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EPIGENETICS IN ECOLOGY AND EVOLUTION – A PRIMER

Linking epigenetics and biological conservation: Towards a *conservation epigenetics* perspective

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Abstract

1. Biodiversity conservation is a global issue where the challenge is to integrate all levels of biodiversity to ensure the long-term evolutionary potential and resilience of biological systems. Genetic approaches have largely contributed to conservation biology by defining "conservation entities" accounting for their evolutionary history and adaptive potential, the so-called *evolutionary significant units* (ESUs). Yet, these approaches only loosely integrate the short-term ecological history of organisms.
2. Here, we argue that epigenetic variation, and more particularly DNA methylation, represents a molecular component of biodiversity that directly links the genome to the environment. As such, it provides the required information on the ecological background of organisms for an integrative field of conservation biology.
3. We synthesize knowledge about the importance of epigenetic mechanisms in (a) orchestrating fundamental development alternatives in organisms, (b) enabling individuals to respond in real-time to selection pressures and (c) improving ecosystem stability and functioning.
4. Using practical examples in conservation biology, we illustrate the relevance of DNA methylation (a) as biomarkers of past and present environmental stress events as well as biomarkers of physiological conditions of individuals; (b) for documenting the ecological structuring/clustering of wild populations and hence for better integrating ecology into ESUs; (c) for improving conservation translocations; and (d) for studying landscape functional connectivity.
5. We conclude that an *epigenetic conservation* perspective will provide environmental managers the possibility to refine ESUs, to set conservation plans taking into account the capacity of organisms to rapidly cope with environmental changes, and hence to improve the conservation of wild populations.

KEYWORDS
conservation, DNA methylation, ecological timescales, epigenetic, evolutionary significant units

1 | INTRODUCTION

Preserving biodiversity is a global and challenging endeavour that relies on innovative approaches. Philosophically, biodiversity conservation has built on four (not mutually exclusive) pillars. First, biodiversity is the legacy of past evolutionary events. Second, biodiversity is the evolutionary fuel for biological systems to resist or be resilient to selection pressures and global change. Third, biodiversity mediates ecosystem functioning and hence services provided to humans. Finally, the current era is referred as the sixth mass extinction of biodiversity on Earth for which anthropogenic impacts are largely responsible (Leakey & Lewin, 1995). Biodiversity, in its conservation meaning, includes levels from genes to populations, species and ecosystems. It is now largely acknowledged that biodiversity conservation should not only focus on rare and iconic species, but also focus on ecosystems as whole unit on the one hand, and on genes as a key element of species' adaptability on the other hand (Eizaguirre & Baltazar-Soares, 2014). Specifically, a consensus has emerged, whereby species are not driven to extinction before genetic factors impact them (Spielman, Brook, & Frankham, 2004). Furthermore, we know rescuing mechanisms linked to plasticity and non-genetic inheritance are also important (e.g. Chevin, Gallet, Gomulkiewicz, Holt, & Fellous, 2013). Here, we define the adaptive potential as the ability of species/populations to respond to selection by means of molecular or phenotypic changes (Eizaguirre & Baltazar-Soares, 2014).

We advocate for biodiversity conservation to become more integrative, even if doing so presents a challenge to current policies (Corlett, 2017). In the last decades, the development of genetic and genomic approaches has revolutionized conservation biology. In particular, genetic tools allow conservation biologists to address key issues such as estimating demographic parameters and adaptive potential, characterizing population structure, delimiting taxonomic groups and *evolutionary significant units* (ESUs), and managing assisted gene flow and population rescue strategies (Eizaguirre & Baltazar-Soares, 2014; McMahon, Teeling, & Höglund, 2014; Shafer et al., 2015). Despite the undeniable input of these genetic tools in conservation biology, we can identify at least four major gaps: (a) conservation genetics studies are mainly based on neutral genetic variation and as such have little direct connection to any functional properties of populations; (b) the short-term interaction between individuals and their environment is mostly ignored because genetics usually represents the long-term history of populations; (c) the evolutionary potential relies on functional diversity that is inherited, but the non-genetic molecular mechanisms of inheritance are still little considered; and (d) the upscaling from genetics to genomics has not yet filled the gap to identify rapid molecular responses to be used in modern conservation.

Here, we argue that epigenetic marks will be useful in the coming future to fill those knowledge and practical gaps, and hence to reintegrate an ecological perspective to the ESU concept. In particular, epigenetic marks – more particularly DNA methylation – and developmental reprogrammation should be considered as an additional conservation level, a so-called *conservation epigenetics*. In fact, DNA methylation is sensitive to the environment and is involved in organisms' plastic and adaptive responses to changing environments. As such, DNA methylation affects ecological and evolutionary processes at all biological levels, from individuals (phenotypic variation) to the ecosystem level (Latzel et al., 2013). More generally, while the genetic background of species/populations mostly reflects their long-term demography and evolutionary history, DNA methylation patterns are more likely to reflect the short-term "ecological background" of individuals. We will first develop the main specificities of DNA methylation that we argue are particularly relevant in a conservation context. We will then outline ways that epigenetic tools should – and can – be practically implemented in biodiversity conservation.

2 | RELEVANCE OF EPIGENETICS IN A CONSERVATION CONTEXT

Epigenetics can be defined as the study of all reversible chemical changes involved in the regulation of gene products, and ultimately of phenotypes, that do not modify the nucleotide sequence of the DNA. So far, three main components for epigenetic information have been characterized including the methylation of nucleic acids (DNA and RNA), covalent modifications at histone tails and non-coding RNAs (Allis & Jenuwein, 2016). These epigenetic elements can act in conjunction with genetic information to modulate phenotypes during development (Allis & Jenuwein, 2016). Moreover, while some epigenetic patterns (i.e. epigenetic status at a given genomic location) are under genetic determinism (Box 1), some others are directly modulated by the surrounding environmental conditions (Feil & Fraga, 2012). Finally, the last decades have flourished with both empirical studies and theoretical models, showing that epimutations (i.e. changes in epigenetic state) can generate phenotypic variants including key morphological, physiological, behavioural and life-history traits upon which both natural selection and sexual selection can act (Danchin, Pocheville, Rey, Pujol, & Blanchet, 2018; Klironomos, Berg, & Collins, 2013; Pál & Miklós, 1999). We argue that the three main characteristics mentioned here make epigenetics particularly relevant in a biological conservation context, and this is what we develop in the next sections. We will specifically focus on DNA methylation since they are the most documented epigenetic marks so far and because more and more analytical and technical tools are being developed for studying DNA methylation patterns in natural populations (Table S1).

