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# Epigenetic memory of environmental organisms: A reflection of lifetime stressor exposures<sup>☆</sup>

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### ABSTRACT

Both genetic and epigenetic responses of organisms to environmental factors, including chemical exposures, influence adaptation, susceptibility to toxicity and biodiversity. In model organisms, it is established that epigenetic alterations, including changes to the methylome, can create a memory of the received signal. This is partly evidenced through the analysis of epigenetic differences that develop between identical twins throughout their lifetime. The epigenetic marks induce alterations to the gene expression profile, which, in addition to mediating homeostatic responses, have the potential to promote an abnormal physiology either immediately or at a later stage of development, for example leading to an adult onset of disease. Although this has been well established, epigenetic mechanisms are not considered in chemical risk assessment or utilised in the monitoring of the exposure and effects of chemicals and environmental change. In this review, epigenetic factors, specifically DNA methylation, are highlighted as mechanisms of adaptation and response to environmental factors and which, if persistent, have the potential, retrospectively, to reflect previous stress exposures. Thus, it is proposed that epigenetic “foot-printing” of organisms could identify classes of chemical contaminants to which they have been exposed throughout their lifetime. In some cases, the potential for persistent transgenerational modification of the epigenome may also inform on parental germ cell exposures. It is recommended that epigenetic mechanisms, alongside genetic mechanisms, should eventually be considered in environmental toxicity safety assessments and in biomonitoring studies. This will assist in determining the mode of action of toxicants, no observed adverse effect level and identification of biomarkers of toxicity for early detection and risk assessment in toxicology but there are critical areas that remain to be explored before this can be achieved.

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## 1. Introduction

Organisms have the ability to respond to environmental stressors such as toxic chemicals and adapt beneficially to new environments. This is accomplished, in part, by altering their epigenomes and subsequently their transcription profiles. Thus, overlaid on the genome are epigenetic marks, particularly methylation and hydroxymethylation of CpG sites that determine, in part, how the genome responds through regulation of transcription [1–3]. It is well established that various environmental stressors, including dietary deficiencies and exposure to a wide range of chemical pollutants, can modulate the epigenome [4–7]. The changes in response to environmental stressors may contribute to an adaptive survival advantage of local populations of organisms

through advantageous gene expression [8,9]. However, in some cases these changes are associated with marked phenotypic endpoints that can be detrimental [10,11]. Such accumulated modifications of DNA, and the consequent changes in gene expression, have important implications in diseases, including cancer [12] and may, in some situations, be persistent through subsequent generations. Furthermore, it is becoming more evident that epigenetic mechanisms are involved not only in adaptive responses in individuals; they also have a significant role in host–pathogen interactions as reviewed by Gomez-Diaz et al. [13]. This demonstrates the role of epigenetic mechanisms in multiple species interactions.

In this review, we explore the epigenetic responses of organisms to environmental stressors with a particular focus on the persistence or “memory” of such modifications and the ways in which this memory can usefully reflect the status of the environment in which humans and other organisms reside. Epigenetic factors, specifically DNA methylation, are introduced as an interface between the genome and the environment, providing partial mechanistic explanations for the sensitivity of organisms to environmental factors. We argue that epigenetic mechanisms such as DNA methylation are essential in determining how organisms respond to

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environmental agents and we present examples of studies in a range of species showing how exposure to chemicals can promote persistent changes in the epigenome with phenotypic outcomes. Furthermore, these studies lead to the concept of “epigenetic footprinting” for retrospective assessment of chemical exposures. The relevance and significance of epigenetic mechanisms in environmental risk assessment and the potential for establishment of suitable biomarkers is discussed in this review. These insights may shape the future of regulatory toxicology and environmental monitoring, especially where there are chronic exposures to pollutants.

## 2. Epigenetics

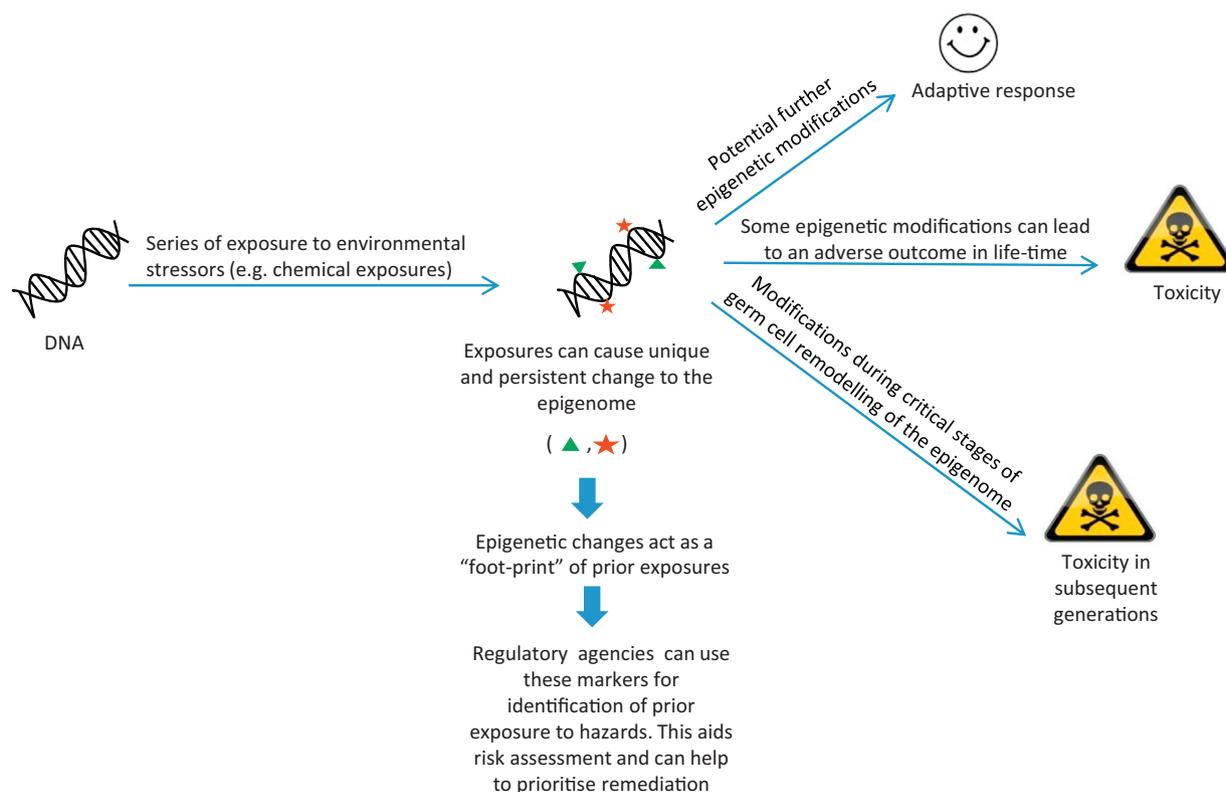
Epigenetics is defined as meiotically and mitotically heritable changes in gene expression that cannot be explained by changes in DNA sequence [14,15]. Such modifications include DNA methylation, post-transcriptional chemical modifications of the N-tail of histones and amino acids within the globular histone domains, binding of non-histone chromatin proteins to DNA or histone modifications (i.e. transcription factors), non-coding RNA, nucleosome positioning and higher order chromatin organisation [10,14,16–19]. Moreover, these modifications are not isolated events and are closely inter-linked by influencing chromatin structure at various levels and by further interactions with the genome [19]. Under normal conditions, cells of an organism display a finely tuned epigenetic equilibrium [1]. However, disruption of the activity of enzymes regulating the epigenome or changes in the levels of the metabolites required for the action of these enzymes can result in alteration of epigenetic marks and the epigenomic equilibrium [10] leading to inappropriate regulation of transcription and potential disorders [14,19]. Methylation of DNA at CpG dinucleotides is the most studied epigenetic modification [1] and it is the principal focus of this review. DNA methylation is the transfer of a methyl group, by DNA methyltransferases (DNMTs), from the universal methyl donor S-adenosylmethionine (SAM) to the 5th carbon position of a cytosine pyridine ring [20,21]. The crosstalk between histone modification and DNA methylation at the transcriptionally active and inactive regions is partly accomplished by a cfp1 (CXXC finger protein 1) and methyl-CpG binding proteins (MeCPs), respectively [22]. These proteins selectively bind to methylation-free and methylated CpG dinucleotides, respectively [22–24], encouraging recruitment of histone acetyltransferases and de-acetyltransferase and other epigenetic and non-epigenetic factors leading to regulation of transcription [23,24]. Although the precise mechanisms of DNA de-methylation are not known, it has been suggested that DNA can also either passively or actively undergo de-methylation. During passive de-methylation, 5-methyl cytosine is removed in a replication-dependent manner by inhibition of DNMTs while active de-methylation depends upon enzymatic removal of 5-methyl cytosine. Recently, TET (ten-eleven translocation) proteins have been found to be involved in regulation of DNA methylation. TET1–3 proteins catalyse the conversion of 5-methyl cytosine to 5-hydroxymethyl cytosine (5-hmC) which is poorly recognised by DNMTs. This results in a passive replication-dependent loss of DNA methylation. Alternatively, 5-hmC can be recognised by the repair machinery and converted to cytosine [25–27]. Thus, although methylation can be removed from CpG islands, this is a highly regulated process and, as discussed below, many such modifications are persistent and “memorised” in cells.

