Facilitation of environmental adaptation and evolution by epigenetic phenotype variation: insights from clonal, invasive, polyploid, and domesticated animals

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Abstract

There is increasing evidence, particularly from plants, that epigenetic mechanisms can contribute to environmental adaptation and evolution. The present article provides an overview on this topic for animals and highlights the special suitability of clonal, invasive, hybrid, polyploid, and domesticated species for environmental and evolutionary epigenetics. Laboratory and field studies with asexually reproducing animals have shown that epigenetically diverse phenotypes can be produced from the same genome either by developmental stochasticity or environmental induction. The analysis of invasions revealed that epigenetic phenotype variation may help to overcome genetic barriers typically associated with invasions such as bottlenecks and inbreeding. Research with hybrids and polyploids established that epigenetic mechanisms are involved in consolidation of speciation by contributing to reproductive isolation and restructuring of the genome in the neo-species. Epigenetic mechanisms may even have the potential to trigger speciation but evidence is still meager. The comparison of domesticated animals and their wild ancestors demonstrated heritability and selectability of phenotype modulating DNA methylation patterns. Hypotheses, model predictions, and empirical results are presented to explain how epigenetic phenotype variation could facilitate adaptation and speciation. Clonal laboratory lineages, monoclonal invaders, and adaptive radiations of different evolutionary age seem particularly suitable to empirically test the proposed ideas. A respective research agenda is presented.

Key words: epigenetic variation; adaptation; general-purpose genotype; speciation; genome reconfiguration; monoclonal invaders.

Introduction

The generation of phenotypic variation by genetic mechanisms and natural selection of the best suited phenotypes is the central tenet of the prevailing neo-Darwinian theory of evolution [1]. However, phenotypic diversity can also result from the production of epigenetic variants from the same genome either via developmental stochasticity or environmental induction [2-6]. Earlier, genetic variation was regarded as the sole type of variation that could produce an evolutionary response. Epigenetic sources of phenotype variation were seen as a one-generation
phenomenon without evolutionary impact because they were considered non-heritable.

The importance of the environment for shaping of the phenotype and evolution has been recognized since Darwin’s times and has been reinforced in recent discussions concerning phenotypic plasticity [7–12]. Meanwhile, there is sound evidence that developmental and environmental influences on phenotype are mediated by epigenetic mechanisms such as DNA methylation, histone modifications and microRNAs [2, 13–15]. The involvement of epigenetic mechanisms in the production of phenotypic diversity is much better investigated in plants than in animals [16–19]. However, if the role of epigenetic phenotype variation is to be understood in ecology and evolution, its significance must also be demonstrated for animals. Although research on environmental and evolutionary epigenetics is still in an early stage there have already been attempts to integrate it into evolutionary theory [3–5, 13, 22–24].

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Most animal populations are obligatory sexual and recombine the phenotype-determining genes in each generation, which makes research on the role of epigenetics in animal ecology and evolution difficult. Genetically identical or genetically depauperate populations may help to overcome this constraint, as will be discussed below. The article starts with a comparison of stochastic developmental and environmentally induced phenotype variation. It then reviews the contribution of asexually reproducing, invasive, domesticated, hybrid, and polyploid animals to the understanding of the role of epigenetic mechanisms in ecological adaptation, consolidation of speciation, and potential triggering of speciation. The last section elaborates on the special suitability of monoclonal invaders for research on environmental and evolutionary epigenetics and proposes a research agenda.

Stochastic Developmental versus Environmentally Induced Phenotype Variation

Phenotypic variation can be subdivided into genetic, stochastic developmental, and environmental components [4, 20, 21]. The two epigenetic proportions are called ‘stochastic developmental phenotype variation’ (SPV) and ‘environmentally induced phenotype variation’ (EPV) in the following. Both epigenetic phenomena are closely intermingled but represent different mechanisms to generate phenotypic diversity [3–5, 13, 22–24].

The difference between SPV and EPV can be best illustrated by raising genetically identical populations either in the same or different environments [24]. SPV (sometimes called ‘developmental noise’) generates a range of epigenetically mediated phenotypes around a mean phenotype (MP) that is determined by the interaction of the genome and the prevailing environment (Fig. 1A). The range of SPV can randomly vary between identically raised populations or between subsequent generations grown in the same environment but the MP is thought to remain constant in both cases (Fig. 1A). This a priori production of a range of epigenetically diverse phenotypes from the same genotype (DNA sequence and its copies) without knowing the future conditions serves for risk-spreading that enhances the chance to stay in the game of life when the environmental conditions change. It is thought to contribute to evolutionary bet-hedging [3–5]. SPV seems to be particularly important for asexual species and lineages but is probably also effective in sexually reproducing species, which use genetic recombination as a major generator of phenotypic diversity.