2.1 | Epigenetic mechanisms as orchestrators of developmental biology

The term epigenetics was first coined in the context of developmental biology to explain differentiation and maintenance of specialized somatic cells within organisms from a unique zygote (i.e. a unique

BOX 1 Source of epigenetic variation: why measuring epigenetic variation in conservation?

Natural epigenetic variation is increasingly reported in wild populations of both plants and animals (Hu & Barrett, 2017). Such variation (often exceeding genetic variation) relies on at least three main sources. First, epigenetic variation is – at least partly – genetically determined. In this regard, the overall epigenetic machineries including enzymes (e.g. dnmt1, dnmt3 and acetyl transferase) and proteins (e.g. Polycomb and Trithorax groups) involved in epigenetic modifications are encoded by specific genes. However, in spite of the numerous advances in determining the molecular mechanisms responsible of epigenetic variation, the genetic basis underlying epigenetic variation remains largely unknown (Taudt, Colomé-Tatché, & Johannes, 2016). Moreover, most of the studies deal with genetic model organisms including humans (e.g. Schmitz et al., 2013) and very few are known in the context of natural populations (Dubin et al., 2015). With the advent of molecular and analytical tools (Table S1), it is very likely that our knowledge on the relative contribution of genetic variation in shaping epigenetic variation in wild populations will increase in the near future.

Second, epigenetic variation may result from epigenetic modifications arising stochastically and irrespective of the surrounding environment (Feinberg & Irizarry, 2010). Such "epigenetic mutations" are known to be more common than genetic mutations and are reversible (Van Der Graaf et al., 2015). Interestingly, some emerging epigenetic modifications can be associated with adaptive phenotypes and hence contribute to the maintenance of populations in changing environments, at least over short term, and possibly over longer time-scales, if transmitted over generations (Feinberg & Irizarry, 2010). This source of adaptive epigenetic variation is particularly relevant in genetically depauperate populations, including small-sized and/or inbred isolated populations or in clonal organisms (Leung, Breton, & Angers, 2016; Verhoeven & Preite, 2014). Moreover, assuming that the molecular mechanisms underlying changes in DNA methylation (and possibly histone modification or RNAs) are property of the genotype (Feinberg & Irizarry, 2010), some genotypes can then be selected for their high epigenetic potential in unpredictable environments (bet-hedging strategy; Angers, Castonguay, & Massicotte, 2010; Leung et al., 2016)).

Third, epigenetic variation can be fostered by environmental conditions (Feil & Fraga, 2012). This environmentally driven epigenetic variation can result from the production of stochastic epigenetic mutations as a genomic response to stressful and unpredictable environment (Feinberg & Irizarry, 2010). In this case, genotypes harbouring an optimal "epigenetic flexibility" might be favoured, hence leading to the selection of a bet-hedging strategy as previously described in the case of purely stochastic epigenetic mutations. Alternatively, environmentally driven epigenetic variation can also result from non-random epigenetic modifications at specific genes to modify the phenotype according to the prevailing environment, hence corresponding to adaptive phenotypic plasticity (Duncan, Gluckman, & Dearden, 2014). Importantly, one might expect that genetic determinism exists for some epigenetically induced phenotypes in response to the environment, that is the genetic determinants of phenotypic plasticity (Pigliucci, 2005). Importantly, selection may favour genetic lines associated with the epigenetic machinery that allows flexibility to encode for some adaptive yet reversible phenotypes in predictable fluctuating environments, that is the genotypes harbouring the optimal adaptive phenotypic plasticity (Duncan et al., 2014).

Despite an increasing interest in depicting natural epigenetic variation, the molecular bases underlying such variation remain largely unknown. Assessing epigenetic variation directly is therefore the most direct proxy for studying the epigenetic potential of organisms as it takes into account both environmentally induced and stochastic sources of variation.

genomic unit) (Waddington, 1940). Indeed, epigenetic mechanisms are fundamental for the reprogramming, differentiation and maintenance of specific cell lineages (Hemberger, Dean, & Reik, 2009). Part of a organism's epigenetic landscape (i.e. the epigenetic status at the genome-wide scale), and particularly that of DNA methylation, can be modulated by environmental factors either biotic (e.g. social environment and parasites) or abiotic (e.g. temperature, drought and chemicals; Bossdorf, Richards, & Pigliucci, 2008; Feil & Fraga, 2012). Thus, in both plants and animals, the surrounding environment can affect DNA methylation patterns during early developmental stages and ultimately modulate phenotypes of individuals, either in a discontinuous or in a continuous fashion (respectively, corresponding to polyphenism and reaction norm) (Chinnusamy & Zhu, 2009; Faulk & Dolinoy, 2011). For instance, environmental sex determination (ESD) in some fish and some reptiles mainly relies on the expression of the *cyp19a1* gene (which encodes for an aromatase enzyme involved in ovarian differentiation) and which expression is controlled by the environmentally driven methylation status of its promoter (Hunt et al., 2013; but see Ge et al., 2018). As a result, some authors argue that given the ongoing global warming, such epigenetically mediated ESD could become an epigenetic trap by altering sex ratio in natural populations (Consuegra & Rodríguez López, 2016; but see Piferrer, 2016). More generally, DNA methylation induced by environmental stressors during development that produces maladaptive phenotypes can have negative consequences in populations (Piferrer, 2016). Thus, accounting for such epigenetic trap effect faced by some populations could be useful in a conservation context. Noteworthy, the role and importance of DNA methylation in development is not universal (Box 2), and hence, not all species are expected to face and suffer from epigenetic traps.

