

## Spotlight

## A conserved genomic code underpins animal DNA methylation patterns

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**Evidence is mounting that non-genetic inheritance impacts evolution, however, how conserved the underlying processes are remains unexplored. Klughammer *et al.* investigated DNA methylation across the animal kingdom, one important mechanism of non-genetic inheritance. Using a dataset encompassing 580 species, the authors identified conserved associations between sequence and DNA methylation.**

Conrad Waddington is considered by many as the father of epigenetics. In the 1940s, he defined epigenetics as ‘the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being’ [1]. Waddington saw epigenetics as a way to bridge the field of embryological development with genetics and ecology. His driving motivation stemmed from his observation that genetic and phenotypic variations could be uncoupled. In epigenetics he saw how an organism can respond plastically to the environment and exhibit a phenotype not purely determined by genetics. By the end of the 20th century, Jablonka and Lamb had recognized the evolutionary potential of epigenetic variation to affect large-scale processes, such as rapid adaptation and speciation [2]. However, they also underlined that this may be limited in animals due to germ line–soma segregation, something that

is not present in plants and fungi. This view has now changed due to strong data showing that epigenetic variation, including epigenetic inheritance, is a near ubiquitous phenomenon in animals [3,4].

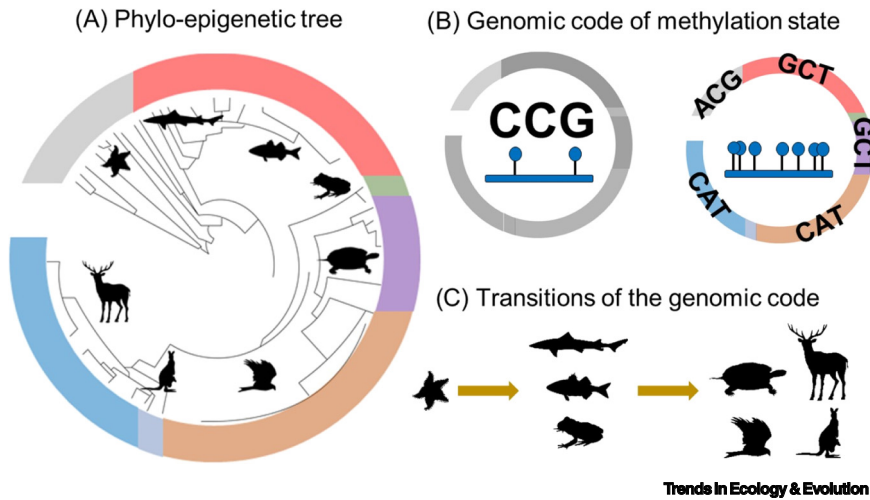
The Klughammer *et al.* study [5] is important because it presents a step change in our knowledge of the distribution and functions of DNA methylation in animals. Most of the previous research on DNA methylation has come from single species studies of abnormal processes, such as toxic exposures, or diseases. These studies have been without doubt revealing but they lacked the power to characterize the evolutionary signal in the epigenetic machinery across animals to glean insights into how epigenetic variation can be produced and inherited. Being equipped with the 580 species strong dataset, it is now possible to ask questions about the large-scale relevance of DNA methylation variation in animals and interrogate their phylo-epigenetic relationships (Figure 1A).

Analyzing the dataset, Klughammer *et al.* [5] were able to reveal that animals have a unique ‘genomic code’ that connects DNA methylation states to the underlying DNA sequence motifs, indicating that this relationship is conserved. This genomic code is composed of three consecutive nucleotides, called 3-mer DNA sequences, and separates regions of low and high DNA methylation states. DNA sequences that start with a C or G nucleotide and end in a CpG, the standard target dinucleotide for DNA methylation, are characterized by low DNA methylation levels across taxonomic groups (Figure 1B). Interestingly, DNA sequences linked to high DNA methylation levels were found to be more diverse and taxon-specific. This suggests that while low methylation regulation has been conserved in animals across evolutionary time, the relationship between DNA sequence and high DNA methylation levels may have been exposed to novel selective

pressures that differ across animal groupings.

