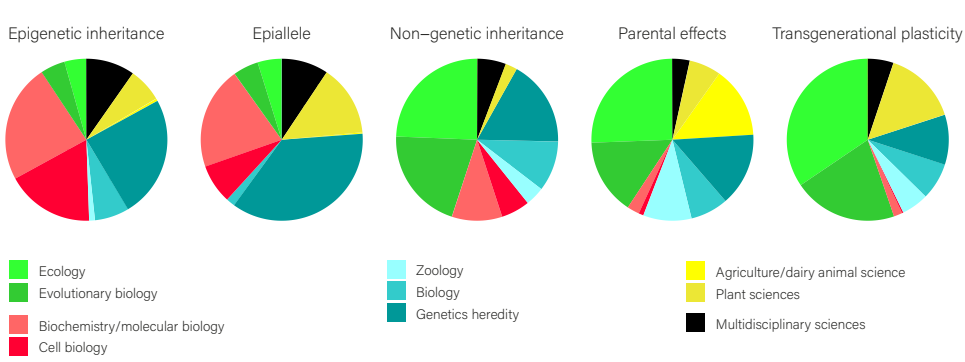

Figure 2. Terminology for 'Non-genetic' Inheritance. A search in the Web of Science reveals that the use of terminology differs by field. Raw data and methods, as well as a detailed analysis of properties, questions, and challenges associated with these and other relevant terms, are provided in Table S1 in the supplemental information online.

Box 2. Modeling the Impact of 'Non-genetic' Inheritance

Mathematical and computational models have played a crucial role in mapping the broader implications of epigenetic modifications, maternal effects and other aspects of non-genetic inheritance (NGI) since the first models (e.g., [55]) revealed their impact on selective dynamics and outcomes. Although the two predominant modeling approaches have provided important insights, their scope is limited since current models either focus on atypical forms of NGI (such as epialleles at a single locus) or neglect the close interdependence of genetic and non-genetic factors (Features 1 and 3 in the main text).

In the first approach, population genetic (PG) and quantitative genetic (QG) models are expanded by including NGI. PG models explicitly include the mechanisms of genetic and NGI; even with simple scenarios (such as epialleles at a single locus, e.g., [140,141]), the resulting models are highly complex and difficult to analyze. To avoid these complexities, some PG models do not model inheritance directly, but instead focus on the fitness landscape [142], making the assumption that selection will shift the population to a fitness peak. In principle, fitness landscape models are versatile and can be applied to various NGI mechanisms [4]. However, current models may make unrealistic assumptions about the fitness landscape (e.g., that fitness can be split into separate genetic and epigenetic components). Moreover, when inheritance is complex, fitness may not increase during selection [143]. QG models (e.g., [55,144,145]) are technically more tractable than PG models, but they are based on strong and empirically untested assumptions such as a normal distribution of genetic and non-genetic effects, with stable variances and covariances. In particular, QG models tend to assume that genetic and non-genetic effects are additive (and therefore independent of each other) or that selection is very weak (implying that nonadditive effects are negligible). Such additivity is not supported by the available data (e.g., [146]) and does not align with an inclusive understanding of NGI as inherited gene regulatory information. By means of a Price equation approach, PG and QG models can be viewed from a unified perspective [7]. This provides useful insights, for instance, that NGI can foster rapid adaptation when a population is far from a fitness peak, while it will often lead to a fitness reduction in an already well-adapted population [56]. To date, however, applications of the Price equation (e.g., [7]) have also relied on potentially misleading simplifying assumptions such as additivity of genetic and non-genetic effects.