BOX 2 Major differences in DNA methylation patterns and reprogramming among taxa

The heterogeneity in genome-wide DNA methylation patterns and reprogrammation among the tree of life has already received considerable attention, and several valuable reviews exist on this topic (Feng, Jacobsen, & Reik, 2010; Head, 2014; Hunt, Glastad, Yi, & Goodisman, 2013; Law & Jacobsen, 2010). In this box, we will briefly recall the major differences in DNA methylation patterns across species that we believe needs to be considered, when studying DNA methylation in a conservation context.

In vertebrates, organisms generally display high levels of methylation distributed in a continuous fashion over the genome except in some specific regions called CpG islands often corresponding to promoters and regulatory sequences of active genes (Feng, Cokus, et al., 2010). The methylation of these particular genomic regions generally inhibits the transcription of the related gene(s), hence ultimately influencing cells' and organisms' phenotypes. As such, DNA methylation is largely involved in individuals' development. In this regard, the specialization of somatic cells during early development of vertebrates requires an extensive erasure and reprogrammation of DNA methylation patterns. Such mechanisms and outcomes of these processes largely differ among vertebrate species. In some vertebrates (e.g. rodents and humans), two extensive DNA methylation erasure occur during gonadogenesis both in parents and in the zygote during early embryogenesis. As a result, transmission of specific DNA methylation profiles is expected to be rare in mammals. In some fish (e.g. zebrafish), the erasure of DNA methylation only occurs during female gonadogenesis while maintained in male gonads (Jiang et al., 2013). This means that the DNA methylation patterns in males potentially influenced by environmental cues are at least partly transmitted to the next generations. In birds, amphibians and reptiles, DNA methylation is also generally distributed over the genome in a continuous fashion, but very little information exists related to DNA methylation reprogrammation and potential transgenerational inheritance (Head, 2014).

Classical genomes of invertebrates are characterized by levels of methylation lower than vertebrates and following a mosaic distribution mostly targeting a subset of transcription units (Head, 2014; Hunt et al., 2013). Several lines of evidence indicate that DNA methylation is involved in the developmental pathways of some insects including caste determination in eusocial insects (Kucharski, Maleszka, Foret, & Maleszka, 2008). However, in some invertebrate species, no DNA methylation (e.g. *Caenorhabditis elegans*) or extremely low levels of DNA methylation (<1% of the genome; e.g. *Drosophila melanogaster*) was detected, clearly indicating that DNA methylation does not constitute a key element for the development in these species (Head, 2014). Very little information exists concerning the reprogramming of DNA methylation patterns during gonadogenesis and/or embryogenesis; however, partial maintenance of epigenetic imprints observed in some species makes transgenerational epigenetic inheritance in some invertebrate species more likely than in vertebrates and more specifically mammals.

In plants, DNA methylation patterns greatly differ from those observed in animals, in particular because DNA methylation occurs in several genomic contexts including on cytosines in CG, CHG and CHH contexts (where H = C, T or A; Feng, Jacobsen, et al., 2010). Moreover, the establishment and maintenance of methylations at some specific genomic locations depend on several mechanisms involving enzymes specific to plants. Surprisingly, however, DNA methylation often occurs in exons as in animals. DNA methylation is involved in gene regulation and in the repression of transposable element activities although the underlying mechanisms somehow differ from animals (Feng, Jacobsen, et al., 2010). One major difference with animals is that germline cells in plants are produced continuously and the differentiation between germline and somatic cells is often confused. Moreover, no erasure of DNA methylation patterns occurs during meiosis (Feng, Jacobsen, et al., 2010), hence meaning that the stability of epimutations over generations is expected to be higher in plants than in animals (Quadrana & Colot, 2016).

2.2 | Epigenetics, phenotypic plasticity and bet hedging

In an eco-evolutionary context, phenotypic plasticity has received increasing attention in the last decades (Bossdorf et al., 2008; Verhoeven, Vonholdt, & Sork, 2016). At the population level, modifications of DNA methylation patterns among individuals in response to changing environment can be associated with a phenotypic shift from suboptimal to optimal value in the resulting environment, hence leading to adaptive phenotypic plasticity (corresponding to the environmentally induced phenotype variation; i.e. EPV; Vogt, 2017). Alternatively, environmental changes can potentially induce spontaneous and random modification in DNA methylation patterns potentially resulting in the broadening of phenotypic values around the original mean phenotype within populations (i.e. corresponding to the stochastic developmental phenotype variation; i.e. SPV; Angers et al., 2010; Vogt, 2017).

Those two above processes can lead to phenotypic diversification, and both empirical and theoretical models indicate that they might be favoured in different ecological contexts (e.g. Klironomos et al., 2013). On the one hand, EPV is expected to be selected when environmental changes are predictable, thus allowing organisms to quickly respond and adjust their phenotypes so as to maximize their fitness (Angers et al., 2010). This type of phenotypic adjustment

implies that the resulting environmentally induced phenotypic shift is encoded either epigenetically or genetically and that selection can act on it. On the other hand, SPV can be considered as a random/non-directional flexibility of the genome expression to new and/or unpredictable environments. SPV constitutes a bet-hedging strategy resulting in the maintenance of few individuals harbouring optimal phenotypes and most individuals expressing suboptimal phenotypes in the new environment (Rey, Danchin, Mirouze, Loot, & Blanchet, 2016). Unlike EPV, the environmentally induced phenotypic shift towards optima is not selected for under unpredictable environments, but selection might favour the epigenetic machinery that maximizes the broadening of phenotypes. Recently Leung et al. (2016) provided an empirical illustration of how EPV and SPV can be associated with adaptive responses to predictable and unpredictable environments, respectively. In particular, they found that asexual lineages of the fish *Chrosomus eos-neogaeus* displayed contrasting genome-wide DNA methylation remodelling in response to environmental changes according to their origins (predicable, i.e. lakes, vs. unpredictable, i.e. intermittent streams). These differences were consistent with theoretical models as higher environmentally induced epigenetic changes (phenotypic plasticity) or stochastic epimutations (diversifying bet hedging), respectively, prevailed in predictable or unpredictable environments.