EPV is synonymous with phenotypic plasticity sensu strictu, the adaptive response of populations to environmental signals [3, 5, 8, 25, 26]. Researchers on phenotypic plasticity have often
subsumed stochastic developmental and environmental influences on phenotype under this term [9]. For reasons of clarity, I use EPV instead of phenotypic plasticity in the following. Cues from different environments can induce different epigenetically mediated phenotypes from the same genotype (Fig. 1B). Snell-Rood [27] defined two forms of phenotypic plasticity: developmental and activational. Developmental plasticity refers to the capacity of a genotype to adopt different developmental trajectories in different environments (not to be confused with SPV). Activational plasticity refers to differential activation of an underlying network in different environments such that an individual expresses various phenotypes throughout its lifetime. In contrast to SPV, EPV is thought to be directional shifting of the MP to different positions on the scale of possible phenotypes (Fig. 1B). The MPs of all environments together form the reaction norm curve for phenotypic traits [28]. SPV and EPV are closely intermingled because the environment determines the position of the MP on the scale of possible phenotypes and sets the frame in which SPV can vary (Fig. 1B).

A special case of environmentally induced phenotype induction is polyphenism, the switch between alternative phenotypes in response to environmental signals [29-31]. A good example is the queens and workers of honey bee, which originate from the same genome but differ in morphology, social behavior, and life span due to different feeding of their larval stages (royal jelly for the designated queens, pollen for the workers). These phenotypic differences are mediated by epigenetic mechanisms. Lyko et al. [31] established that DNA methylation in brains of queens and workers of bee differs in more than 550 genes, including genes involved in metabolism, brain development, and neural functions. Herb et al. [32] revealed that DNA methylation is even involved in the reversible switch of honey bee workers between nursing and foraging periods.

All traits in animals seem to vary due to SPV but at different degrees, depending on species and condition [4]. As a rule of thumb, morphological traits show the lowest degree of SPV whereas behavioral traits show the highest degree [4]. Probably, only few of these variations have an adaptive potential, among them traits involved in feeding, camouflage, or temperature resistance. Examples of traits without adaptive potential are the fingerprints of primates, which are highly variable among genetically identical twins [4]. Similarly, the majority of environmental cues do not induce EPV. Many result in a short-term physiological response and many in no response at all. Most examples on EPV published so far for animals concern environmental toxicants [33, 34]. Food shortage, predator pressure, and harsh environmental conditions are thought to be the main elictors of non-pathological EPV [3, 26, 35].

Animals seem to have sensitive windows for the susceptibility to SPV, which depends much on trait and life history of the species considered [4]. As a rule of thumb, embryonic development seems to be of prime importance in determinately growing mammals and insects, whereas the adult life period appears equally important in indeterminately growing crustaceans, molluscs, and fish [4]. For example, differences in adult body weight among identically raised littermates of inbred rat were shown to have their roots in stochastic events in the zygote and first cleavage stages [36], whereas in isogenic batch-mates of marbled crayfish such differences were more attributable to the adult life [24]. SPV-related differences in ageing and diseases of humans can have their origin in both early development and the later life [37, 38]. Similar sensitive windows also exist for EPV [3, 33, 39].

The environmental exposure at a critical window can alter the epigenetic programming and subsequently change gene expression [33].

Facilitation of Ecological Adaptation by Epigenetic Phenotype Variation Exemplified by Invasions

Investigation of natural animal populations revealed that epigenetic diversity is usually higher than genetic diversity. This was shown for species diverse as sandhoppers, fish, and bats [40-42]. These findings suggest that wild animals might use epigenetic phenotype variation in addition to genetic variation to adapt to different environments. In sexually reproducing species, the generation of different phenotypes via genetic recombination and selection of the best suited phenotypes is regarded as the prime mechanism of ecological adaptation. However, this mode of adaptation to new environments is not applicable for asexually reproducing populations and genetically uniform invasive groups. The generation of a range of epigenetically different phenotypes from the same genome is probably an alternative in these organisms. Epimutations are apparently much more frequent than genetic mutations, e.g. $3 \times 10^{-4}$ methylation gains or losses per CpG and generation compared with $7 \times 10^{-6}$ base substitutions per site and generation in the model plant Arabidopsis thaliana [43, 44]. One may conclude from this fact that the generation of phenotypic diversity by epigenetic mechanisms occurs with much higher speed when compared with random mutation, which is the only genetic mechanism to generate phenotypic diversity in asexual lineages. However, it is largely unknown how many single nucleotide polymorphisms (SNPs) or single base methylation polymorphisms (SMPs) are required to change phenotypic traits. The relationship between genetic, epigenetic, and phenotypic variation is presently investigated in animals, plants, and humans by genome-wide association studies [45-48]. Individual SMPs seem to have no direct phenotypic effect [49] but many of them together produce differentially methylated regions (DMRs) and epialleles, which have been shown to underlie phenotypic changes [48-52]. Epialleles are often considered to have a low stability as will be discussed in detail later on, and some theoretical models suggest that epigenetic variation may be less responsive to natural selection than genetic variation, even in cases where levels of heritability appear similar [53]. These argument seem to speak against a significant role of epigenetic phenotype variation in adaptation.

