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A comparative perspective on epigenetics

Ever since Watson and Crick signed off their 1953 *Nature* paper with the closing line, 'It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material', we have lived in an age of genes and genomes. And before the human genome was decoded in 2000, it was common to hear talk of specific genes for this and that. However, more recently, it has become apparent that the relationship between genes and the phenotypes that they express is significantly more complex. 'Additional mechanisms come into play when there is an interaction between the genotype and the environment', says Hans Hoppeler, Editor-in-Chief of *The Journal of Experimental Biology*. One of the key regulatory mechanisms that has become increasingly apparent in recent decades is epigenetics, where non-genetic changes to DNA (alterations that do not affect the nucleotide sequence of a gene) result in specific cellular and physiological traits and these epigenetic alterations are also transmissible across generations, contributing to evolution and comparative physiology.

Given the potential impact of this form of phenotypic regulation, Hoppeler commissioned a special selection of review articles for *The Journal of Experimental Biology* discussing epigenetics from the perspective of the fundamental mechanisms that alter phenotypes through to examples of intergenerational effects and dramatic life history alterations that are epigenetically regulated. In the first review, Denis Noble from the University of Oxford, UK, argues for a fundamental shift in our understanding of inheritance and biological change that encompasses epigenetics, and he proposes that the current dogma – which places the nucleotide sequence of a gene as a template for proteins at its heart – is replaced with a more integrative approach (p. 7). Explaining that the reductionist definition of biology that considers the genome as a blueprint that contains all of the essential information required to reconstruct an organism, Noble proposes an alternative framework: where genes are considered simply as templates that are activated when required by the cell network. Noble also proposes that adaptation and evolution emerge through network-wide responses to change at all levels of biological organisation, from proteins and membranes to organelles and cells, rather than being driven by individual genes. 'Active causation resides in the networks, which includes many components for which there are no DNA templates', says Noble, concluding that this novel approach provides 'an integrated systems view of evolution'.

Epigenetics: scope and mechanisms

In the first review of the section dedicated to epigenetic mechanisms, Gabriella Ficz from Barts Cancer Institute, UK, explains that the human body is composed of approximately 200 different cell types, yet each cell originates from the same zygote and carries the same DNA. Describing how every cell is locked into its cell type by a unique epigenetic signature, Ficz discusses the role of DNA methylation in epigenetic regulation. Although CpG dinucleotides are the rarest dinucleotides in genomes, Ficz says that 75–85% of these dinucleotides are methylated in mammals (p. 14). However, when they group together to form CpG islands at promoter regions, they are generally unmethylated. Adding that this so-called 'hypermethylation' effectively shuts down genes, Ficz says that promoter hypermethylation is rare during development, yet increases with age and in cancer. Describing the signalling pathways and enzymes that regulate CpG methylation, Ficz explains that altered DNA methylation patterns are potential key trigger factors in many cancers, saying, 'It is highly likely that epigenetic aberrations are at the root of subsequent genomic instabilities that enable genetic mutations to occur'.

Moving on to the role of epigenetics in depression, Florian Duclot and Mohamed Kabbaj from Florida State University, USA, discuss the epigenetic regulation of a key factor – brain-derived neurotrophic factor (Bdnf) – in depression and the effect of classical antidepressants via epigenetic regulation on brain and serum Bdnf levels (p. 21). Duclot and Kabbaj explain that DNA methylation and post-translational histone modifications are altered at the *Bdnf* gene in both depressed patients and animal models of depression.

Epigenetic regulation of stress has been implicated in chicken domestication. Photo credit: Per Jensen.

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They also describe how the same epigenetic modifications are targeted by antidepressant treatments. Reviewing the different DNA methylation patterns and histone modifications that are associated with Bdnf in blood and several regions of the brain in response to stress and depression in humans and rodents, the duo also describe how treatment with antidepressants, such as imipramine and fluoxetine, influences histone modification and DNA methylation to restore Bdnf levels. In addition, the duo describe how environmental enrichment can alter methylation of histones, including histone 3 at Lys 27 – which is associated with suicide completion in humans – in depressed rats and they say, 'These findings illustrate how epigenetic mechanisms translate environmental cues into long-lasting changes in gene expression'.