## 3. Environmental sensitivity of the epigenome throughout life time and retention (memory) of environmentally-induced changes

Adaptive responses and sensitivity of an organism to environmental stimuli (e.g. chemical contaminants, diet and stress) are

observed throughout the lifetime of an organism. However, a key question is what are the molecular mechanisms leading to changes in the expression of the genome? For example, why do homozygous twins have different disease susceptibilities in the absence of genetic variation? As introduced above, it is becoming more evident that, although epigenetic marks are stable enough to regulate gene expression, they are also susceptible to change by environmental signals. This means that the epigenome can change as a response to environmental stimuli, which then can lead to alteration in the phenotype [15]. In a way, the epigenome can act as the link between environmental cues (external and internal) to the organism and phenotype by translating the environmental signals to phenotypic responses through altered gene expression profiles. For example, in response to their immediate environment, a fraction of pluripotent stem cells will differentiate and form distinct cell types with a characteristic gene expression profile. The tissue-specific expression patterns are generally maintained throughout the lifetime of the individual. The differences in transcription profiles of the differentiated cells are then attributed to their different heritable epigenetic profiles. Hence, tissue-specific epigenetic profiles provide a method of sustaining the memory of the differentiation process in the absence of the initiating signal [12,28–30]. One of the best examples of the influence of environment on the epigenome and subsequent changes in gene expression is the response of *Arabidopsis* to prolonged exposure to cold weather (vernalisation). Following prolonged exposure to cold weather (an environmental factor), flowering locus C, a repressor of flowering, becomes epigenetically silenced. This results in coordination of flowering time (phenotype) with spring and summer [31–33].

Although the epigenome is sensitive to the environmental stimuli throughout an individual's lifetime, there are critical windows during development that the epigenome is at its most sensitive with lasting transcriptional effects. For example, genes such as oestrogen receptor (ER) and glucocorticoid receptor (GR) are regulated, in part, through DNA methylation of their promoter regions. The methylation and subsequently the transcription levels of these genes are gender- and region-specific. Furthermore, DNA methylation of these genes is substantially influenced by environmental factors, such as maternal care and exposure to chemicals, encountered during embryogenesis and early postnatal stages (reviewed in [34–36]). Another good example of developmental sensitive windows of alterations in the epigenome comes from the studies conducted in fish species looking at the effect of temperature on gender. Sex determination in many fish and reptile species is influenced by many factors including the temperature of the water during early stages of larval development. In European sea bass, high temperatures (21 °C) and low temperatures (15–16 °C) increase the number of male fish and female fish, respectively. It was demonstrated that exposure to high temperature at critical stages of larval development increased the DNA methylation level of the *aromatase* (*cyp19a1*) promoter in female gonads prior to formation of gonadal ridge and differentiation of the gonads. Aromatase converts androgen to oestrogen. A decrease in the expression of this gene as a result of high temperatures and subsequent methylation and suppression of the promoter region of this gene results in increased levels of androgen, differentiation of the gonads, formation of testis and a male-biased sex ratio [37]. Many other studies, demonstrate that environmental agents, independent of inducing mutations, can alter transcription profile and subsequently the phenotype of an individual by altering its epigenome [11,39]. Such changes in the epigenome profile can act as a memory (Fig. 1). However, although gender-dependent DNA methylation of the promoter region of the *aromatase* gene could explain differential expression of the *aromatase* gene in brain, liver and gonads of Japanese medaka, this correlation was not apparent following treatment with 17 $\beta$ -estradiol [38].



**Fig. 1.** "Epigenetic memory" as an indicator of prior exposure. Epigenetic alterations induced by environmental stressors, including changes to the normal DNA methylation pattern, can create a persistent memory of the received signal. Most interestingly, it is proposed that each class of chemicals can induce class-specific alterations to the normal pattern of DNA methylation (epigenetic foot-print). These changes will further induce alterations to the gene expression profile, which can promote change in organism's traits either immediately or at a later stage of development. Such persistent modulations of the epigenome offer a unique opportunity to provide a life-time history of an organism's prior exposure to factors influencing the epigenome. There is also potential for germ-line modifications to persist into subsequent generations depending on the nature and critical timing of exposure.

Furthermore, several studies have shown phenotypic plasticity driven by epigenetic changes. The differences in morphology, behaviour and reproductive ability of genetically identical female worker and queen honey bees (*Apis mellifera*) have been explained by distinct methylation profiles of their brain DNA and subsequent impacts on transcription. The variation in their methylation profiles has been linked, in part, to their different diets during larval development. [40]. Likewise, early maturation of male Atlantic salmon (*Salmo salar*) has evolved in response to lower population densities. Moran and Perez-Figueroa [41] demonstrated that transcription levels vary between brain and testes of mature and immature salmon in the absence of genetic variations. Furthermore, maturation stage correlated with differences in DNA methylation profiles of mature and immature fish, providing evidence of epigenetically driven phenotypic differences as a response to an environmental factor in the absence of genetic variations.

Of particular relevance to evidence of epigenetic "memory" are several studies in genetically identical human twins that have clearly demonstrated a link between environmental factors, change in the epigenome and different susceptibility to disease. One of the first studies that demonstrated this link was conducted by Fraga et al. [42]. In this study, it was shown that differences in the epigenomic profiles of monozygotic (MZ) twins accounts for their different phenotype (i.e. disease) in response to environmental factors over time. Indeed, these epigenetic differences appeared more prevalent with increased age of twins with different lifestyles (i.e. diet, smoking and physical activity). This demonstrates a strong link between accumulation of epigenetic changes over time and altered phenotype.

#### 4. Examples of the range of environmental stressors that can alter the epigenome

Direct exposure to chemicals, such as chronic exposure to persistent lipophilic compounds or metals in the environment, can cause adverse effects by inducing change in the epigenome ([7], reviewed in [43,44]). For example, the carcinogenicity of some environmental contaminants such as endocrine disruptors, nickel, cadmium, chromium and arsenic cannot entirely be explained through genetic based mechanisms [45]. Alterations to the epigenome through exposure to endocrine disruptors have been linked, for example, to negative impacts on neuroendocrine systems [34,46], altered behavioural neuroendocrinology [21,47] and higher rates of tumourigenesis at environmentally relevant concentrations in mice [48]. Metals can interfere with the activity of DNA methyltransferases either directly or through production of reactive oxygen species. This leads to an altered DNA methylation profile and subsequent alterations in gene expression. For instance, it has been proposed that cadmium (Cd)-induced global DNA hypomethylation may be due to Cd interaction with DNA methyltransferases (DNMTs) and subsequent interference with their methylation activity. Detoxification of inorganic arsenic (As) is dependent on its enzymatic methylation via the universal methyl donor *S*-adenosylmethionine (SAM), thus reducing the amount of available SAM for DNA methylation reactions. Hence, epigenetically-induced deregulation of key signalling pathways can result from exposure to contaminants detected in the environment.