Arguments for a supportive role of epigenetic phenotype variation in environmental adaptation of animals come from biological invasions [54]. Adaptation is the process by which species become fitted to their environment. It is the result of the action of natural selection upon heritable variation. Invaders can show two different types of adaptation, plastic and genetic [26, 55]. Plastic adaptation is based on EPV plus SPV and is mediated by epigenetic mechanisms. Genetic adaptation is based on genetic variation and selection. The relationship between the two is complex and poorly understood [26]. Interestingly, invasive groups can be ecologically and evolutionarily very successful even when genetically impoverished. This phenomenon is well known as ‘invasion paradox’ but the underlying mechanisms are largely unknown [56-59]. Particularly the first steps of invasion, the survival of the invading group and the establishment of a founder population, may depend much on epigenetic variation.
An animal example of the involvement of epigenetic mechanisms in adaptation to new environments is the invasion of house sparrow, *Passer domesticus*, in Kenya [60]. House sparrows were introduced to Mombasa in the 1950s and since evolved significant phenotypic variation. Analysis of DNA methylation sensitive-amplified fragment length polymorphisms (MS-AFLP) and microsatellite loci revealed that Kenyan house sparrow populations have a low genetic diversity but a high epigenetic diversity. There was a significant negative correlation between epigenetic and genetic diversity (Fig. 2) and a positive correlation between epigenetic diversity and the degree of inbreeding, suggesting that DNA methylation may help to overcome genetic bottlenecks and inbreeding.

The production of epigenetically diverse phenotypes from the same genome may also explain why many clonal animals and plants are able to live in a wide range of habitats and geographical regions, despite their genetic uniformity. This phenomenon is often explained by the existence of so-called ‘general-purpose genotypes’ or ‘superclones’, which are thought to be able to cope with a broad spectrum of environmental challenges [61–63]. An interesting animal example is the gynogenetic all-female fish *Chrosomus eos–neogaeus*, which occurs in North America in 14 genetically identical lineages originating from different hybridization events between the redelly dace *C. eos* and the fine-scale dace *C. neogaeus* [64]. Dating of hybridization events suggested a relatively recent origin (<50 000 years ago). Each hybrid lineage apparently originated from a single hybrid zygote and is genetically uniform with the exception of random mutations that accumulated over time. These lineages frequently display a large geographical distribution at a regional scale. The analysis of DNA methylation polymorphisms within such lineages revealed epigenetic similarity of individuals in a given lake but significant differences between lakes, suggesting that epigenetic phenotype diversification may be the mechanistic explanation of the ‘general purpose genotype’ [65].

Investigation of clonal populations has the advantage that genetic variability is usually much smaller than in sexually reproducing populations but it can still be present. Thus, the observed epigenetic variation might mimick genetic variation to some extent (see discussion on polyclonal and monoclone invaders below).

Recent analysis of DNA methylation in *C. eos–neogaeus* lineages from predictable and unpredictable environments (lakes versus intermittent headwater streams) in Canada identified the relative contributions of SPV and EPV to phenotype modification [5]. Comparison of clones from the wild revealed that risk-spreading SPV prevails in unpredictable environments whereas directional EPV is predominant in predictable environments (Fig. 3). Differences in environmental effects on epigenetic variation between sympatric lineages (Fig. 3, LR1 and ET2) further showed that the epigenetic response to environmental signals is strongly influenced by the genotype. Common garden experiments revealed that the proportion of pure environmental effects can considerably change when clone members are transplanted into a new environment (Fig. 3, LR1 and ET2 under natural and experimental conditions). The example of *C. eos–neogaeus* demonstrates that SPV and EPV always act conjointly but have different weighting in different environments.

**Facilitation of Evolution by Epigenetic Mechanisms Exemplified by Hybrids, Polyploids, Adaptive Radiations, and Domesticated Animals**

Even if considered as a one-generation phenomenon only, the production of a range of epigenetically determined phenotypes from the same genetic template would have some evolutionary consequences since it helps populations, particularly clonal ones, to stay in the game of life when the environmental conditions change [2, 4, 66]. The evolutionary relevance of epigenetically mediated phenotypes would even be far greater if the best suited ones were inherited across generations, selected, and genetically fixed on the long range. In the following, I will present some examples on the consolidating role of epigenetic
mechanisms in speciation and then discuss the possibility of epigenetic mechanisms as triggers of speciation.