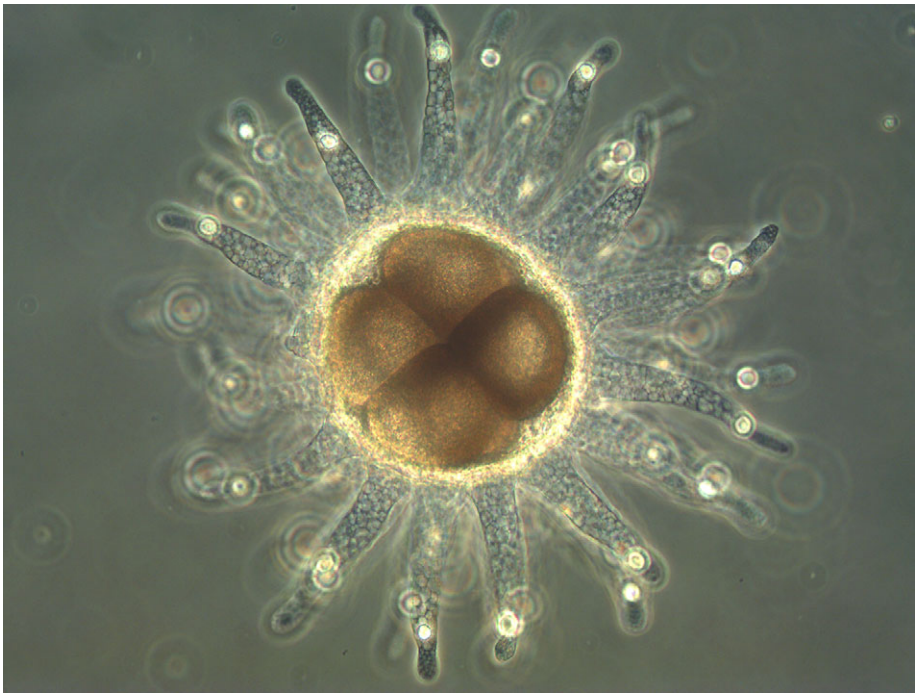
In addition to its role in depression, stress – and the ability to regulate the stress response through epigenetics – has been implicated in animal domestication. Per Jensen from Linköping University, Sweden, explains that tameness usually implies reduced sensitivity to stress and adds that many of the other traits that are associated with domestication are simply correlative. As such, Jensen and co-workers have investigated the stress response of the modern domestic chicken (White Leghorn laying hens) and compared it with the stress response of their ancestors, Red Junglefowl. Jensen has shown that the domesticated animals are less fearful of humans and less stressed than their ancestors. The team and other scientists have also compared the gene expression profiles of the domesticated birds with their ancestors and found differences in the expression of genes linked with stress that are associated with a sizeable increase in DNA methylation. Jensen concludes by pointing out that environmentally induced modifications can be transmitted to subsequent generations via several epigenetic mechanisms (p. 32) and says, 'The interaction between the genome and the environment is far more dynamic and complex than previously thought'.

Switching from the role of epigenetics in domestication to epigenetic mechanisms in the nematode *Caenorhabditis elegans*, Catharine Rankin from the University of British Columbia, Canada, says, 'With its sequenced genome, well-studied genetics and 3 day generation time, *C. elegans* offers opportunities to investigate mechanisms that mediate transgenerational epigenetics' (p. 41). She describes research showing that some epigenetic silencing lasts for tens of generations. Outlining the mechanisms of histone modification and RNA interference (which silences genes), Rankin goes on to explain how histone methylation and demethylation are associated with transcriptional heritability in the germline of the nematodes and that the effects of histone methylation on longevity are heritable across three to four generations. She also reviews how olfactory imprinting can be inherited epigenetically by subsequent generations – although the mechanism of transmission is yet to be determined.