2.3 | Epigenetics and adaptation

Some DNA methylation patterns can be transmitted from one generation to another and hence can be maintained within populations over a few to several hundred generations in plants (e.g. Cubas, Vincent, & Coen, 1999) and to a lower extent in animals (Box 2). When such heritable DNA methylation profiles are associated with phenotypes under selection, they behave as beneficial mutations and hence provide a source for natural selection. Importantly, however, epigenetic mutations are expected to be more common than genetic mutations (Van Der Graaf et al., 2015). Moreover, unlike genetic mutations, epimutations (i.e. change in methylation state at a given genomic region) can be reversible (i.e. the probability that a reverse genetic mutation occurs at a newly arisen genetic mutation is negligible). This means that a newly emerged adapted phenotype induced by a modification of DNA methylation profile is at least partially reversible. This attribute is particularly relevant in habitats characterized by environmental fluctuations over large time-scales (Rey et al., 2016).

The importance of variation in DNA methylation profiles relative to genetic variation through either mutations or recombination in adaptation still needs to be empirically quantified in natural populations (Verhoeven et al., 2016). Because the distribution, function and reprogrammation of DNA methylation greatly vary among species (Box 2), its relative role in adaptation is not expected to be equally important among taxa. Moreover, at the intraspecific level, the adaptive potential of epigenetic variation is likely to be particularly relevant in genetically depauperate populations, including endangered small (and possibly inbred) populations, clonal lineages or recently established invasive populations (Sheldon, Schrey, Andrew, Ragsdale, & Griffith, 2018; Thorson et al., 2017; Verhoeven & Preite, 2014). For instance, Liebl, Schrey, Richards, and Martin (2013) found a negative correlation between genetic diversity and DNA methylation diversity in invasive house sparrow populations along their gradient of invasion. Although not empirically tested, the authors suggest that variation in DNA methylation profiles represents a compensatory mechanism for a loss of genetic diversity. These considerations are extremely relevant in a biological conservation context since conservation issues generally focus on genetically depauperate populations.

Another important factor that could influence the relative importance of epigenetic versus genetic adaptive variation in adaptation is the stability of the environment surrounding organisms/populations (Beauregard & Angers, 2018). In stable environments, selection is likely to be more efficient on genetic variation compared with epigenetic variation. Conversely, epigenetic variation might be of prime interest in fluctuating environment, hence increasing the effect of selection on epigenetic compared with genetic variation in these environments (Angers et al., 2010).

2.4 | Epigenetics and biodiversity functioning

A key aspect of biodiversity conservation concerns the potential pervasive influence of human societies on biodiversity. In the 2000s, a series of empirical and theoretical studies have demonstrated that losing biodiversity may lead to losing key ecosystem services to humans, such as plant productivity or natural medication (Hooper et al., 2012; Loreau, 2000). Arguably, the strongest demonstration of a positive link between biodiversity and ecosystem services is that of a high plant species diversity in a given area being associated with high plant productivity in this area (Grace et al., 2016). More recently, studies have demonstrated that similar positive relationships between biodiversity and ecosystem functions might operate at the intraspecific level (Raffard, Santoul, Cucherousset, & Blanchet, 2018). The basis for biodiversity–function positive relationships is that intraspecific diversity within populations should promote functional complementarity and reduce functional redundancy among individuals, hence optimizing the use of resources in ecosystems. This is because individuals are not ecologically equivalent within populations, and the higher the functional richness of a population, the higher the efficiency of that population for resource consumption and for energy fluxes among trophic levels. Up to now, most studies investigating intraspecific biodiversity–function have manipulated the genetic richness of populations (reviewed in Raffard et al., 2018). Yet, genetic diversity is probably not the only proxy for representing the functional richness of populations, and epigenetic diversity is likely to represent a novel proxy relating "ecological" richness at the intraspecific level and genomic architecture (Richards et al., 2017). Indeed, epigenetic has the potential to lead to within-generation accommodation and/or rapid adaptation, which should improve further the diversification of resource acquisition and exploitation within populations. If true, we expect strong relationships between

epigenetic diversity and ecosystem functioning in wild populations. To the best of our knowledge, a single study has investigated the relationships between epigenetic diversity and ecosystem functions, demonstrating that populations of *Arabidopsis thaliana* that display more DNA methylation variation were more productive and capable of controlling the presence of a competitor (Latzel et al., 2013). Interestingly, the positive effect of epigenetic diversity on primary productivity was stronger under stressful conditions (i.e. presence of pathogens and competitors). Finally, in most experimental treatments, the shape of the relationship between epigenetic diversity and primary production followed a saturated curve, suggesting that complementarity among epigenotypes explained the initial increase in primary productivity, while the plateau likely represents the redundancy present in the system. Although more studies are needed, many lines of evidence strongly support the idea that epigenetic diversity (at the intraspecific level) is a relevant facet of biodiversity for understanding and predicting the functioning of ecosystems and that such level of diversity needs to be integrated into management policy. Noteworthy, because the precise genetic determinisms of DNA methylation patterns and dynamics in space and time within organisms are not fully identified, studying DNA methylation is currently the most direct way to study the epigenetic potential of organisms at all levels of organization (Box 1).

3 | TOWARDS CONSERVATION EPIGENETICS: A ROADMAP

There are four main aspects of conservation where studying DNA methylation can make important contributions, including (a) the development of biomarkers, (b) the study of wild populations' ecological structuring, (c) the improvement of population reinforcement strategies through conservation translocation and (d) the study of landscape functional connectivity. Each of these four aspects is illustrated by recent empirical studies.