The second approach includes models that conceptualize the interplay of 'information channels'. Here, genetic and non-genetic factors are viewed as cues providing potentially adaptive information about the state of the environment (e.g., [5,147]). These models seek to ask what kinds of cues (i.e., inherited parental effects versus an individual's current information) will evolve to be used in a given scenario, depending on such factors as temporal versus spatial environmental fluctuations and transgenerational correlation. In contrast to most PG and QG models, the information channel approach explicitly models the machinery integrating and interpreting different kinds of information. This allows an important additional question to be addressed: how do these information-integrating systems themselves evolve? At present, however, information channel models make similar simplifying assumptions as other current models: the information-processing machinery is represented by weighing factors, and the phenotype results from the weighted summation of genetic and non-genetic cues.

To our knowledge, a mechanistic model for the evolutionary causes and consequences of NGI that addresses the three key features of these systems (see main text) has not yet been proposed. In Supplemental Figure S1, we sketch a gene regulatory network model consistent with an inclusive IGR perspective that explicitly incorporates nonadditive interactions and feedbacks among genetic, epigenetic, and environmental factors.

methylation marks embedded in coding sequences are associated with the timing of transcription initiation events, and marks in intergenic regions have little impact on genome activity [73].

At the same time, the molecular mechanisms underlying IGR feature elements of sequence independence – for example, through environmentally determined changes in the activity of pathway components, or through 'read–write' mechanisms which recognize a modification and reiterate or amplify it [74,75]. Such mechanisms account for pathway-specific fidelity properties [62]; they allow epigenetic marks to spread along chromosomes [76], to be copied to the new DNA strand during cell division [77], and to mediate the prolonged inheritance of environmental signals [78,79]. Overall, the molecular mechanisms underlying IGR operate on a continuum from entirely sequence-determined to completely sequence-independent (also [53,56]). Importantly, the location on this continuum is not fixed for any mechanism, nor for any gene. Genetic versus regulatory aspects may gain and lose importance depending on genomic location, developmental timepoint, tissue, and environmental conditions.