Epigenetics of nutrition and environmental effects

Having discussed the mechanisms of epigenetic regulation and transmission, the collection moves on to address the role of epigenetics in physiological responses to nutrition and the environment. Anne Gabory and colleagues from UMR1198 Biologie du Développement et Reproduction, France, discuss the impact of nutrition and obesity on the placenta and developmental programming in the fetus. Reviewing the effects of placenta size and structure on fetal development and health in later life, Gabory explains that many nutrients and their metabolites are the sources of the moieties that epigenetically modify DNA and histones to provide a direct link between diet and epigenetics (p. 50). The team then discuss the impact of obesity and gestational diabetes mellitus in humans, intrauterine caloric restriction in humans and rodents, and high fat diets in rodents on DNA methylation in placentas. They report that maternal glucose and gestational diabetes result in epigenetic modifications to genes involved in fetal growth and metabolism, while an increase in DNA methylation has been reported in the placentas of calorie-restricted mice that was associated with several metabolic syndromes in the adult offspring. A high fat diet also affected methylation of genes related to cellular, metabolic and physiological functions in the placentas of rat mothers. Gabory says, 'The data presented in this review converge to the fact that adaptation in placental phenotype in response to maternal diet and metabolic status… alter[s] fetal nutrient supply'.

Michael Daniel and Trygve Tollefsbol then discuss the effects of nutrition on ageing and cancer from an epigenetic perspective (p. 59). Having first outlined the epigenetic factors (DNA methylation, histone modification and microRNA) that



Epigenetic factors impact fertilisation and offspring fitness in broadcast fertilisers. Photo credit: Amy Hooper.

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have been implicated in various cancers, the duo go on to introduce some of the key components (*p16*, *p53*, telomerase) that regulate cancer formation. They then discuss the effect of caloric restriction on cancer, metabolic disease, heart disease and ageing, and point out that although the mechanism is not known, there is mounting evidence that epigenetic modification of the genome offers some protection from these diseases. The duo then discuss the 'epigenetic diet', which includes foods such as soy, cruciferous vegetables and green tea. The diet provides a rich source of various compounds that are known to protect against cancer through epigenetic mechanisms. They conclude, 'New discoveries are continually supporting the epigenetic reversal of aberrant mechanisms by dietary control and caloric restriction, and linking them to a decreased incidence of cancer and an extended lifespan'.

Shifting focus from the impact of diet in the epigenetics of disease, Carmen Marsit addresses the effect of the environment on the human epigenome (p. 71). Explaining that arsenic is a potent pollutant often found in well water in developing countries, Marsit discusses the various epigenetic factors, such as DNA methylation, histone modification and the expression of microRNA, that can be modified in response to arsenic exposure and the diseases that result. Although the mechanisms that produce these epigenetic alterations are not known, Marsit says that oxidative stress is implicated. He then discusses the challenges faced by researchers studying epigenetic epidemiology – including the tissue-, cell- and context-specific nature of the epigenome – and the approaches that are being used to assess how the epigenome is modified as well as the use of epigenome-wide associated studies (EWAS) that link alterations in DNA methylation at specific sites to their physical outcomes.

Epigenetics in comparative physiology

Epigenetics is one of the fundamental processes that underpins physiological responses on an adaptive and evolutionary scale to environmental change. 'Having the option to modify a phenotype epigenetically would be a very good way for an organism to be resilient', says Hoppeler. However, Warren Burggren from the University of North Texas, USA, explains that epigenetic phenomena should not be thought of in an evolutionary context as simply 'on' or 'off' – when an adaptation might be considered to be 'on' in one generation but switched off in subsequent generations (p. 80). 'Less frequently have studies considered whether the phenomenon is graded in some form within or across generations', says Burggren. Proposing that epigenetic phenomena decline (wash out) or even build up gradually (wash in) over generations, Burggren describes the transgenerational effects of dioxin exposure in rats and tributylitin exposure in the aquatic larvae of *Chironomus riparius*. He also explains that epigenetic phenotypic effects can be lost across broods of the same generation. For example, the first offspring produced by the water flea *Daphnia magna* after exposure to severe hypoxia are small; however, the effect is lost by the third brood of the first post-hypoxia generation. Suggesting that a progressive reduction in DNA methylation could underpin the gradual loss of a physiological modification within a generation, Burggren wonders whether phenotype washout occurs actively over the course of time by dilution across the generations.