In addition to the significant effects of chemical exposures on the epigenome, diet-induced epigenetic alterations can also have severe, persistent health effects because the activities of

epigenetically modifying and de-modifying enzymes depend upon levels of cofactors and metabolites present in the intra- and extra-cellular environment [10,44]. Several studies have demonstrated that alterations in the levels of nutrients such as folate, choline and methionine in diet or alterations to other components of the one-carbon cycle such as the methyl-donor SAM can also have significant health effects by inducing aberrant DNA methylation. In these studies, a link between diets deficient in choline or other primary methyl donors, DNA hypomethylation and development of tumours in rodents, humans and fish has been established [4–6,49–54].

### 5. Epigenetic memory and its link with adult-onset of disease

There are two DNA methylation re-programming events associated with development in mammals. During embryogenesis, there is susceptibility to modifications due to environmental stressors that can influence phenotype and potential disease later in life. A second stage re-programming in germ cells of the F1 generation, at least in mammals, is also susceptible to interference that could influence phenotype but also can involve modifications to genes such as those that are imprinted and that influence sex determination. Disruption of epigenetic mechanisms during these two critical stages of development has been recognised, in part, as a factor affecting development of certain adulthood phenotypes long after the stimulating factor has been removed. These include linkage of various cancers, diabetes, obesity and behavioural and neurodegenerative disorders with environmental factors encountered during prenatal and early post-natal periods in mammals. This is known as “foetal basis or early-life programming of adult-onset of disease” [15,55–58]. The epigenetic marks inflicted upon the genome by environmental factors very early on during an individual’s life act as an “epigenetic memory” of the exposure. These epigenetic memories can manifest in adults as a pathological phenotype often following a secondary trigger such as ageing or changes in hormone levels. For example, in the 1950s diethylstilbestrol (DES) was used during pregnancy to prevent spontaneous abortions. DNA methylation changes induced by this agent during embryogenesis have been identified as the cause of a range of disorders such as increased breast and testicular cancer in adult female and male offspring [59]. Exposure to oestrogens and a range of endocrine disruptors in early development has been shown to predispose to cancer in rodents and humans [60–62], alter hormonal responses later in life in the frog (*Xenopus laevis*) [63] and cause a range of reproductive and behavioural effects in rodents (reviewed in [34–36]). Specifically, methylation changes caused by diethylstilbestrol to *c-fos* and *lactoferrin* genes at the neonatal stage in mice contribute to an increased incidence of uterine cancer [64].

Studies using the agouti ( $A^{vy}$ ) mouse model have clearly demonstrated a link between adult onset of disease, DNA methylation-induced changes in the activator binding protein–intra-cisternal A particle (IAP) transposon located in the *Avy* allele during embryogenesis and environmental exposure of the gestating female mice [56,65,66]. For example dietary uptake of genistein in gestating agouti mice, at levels comparable to the diet of a human with high soy consumption, results in hypermethylation of the  $A^{vy}$  allele and generation of pseudo-agouti offspring with a lower risk of development of obesity in adulthood [66].

Furthermore in our recent publications [54,67] we established a link between exposure to marine pollutants, alteration in DNA methylation patterns and liver tumours dissected from the flatfish dab (*Limanda limanda*) captured from waters around the UK. Based on the finding of significant epigenetic modifications and

disruption of metabolites of the 1-carbon pathway in non-tumour hepatic tissue of adenoma-bearing fish, our study lends support to the epigenetic progenitor model of cancer. Disruption of epigenetic mechanisms can cause stable, heritable changes in gene expression that are independent from mutations. These changes can occur prior to mutations [68]. Therefore, in this model, it is proposed that epigenetic changes inflicted as a result of chemical exposures can initiate carcinogenesis. Thus epigenetic “memory” may predispose to cancer highlighting the significant impacts that disruption of epigenetic mechanisms can have on the health of an exposed individual. In the light of this, the value of assessment of epigenetic changes in environmental biomonitoring and in the early detection of adverse health effects becomes evident. Importantly, even minor epigenetic changes as a response to environmental factors can accumulate over time, leading to gradual alteration of the phenotype [7]. However, it is important to bear in mind that establishing a cause- and effect-relationship between exposure to environmental factors, changes in the epigenome and disease is challenging.

### 6. Transgenerational epigenetic “memory”

Perhaps the most concerning aspect of epigenetic modulation by environmental toxicants is the potential for modulation of the programming of the germ line, causing a transgenerational “memory” (Fig. 1) [58,69]. Transmission of a phenotype to a following generation is accomplished through germ lines. Therefore environmentally induced-epigenetic changes in imprinted genes during the re-programming at the critical germ cell stage can influence both sex determination and can potentially be inherited leading to transgenerational modifications. During the germ cell re-programming event in mammals, most epigenetic marks are removed and reset in a gender-dependent manner. Certain epigenetic marks that are sex-specific, such as imprinted genes established during germ cell reprogramming, escape the second wave of DNA methylation changes that occur in pre-implantation embryos [2,70]. Transgenerational epigenetic inheritance or the epigenetic basis for inheritance of a trait [56,71] provides a further aspect of “epigenetic memory” relevant to this review.

Importantly, it is necessary to distinguish between epigenetic transgenerational effect and epigenetic transgenerational inheritance. The former is a broad term incorporating all phenotypes in following generations that are not genetically determined [69]. For example, stressed female rats have a reduced maternal licking/grooming and arched back nursing (LG-ABN) behaviour towards their neonates. A lower level of LG-ABN behaviour results in epigenetic silencing of the glucocorticoid receptor (*GR*) gene, resulting in fearful behaviour in the F2 generation, and persistence and transfer of the phenotype to subsequent generations. The acquired phenotype is epigenetically maintained in multiple generations. However, cross nurturing of the F3 generation born from the low LG-ABN F2 group results in hypomethylation of the *GR* gene and normal LG-ABN behaviour in these mice [72–74]. Therefore, transfer of the acquired epigenetic phenotype is not through gametes and it is dependent upon consistency of the environmental condition [70]. In contrast, transgenerational epigenetic inheritance relies on inheritance of the epigenetically acquired phenotype through gametes. This requires persistence of epigenetic marks through the reprogramming events. [75]. Also important is the realisation that the DNA re-methylation of germ cells is influenced by the microenvironment afforded by the surrounding somatic cells and by signals received directly from environmental factors. As a consequence, environmental factors can both directly or indirectly influence the re-methylation of germ cells [76].