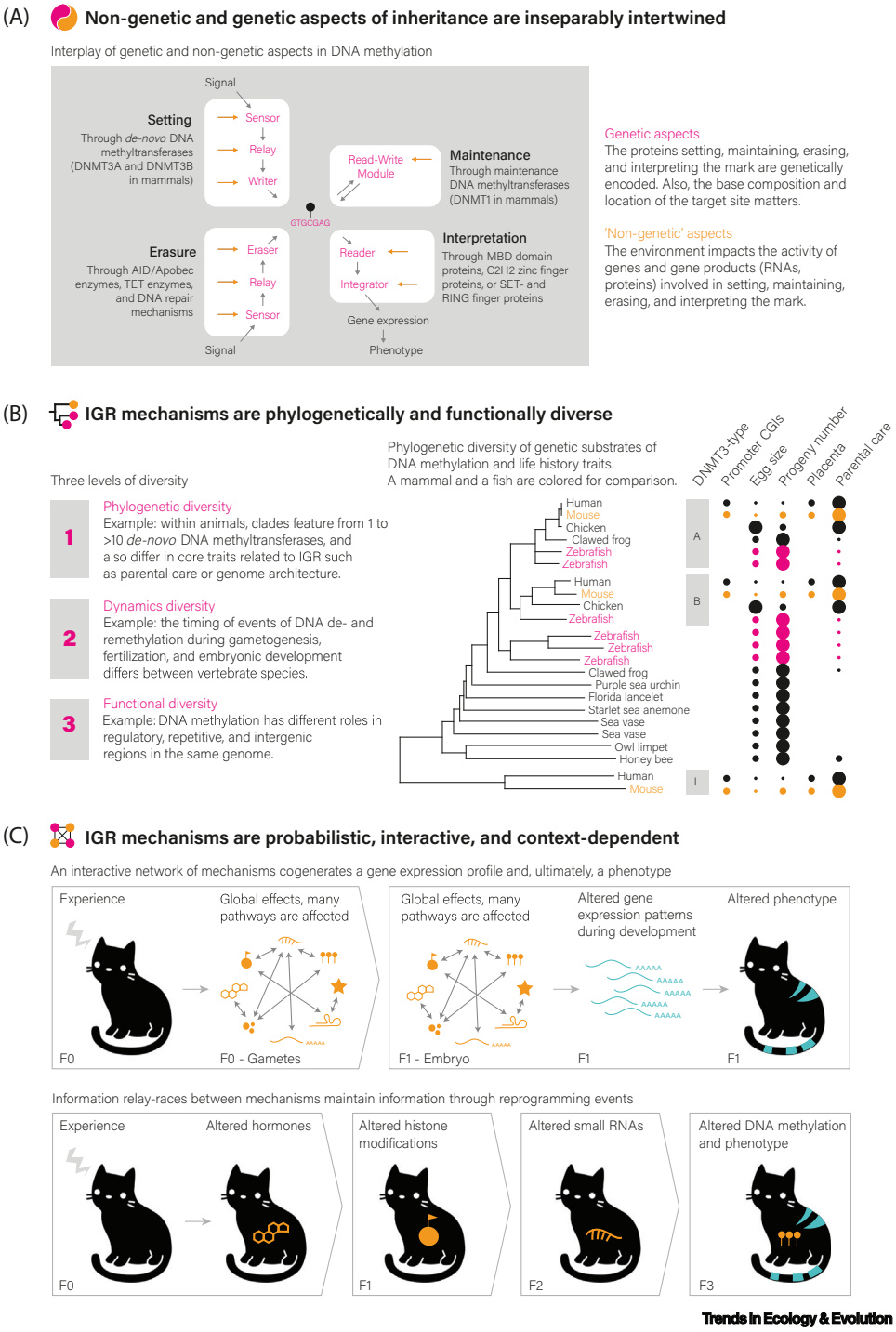

Figure 3. Fundamental Features of Inherited Gene Regulation (IGR). (A) In IGR, 'non-genetic' and genetic aspects are inseparably intertwined. The pathways setting, maintaining, erasing, and interpreting a particular mark receive input from genetic and regulatory (including environmental induction) sources (shown here for DNA methylation, but the concept applies equally to other mechanisms). (B) IGR mechanisms are phylogenetically and functionally diverse. This diversity stems from multiple levels: (i) the phylogenetic level (different species feature different gene numbers and types, and

(*Figure legend continued at the bottom of the next page.*)

The complex interplay of heritable regulatory factors and DNA sequence information poses considerable conceptual and experimental challenges. To face these challenges, we offer four recommendations. First, assume from the start that genetic and 'non-genetic' factors both contribute to any inherited phenomenon, and only drop this assumption in the case of unequivocal evidence to the contrary. IGR genes act pleiotropically, and even a single undetected base pair change (for example, in a master regulatory gene for DNA methylation) can potentially alter the epigenetic landscape in strains believed to be isogenic. If complete genome sequences are unavailable, and/or the effect of sequence differences is not investigated through crosses or transgenics, the relative impact of genetic and 'non-genetic' effects cannot be precisely quantified.

Second, explore how genetic variation affects IGR in your particular experimental model. Genotype or strain-specific capacities for IGR exist (see also Feature Two, later) but are poorly characterized, yet identifying loci that affect IGR capacity in natural populations could greatly increase our understanding of IGR in evolution and potentially in human disease. Third, consider a fine-grained approach to the genome when assessing epigenetic and genetic variance by separately analyzing functional units such as exons, introns, promoters, enhancers, repetitive elements, and intergenic regions, or by focusing on genome regions of known phenotypic effect. A basic functional genome annotation can be created through methods such as RNA expression time courses, ATAC-seq (assay for transposase-accessible chromatin using sequencing) analyses [80], Hi-C approaches [81] or histone profile mapping [82] (also [83,84]). Technological advances bring such annotation within reach even for non-model species. A fourth alternative is to 'zoom out' rather than to 'zoom in' on the molecular picture: it may not be necessary to distinguish heritable regulatory factors from sequence information as distinct aspects of gene expression regulation, and an integrative, systems biology approach might more accurately capture a biological reality. In the theoretical domain, evolutionary models for gene-regulatory networks can be designed that explicitly address the interaction of these two components (Box 2 and Figure S1 for a sketch of such a model). Experimentally, this integration can be achieved by replacing approaches focused solely on one aspect of gene regulation, such as DNA methylation profiling, with broader measures of genome activity such as gene expression (RNA-seq), chromosome-conformation analyses (Hi-C), or overall chromatin accessibility (ATAC-seq; see references earlier). These techniques have been greatly optimized in terms of sensitivity, sample requirements and technical difficulty, making them accessible for the first time to non-experts in molecular research.