The switch from the solitary to gregarious life phase in *Schistocerca gregaria* is epigenetically regulated. Photo credit: Tom Fayle.

One spectacular example of an animal that expresses vastly different phenotypes from a single genotype is the locust. In fact, Liliane Schoofs and colleagues from KU Leuven, Belgium, explains that the locust was so successful that it succeeded in fooling generations of scientists into thinking that the insect's solitary and gregarious (swarming) life phases were two completely different species. Describing the dramatic physical and physiological differences between the two morphs, Schoofs explains how the switch from a solitary life phase to a gregarious lifestyle occurs in a matter of hours, while the return to the solitary life phase occurs gradually over multiple generations. The team list the molecular mechanisms that underpin aspects of both life phases before discussing evidence for the role of epigenetic mechanisms in the transitions (p. 88). The team also discusses the use of single base resolution methylome analysis, which could reveal more details of the epigenetic machinery of locust phase transitions. In addition, Schoofs hopes to use pharmacological approaches that specifically manipulate DNA methylation and histone modification, to 'unravel how the different epigenetic pathways interact to bring about the phenomenon of phase transition'.

Moving on from the role of epigenetics in life phase transitions to epigenetic effects

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in the evolution of bat populations in response to environmental change, Jiang Feng and colleagues from Northeast Normal University, China, explain that it is essential to look at the role of epigenetics in adaptation in wild populations, as many of the earlier analyses of epigenetic effects were conducted in highly selected model organisms (p. 100). Explaining that bat populations are critical to their ecological niches and are extremely sensitive to environmental change, the team report that they have identified high levels of epigenetic polymorphisms within and between female populations of roundleaf bats, least horseshoe bats and eastern bent-winged bats across southern China. The team suggest that this epigenotypic variation 'could support the "raw material" for adaptive evolution'. Having outlined the genetic and environmental factors that contribute to the epigenetic diversity that they have identified, the team conclude, 'Heritable environment-induced epigenetic variation may be beneficial or harmful for bat populations and could be one of the driving forces in bat evolution'.

Concluding the section on epigenetics in comparative physiology, Dustin Marshall discusses paternal influence on offspring physiology (p. 107). He explains that non-genetic factors that affect sperm size, age and condition vary in species that practise broadcast fertilisation, and these factors affect fertilisation success and the resulting offspring. Environmental factors, such as salinity, can also select a subset of the sperm that achieve fertilisation, and when Marshall and Hannah Ritchie collected sperm from the marine invertebrate *Galeolaria geminoa* and exposed half of the sperm to low salinity, they found that the low salinity offspring behaved differently from the normal salinity offspring, even though they were full genetic siblings. Explaining that similar epigenetic effects can also occur in internal fertilisers, Marshall says, 'Environmentally induced phenotypic covariance between sperm and offspring represents an important, yet largely unexplored, source of variation in offspring phenotypes'. However, he warns that contrasting sperm and offspring performances – such as changes that improve sperm success while compromising offspring fitness – could restrict sperm plasticity on some occasions.

Epigenetics in phenotypic plasticity and heritability

Ultimately, the main goal of comparative physiologists is to understand how the environment influences biological phenotypes, and Hoppeler says, 'Modifying a phenotype epigenetically is a very good way for an organism to be resilient'. Roberto Bonasio from the University of Pennsylvania, USA, explains that although there are many technical advantages to studying fundamental questions about the mechanisms of epigenetics in model organisms, many questions that remain unresolved – such as life expectancy, and how different life stages arise from a single genome – would be better addressed in non-model organisms (p. 114). Discussing phenotype changes that occur in various organisms, for example sex change in the half-smooth tongue sole (*Cynoglossus semilaevis*), caste change in eusocial insects and tissue regeneration in flatworms, Bonasio provides a fascinating insight into some of the dramatic phenotype changes that can emerge from a single genotype during the course of an animal's life and the intriguing lessons that they can teach us about the role of epigenetics in plasticity and heritability.