This discovery provides new insights into the intriguing interplay between DNA sequence variation and DNA methylation. In 2006, Richards [6] already highlighted that DNA methylation can be completely determined by genetics to be entirely independent from it, with intermediate dependencies also possible. A clear example of the tight relationship concerns the simple presence of CpG dinucleotides. CpG dinucleotides provide the major docking sites for methyl groups and thus determine variation in DNA methylation. Consequently, if a mutation results in the disappearance of the C base pair from the CpG nucleotide, then the methyl group has lost its docking site and the stretch of sequence cannot become methylated any longer. Additional examples where genetic sequence variation is functionally linked to the DNA methylation status concern the protein machinery needed for the reading, writing, and erasing of DNA methylation, which is also fully encoded in the genome [7]. Unravelling DNA methylation changes that have evolved more independently from the genetic code provides insights into the other extreme of this relationship. This search is motivated by an attempt to identify DNA methylation patterns that have been shaped by deep phylogenetic processes, in addition to more short-term DNA methylation changes that can arise plastically in response to environmental change.

Klughammer *et al.* [5] also uncovered group-specific phylogenomic codes that can be used to predict DNA methylation levels in other groups. An important aspect of this finding is that the predictive ability decays when phylogenetically older lineages are used to estimate DNA methylation levels of younger lineages (Figure 1C). For example, invertebrates, fish, and amphibians can be used to predict DNA



**Figure 1. DNA methylation patterns across the animal kingdom.** (A) Evolutionary relationships are reflected in the phylo-epigenetic tree redrawn from Klughammer *et al.* [5]. (B) Conserved sequences of three nucleotides are associated with low DNA methylation (left panel) and more diverse sequences of three nucleotides are associated with high DNA methylation (right panel). Outer circles represent the phylo-epigenetic tree, with gray indicating sequence conservation, whereas colored ring segments correspond to sequences specific to phylogenetic groups. Lollipop represents sites of DNA methylation. (C) Two major transitions, with the emergence of vertebrates and reptiles, can be inferred based on predictive relationships of the genomic code of DNA methylation state among groups. Animal silhouettes are from <https://www.phylopic.org/> under CC licenses.

methylation levels in the evolutionary younger reptiles, birds, and mammals, but not vice versa. When applying this rationale and reading the DNA methylation genomic code, two major transitions can be inferred, one coinciding with the emergence of vertebrates and another one coinciding with the emergence of reptiles. Thus, their study highlights that the evolutionary relationships of animals are reflected in the association of DNA sequence variation with DNA methylation.

Surprisingly, they also found that some species of invertebrates, fish, amphibians, and reptiles do not follow the general pattern and show an inversion of the genomic code. Low and high DNA methylation patterns are predicted by the opposite 3-mer DNA sequences in the majority of other species. For example, this is observed in the white hake (*Urophycis tenuis*), where high DNA methylation levels are associated with specific 3-mer DNA sequences. These sequences cluster near predominantly repetitive elements, which are CpG rich and may have introgressed recently

and therefore require suppression. This indicates that the levels and genomic patterns of DNA methylation are evolutionarily labile, with further research needed to elucidate the causes, mechanisms, and consequences. A future challenge will, therefore, be to decipher the epigenetic evolutionary history of these outlier animal groups to elucidate trajectories of large-scale events in conjunction with genetic relationships.

While epigenetic research was for a long time seen as a marginal and immature field of science, recent years have witnessed an accelerating interest in epigenetics, centering around the realization that epigenetic variation is a fundamental substrate of evolution. Epigenetic variation can act as a crucial mediator between the genome and the environment, maximizing variability where and when it is most likely to yield positive changes, while minimizing phenotypic variability when and where it is not needed. It is therefore not surprising that considerations of non-genetic inheritance are progressively incorporated

into eco-evolutionary thinking, particularly in cases where immediate multilevel changes (molecular/cellular/morphological/physiological/behavioral) are required, such as in the case of species response to rapid global warming. What this study has clearly brought to the table is that new animal DNA methylomes are not just ‘old wines in new bottles’ but that new and fascinating lessons can be learned about the core workings of the evolutionary machinery itself [8]. Future studies need to extend this work beyond the study of epigenetic mechanisms at the genome level and investigate epigenetic outcomes at the level of both the individual organism and across animal populations. We are certain that the study of epigenetics will remain exciting and that these latest discoveries of the intricate relationship between DNA sequence variation and DNA methylation variation are just the latest chapter in a large and detailed book on the role of epigenetics in ecology and evolution that remains to be completed.

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**Declaration of interests**

No interests are declared.

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