In viviparous species, for a biological trait to be categorised as inherited, the phenotype must be maintained to at least the F3 generation. This is because exposure of the gestating female (F0) to chemicals that modify the epigenome could result in simultaneous direct exposure of the developing embryo (F1) and the developing germ line of the embryo (F2) [56,76]. Several studies have demonstrated the possibility of epigenetic inheritance of a phenotype in multiple generations in mammals, plants and flies (for reviews see [58,69]). Of particular interest is the demonstration that, following intra-peritoneal exposure of gestating outbred Harlan Sprague-Dawley female rats to the anti-androgenic endocrine disrupter vinclozolin (100 mg/kg body weight (bw)/day), epigenetically male germ cell transmitted phenotypic characteristics are induced during critical stages of sex determination (E12–E15), up to at least the F3 generation in male rat offspring. The characteristics include, for instance, increased spermatogenic cell apoptosis, decreased sperm motility and numbers, prostate abnormalities, tumours and renal lesions. The reproducibility and frequency of the vinclozolin-induced phenotypes (i.e. rate of tumours) and identification of genes with altered methylation in the affected individuals compared to controls indicated that mutations are not the most likely cause of this abnormality [60,75–80]. Furthermore, using a rat model exposed to several chemicals, it has been demonstrated that the ovarian disease can be epigenetically inherited across several generations [81]. However, it has been demonstrated that the effect and epigenetic inheritance of changes induced by vinclozolin are highly dependent on dose [58], animal strain [82] and route of exposure [83]. Oral administration of vinclozolin (100 mg/kg bw/day) in outbred Wistar rats [83] and intra-peritoneal (IP) injection of vinclozolin (100 mg/kg bw/day) in inbred CD-Sprague Dawley rats [82] during the sex determination stage failed to induce inherited phenotypic effects. However, there is a great deal of controversy surrounding this area of research. For example, a recent publication failed to demonstrate any transgenerational inheritance after IP administration of vinclozolin (0, 4, 100 mg/kg bw/d) on gestation days 6–15 in outbred Wistar rats [84].

In contrast to various studies in rodents, transgenerational epigenetics remains a fairly unexplored field in other species. Epigenetic changes have been studied in two generations of the water flea (*Daphnia magna*) following exposure to a range of environmentally relevant compounds ([85–88], reviewed in [89]). 5-Azacytidine, a demethylating drug, induced significant DNA hypomethylation in non-directly exposed F1 and F2 *D. magna* offspring as well as the exposed F0 generation [88]. Although it is possible that these effects are epigenetically inherited, *D. magna* reproduces both sexually and asexually and therefore observation of similar changes in the phenotypes in F3 generation are required prior to concluding that the observed effects as truly transgenerational [90].

In the non-eutherian fish, only the F0 and the gamete/oocyte of the F1 generation are directly exposed. Therefore, after elimination of the potential exposure directly to the eggs following spawning, it would be possible to categorise any epigenetically inherited phenotype in the F2 generation as a true transgenerational epigenetic effect. Being non-eutherian, fish do not require imprinting to prevent direct “parent conflict” and it is not known if the reprogramming event in germ cells during the sex determination stage (a critical stage for transgenerational effects in mammals) occurs in fish. This is not to say that methylation does not play a role during sex determination in fish since the regulation of aromatase, involved in sex determination in fish, is partly through DNA methylation [37,38]. Furthermore the methylation, and as a result the expression of this gene, is sensitive to compounds such as 17 $\alpha$ -ethinylestradiol [38]. Thus, the existence of the environmentally-sensitive differentially methylated aromatase

gene in male and female fish further justifies the investigation of transgenerational epigenetic mechanisms and the occurrence of DNA methylation reprogramming during the sex determination stage in non-mammalian species.

## 7. Implications of “epigenetic memory” for chemical safety assessment and environmental biomonitoring and future directions

In the context of toxicity testing, whether in laboratory animals to assess the risk to humans or in species relevant to the natural environment, epigenetic changes are not currently a standard feature of safety assessment. Part of the reason for this is the inability to interpret the findings in relation to potential adverse outcome without a more complete knowledge of the fundamental relationships between specific changes and disease. However, the evidence for changes in DNA methylation in early development influencing disease, including cancer, in later life or beyond into subsequent generations should be borne in mind.

Currently, standard carcinogenicity testing does not usually include exposure to the test compound during early development. The evidence accruing for the contribution of change in DNA methylation to cancers produced by numerous non-genotoxic and genotoxic carcinogens [91–93] and the established carcinogenic effect of DNA methylation changes following deficiencies in choline and other primary methyl-donors [49–52], indicates that such potential mechanisms should not be ignored. However, unlike the assumptions made about genotoxic carcinogens, a pragmatic threshold level of exposure to non-genotoxic carcinogens or dietary deficiency may be required for a clear impact on cancer development.

The fact that methylation of CpG islands increases the rate of mutations at these sites by around 10-fold [94] raises implications not only for cancer but also for reproductive deficiencies that could impact on ecosystems [95]. Moreover, environmentally-induced alterations in the methylation of sex determining genes may have a significant impact on the population and health of species (e.g. impaired rate of fertilisation). As noted above, although so far only demonstrated in laboratory maintained and in farmed fish, the ratio of fish embryos that develop into female or male fish can be influenced by the methylation level of the aromatase gene [37]. Therefore it is possible that environmentally-induced DNA methylation changes, as well as contributing towards adaptive responses of various species to their ever changing environment and species biodiversity, can have a significant contribution towards some of the adverse effects observed in response to environmental stressors immediately or at a later stage in life (a concept referred to as epigenetic predisposition and adult-onset of disease). For example, epigenetic mechanisms, as well as single nucleotide polymorphisms (SNPs), can prove to contribute to the mechanisms involved in the sex differentiation and sex ratio shifts observed in response to changes in the temperature of the water and the plasticity of this response in wild sea bass from different geographical locations.

In addition to the concerns about the potential of pollutant-induced epigenetic modulation to impact on disease and biodiversity, those modulations that are persistent offer a unique opportunity to provide a reflection of an organism's prior exposure to factors influencing the epigenome. This fact is well established through the discovery of differential epigenetic changes that develop in genetically identical human twins (see above). Such persistent modifications thus provide the exciting opportunity to assist in retrospective environmental monitoring of organisms to such exposures including pollutants. We propose that epigenetic “foot-printing” of organisms could identify classes of chemical

contaminants to which they have been exposed throughout their life-time. Such epigenetic variability has been shown to occur in natural populations of the clonal fish *Chrosomus eos-neogaeus* [39]. The adaptive changes observed can help identify populations vulnerable to environmental change. To study this effectively, genetically identical organisms can provide a stable background upon which accumulation of epigenetic changes can be measured and we are currently assessing this in clonal vertebrate and invertebrate species.

The classical ecological endpoints for ecotoxicology may not be sufficient in that, as mentioned above, early life stage changes in the epigenome following acute exposure have the potential to cause disease later in life and potentially through generations. This raises the possibility of benefits of investigating epigenetic marks in the context of environmental monitoring and ecotoxicological assessments. A limitation of the current practices for ecotoxicological assessments and a major difficulty for regulators in the context of water quality regulations such as the EU Water Framework Directive [96], is the inability to make assessments of exposures and their effects other than through “snap-shot”, expensive analyses of organism biodiversity, individual organism health, chemical measurements and a few biomarkers of limited diagnostic value. We propose a novel mechanism that allows researchers to retrospectively interrogate exposure history and chemical effects on the epigenome, thus potentially helping to provide a mechanism-based assessment of the quality and impact of the environment. The epigenetic “memory” can inform on the life-time exposure to stressors that modify the epigenome (Fig. 1). Irrespective of whether such changes may be indicative of toxicity per se, the signature has the potential to act as a surrogate for assessment of toxic exposures and other environmental stressors that could manifest as disease through alternative mechanisms for the same agents. For example, oxidative stress is known to be caused by a range of organic chemicals and metals and is frequently detected in animals exposed to aquatic pollutants [97]. Oxidative stress can lead to DNA hypomethylation through the inhibition of methyltransferases [7] and this may, at least in part, explain the changes in DNA methylation in the liver of fish [98] and in *Daphnia* [85–88] treated with certain metals. A good example of the potential use of methylation in environmental monitoring comes from the effects of endocrine disruptors. The 5' flanking region of the un-transcribed vitellogenin (*vtg1*) gene in the brain of the male and female adult zebrafish and the liver of the male adult zebrafish is highly hypermethylated compared to the *vtg1* in the liver of female zebrafish, where it is very highly expressed in response to endogenous oestrogen during oocytogenesis. [99]. *Vtg1* is not expressed normally in the liver of male fish but, upon exposure to exogenous estrogenic compounds, *vtg1* becomes highly expressed and is associated with feminisation of male fish. Therefore, expression of vitellogenin protein in male fish is used as a standard biomarker of exposure to oestrogens [100]. It has been shown that the 5' flanking region of the *vtg1* gene in male fish exposed to oestrogens is significantly hypomethylated, corresponding with its induction [99]. Therefore, it is reasonable to suggest that DNA methylation changes have biomarker potential. There is also the possibility that a specific pattern of gene methylation represents an indicator of the type of exposures. Although the degree and mechanisms of specificity are not established, there is evidence to suggest its occurrence. For example, phenobarbital is a non-genotoxic compound that can cause tumours through the constitutive androstane receptor pathway (CAR) in the liver of treated rodents. Lempiainen et al., [101] demonstrated that treatment with phenobarbital causes non-random and tissue-specific changes in DNA methylation and transcription. While Cyp2b10, one of the main genes affected through the CAR pathway, was hypomethylated and over-expressed in the liver of treated mice, it was not affected in non-tumour bearing kidney. Furthermore, there was