While many animals respond to environmental change through out life, Nadine Provençal, Linda Booij and Richard Tremblay report the impact of early social experience on a small percentage of the human population that display chronic physical aggression through adult life. Listing the risk factors that are implicated in chronic physical aggression – maternal parenting and maternal, family and child characteristics – the trio go on to discuss how early life experiences alter the methylation patterns of a key player in the stress response system (the glucocorticoid receptor) and how alterations to DNA methylation patterns associated with social adversity are found throughout the tissues of the body and are strongly associated with components of the stress response (p. 123). They say, 'Results from these studies suggest that early adversity produces acute and long-lasting epigenetic alterations. These alterations may influence brain development, and the ability to learn to control aggressive behaviour.' They also note, 'Genetic resistance to epigenetic alterations could explain why some children will be resilient to the deleterious effects of early life adversity'.

Another group of individuals that could provide remarkable insight into the epigenetic mechanisms of diseases associated with development and the ageing process is monozygotic – identical – twins, which share the same genome and environment from the uterus through to leaving home. Qihua Tan and colleagues from the University of Southern Denmark explain how comparing the DNA methylation patterns of identical twins allows scientists to dissect out the role of environmental factors in disease (p. 134). Listing the diseases that have been shown to have some degree of epigenetic regulation, they also discuss the role of environmental factors that alter DNA methylation patterns with age. Tan says, 'With proper study design and analytical approaches, twin studies can help with the identification of novel epigenetic marks and the linking of these with

Harpegnathos saltator workers collecting newly laid eggs. Photo credit: Daniel F. Simola.

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Altered microRNA expression is a component of freeze tolerance by wood frogs (*Rana sylvatica*). Photo credit: J. M. Storey.

environmental exposures'. They conclude, 'It is highly expected that the valuable sample of twins is going to make new contributions in unravelling and understanding the epigenetic basis of the development of human complex diseases and traits'.

However, epigenetic regulation is not restricted to physiological phenomena. 'Social plasticity is achieved by rewiring or by biochemically switching nodes of a neural network underlying social behaviour in response to perceived social information', says Rui Oliveira and colleagues from the ISPA – Instituto Universitário, Portugal. Explaining that social plasticity comes about thorough the remodelling of brain structures, biochemical switching in neurons and epigenetic modifications, the team go on to list examples of social phenotypes, in animals ranging from insects (fire ants and honey bees) to fish (salmon and cichlids), some of which are fixed while others vary in response to the social context or throughout life history (p. 140). After outlining the molecular mechanisms (activation of proteins, transcriptional activation and microRNA regulation) that translate social information into genetic and neural changes, Oliveira describes and gives examples of how epigenetic mechanisms activate gene expression gradually over time and in response to environmental factors in various social species (locusts, prairie voles and honey bees).

Finally, Ken Storey focuses on epigenetic mechanisms that have been implicated in many hypometabolic states – for example, hibernation, aestivation and anoxia tolerance – where animals conserve energy and protect cellular structures to survive stressful situations such as low temperature, drought and hypoxia (p. 150). In addition to reviewing the roles of DNA methylation and histone modification in mammalian hibernation, anoxia tolerance in turtles and aestivation in frogs, Storey discusses novel regulatory mechanisms, such as O-GlcNAcylation of epigenetic and transcription factors and inhibition of protein activity by SUMOylation (the conjugation of small ubiquitin-related modifiers to other proteins). Also reviewed is the role of mRNA storage in metabolic suppression in hibernating ground squirrels and dormice, the role of microRNAs in ground squirrels and other hibernators, and aestivation in the marine sea cucumber. Finally, Storey shows that mechanisms such as protein acetylation – that have long been associated with epigenetics – are now proving to be crucial regulators of many metabolic enzymes, providing potential explanations for how both gene and metabolic processes are coordinated in response to environmental stress.

Concluding remarks

Having edited many of the manuscripts in this themed collection of reviews, Hoppeler says, 'I really hope that the awareness of the possibility that epigenetic mechanisms modify the situation of a species under certain conditions is providing a better understanding of how a phenotype can be morphed and may morph over several generations'. He is also optimistic that comparative physiologists will continue to embrace the potential that an epigenetic approach offers for interpreting the complex interaction between our ever changing environment and the organisms that inhabit it.

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