3.1 | Epigenetic patterns as biomarkers

Several stressors, including biotic (e.g. social and parasitic) and abiotic (e.g. thermal, mechanic and chemical) stresses, can induce modifications of DNA methylation profiles (Feil & Fraga, 2012). These environmentally sensitive labile marks hence constitute good molecular biomarkers to evaluate environmental stress experienced by organisms (Mirbahai & Chipman, 2014). The usefulness of epigenetic biomarkers was recently highlighted in an agronomic context for plant cultivars, whereby the pruning systems used in vineyards induce detectable DNA methylation signatures in vines even at narrow geographical scales (Xie et al., 2017). Based on these findings, specific DNA methylation profiles patterns could be used as biomarkers to characterize “terroirs” not only by allocating the geographical and genetic origin of vines but also by determining the pruning systems used in vineyards. In a conservation perspective, this example illustrates how DNA methylation can be used to determine conservation units (for instance here the vine terroirs) accounting not only for the long-term evolutionary history of organisms but also for some important fractions of their current ecological context. Importantly, some environmentally induced modifications in DNA methylation patterns can be transmitted over several generations (Mirbahai & Chipman, 2014). It is thus likely that long-lasting epigenetic biomarkers give information on the past ecological conditions in the last generations. In a practical perspective, this requires the identification of specific DNA methylation patterns that are induced by certain environmental cues and that are transmitted across generations. However, direct investigations for such prediction are, so far, lacking, and stable DNA methylation changes over generations have been identified for very few model organisms so far (see Section 4).

Additionally, several intrinsic individual biological traits also influence the overall epigenetic state of organisms, suggesting that epigenetics could also be used to determine the physiological/biological states of some targeted individuals. For instance, some genes (e.g. *TET2; CDKN2A/CDKN2B*) undergo a gradual hypo- or hypermethylation during ontogeny in several mammals, hence constituting compelling non-disruptive molecular age biomarkers (MABs) particularly in long-lived organisms (Jarman et al., 2015). For instance, efficient epigenetic MABs were developed by Polanowski, Robbins, Chandler, and Jarman (2014) to estimate the age of wild humpback whales using non-invasive skin biopsy samples. Chronological age influences several ecological traits of animals, including reproduction success and survival rate, both of which being of prime interest in conservation biology.

Specific DNA methylation variants at some specific genes also correlate with personality/behavioural traits in several species including fish, birds and mammals (Ledon-Rettig, Richards, & Martin, 2013; Verhulst et al., 2016), two major traits that are increasingly considered in the management of captive and free-ranging wildlife (Powell & Gartner, 2011). For instance, Saino et al. (2017) identified specific DNA methylation patterns at some photoperiodic genes that allow predicting migratory phenology and ultimately the seasonal breeding success of wild barn swallows from blood samples. In conservation, using such epigenetic biomarkers for predicting the migratory behaviour of individuals could greatly improve conservation planning for mobile species (Runge, Martin, Possingham, Willis, & Fuller, 2014).

3.2 | Epigenetics reflect “ecological populations”

The genome-wide DNA methylation patterns of organisms are influenced by their contemporary environment and also by the surrounding environment experienced by their recent ancestors (Mirbahai & Chipman, 2014). Thus, DNA methylation profiles also reflect the environmental context in which organisms' lineages evolved on a short ecological time-scale. Accordingly, studying DNA methylation diversity among wild populations constitutes an opportunity to further characterize “ecological populations”. How populations are ecologically structured is crucial in conservation biology and more particularly to define conservation units. We here propose an integrative

BOX 3 Quantifying epigenetic variation for conservation biology

Investigating the contribution of epigenetic modifications on phenotypic variation could be an invaluable tool to identify which species can cope in time or are vulnerable to environmental changes. This can provide useful insights into conservation and management programmes. The addition of a methyl group to cytosine nucleotides (that can occur in three sequence contexts: CpG, CHG or CHH) is by far the best characterized epigenetic mark, primarily, due to advances in next-generation sequencing (Table S1). Current genome-wide DNA methylation methods typically use bisulphite conversion, methylation-sensitive restriction enzymes or affinity enrichment (Table S1). But the future of ecological epigenetics is in bisulphite sequencing-based technologies (BS-seq), as they provide high-resolution information of cytosine methylation and the genomic and sequence context, whereas more and more methylome data of populations become available. Perhaps most importantly, bisulphite sequencing methods can integrate population genomic approaches to evaluate population structure and differentiation and infer population dynamics, using single methylation polymorphisms (Sumps) (e.g. Liebl et al., 2013).

Originally, whole-genome bisulphite sequencing (WGBS) is the recommended approach for the detection of widespread CpG methylation sites at single-nucleotide resolution. But its cost and long analysis time limit its broad use for studying wild populations. Recently, targeted BS-seq approaches, aiming to cover either the most differentially methylated regions (such as the dynamic methylome (DyMe-Seq); Ziller, Stamenova, Gu, Gnirke, & Meissner, 2016) or the RainDrop BS-seq (Paul et al., 2014)) or amplify specific loci (such as the BisPCR^2; Bernstein, Kameswaran, Le Lay, Sheaffer, & Kaestner, 2015) and the bisulphite amplicon sequencing (Masser, Stanford, & Freeman, 2015) and reduced representation technologies (such as reduced representation bisulphite sequencing (RRBS; Gu et al., 2011) and bisulphite-converted restriction site-associated DNA sequencing (bsRADseq; Trucchi et al., 2016)) presented more cost-efficient methods that follow the same principle as WGBS.

Like conservation genomics, ecological epigenetics require quantifying epigenetic variation to account for environmental and genetic effects. Since genetic variation typically measures allele frequency, whereas epigenetic accounts for the presence or absence of an epigenetic mark (herein DNA methylation), genetic and epigenetic estimates of variation can be fundamentally different. Yet, some measures used in evolutionary or population genetics can be transferred to ecological epigenetics and recent studies have developed several statistical approaches to quantify for epigenetic variation (Table S1). Liebl et al. (2013) calculated and epi-F_{ST} statistic measure to describe levels of differentiation between populations due to epigenetic variation, while Wang and Fan (2014) developed a neutrality test (D^m) to detect selection forces shaping DNA methylation pattern within a population. However, to fully unravel the meaning of epigenetic variation and its role in conservation more efforts are required to develop measures of diversity.

approach to better integrate the ecological structuring of wild organisms when identifying ESUs. Combined with genetic approaches, the study of epigenetic structure and diversity in wild populations allows a better definition of the overall eco-evolutionary background of natural populations and eventually ESUs. We develop this idea by defining several scenarios expected from such combined genetic–epigenetic studies in wild populations and how these scenarios can be useful for refining ESUs (Figure 1).