no significant overlap between DNA methylation or transcriptional changes observed in the liver and kidney of the treated mice. Several other studies have also indicated that at various stages of phenobarbital-induced tumorigenesis DNA methylation and transcription changes are non-random (in the absence or presence of mutagenic compounds) [102–104]. Further evidence on the specificity of the DNA methylation profile on the causative agent comes from a study demonstrating that hepatocellular carcinoma induced by hepatitis C virus (HCV) or hepatitis B virus (HBV) have different methylation profiles [105]. These studies all support the idea that each category of compounds can give rise to a specific DNA methylation “footprint” which will not only inform on the potential for adverse effects during life-time, but most importantly will also establish and introduce a novel retrospective and predictive monitoring tool to assess environmental quality. Therefore, in the same way that genetic polymorphisms can influence the susceptibility of individual organisms to toxicity, differences in the epigenome that emerge throughout the life-time of an individual may also have the same effects [12]. Indeed, modulation of methylation as a result of environmental stresses such as metals, could, through selection, afford local populations of organisms a survival advantage through consequent advantageous gene expression changes [8]. However, in identification of suitable epigenetic biomarkers, it is important to differentiate between the initial DNA methylation changes (referred to as driver methylation) and the DNA methylation changes that are triggered as a consequence of a change (e.g. formation of tumours). The latter is referred to as passenger methylation [25] and is less relevant to environmental monitoring. Furthermore, it is important to bear in mind that some DNA methylation changes may simply be biomarkers indicative of exposure rather than necessarily predictive of an adverse effect [91].

Furthermore, the field of paleoecology (resurrection ecology) may provide a unique opportunity for investigating the role of epigenetic mechanisms in population diversity and adaptation to environmental changes. *Daphnia* species can produce diapausing eggs viable for decades [8]. Sampling of these eggs from sediment cores from ponds and lakes, alongside sediment chemistry data, provide a unique opportunity for unveiling ecological and evolutionary changes as a result of environmental changes throughout several decades [106,107]. In a review by Eads et al. [108], several studies have been highlighted looking at phenotypic plasticity and genetic difference as a result of environmental changes (e.g. chemicals, introduction of a new predator) using *Daphnia* resting eggs dating back several decades. However, the focus of the majority of such studies is genomic variation (e.g. copy number variation) and the relationship to phenotypic plasticity and adaptation. Considering the well-established role of epigenetic mechanisms in regulating the responses of organisms to environmental factors, adaptation and evolution [9,109–112], it seems highly possible that epigenetic mechanisms have a substantial role in the evolution and adaptation that are observed throughout decades, for example in the resting eggs of *Daphnia*. Certainly, the resting eggs provide an exceptional opportunity for studying the concept of epigenetically-driven phenotypic evolution and adaptation.

In conclusion, the advances in knowledge of epigenetic mechanisms and the potential for such changes to persist throughout life-time, and even beyond into subsequent generations, give concern as to whether such changes imposed by environmental stressors impact on health despite beneficial adaptive consequences. There are opportunities to exploit epigenetic memory, using already available techniques such as bisulfite and modified bisulfite sequencing, as a reflection of prior stressor exposures and it is anticipated that, with improved knowledge, such changes will become an important component of safety assessment and in environmental monitoring.

## Conflict of interest

The authors declare that there are no conflicts of interest.