Stabilization of New Lineages and Species by Epigenetic Mechanisms

Epigenetic mechanisms are thought to contribute to speciation by stabilizing the genomes of new species [67, 68]. The examples presented below show that epigenetic patterns can markedly change during speciation, particularly in hybrids and polyploids, in which genome rearrangements are most intense. However, these examples do not conclusively answer the question whether the observed epigenetic alterations passively follow genetic changes or whether they play an active role in restructuring of the genome, which I consider possible. The relationship between epigenetics and speciation is better investigated in plants than in animals and is particularly pronounced in polyploids and domesticated species [67–73].

Polyploid speciation is usually saltational and accompanied by reproductive isolation, alteration of life history traits, and modifications of DNA content and DNA methylation level. An example is the marbled crayfish Procambarus virginalis (Fig. 4A), which originated in evolutionarily recent times from slough crayfish P. fallax by autotriploidization and concomitant change in the sexual system from gonochorism to parthenogenesis. The global DNA methylation levels in polyploids can either be higher or lower when compared with the parent species. For example, after doubling of the genome in the stem line of fishes, 70–80% of duplicated genes have been lost over time [83]. DNA loss mostly concerns redundant genes. For example, in hybrids of kangaroo Macropus eugenii and Wallabia bicolor, in which the reproductive barrier is provided by epigenetic imprinting of genes involved in placentation [80]. New auto-polyploid and allopolyploid (hybrid) genomes are usually unstable and require chromatin rearrangement, which is apparently achieved by the interaction of genetic and epigenetic mechanisms [67, 81, 82]. A common feature in polyploid neo-species is the reduction of the genome size, in extreme cases back to diploidy [83]. DNA loss mostly concerns redundant genes. For example, after doubling of the genome in the stem line of fishes 70–80% of duplicated genes have been lost over time [83].

The global DNA methylation levels in polyploids can either be higher or lower when compared with the parent species. For example, hybrids of kangaroo Macropus eugenii and Wallabia bicolor and autotriploids of crayfish P. virginalis showed genome-wide undermethylation [76, 81], whereas hybrids of red crucian carp Carassius auratus and common carp Cyprinus carpio displayed hypermethylation [84]. In the kangaroo hybrids, the removal of DNA methylation from retrotransposons facilitated their amplification and caused gross changes in genome structure.

In hybrids of the frogs Xenopus laevis and Xenopus muelleri, 364 out of 546 MS-AFLP markers were effective in elucidating the difference in methylation patterns between hybrids and parental species. Hybrids exhibited a significantly higher proportion of methylated fragments relative to both parental species,
which may translate into changes of gene expression profiles. Moreover, 76 methylated fragments were diagnostic of hybrids only (Fig. 5). These new epigenetic patterns may indicate the involvement of epigenetic mechanisms in restructuring of the hybrid genome [85]. The parental species and hybrids are all tetraploids but X. muelleri and the hybrids have a DNA content of 113.5% and 112.3% when compared with X. laevis, indicating that DNA methylation levels are not strictly associated with genome size. Hybrid females are fertile and hybrid males are sterile. Differential methylation between sexes (Fig. 5) and misexpression of genes responsible for reproduction in males may account for these differences [85, 86].

Polyploids often have life history traits that are different from those of the parent species as shown for marbled crayfish (Fig. 4B). Growth, number of offspring, and other quantitative traits can either be decreased or increased when compared with the diploid ancestors [76, 87, 88]. In allopolyploids, the increase of life history traits is usually explained as the result of heterozygosity. This explanation is not applicable for autopolyploids, which have the same set of genes as their parent species. In autopolyploids, trait alteration is caused by changes of gene dosage, rearrangement of gene-networks, and modulation of gene expression. All of these features apparently require the contribution of epigenetic mechanisms. As an example, during ancient mammalian gene duplication, DNA methylation apparently played a dominant role in dosage rebalance by inhibiting transcription initiation of duplicate genes [89].

Domesticated animals are particularly suitable for evolutionary epigenetics because their evolution (breeding history) is short and much better known when compared with wild species. Moreover, domesticated species show an exceptionally broad variation in phenotypic traits and their genetics is comparably well investigated. In dogs (Canis familiaris), which descended from the grey wolf (Canis lupus), some of the phenotypic changes have already been linked to specific genes. For example, different alleles involved in the fight-or-flight response have been subject to strong selection and resulted in behavioral differences between dogs and wolves [72]. Janowitz Koch et al. [73] established that domestication of dogs has also been associated with epigenetic alterations. They analyzed methylation differences in >24 000 cytosines distributed across the genomes and revealed species-specific patterns of DMRs at 68 sites. The majority (>66%) of DMRs were associated with repetitive elements, indicative of a genotype-mediated trend. However, DMRs were also often linked to functionally relevant genes (e.g., neurotransmitters), suggesting that selection has not only acted on genes but also on methylation patterns [73].