Feature Two: IGR Mechanisms Are Phylogenetically and Functionally Diverse

The mechanisms underlying IGR are evolutionarily ancient, and it is tempting to treat them as if they were as universal as the principles underlying DNA-based inheritance. However, this may be misleading, as NGI mechanisms are highly diverse. For instance, both models and experiments often assume that DNA methylation is a singular mechanism, with a uniform mode of action across the genome and in different taxa, even though differences between mammals, reptiles, and insects had been recognized by the 1980s [85]. Recognizing and deliberately

different life history traits related to reproduction and inheritance), (ii) from the level of dynamics (the same pathway may display different dynamics across fertilization in two species), and (iii) from the functional level (the same mark may have different functions in the same species, depending on the genomic context). A phylogenetic tree of *de novo* DNA methyltransferase genes (modified from [148]) illustrates this diversity: two of the best-studied animal models, mouse and human, feature *DNMT3L* genes which are absent from non-mammals. Similarly, the assignment of *de-novo* methyltransferases from many animals to the two 'canonical' types A and B is not straight-forward [149]. (C) IGR mechanisms are probabilistic, interactive, and context dependent. In IGR, molecular mechanisms form a 'gene-regulatory landscape' that culminates in a particular gene expression pattern and phenotype. Regulatory information can be preserved across time and generations in the absence of stable mark inheritance due to reprogramming. For a legend of symbols, see Figure 1. Abbreviations: AID, activation-induced cytidine deaminase; CGI, CpG island; DNMT, DNA methyltransferase.

incorporating the diversity of these mechanisms into research designs can lead to important progress in understanding eco-evolutionary implications.

Diversity in IGR mechanisms is relevant at three levels (Figure 3B). First, although certain features may be broadly conserved, there is striking phylogenetic diversification in the molecular machinery for setting, maintaining, reading, and erasing epigenetic marks. For example, mammalian genomes feature three unique DNA methyltransferase *DNMT* genes, but teleost fishes have between five and 12 such genes ([86–90]; [91–93] for other eukaryotic examples). Phylogenetic variation has also been described for chromatin modifiers [94], histones and their variants [95,96], and chromatin modification readers [97]. Second, even if different taxa feature roughly similar gene complements, the function of certain genes and the temporal or spatial dynamics of potential epigenetic modifications may differ. Such differences are particularly apparent in soma-germ line transitions in vertebrates, where the reprogramming dynamics of epigenetic setting and erasure are intensively studied. For example, modified histones are largely removed during mouse *(Mus musculus)* spermatogenesis but retained to some extent in human *(Homo sapiens)* sperm [98]. Similarly, parental DNA methylation is erased during mammalian germ cell development, but paternal methylation is largely retained in zebrafish *(Danio rerio)* [99,100], although less so in other fish species [101,102]. And while all taxa feature maternal RNA, clearance and maintenance dynamics differ widely [63,103–106]. A third relevant level of diversity pertains to the functional consequences of epigenetic marks, since biochemically identical marks can have very different 'meanings' in different taxa. This can be inferred at the among-species level from pattern comparisons: DNA methylation marks are concentrated within actively expressed genes in teleost fish, but are found upstream of inactive genes in mammals [107,108]. Within a species, the genomic location of a given type of mark affects its impact: functional assays indicate that DNA methylation upstream of genes in many cases inhibits gene expression, while methylation that occurs within active genes can affect the choice of transcription initiation site [109].