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## References

- [1] M. Esteller, J.G. Heman, Cancer as an epigenetic disease: DNA methylation and chromatin alterations in human tumours, *J. Pathol.* 196 (2002) 1–7.
- [2] E. Kriukiene, Z. Liutkeviciute, S. Klimasauskas, 5-Hydroxymethylcytosine: the elusive epigenetic mark in mammalian DNA, *Chem. Soc. Rev.* 41 (2012) 6916–6930.
- [3] T.Y. Xing, W. Ding, Recent advance in DNA epigenetic modifications—the sixth base in the genome, *Sheng Li Ke Xue Jin Zhan* 43 (2012) 164–1670.
- [4] J.-L. Gueant, F. Namour, R.-M. Gueant-Rodriguez, J.-L. Daval, Floate and fetal programming: a play in epigenetics? *Trends Endocrinol. Metab.* (2013), <http://dx.doi.org/10.1016/j.tem.2013.01.010>.
- [5] P. Dominguez-Salas, S.E. Cox, A.M. Prentice, B.J. Hennig, S.E. Moore, Maternal nutritional status, C1 metabolism and offspring DNA methylation: a review of current evidence in human subjects, *Proc. Natl. Acad. Sci.* 71 (2012) 154–165.
- [6] J.A. Alegria-Torres, A. Baccarelli, V. Bollati, Epigenetics and lifestyle, *Epigenomics* 3 (2011) 267–277.
- [7] A. Baccarelli, V. Bollati, Epigenetics and environmental chemicals, *Curr. Opin. Pediatr.* 21 (2009) 243–251.
- [8] A.J. Morgan, P. Kille, S.R. Sturzenbaum, Microevolution and ecotoxicology of metals in invertebrates, *Environ. Sci. Technol.* 41 (2007) 1085–1096.
- [9] K.B. Flores, F. Wolschin, G.V. Amdam, The role of methylation of DNA in environmental adaptation, *Integr. Comp. Biol.* (2013), PMID: 23620251.
- [10] B.M. Turner, Epigenetic responses to environmental change and their evolutionary implications, *Philos. Trans. R. Soc. Lond. B: Biol. Sci.* 364 (2009) 3403–3418.
- [11] D.C. Dolinoy, R. Das, J.R. Weidman, R.L. Jirtle, Metastable epialleles, imprinting, and the foetal origins of adult diseases, *Pediatr. Res.* 61 (2007) 30–37.
- [12] B.C. Christensen, E. Andres Houseman, C.J. Marsit, S. Zheng, M.R. Wrensch, J.L. Wiemels, H.H. Nelson, M.R. Karagas, J.F. Padbury, R. Bueno, D.J. Sugarbaker, R. Yeh, J.K. Wiencke, K.T. Kelsey, Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context, *PLOS Genet.* 5 (2009) 1–13.
- [13] E. Gomez-Diaz, M. Jorda, M.A. Peinado, A. Rivero, Epigenetics of host–pathogen interactions: the road ahead and the road behind, *PLoS Pathog.* 8 (2012) e1003007.
- [14] G. Egger, G. Liang, A. Aparicio, P.A. Jones, Epigenetics in human disease and prospects for epigenetic therapy, *Nature* 429 (2004) 457–460.
- [15] R. Jirtle, A. Bird, Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals, *Nat. Genet.* 33 (2003) 245–254.
- [16] B.M. Turner, Defining an epigenetic code, *Nat. Cell Biol.* 9 (2007) 2–6.
- [17] T.A. Chan, S. Glockner, J.M. Yi, W. Chen, L. Van Neste, L. Cope, J.G. Herman, V. Velculescu, K.E. Schuebel, N. Ahuja, S.B. Baylin, Convergence of mutation and epigenetic alterations identifies common genes in cancer that predict for poor prognosis, *PLOS Med.* 5 (2008) 823–838.
- [18] P. Cui, L. Zhang, Q. Lin, F. Ding, C. Xin, X. Fang, S. Hu, J. Yu, A novel mechanism of epigenetic regulation: nucleosome-space occupancy, *Biochem. Biophys. Res. Commun.* 391 (2010) 884–889.
- [19] A.V. Probst, E. Dunleavy, G. Almouzni, Epigenetic inheritance during the cell cycle, *Nat. Rev. Mol. Cell Biol.* 10 (2009) 192–206.
- [20] I.P. Pogribny, Epigenetic events in tumorigenesis: putting the pieces together, *Exp. Oncol.* 32 (2010) 132–136.
- [21] K. Gronbaek, C. Hother, P.A. Jones, Epigenetic changes in cancer, *Agric. Prod. Market Inform. Syst.* 115 (2007) 1039–1059.
- [22] J.P. Thomson, P.J. Skene, J. Selfridge, T. Clouaire, J. Guy, S. Webb, A.R.W. Kerr, A. Deaton, R. Andrews, K.D. James, D.J. Turner, R. Illingworth, A. Bird, CpG islands influence chromatin structure via the CpG-binding protein Cfp1, *Nature* 464 (2010) 1082–1087.
- [23] J.S. Butler, J.H. Lee, D.G. Skalnik, CFP1 interacts with DNMT1 independently of association with the Setd1 histone H3K4 methyltransferase complexes, *DNA Cell Biol.* 27 (2008) 533–543.
- [24] C.M. Tate, J. Lee, D.G. Skalnik, CXXC finger protein 1 restricts the Setd1A histone H3K4 methyltransferase complex to euchromatin, *FASEB J.* 27 (2010) 210–223.
- [25] Z. Herceg, T. Vaissiere, Epigenetic mechanisms and cancer. An interface between the environment and the genome, *Epigenetics* 6 (2011) 804–819.
- [26] M. Tahiliani, K. Peng Koh, Y. Shen, W.A. Pastor, H. Bandukwala, Y. Brudno, S. Agarwal, L.M. Iyer, D.R. Liu, L. Aravind, A. Rao, Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1, *Science* 324 (2009) 930–935.
- [27] K. Williams, J. Christensen, M. Terndrup Pedersen, J.V. Johansen, P.A.C. Cloos, J. Rappilber, K. Helin, TET1 and hydroxymethylcytosine in transcription and DNA methylation fidelity, *Nature* 473 (2011) 343–349.
- [28] A. Bird, DNA methylation patterns and epigenetic memory, *Genes Dev.* 16 (2002) 6–21.
- [29] R. Holliday, DNA methylation and epigenotypes, *Biochemistry (Mosc.)* 70 (2005) 500–504.
- [30] M. Godmann, R. Lambrot, S. Kimmins, The dynamic epigenetic program in male germ cells: its role in spermatogenesis, testis cancer, and its response to the environment, *Microsc. Res. Tech.* 72 (2009) 603–619.
- [31] Y. He, S.D. Michaels, R.M. Amasino, Regulation of flowering time by histone acetylation in *Arabidopsis*, *Science* 302 (2003) 1751–1754.
- [32] R. Bastow, J.S. Mylne, C. Lister, Z. Lippman, R.A. Martienssen, C. Dean, Vernalization requires epigenetic silencing of FLC by histone methylation, *Nature* 427 (2004) 164–167.
- [33] J.E. Burn, D.J. Bagnall, J.D. Metzger, E.S. Dennis, W.J. Peacock, DNA methylation, vernalization, and the initiation of flowering, *Proc. Natl. Acad. Sci.* 90 (1993) 287–291.
- [34] M.E. Wilson, T. Sengoku, Developmental regulation of neuronal genes by DNA methylation: environmental influences, *Int. J. Dev. Neurosci.* (2013), PMID: 23501000.
- [35] D. Crews, Epigenetics, brain, behaviour, and the environment, *Hormones (Athens)* 9 (2010) 41–50.
- [36] M.M. McCarthy, D. Crews, Epigenetics—new frontiers in neuroendocrinology, *Front. Neuroendocrinol.* 29 (2008) 341–343.
- [37] L. Navarro-Martin, J. Vinas, L. Ribas, N. Diaz, A. Gutierrez, L. Di Croce, F. Piferrer, DNA methylation of the gonadal aromatase (cyp19a) promoter is involved in temperature-dependent sex ratio shifts in the European sea bass, *PLOS Genet.* 7 (2011) 1–15.
- [38] R.G. Contractor, C.M. Foran, S. Li, K.L. Willett, Evidence of gender- and tissue-specific promoter methylation and the potential for ethinylestradiol induced changes in Japanese medaka (*Oryzias latipes*) estrogen receptor and aromatase genes, *Environ. Health* 67 (2004) 1–22.
- [39] R. Massicotte, E. Whitelaw, B. Angers, DNA methylation. A source of random variation in natural populations, *Epigenetics* 6 (2011) 421–427.
- [40] F. Lyko, S. Foret, R. Kucharski, S. Wolf, C. Falckenhayn, R. Maleszka, The honey bee epigenomes: differential methylation of Bbrain DNA in queens and workers, *PLoS Biol.* 8 (2010) 1–12.
- [41] P. Moran, A. Perez-Figueroa, Methylation changes associated with early maturation stages in the Atlantic salmon, *BMC Genet.* 12 (2011) 1–8.
- [42] M.F. Fraga, E. Ballester, M.F. Paz, S. Ropero, F. Setien, M.L. Ballestar, D. Heine-Suner, J.C. Cigudose, M. Urioste, J. Benitez, M. Boix-Chornet, A. Sanchez-Aguilera, C. Ling, E. Carlsson, P. Poulsen, A. Vaag, Z. Stephan, T.D. Spector, Y. Wu, C. Plass, M. Esteller, Epigenetic differences arise during the lifetime of monozygotic twins, *Proc. Natl. Acad. Sci.* 102 (2005) 10604–10609.
- [43] I.P. Pogribny, F.A. Beland, DNA methylome alterations in chemical carcinogenesis, *Cancer Lett.* 334 (2013) 39–45.
- [44] V.K. Cortessis, D.C. Thomas, A.J. Levine, C.V. Breton, T.M. Mack, K.D. Siegmund, R.W. Haile, P.W. Laird, Environmental epigenetics: prospects for studying epigenetic mediation of exposure–response relationships, *Hum. Genet.* 131 (2012) 1565–1589.
- [45] R. Martinez-Zamudio, H.C. Ha, Environmental epigenetics in metal exposure, *Epigenetics* 6 (2011) 820–827.
- [46] A.C. Gore, Developmental programming and endocrine disruptor effects on reproductive neuroendocrine systems, *Front. Neuroendocrinol.* 29 (2008) 358–374.
- [47] D. Crews, Epigenetics and its implications for behavioral neuroendocrinology, *Front. Neuroendocrinol.* 29 (2008) 344–357.
- [48] S. Ho, W. Tang, J.B. de Frausto, G.S. Prins, Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4, *Cancer Res.* 66 (2006) 5624–5632.
- [49] V. Michel, Z. Yuan, S. Ramsuvar, M. Bakovic, Choline transport for phospholipid synthesis, *Exp. Biol. Med.* 231 (2006) 490–504.
- [50] J. Locker, T.V. Reddy, B. Lombardi, DNA methylation and hepatocarcinogenesis in rats fed a choline-devoid diet, *Carcinogenesis* 7 (1986) 1309–1312.
- [51] M.J. Wilson, N. Shivapurkar, L.A. Poirier, Hypomethylation of hepatic nuclear DNA in rats fed with a carcinogenic methyl-deficient diet, *Biochem. J.* 218 (1984) 987–990.
- [52] A.K. Ghoshal, E. Farber, The induction of liver cancer by dietary deficiency of choline and methionine without added carcinogens, *Carcinogenesis* 5 (1984) 1367–1370.
- [53] S. Sibani, S. Melnyk, I.P. Pogribny, W. Wang, F. Hiou-Tim, L. Deng, J. Trasler, S.J. James, R. Rozen, Studies of methionine cycle intermediates (SAM, SAH), DNA methylation and the impact of folate deficiency on tumour numbers in Min mice, *Carcinogenesis* 23 (2002) 61–65.
- [54] L. Mirbahai, A.D. Southam, U. Sommer, T.D. Williams, J.P. Bignell, B.P. Lyons, M.R. Viant, J.K. Chipman, Disruption of DNA methylation via S-adenosylhomocysteine is a key process in high incidence liver carcinogenesis in fish, *J. Proteome Res.* (2013), PMID: 23611792.
- [55] S.P. Barros, S. Offenbacher, Epigenetics: connecting environment and genotype to phenotype and disease, *J. Dent. Res.* 88 (2009) 400–408.
- [56] R.L. Jirtle, M.K. Skinner, Environmental epigenomics and disease susceptibility, *Nat. Rev. Genet.* 8 (2007) 253–262.
- [57] S. Li, S.D. Hursting, B.J. Davis, J.A. McLachlan, J.C. Barrett, Environmental exposure, DNA methylation, and gene regulation: lessons from