**Triggering of Evolutionary Change by Epigenetic Mechanisms—A Provocative Idea**

Genetics and epigenetics are closely linked, simply because epigenetic marks are on the nucleotides and histones that built up the DNA and chromatin. Therefore, when analyzing genetic and epigenetic diversity in populations it is difficult to identify what was first, genetic variation or epigenetic variation. Probably, most scientists tend to interpret epigenetic changes as followers of genetic changes [90, 91] rejecting the possibility of a leading role of epigenetic mechanisms in evolution (epigenetics-first hypothesis). However, some authors including myself consider the latter alternative possible [4, 7, 18, 92] (epigenetics-first hypothesis), which would considerably enhance the relevance of development and environment for evolution.

Support for the genetics-first hypothesis comes from the methylome analysis of a geographically dispersed Arabidopsis thaliana population of near-isogenic lines that diverged for at
least a century from a common ancestor [91]. Methylome variation largely reflected genetic distance and was in many aspects similar to that of lines raised in uniform conditions. The authors concluded from their results that even when plants are grown in varying and diverse natural sites genome-wide epigenetic variation accumulates in a clock-like manner, suggesting that epigenetic divergence parallels the pattern of genome-wide DNA sequence divergence.

Support for the epigenetics-first hypothesis comes from the investigation of the well-known radiation of Darwin’s finches [92]. Darwin’s finches evolved over a period of 2–3 million years from a single invader and yielded 14 extant species that fill distinct ecological niches. In the five species investigated, epimutations (DMRs) were more common than genetic mutations (copy number variants, CNVs). Moreover, the number of DMRs increased monotonically with phylogenetic distance, whereas the number of CNVs did not (Fig. 6). The number, chromosomal locations, regional clustering, and lack of overlap of epimutations and genetic mutations suggest that epigenetic changes are distinct and may cause evolutionary change independently from genetic change.

The idea of epigenetic variation as a driving force of evolution requires transgenerational inheritance of adaptive epigenetic patterns, selection of the epigenetically determined phenotypes, and genetic fixation of these phenotypes on the long term. The concept of transgenerational epigenetic inheritance (TEI) is still controversial [99] but there are several theoretical models and convincing empirical examples published for plants and animals [7, 94–98]. Major arguments against the involvement of epigenetics in animal evolution come from Weissmann’s germ plasm theory [94, 99] and the observation that DNA methylation is erased in the primordial germ cells and the zygote of mammals [100, 101], preventing epigenetic profiles from being inherited to the next generation. However, both objections were inferred from mammals only and do not apply to all animal groups. In nematodes, insects, and vertebrates, there is indeed an early separation of the germline from the soma [102], but in sponges, cnidarians, bryozoans, flatworms, annelids, ascidians, and echinoderms, somatic cells can transform into germ cells in later life stages, and thus, somatic epimutations acquired during early life stages may reach the germline to be transgenerationally inherited. Moreover, many animals can reproduce asexually by budding, fragmentation, and parthenogenesis [103, 104], sometimes alternating with sexual reproduction. Genome-wide DNA demethylation and remethylation has so far only been demonstrated for the germ cells and zygote of sexually reproducing mammals but not for the germ cells and zygote of sexually reproducing invertebrates and the reproductive units of asexually reproducing animals. And even in mammals, there are increasing examples of incomplete DNA methylation in the zygote [105] and epigenetic inheritance via microRNAs and DMRs in sperm [35, 106–108].

Natural selection acts on phenotypes, and therefore, epigenetic variation is thought to provide a substrate for selection similarly to genetic variation [109]. Ideally, only those epigenetic profiles should be preserved and selected that accomplish adaptation to new environmental conditions or enhance fitness in the prevailing environment. Herman et al. [110] emphasized that the stability of epigenetic states beyond a single generation depends on the degree of environmental variation and the costs of epigenetic resetting. Becker et al. [111] investigated spontaneous epimutations in 10 lineages of A. thaliana over 30 generations under constant greenhouse conditions and found that under these conditions not many epimutations were inherited over the long term. Some sites seemed to go through recurrent cycles of forward and reverse epimutations. This seemingly low stability of epimutations in constant environments was often used to argue against an evolutionary role of epigenetic inheritance.