3.2.1 | Case 1 (a in Figure 1)

Geographically isolated and genetically differentiated populations inhabit different ecological habitats. Both genetic differentiation and DNA methylation differentiation are expected between populations. Patterns of genetic and DNA methylation differentiation can coincide if the variance in DNA methylation profiles is under strong genetic determinism or if potential local adaptation involved the co-segregation of some genetic and DNA methylation patterns. For instance, Liu et al. (2012) found a strong correlation between DNA methylation and genetic variation in wild populations of the great round leaf bats (*Hipposideros armiger*). The authors suggest that such correlation likely results from a strong genetic determinism of DNA methylation profiles although other factors could lead to such co-segregation pattern (e.g. inbreeding depression). Under a conservation perspective, the ecological background of these bat populations did not lead to an observable epigenetic structure independent of the genetic background. In this case, the added value of epigenetic compared to genetic information is not trivial for distinguishing ESUs.

Alternatively, patterns of genetic and DNA methylation differentiation can diverge in particular if recent ecological divergence occurred irrespective of the long-term demographic history of populations and if organisms' DNA methylation profile is highly influenced by the surrounding environment. This pattern is well illustrated by some populations of the perennial herb *Helleborus foetidus* in the Sierra de Cazorla, south-eastern Spain (Herrera, Medrano, & Bazaga, 2017). The genetic, epigenetic and phenotypic structures of subpopulations were established on 10 geographically distant sites characterized by diverging environmental conditions. The authors reported that the genetic structure followed a classical isolation-by-distance pattern (i.e. IBD), while the epigenetic structure clearly followed an isolation-by-environment

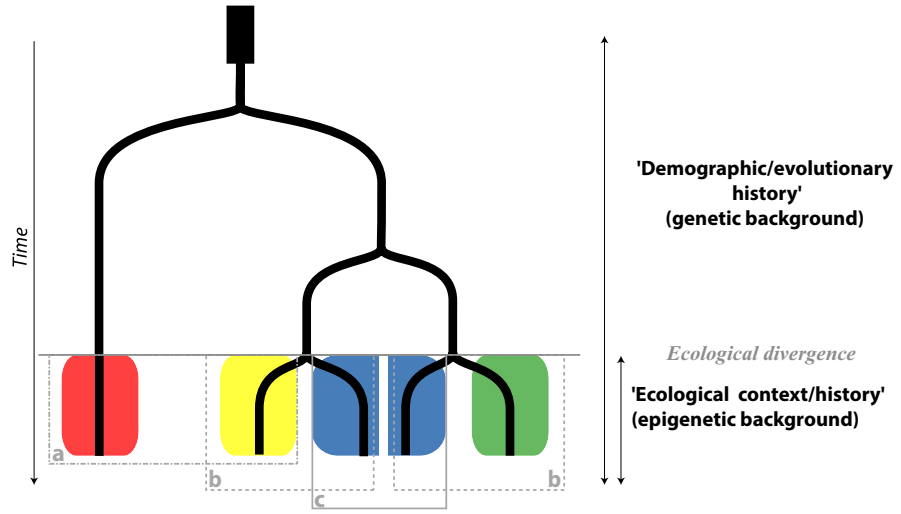

FIGURE 1 Importance of genetics and epigenetics for documenting the long-term demographic and evolutionary history and the contemporaneous ecological context of organisms. Three main hypothetical scenarios of genetic/epigenetic signatures are here presented. In (a) genetically differentiated populations inhabit different ecological habitats. In this case, both genetic differentiation and epigenetic differentiation are expected. Correlated genetic and epigenetic differentiation is expected if there is a strong genetic basis for epigenetic variation. Conversely, no correlation is expected if epigenetic pattern is only loosely genetically determined and/or under strong environmental determinism. In (b), non-genetically differentiated subpopulations occupy different ecological habitats. In this case, epigenetic differentiation between the two subpopulations (i.e. "ecological populations") is expected especially due to environmentally induced epigenetic shifts, while no genetic differentiation should be detected. Most of the empirical studies conducted so far to compare genetic and epigenetic differentiation support this hypothesis (see full text). (c) In this case, genetically differentiated populations are sympatric in a common ecological habitat. Here, genetic differentiation is expected to be greater than epigenetic differentiation. Indeed, while the genetically fixed part of the epigenome is expected to be congruent with the genetic signatures, the portion of the epigenome which is sensitive to the environment should be similar in both populations, hence reducing epigenetic differentiation between populations

pattern (i.e. IBE). These results indicate that while the observed IBD genetic signature mostly reflects the long-term evolutionary dynamics of *H. foetidus* in this geographical region (e.g. limited gene flow and genetic drift), the epigenetic structure better reflects the ecological processes that have shaped population phenotypic differentiation (Herrera et al., 2017). In the same vein, Sheldon et al. (2018) found similar degrees of genetic and DNA methylation differentiation between three invasive populations of house sparrow (*Passer domesticus*) in Australia originating from three independent introduction events. However, the authors did not find significant correlation between pairwise site comparisons of genetic and DNA methylation differentiation indexes (F_{ST}). In this particular case, populations could be considered as two distinct ESUs with limited exchangeability at both the genetic and the ecological levels.

3.2.2 | Case 2 (b in Figure 1)

Non-genetically differentiated "subpopulations" have experienced an ecological divergence event. Here, diverging environments may independently modulate DNA methylation patterns of individuals in each "ecological populations" either stochastically or "directed" by the environment (Leung et al., 2016). Differentiation in DNA methylation profiles is thus expected between "ecological populations" despite the absence of genetic differentiation. Most empirical studies that compared genetic and DNA methylation differentiation in wild populations support this scenario in both plants and animals (Hu & Barrett, 2017). One example that well illustrates this scenario concerns wild populations of asexual organisms (Thorson et al., 2017; Verhoeven & Preite, 2014). For instance, Thorson et al. (2017) studied the morphological divergence and natural DNA methylation variation in "ecological populations" of the invasive freshwater snail *Potamopyrgus antipodarum*, originating from a single clonal genotype and established in diverging habitats (two lakes vs. two rivers). The authors found a strong DNA methylation differentiation between populations exposed to contrasting habitat types (i.e. lake vs. river) along with an adaptive difference in shell morphology according to habitat types. DNA methylation variation observed between populations from these two habitats was greater than that observed within a habitat type (i.e. lake or river), suggesting that DNA methylation differentiation likely results from a direct effect of the environment and not from purely stochastic processes (i.e. "population epigenetic drift"). Although a genetic basis underlying such adaptive change in shell morphology cannot completely be ruled out, these findings strongly support the emerging idea that, in some cases, variation in DNA methylation patterns can compensate for a lack of genetic variation and may provide non-negligible support for adaptation (Verhoeven & Preite, 2014).