- diethylstilbesterol-induced cancers, *Ann. N. Y. Acad. Sci.* 983 (2003) 161–169.
- [58] M.K. Skinner, M. Manikkam, C. Guerrero-Bosagna, Epigenetic transgenerational actions of environmental factors in disease etiology, *Trends Endocrinol. Metab.* 21 (2010) 214–222.
- [59] G.S. Prins, Estrogen imprinting: when your epigenetic memories come back to haunt you, *Endocrinology* 149 (2008) 5919–5921.
- [60] M.D. Anway, M.A. Memon, M. Uzumcu, M.K. Skinner, Transgenerational effect of the endocrine disruptor vinclozolin on male spermatogenesis, *J. Androl.* 27 (2006) 868–879.
- [61] R.R. Newbold, R.B. Hanson, W.N. Jefferson, B.C. Bullock, J. Haseman, J.A. McLachlan, Proliferative lesions and reproductive tract tumours in male descendants of mice exposed developmentally to diethylstilbesterol, *Carcinogenesis* 21 (2000) 1355–1363.
- [62] G.S. Prins, L. Birch, W.-Y. Tang, S.-M. Ho, Developmental estrogen exposures predispose to prostate carcinogenesis with aging, *Reprod. Toxicol.* 23 (2007) 374–382.
- [63] A. Andres, D.B. Mueller, G.U. Ryffel, Persistence, methylation and expression of vitellogenin gene derivatives after injection into fertilized eggs of *Xenopus laevis*, *Nucleic Acids Res.* 12 (1984) 2283–2302.
- [64] S. Li, K.A. Washburn, R. Moore, T. Uno, C. Teng, R.R. Newbold, J.A. McLachlan, M. Negishi, Developmental exposure to diethylstilbesterol elicits demethylation of estrogen-responsive lactoferrin gene in mouse uterus, *Cancer Res.* 57 (1997) 4356–4359.
- [65] D.C. Dolinoy, The agouti mouse model: an epigenetic biosensor for nutritional and environmental alterations on the foetal epigenome, *Nutr. Rev.* 66 (2008) 1–8.
- [66] D.C. Dolinoy, J. Wiedman, R. Waterland, R.L. Jirtle, Maternal genistein alters coat colour and protects Avy mouse offspring from obesity by modifying the foetal epigenome, *Environ. Health Perspect.* 114 (2006) 567–572.
- [67] L. Mirbahai, G. Yin, J.P. Bignell, N. Li, T.D. Williams, J.K. Chipman, DNA methylation in liver tumourigenesis in fish from the environment, *Epigenetics* 6 (2011) 1319–1333.
- [68] A.P. Feinberg, R. Ohlsson, S. Henikoff, The epigenetic progenitor origin of human cancer, *Nat. Rev. Genet.* 7 (2006) 21–33.
- [69] N.A. Youngson, E. Whitelaw, Transgenerational epigenetic effects, *Annu. Rev. Genomics Hum. Genet.* 9 (2008) 233–257.
- [70] W. Reik, J. Walter, Evolution of imprinting mechanisms: the battle of the sexes begins in the zygote, *Nat. Genet.* 27 (2001) 255–256.
- [71] N.C. Whitelaw, E. Whitelaw, Transgenerational epigenetic inheritance in health and disease, *Curr. Opin. Genet. Dev.* 18 (2008) 273–279.
- [72] D. Francis, J. Diorio, D. Liu, M.J. Meaney, Nongenomic transmission across generations of maternal behaviour and stress responses in the rat, *Science* 286 (1999) 1155–1158.
- [73] I.C.G. Weaver, N. Cervoni, F.A. Champagne, A.C. D' Alessio, S. Sharma, J.R. Seckl, S. Dymov, M. Szyf, M.J. Meaney, Epigenetic programming by maternal behaviour, *Nat. Neurosci.* 7 (2004) 847–854.
- [74] I.C.G. Weaver, A.C. D'Alessio, S.E. Brown, I.C. Hellstrom, S. Dymov, S. Sharma, M. Szyf, M.J. Meaney, The transcription factor nerve growth factor protein A mediates epigenetic programming: altering epigenetic marks by immediate-early genes, *J. Neurosci.* 27 (2007) 1756–1768.
- [75] M.D. Anway, A.S. Cupp, M. Uzumcu, M.K. Skinner, Epigenetic transgenerational actions of endocrine disruptors and male fertility, *Science* 308 (2005) 1466–1469.
- [76] M.D. Anway, M.K. Skinner, Epigenetic transgenerational actions of endocrine disruptors, *Endocrinology* 147 (2006) S43–S49.
- [77] M.D. Anway, M.K. Skinner, Transgenerational effects of the endocrine disruptor vinclozolin on the prostate transcriptome and adult onset disease, *Prostate* 68 (2008) 517–529.
- [78] M.D. Anway, S.S. Rekow, M.K. Skinner, Transgenerational epigenetic programming of the embryonic testis transcriptome, *Genomics* 91 (2008) 30–40.
- [79] M.D. Anway, S.S. Rekow, M.K. Skinner, Comparative anti-androgenic actions of vinclozolin and flutamide on transgenerational adult onset disease and spermatogenesis, *Reprod. Toxicol.* 26 (2008) 100–106.
- [80] C. Stouder, A. Paoloni-Giacobino, Transgenerational effects of the endocrine disruptor vinclozolin on the methylation pattern of imprinted genes in the mouse sperm, *Reproduction* 139 (2010) 373–379.
- [81] E. Nilson, G. Larsen, M. Manikkam, C. Guerrero-Bosagna, M.I. Savenkova, M.K. Skinner, Environmentally induced epigenetic transgenerational inheritance of ovarian disease, *PLoS ONE* 7 (2012) e36129.
- [82] K. Inawaka, M. Kawabe, S. Takahashi, Y. Doi, Y. Tomigahara, H. Tarui, J. Abe, S. Kawamura, T. Shirai, Maternal exposure to anti-androgenic compounds, vinclozolin, flutamide and procymidone, has no effects on spermatogenesis and DNA methylation in male rats of subsequent generations, *Toxicol. Appl. Pharmacol.* 237 (2009) 178–187.
- [83] S. Schneider, W. Kaufmann, R. Buesen, B. Van Ravenzwaay, Vinclozolin—the lack of a transgenerational effect after oral maternal exposure during organogenesis, *Reprod. Toxicol.* 25 (2008) 352–360.
- [84] S. Schneider, H. Marxfeld, S. Groters, R. Buesen, B. van Ravenzwaay, Vinclozolin—No transgenerational inheritance of anti-androgenic effects after maternal exposure during organogenesis via the intraperitoneal route, *Reprod. Toxicol.* 37 (2013) 6–14.
- [85] M.B. Vandegehuchte, D. De Coninck, T. Vandenbrouck, W.M. De Coen, C.R. Janssen, Gene transcription profiles, global DNA methylation and potential transgenerational epigenetic effects related to Zn exposure history in *Daphnia magna*, *Environ. Pollut.* 158 (2010) 3323–3329.