Recent theory established that epigenetic flexibility can buy time for a genetic response to evolve. Epigenetic variants can be exposed to selection so that they follow dynamics similar to those of ordinary genetic variants [112–115]. However, under some circumstances epigenetic inheritance can impede adaptation. Kronholm and Collins [114] modeled cases where epigenetic mutations speed adaptation resulting in populations with higher fitness and cases where they slow adaptation resulting in populations with lower fitness. The effect of epigenetic mutations on adaptation seems to depend on their stability and fitness effects relative to genetic mutations. Geoghegan and Spencer [116] investigated the conditions under which an epigenetically modifiable allele can invade a population. They found that epialleles were less likely to invade when mismatch between environment and phenotype was too costly. Moreover, for a wide range of parameters, a higher rate of germline epigenetic resetting decreased the likelihood of epiallele invasion. Furrow and colleagues [53, 117] predicted from their model that epigenetic variation may be less responsive to natural selection than genetic variation. On the other hand, simple deterministic selection models showed that newly arising epimutations are stable enough to respond effectively to long-term selection, yielding epimutation-selection equilibria that are close to those expected for DNA sequence mutation rates [43]. Appropriate experiments should be designed to empirically test these conflicting model predictions.

Epigenetically determined phenotypes can be genetically accommodated or assimilated on the long term by a series of quantitative genetic changes [9, 10, 118–120]. This mechanism requires pre-existing genetic variation in the population and is thus not applicable to genetically uniform populations. There, epigenetically caused phenotypic diversity may be genetically fixed either by awaiting suitable random mutations or, probably with much higher speed, by facilitated mutations at epigenetically modified sites. Potential mechanisms are the well-established high mutability of methylated CpGs [121], the transposable element-epigenetic component engine [122], or the promotion of genetic mutations in CNVs via epimutations [92]. Methylated CpGs have a much higher mutation rate when compared with cytosine in other contexts. Such sites can even have mutational effects on the surrounding DNA [121]. Transposons are often silenced by DNA methylation, which is sensitive to environmental cues. If activated by new environmental signals they can multiply and shift to new sites in the genome generating genetic diversity [68, 123]. Transgenerationally inherited epigenetic features in rat were shown to promote genome instability such that CNV mutations were acquired in later generations [92].

The potential role of TEI for evolution is discussed in depth in Jablonka and Raz [94]. The authors stressed that epigenetic control mechanisms and heritable epigenetic variations are relevant for evolution because they affect both the processes of adaption and divergence. Adaptation can occur through the selection of heritable epialleles without any genetic change, which is of particular importance when populations are small and lack genetic variability. Bonduriansky et al. [124] reviewed models and empirical examples of adaptation to changing environments that included environmentally induced as well as spontaneously arising phenotypic variants. They predicted that
non-genetic inheritance can increase the rate of both genetic and phenotypic change and, in some cases, alter the direction of change. Empirical evidence confirms that a diversity of epigenetically determined phenotypes, spanning a continuum from adaptive to pathological, can be transmitted non-genetically [124].

Branciamore et al. [125, 126] revealed with their model that epigenetic modifications of gene duplicates and transposons with rates consistent with experimental observation can have significant micro- and macro-evolutionary effects. Epigenetic modifications can drive neo- and subfunctionalization of gene duplicates thus dramatically improving the efficacy of evolution. Epigenetic mechanisms can also rapidly fix transposons and cis-located gene alleles. Interestingly, the model further suggests that epigenetic silencing, even if strictly transient (being reset at each generation), can still have significant macro-evolutionary effects.

**Clonal Invaders as Promising Models for Environmental and Evolutionary Epigenetics: Theoretical Considerations and Research Agenda**

The different outcomes and interpretations of theoretical models, laboratory experiments, and field studies complicate our understanding of the role of epigenetics in ecology and evolution, requiring further studies with simple model systems. Clonal invaders seem to be particularly useful because they combine the advantages of clonal organisms and invaders and can adapt to different environments despite genetic uniformity. There are several interesting clonal animal invaders known, among them crustaceans, insects, snails, and fishes [5, 64, 127–131]. In this section, I will first highlight the advantages of clonal species and successful invaders for research on environmental and evolutionary epigenetics. Thereafter, I will sketch a simple scenario on the invasion of a new environment by a monoclonal invader to hypothesize about the role of epigenetic phenotype diversification in adaptation and speciation. Finally, I will describe two highly successful monoclonal invaders, which I regard particularly suitable to empirically test some of the controversial issues of environmental and evolutionary epigenetics.

**The Advantages of Clonal Lineages**

The epigenetic proportion of phenotypic variation can more easily be determined in clonal lineages than in sexually reproducing species because of the paucity or absence of genetic variation. Therefore, asexually reproducing plants and animals have repeatedly been used to investigate the role of epigenetics in ecology and evolution [2, 64, 127–133]. Natural asexual populations are mostly polyclonal and only rarely monoclonal. Polyclonality is the result of repeated hybridization events, backcrossing with related sexual species, and long-lasting accumulation of genetic mutations [64, 134]. Monoclonal populations are of single origin and genetically identical with the exception of random mutations. In evolutionarily young monoclonal populations genetic diversity is virtually absent.