3.2.3 | Case 3 (c in Figure 1)

Genetically differentiated populations occupy similar ecological habitats. In this case, genetic differentiation is expected to be

greater than DNA methylation differentiation when the latter is more influenced by the environment than by drift or other stochastic events (i.e. environmentally induced epigenetic convergence). One empirical study has documented this scenario in endangered populations of the toller violet *Viola eliator* (Schulz, Eckstein, & Durka, 2014). Schulz and collaborators studied patterns of genetic and DNA methylation diversity and differentiation between wild populations from adjacent habitat types with respect to light availability (i.e. floodplain meadow vs. alluvial woodland fringe). They found a strong genetic structure between *V. eliator* populations irrespective of the geographical distances (i.e. no IBD pattern) most likely due to high selfing rates and small population sizes, both factors promoting genetic drift. Conversely, differentiation in DNA methylation patterns between populations was significantly lower and better related to habitat conditions, which strongly suggests an environmentally induced epigenetic convergence between populations. In a conservation context, these populations should be considered as different ESUs that can be ecologically exchangeable.

3.3 | Ecological exchangeability and population reinforcement

Conservation translocation consists of the movement and release of organisms for conservation reasons. Depending on the conservation status of the recipient population, population reinforcement can take different forms, such as genetic rescue, assisted gene flow or stocking (Corlett, 2016). Genetic rescue refers to the situation where a small and inbred recipient population requires a dramatic increase in standing genetic variation to promote heterosis and increase its adaptive potential (Harrisson et al., 2016). Assisted gene flow relates to a case where a recipient population is anticipated to be threatened by environmental changes and would benefit from the increase in the frequency of some preadapted alleles (Aitken & Whitlock, 2013). Lastly, when the recipient population is regularly harvested, population reinforcement takes the form of stocking (Griffith, Scott, Carpenter, & Reed, 1989). We argue that population reinforcement through conservation translocation may benefit from the assessment of epigenetic backgrounds and ecological exchangeability between the donor and the recipient populations. For instance, the success of genetic rescue may be enhanced by translocating individuals originating from populations that are genetically (though moderately) distinct from the recipient population (Harrisson et al., 2016). In doing so, this could allow increasing genetic diversity within the recipient population while preserving a similar environmentally induced epigenetic background, so that released individuals are preadapted to local environmental conditions (case 3; Figure 1c). Of course, the concomitant increase in epigenetic variation (stemming from the translocation of similar but not clonal individuals) would simultaneously buffer the recipient population against rapid environmental changes and/or environmental unpredictability. On the contrary, the success of assisted gene flow operations may be enhanced by translocating individuals originating from populations sharing a common genetic background with the recipient population, so as to avoid outbreeding depression and/or gene swamping (Aitken & Whitlock, 2013), but also showing a distinct epigenetic background, so that the recipient population can cope with anticipated environmental changes through the increase in the frequency of some identified preadapted epi-alleles (case 2; Figure 1b). For instance, the heritable "toad-smart" behaviour of the northern quoll *Dasyurus hallucatus* identified by Kelly and Phillips (2018) in populations recently exposed to the cane toad *Rhinella marina* may have an epigenetic basis (Ledon-Rettig et al., 2013): translocating "toad-smart" individuals into soon to be impacted but genetically similar recipient populations may help northern quolls resist toad invasion while limiting risks of outbreeding depression.

Noteworthy, the success of stocking operations may be enhanced by translocating individuals originating from populations that are both genetically and ecologically exchangeable with the recipient population. For instance, Le Luyer et al. (2017) investigated why hatchery-reared coho salmons (*Oncorhynchus kisutch*) experience reduced fitness once released in the wild, despite improved production strategies, notably based on the use of local broodstock. They measured genome-wide variation at both the genetic and the DNA methylation levels between hatchery-reared juvenile fish and their wild counterpart originating from two geographically distant rivers in British Columbia (Canada). Despite a non-significant genetic difference between hatchery and wild salmons originating from the same river drainage, the authors identified hypermethylated genome regions associated with key biological functions such as stress tolerance and locomotion patterns in hatchery-reared individuals, suggesting that rapid epigenetic modifications induced by rearing conditions may be sufficient to decrease stocking success. This study nicely illustrates the importance of considering patterns of environmentally induced epigenetic variation when planning conservation translocation.

3.4 | Epigenetic spatial variation and landscape functional connectivity

The comparison of DNA methylation patterns among populations may also be worth considered when studying landscape functional connectivity. Genetic and genomic data are now routinely used to measure dispersal rates among populations and/or to assess the influence of landscape configuration on dispersal, using approaches such as assignment analyses or linked-based methods (Cayuela et al., 2018). However, these molecular tools are not without drawbacks. For instance, pairwise measures of genetic differentiation used in linked-based methods may be affected by important temporal lags between the decrease in dispersal rates, occurring at ecological timescales (e.g. resulting from human-induced landscape fragmentation) and the corresponding genetic response (genetic drift and subsequent population differentiation), occurring at evolutionary timescales (Landguth et al., 2010). If assignment analyses may contrarily allow identifying contemporary dispersal events (Manel, Gaggiotti, & Waples, 2005), they also require contrasted genetic allelic frequencies among patches, confining their use to spatially structured populations (Lowe & Allendorf, 2010). We argue that spatial variations

in epi-allele frequencies could be considered in complement to the classical study of spatial variations in (genetic) allelic frequencies to improve the inference accuracy of current molecular tools, in a way similar to the proposed use of isotopic signatures (e.g. Ruegg et al., 2017). Spatial variations in epi-allele frequencies, induced by environmental heterogeneity, may appear both faster (Duckworth, 2013) and at shorter lag distances than spatial variations in allelic frequencies (e.g. Herrera, Medrano, & Bazaga, 2016). Provided that correlation between genetic and DNA methylation variation is taken into account (e.g. Foust et al., 2016), it may allow refining outcomes from linked-based methods (for instance using both pairwise measures of genetic and epigenetic differentiation) and assignment analyses (based on the comparison of both genetic and epigenetic spatial patterns of variation), hence paving the way to a landscape epigenetics toolbox for conservation planning.