- [86] M.B. Vandegehuchte, T. Kyndt, B. Vanholme, A. Haegeman, G. Gheysen, C.R. Janssen, Occurrence of DNA methylation in *Daphnia magna* and influence of multigeneration Cd exposure, *Environ. Int.* 35 (2009) 700–706.
- [87] M.B. Vandegehuchte, F. Lemiere, C.R. Janssen, Quantitative DNA methylation in *Daphnia magna* and effects of multigeneration Zn exposure, *Comp. Biochem. Physiol.* 150 (2009) 343–348.
- [88] M.B. Vandegehuchte, F. Lemiere, L. Vanhaecke, W. Vanden Berghe, C.R. Janssen, Direct and transgenerational impact on *Daphnia magna* of chemicals with a known effect on DNA methylation, *Comp. Biochem. Physiol.* 151 (2010) 278–285.
- [89] M.B. Vandegehuchte, C.R. Janssen, Epigenetics and its implications for ecotoxicology, *Ecotoxicology* 20 (2011) 607–624.
- [90] K.D.M. Harris, N.J. Bartlett, K.L. Vett, *Daphnia* as an emerging epigenetic model organism, *Genet. Res. Int.* 2012 (2012) 1–8.
- [91] J.I. Goodman, K.A. Augustine, M.L. Cunningham, D. Dixon, Y.P. Dragan, J.G. Falls, R.J. Rasoulpour, R.C. Sills, R.D. Storer, D.C. Wolf, S.D. Pettit, What do we need to know prior to thinking about incorporating an epigenetic evaluation into safety assessments? *Toxicol. Sci.* 116 (2010) 375–381.
- [92] J.G. Mogg, J.I. Goodman, J.E. Trosko, R.A. Roberts, Epigenetics and cancer. Implications for drug discovery and safety assessment, *Toxicol. Appl. Pharmacol.* 196 (2004) 422–430.
- [93] J. Legler, Epigenetics: an emerging field in environmental toxicology, *Integr. Environ. Assess. Manag.* 6 (2010) 314–315.
- [94] J. Sved, A. Bird, The expected equilibrium of the CpG dinucleotide in vertebrate genomes under a mutation model, *Proc. Natl. Acad. Sci.* 87 (1990) 4692–4696.
- [95] C. Guerrero-Bosagna, P. Sabat, L. Valladares, Environmental signaling and evolutionary change: can exposure of pregnant mammals to environmental estrogens lead to epigenetically induced evolutionary changes in embryos? *Evol. Dev.* 7 (2005) 341–350.
- [96] <http://www.wfduk.org/faq/HowAreTheNewClassificationSystemsDifferentFromPreviousClassificationSystems>
- [97] I. Ahmad, M. Pacheco, M.A. Santos, *Anguilla anguilla* L., oxidative stress biomarkers: an in situ study of freshwater wetland ecosystem (Pateira de Fermentelos, Portugal), *Chemosphere* 65 (2006) 952–962.
- [98] X.W. Zhou, G.N. Zhu, M. Jilisa, J.H. Sun, Influence of Cu, Zn, Pb, Cd and their heavy metal ion mixture on the DNA methylation level of the fish (*Carassius auratus*), *J. Environ. Sci. (China)* 21 (2001) 549–552.
- [99] M. Stromqvist, N. Tooke, B. Brunstrom, DNA methylation levels in the 5' flanking region of the vitellogenin I gene in liver and brain of adult zebrafish (*Danio rerio*)-sex and tissue differences and effects of 17 $\alpha$ -ethinylestradiol exposure, *Aquat. Toxicol.* 98 (2010) 275–281.
- [100] A.P. Scott, M. Sanders, G.D. Stentiford, R.A. Reese, I. Katsiadaki, Evidence for estrogenic endocrine disruption in an offshore flatfish, the dab (*Limanda limanda*), *Mar. Environ. Res.* 64 (2007) 128–148.
- [101] H. Lempiainen, A. Muller, S. Brasa, S. Teo, T.C. Roloff, L. Morawiec, N. Zamurovic, A. Vicart, E. Funhoff, P. Couttet, D. Schubeler, O. Grenet, J. Marlowe, J. Mogg, R. Terranova, Phenobarbital mediates an epigenetic switch at the constitutive androstane receptor (CAR) target gene *Cyp2b10* in the liver of B6C3F1 mice, *PLoS ONE* 6 (2011) 1–14.
- [102] J.M. Phillips, L.D. Burgoon, J.I. Goodman, Phenobarbital elicits unique, early changes in the expression of hepatic genes that affect critical pathways in tumour-prone B6C3F1 mice, *Toxicol. Sci.* 109 (2009) 193–205.
- [103] J.M. Phillips, J.I. Goodman, Multiple genes exhibit phenobarbital-induced constitutive active/androstane receptor-mediated DNA methylation changes during liver tumourigenesis and in liver tumours, *Toxicol. Sci.* 108 (2009) 273–289.
- [104] A.N. Bachman, J.M. Phillips, J.I. Goodman, Phenobarbital induces progressive patterns of GC-rich and gene-specific altered DNA methylation in the liver of tumour-prone B6C3F1 mice, *Toxicol. Sci.* 91 (2006) 393–405.
- [105] Y. Pei, T. Zhang, V. Renault, X. Zhang, An overview of hepatocellular carcinoma study by omics-based methods, *Acta Biochim. Biophys. Sin. (Shanghai)* 41 (2009) 1–15.
- [106] N. Brede, C. Sandrock, D. Straile, P. Spaak, T. Jankowski, B. Streit, K. Schwenk, The impact of human-made ecological changes on the genetic architecture of *Daphnia* species, *Proc. Natl. Acad. Sci.* 106 (2009) 4758–4763.
- [107] W.C. Kerfoot, L.J. Weider, Experimental paleoecology (resurrection ecology): chasing Van Valen's Red Queen hypothesis, *Limnol. Oceanogr.* 49 (2004) 1300–1316.
- [108] B.D. Eads, J. Andrews, J.K. Colbourne, Ecological genomics in *Daphnia*: stress responses and environmental sex determination, *Heredity* 100 (2008) 184–190.
- [109] C. Furusawa, K. Kaneko, Epigenetic feedback regulation accelerates adaptation and evolution, *PLoS ONE* 8 (2013) e61251.
- [110] O. Pau, Bateman, R.M. Fay, M. Hedren, L. Civeyrel, M.W. Chase, Stable epigenetic effects impact adaptation in allopolyploid orchids (Dactylorhiza: Orchidaceae), *Mol. Biol. Evol.* 27 (2010) 2465–2473.
- [111] K. Brautigam, K.J. Vining, C. Lafon-Placet, C.G. Fossdal, M. Mirouze, J.G. Marcos, S. Fluch, M.F. Fraga, M.A. Guevara, D. Abarca, O. Johnsen, S. Maury, S.H. Strauss, M.M. Campbell, A. Rohde, C. Diaz-Sala, M.T. Cervera, Epigenetic regulation of adaptive responses of forest tree species to the environment, *Ecol. Evol.* 3 (2013) 399–415.
- [112] S. Hirsch, R. Baumberger, U. Grossniklaus, Epigenetic variation, inheritance, and selection in plant populations, *Cold Spring Harb. Symp. Quant. Biol.* (2013), PMID: 23619013.