The number of potentially disturbing genetic mutations is particularly small among asexually produced batch-mates. Therefore, they are preferred subjects for laboratory studies on epigenetic variation that is independent of genetic variation. SPV and EPV can be distinguished by raising batch-mates either in the same or different environments. Laboratory experiments with clonal lineages are also suitable to identify the environmental cues that induce alterations of epigenetic profiles and the conditions under which phenotype modulating epigenetic marks are transgenerationally inherited. Moreover, they are useful to test whether epigenetic diversity can be transformed into genetic diversity in the long term. The investigation of wild clonal populations, particularly monoclonal ones, gives an idea on the extent and relevance of epigenetic phenotype variation under real life conditions.

Latzel et al. [135] presented evidence from greenhouse experiments with genetically identical but epigenetically diverse populations of Arabidopsis thaliana that the generation of epigenetically different phenotypes from the same genome can markedly modify fitness and functional diversity. They recorded up to 40% more biomass in epigenetically diverse populations when compared with epigenetically uniform populations. Interestingly, the positive effects of epigenetic diversity were strongest under stress conditions, for instance, when populations were grown together with competitors or infected with pathogens. Such studies are not yet available for animals. Dodd and Douhovnikoff [136] speculated that the expansion of phenotypic diversity by epigenetic mechanisms may provide a buffer against the hazards of global warming.

**The Advantages of Invasions and Adaptive Radiations**

Above, I have already elaborated on the advantages of animal invasions for ecological epigenetics. Respective references on plants invasions are found in [137, 138]. In this section, I want to highlight the advantages of invasions and adaptive radiations for evolutionary epigenetics.

Phylogenetically young invasions are expected to provide information on the role of epigenetic mechanisms in adaptation of the first generations to the new environment whereas older radiations may provide information on the role of epigenetics in evolutionary adaptation and speciation. Examples of relatively young animal invasions are the marbled crayfish in Europe and Madagascar (~10 years) [131], the snail Potamopyrgus antipodarum in America (~30 years) [129], the house sparrows in Kenya (~50 years) [60], the American water flea Daphnia pulex in Africa (~90 years) [128], the house sparrows in Florida (~150 years) [139], and P. antipodarum in Europe (~175 years) [127]. The ages of these invasions are proven by historical documents. Evolutionarily older invasions and radiations are easiest found on islands and in lakes of known geological age. Examples are the cichlid fish Amphiliopus citrinellus in crater lakes in Nicaragua (~20 000 years) [140], the cyprinid fish Chrosomus eos-neogaeus in lakes and streams of Canada (~54 000 years) [64], the Darwin’s finches on Galapagos (~2 million years) [92], and the cichlid fish in East Africa’s large rift lakes (500 000–12 million years, depending on lake) [141].

Preliminary genetic and epigenetic analyses are available for only three of these examples, the Kenyan house sparrows, the Canadian C. eos-neogaeus and the Darwin’s finches [5, 60, 92]. The results seem to suggest that epigenetic diversity may compensate for reduced genetic diversity in the first period after invasion and promote speciation later on. All examples show that alleles and epigenetic patterns can vary independently from each other to some degree. Support for the independent variation also comes from the radiation of human populations [48], suggesting that epigenetic variation may contribute to adaptation and evolution. However, all of these studies lack information on the dynamics of genetic and epigenetic patterns over time, which makes it difficult to decide what was first, genetic or epigenetic variation. This issue could be best investigated by
long-term laboratory experiments or field studies with animals that deposit dormant stages, which remain viable for decades and centuries, as detailed below.

Scenario on the Invasion of a New Geographical Area by a Monoclonal Invader and Subsequent Radiation

In order to illustrate the possible role of epigenetic phenotype variation for adaptation and evolution I use a simple thought experiment, namely the invasion of a new geographical region by a single gravid apomictic parthenogenetic female. The embryos carried by this female consist of genetically identical but epigenetically different phenotypes that have been generated by SPV under the conditions of the old environment (Fig. 7A). If the epigenetic differences concern ecologically relevant traits like food processing efficiency or tolerance to extreme temperatures, then they increase the chance of survival of the first generation and facilitate establishment of a founder population.

Differences in the preference of food, substrate or temperature may then lead to the occupation of different habitats and ecological niches (Fig. 7B). Exposure to different environmental cues may speed up phenotype diversification via EPV. In the following generations, the interplay of EPV and SPV, TEI of evolutionarily relevant epigenetic signatures, and differential selection (DS) on the epigenetically mediated phenotypes (D) may lead to the occupation of different habitats and the establishment of ecological barriers. It may then lead to the origin of new species.