4 | LIMITATIONS AND PERSPECTIVES

In this study, we reviewed evidence that epigenetic approaches using DNA methylation constitute promising tools to characterize the ecological background of organisms, a crucial yet overlooked aspect in conservation biology. In particular, while studying genetic diversity is a valuable option to decipher long-term evolutionary changes, epigenetic should be considered as an option to inform on short-term/immediate responses to contemporaneous environmental changes.

However, for several reasons, it is presently difficult to evaluate the full range of organisms for which studying DNA methylation patterns and diversity are effectively applicable in a conservation context. First, the distribution of DNA methylation at the genomic scale among taxa is still incompletely documented. So far, DNA methylation was detected in most, but not all (e.g. *C. elegans*), species in which it has been directly investigated (Box 2) and highly variable amount of methylation levels also exists at the intraspecific level (e.g. population and life stage; Suzuki & Bird, 2008; Yi & Goodisman, 2009; see Box 3). More generally, four general DNA methylation distribution patterns were identified (i.e. mosaic vs. global and targeted to either genes or transposable elements) irrespective of the phylogenetic relationship between organisms, meaning that phylogenetic proximity cannot be used to predict the genome-wide methylation patterns of non-model organisms (Aliaga, Bulla, Mouahid, Duval, & Grunau, 2019; Suzuki & Bird, 2008). Interestingly, however, indirect methods based on the estimation of CpG observed/expected ratio (CpG o/e) can be used as a proxy of genome-wide methylation levels of organisms in non-model organisms (Aliaga et al., 2019). Noteworthy, alternative epigenetic components (e.g. histone tail modifications) ensure proper developmental processes and the shaping of phenotypic variation and more particularly when DNA methylation is absent or poorly present in organisms' genomes (Glastad, Hunt, & Goodisman, 2019). In these species, other epigenetic components should be accounted for in conservation epigenetics.

Second, the consequences (in terms of developmental pathways) of epigenetic variation on phenotypes remain unknown in many organisms (Verhoeven et al., 2016). Several studies have documented strong associations between the diversity and structure of DNA methylation patterns in wild populations and the environmental conditions in which these populations are established, mainly in plants and to a lower extent in animals (Hu & Barrett, 2017, see empirical examples cited in this study). Importantly, however, these studies are mainly based on correlative approaches and the direct effect of the environment in shaping DNA methylation patterns and ultimately epigenetically induced (potentially adaptive) phenotypes of organisms is not functionally demonstrated. This might be partly explained by the fact that global DNA methylation patterns in wild populations are generally investigated using "blind" approaches (e.g. MS-AFLP; Table S1), that is meaning that no information is available on the identity and function of the targeted genomic regions that display variation in DNA methylation levels (but see Gugger, Fitz-Gibbon, Pellegrini, & Sork, 2016; Lea, Altmann, Alberts, & Tung, 2016). The recent advents in sequence-based approaches that allow simultaneously quantifying epigenetic diversity and structure among wild populations and identifying the targeted genomic regions (e.g. RRBS, epiGBS, Table S1) will clearly improve our understanding on how the environment shapes DNA methylation patterns and possibly (adaptive) phenotypes in wild populations in the next future. In this regard, depending on the genome-wide DNA methylation profile of organisms (i.e. mosaic or global and targeted to genes or transposable elements) some predictions can be made. For instance, one might expect that in organisms with methylation being directed towards transposable elements such as in plants, patterns of DNA methylation diversity/structure can reflect ecological conditions but will not necessarily be associated with specific adaptive phenotypes. Conversely, in organisms that display mosaic/global DNA methylation patterns targeted on genes and/or regulatory elements (these genomic elements being also targetted by selection), the potentially identified environmentally induced DNA methylation patterns might be associated with adaptive phenotypic responses in the respective environment.

5 | CONCLUSIONS

Certainly, the greatest recent revolution in conservation biology has been the implementation of genetic and genomic approaches to account for the evolutionary history and evolutionary potential of wild lineages, for defining entities to be preserved, to predict demographic and evolutionary consequences of environmental changes and to develop concrete management actions (Olivieri, Tonnabel, Ronce, & Mignot, 2016). Yet, linking the long-term evolutionary history of organisms to their responses to changing environments on short-term ecological time-scales is still challenging. We anticipate that epigenetics could fill this gap and constitute an unprecedented opportunity to account for the organisms' ecological background, a key component of organisms. We specifically highlighted how integrating epigenetics, and more specifically analyses of DNA methylation profiles in conservation biology, is promising to give precise insights on the

physiological, biological and ecological status of targeted organisms, refine – by going back to its original definition that explicitly included ecological/life-history traits – the "evolutionary significant units" concept, improve conservation translocation managements and identify landscape functional connectivity.

Epigenetics, just like genomic approaches, are currently mainly confined to academic research and may appear at a first glance inaccessible to conservation managers. However, the last decades have flourished with several methodological and analytical studies specifically dedicated to epigenetic studies, which makes these approaches increasingly accessible. Moreover, we are currently witnessing a democratization of some normalized sequencing protocols available for studying DNA methylation in wild populations (Table S1), hence greatly facilitating their implications in ecology and evolution and in the near future in conservation biology.

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Original ideas were conceived by O.R. and S.B. All authors contributed critically to the drafts in their respective expertise and gave final approval for publication.

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