In summary, while most features of DNA inheritance are exceedingly highly conserved, NGI is a language with many dialects. Caution is therefore warranted when extending insights from model organisms to other taxa or strains with different evolutionary histories. This point leads to several recommendations. First, whenever possible, generalizations from distantly related species should be replaced by known properties of the species – or at least the clade – of interest. This entails addressing several questions. What is the species' phylogenetically based capacity for IGR – does the species or its clade feature RNA silencing pathways, how many DNA (de)methylases are there, does the genome feature the CpG islands in promoter regions that are most prone to methylation? What are the temporal and spatial dynamics of the mechanism of interest – are histones retained in sperm, are hormones deposited in the egg cell? Is the mechanism of interest known to be linked to gene expression in this species? Even when the answers are not all known, posing these questions can be a first step to more focused interpretation and to guide further research steps.

A related recommendation is to use a phylogenetic perspective in choosing specific research techniques. For example, in a species (such as the nematode *Caenorhabditis elegans*) that features dozens of Argonaute RNA-silencing genes, targeting small RNAs may be promising, but RNA isolation protocols must be fine-tuned to account for diverse small RNA types. In fish, maternal RNA is of interest, but it is important to be aware of the absence of poly-A tails on maternal messages because standard sequencing protocols rely on their presence. Affinity-based techniques to measure DNA methylation [such as MeDIP (methylated DNA immunoprecipitation) or methyl-CpG-binding domain (MBD) capture] do not perform well on loosely interspersed DNA methylation and should be avoided in species such as fish or invertebrates [110] that lack typical

CpG islands [111–113]. We also encourage biomolecular researchers interested in IGR to incorporate evolutionary and ecological considerations into their research design. This entails using IGR triggers that are relevant to a species' naturally occurring conditions and selective forces (such as treatments that impose resource limits or biotic stresses like competition and pathogens), or choosing taxa that have evolved in response to known environmental changes [114,115]. These research criteria may seem obvious to the evo-ecologist but are somewhat undervalued in the molecular field.

Feature Three: IGR Mechanisms Are Probabilistic, Interactive, and Context Dependent

Views of NGI – and consequently, experiments and theoretical models – are largely shaped by classic cases such as paramutation of the Kit tail color gene in mice [116], flower morphology in toadflax *(Linaria vulgaris)* [117], or mammalian imprinting [118]. These examples are used as research models because they are robustly heritable, deterministic, and 'allelic'. However, these cases may be quite exceptional [119]. The molecular mechanisms involved in IGR most often act in a probabilistic, facultative, and context-dependent manner. Accounting for these nondeterministic properties is an important step in advancing understanding.

In what ways are IGR mechanisms nonlinear, probabilistic and context dependent? First, IGR mechanisms do not operate at the level of nucleotide resolution, but rather they integrate across larger genomic regions. In the example of a CpG-rich mammalian promoter, the combined overall state of several hundred base pairs is related to gene activity (e.g., [120]), but the methylation state of any particular nucleotide in the promoter is usually irrelevant. Treating individual occurrences of epigenetic marks like nucleotide polymorphisms (i.e., as epialleles), and analyzing 'epimutations' rather than the overall state of a genome region poorly captures the biological process [120], and might detect statistically significant but functionally uninformative differences. Second, IGR integrates information across several distinct mechanisms that spatially and temporally co-occur, work in concert, and influence each other [121–129]. This complex 'chromatin landscape' creates a gene expression profile that could not have been generated by any one mechanism alone (Figure 3C). An example is 'poised' promoters, which feature the paradoxical combination of activating and repressing histone marks on the same nucleosome [130]. Third, the stability of information is not necessarily linked to the stability of any individual mark. For example, in mice, a protein binding to previously methylated regions preserves the 'memory' of DNA methylation through meiotic demethylation in the absence of the mark itself [131–134]. Similarly, feedback loops between small RNAs and histone marks maintain information in fission yeast *(Schizosaccharomyces pombe)* in a phenotypically neutral state, allowing later generations to benefit from the information when required [135]. Other examples of such 'information relay races', where information about (grand)parental conditions persists in the absence of robust mark inheritance, include paternal heat exposure in wild guinea pigs *(Cavia aperea)* through nonconstant DNA methylation patterns [136] and offspring pathogen avoidance mediated through sequential neuronal signaling and small RNAs in *C. elegans* [137], see also [138]. Hence, robust inheritance of any single mark across more than one generation is not necessarily required for multigenerational effects on the phenotype (Figure 3C).

In sum, IGR mechanisms act as a cluster of interdependent molecular nudges that together enhance the likelihood of a particular outcome on the gene expression level. This complexity creates challenges for empirical studies, which often aim to track down the effect of a single type of mechanism or a single localized change. How can we address this challenge in designing eco-evolutionary studies of NGI? One approach is to design experiments to investigate the 'cluster of nudges' in its entirety, for example, by starting investigations at the integrative level of gene

expression (e.g., with RNA-seq approaches), or by investigating multiple pathways (DNA methylation, small RNAs, histone modifications, nutrients, metabolites, hormones) in parallel. Key decisions must also be made in assessing the outcome. IGR may manifest in developing offspring only under certain conditions [29], and it may be useful to measure gene expression at early life-cycle points (e.g., embryos or even gametes) in which developmental responses to laboratory conditions may be less likely to overwrite any inherited alterations [139]. Functional analyses of early-life gene expression can also help guide the choice of adult traits to study. For example, differential expression analyses of the offspring of drought-stressed plants might reveal down-regulation of small RNAs targeting root-related processes, suggesting a focus on root extension and uptake rates rather than on leaf traits or transition to flowering. Importantly, tissue-specific approaches to gene expression analyses should always be preferred (even if they require delicate dissections) to avoid losing signals that manifest only in particular tissues.

Concluding Remarks

A key contemporary research challenge is incorporating inheritance mechanisms beyond DNA sequence into evolutionary and ecological investigations. Some simplification of these dauntingly diverse and functionally complex mechanisms is reasonable and indeed necessary for this effort. By drawing on molecular insights to these mechanisms, this can be done in ways that maintain rather than distort key aspects of their functionality. 'Cross-talk' between evolutionary and molecular biologists provides a way to bridge this gap in understanding. Recognizing the common effect of highly diverse molecular mechanisms as IGR is a first step toward identifying general features of NGI systems. Characterizing such features can help replace some initial misconceptions with a more solid mechanistic foundation to inform ecology and evolution theory and research programs. IGR seems a quintessential area where collaboration between molecular specialists and those with knowledge of ecological and evolutionary issues can be especially productive on both sides (see also Outstanding Questions).

Outstanding Questions

What are the key functional features of inheritance systems beyond DNA sequence with regard to adaptive variation and evolution? Studies of induction and reversal cues and dynamics, phenotypic impact, and potential cumulative effects across generations are of particular relevance.

Given the interactive and reciprocating functionality of genetic and 'non-genetic' factors, how can these aspects of inheritance most productively be addressed? Treating them as separate elements may be less informative for understanding gene regulatory effects than an integrated approach, which will require changes to experimental design.

How can the interplay of genetic and 'non-genetic' factors be incorporated in eco-evolutionary models in a realistic manner? The close interaction of genetic and 'non-genetic' regulatory elements calls for the development of regulatory network models, together with new tools for deriving overarching principles from such mechanistic models.

How do specific IGR mechanisms vary in different phylogenetic, environmental, and tissue contexts? Accounting for this diversity and complexity calls for expanding empirical studies far beyond the most well-known model organisms and standard laboratory conditions.

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