Figure 7: Thought experiment on the role of epigenetic phenotype variation in environmental adaptation and evolution. (A) Starting point is the invasion of a new geographical region by a gravid parthenogenetic female and the subsequent release of genetically uniform but epigenetically and phenotypically diverse progeny (P1–P10). The diversity of the offspring was produced by SPV under the conditions of the old environment. (B) The epigenetically different offspring then occupy different habitats (H1–45) and are now exposed to different environmental cues. (C) In the following generations, different ecotypes (ET1–ET5) evolve by the interplay of EPV and SPV, TEI of evolutionarily relevant epigenetic signatures, and differential selection (DS) on the epigenetically mediated phenotypes. (D) The conversion of epigenetic diversity (ED) into genetic diversity (GD) and the establishment of ecological barriers may finally lead to the origin of new species.

Research Agenda with Monoclonal Invaders

Long-term laboratory experiments with monoclonal lineages combined with the analysis of respective wild populations should reveal whether the epigenetics-first scenario is principally possible or not. This approach is also expected to solve the chicken and eggs dilemma described above by comparing the chronology of genetic, epigenetic, and phenotypic changes in a population. It should also provide information on the extent of stochastic and environmentally induced epigenotype diversification and the heritability and selectability of epigenetic patterns. Finally, it should reveal whether epimutations can induce genetic mutations, which permanently fix phenotypes that were initially epigenetically determined. In the following, I will introduce two highly successful monoclonal invaders that I consider particularly suitable to test the first steps of the scenario in Figure 7 and several of the above questions. In order to highlight the special advantages of these model systems I need to describe their biology and invasion history in some detail.

Obligately Parthenogenetic Crayfish Clone Invasive in Europe and Madagascar

The parthenogenetic marble crayfish Procambarus virginalis has evolved from sexually reproducing slough crayfish P. fallax by
autotriploidy as described earlier. It is regarded as an independent species for reasons detailed in [76]. In the year 2013, we have launched a project at the German Cancer Research Center (DKFZ, Heidelberg) to establish the complete genome sequence and a genome-wide reference methylome [152], which are basic requirements for using this clonal species for research in ecological and evolutionary epigenetics. Whole genome and whole methylome comparison between marbled crayfish and slough crayfish is expected to reveal how much genetic and epigenetic change is necessary to create a new species and which genes and epigenetic markers underlie the transition from gonochorism to parthenogenesis. Comparison of the genomes and methylomes of marbled crayfish adapted to different environments is expected to establish whether DNA methylation is involved in ecological adaptation and the evolution of different ecotypes from the same genotype.

Meanwhile, both crayfish have been sequenced on an Illumina HiSeq platform for de novo assembly of the genomes and whole-genome bisulfite sequencing has been applied to establish genome-wide methylation maps at single-base resolution [76, 153, 154]. The genome of the marbled crayfish has a size of 3.5 GB and includes more than 21,000 genes [153]. A draft genome is accessible at the Marbled Crayfish Genome Server (http://marmorkrebs.dkfz.de/). The transcriptome revealed 22,338 transcripts of which 12,855 could be automatically annotated. Proteome analysis yielded a total of 43,783 peptides [154]. The global DNA methylation level is 2.4% (Fig. 4D) [76]. Genomewide methylome analysis revealed mosaic methylation, gene body methylation, and hypomethylation of repeats. Methylation is particularly prominent in housekeeping genes, suggesting a role in fine-tuning of gene expression and perhaps environmental adaptation [154].

Marbled crayfish have an adult size of 4–11 cm, a generation time of 6–7 months and a maximum age of about 4.5 years [77, 155–159]. The relatively large size allows whole genome and methylome analyses of individuals and even individual organs. Marbled crayfish produce up to seven clutches per lifetime, each clutch comprising some 50–400 genetically identical siblings (Fig. 8A) [24, 78, 160]. The high number of batch-mates enables intense experimentation with isogenic specimens. Comparison of genomes and methylomes among brooded batch-mates that have not yet experienced different environments (Fig. 8A) are particularly suitable to obtain information on the frequency of random mutations and stochastic epimutations per generation. Since marbled crayfish can be raised in a variety of housing systems at water temperatures between 3 °C
and 30 °C and fed with a variety of food [161, 162], they are also well suitable for the investigation of environmentally induced phenotype variation. Long term experiments in the laboratory under different stable and unstable conditions are expected to reveal whether epigenetic profiles can be inherited and genetically fixed and under which conditions this might happen.

In order to investigate whether epigenetic mechanisms facilitate ecological adaptation, laboratory-raised reference lineages should be compared with wild populations of known age that have evolved under clearly different conditions. In the years 2003 and 2004, I have founded two indoor lineages from single individuals, which are well suitable as reference lineages [76]. These lineages (Heidelberg and Petshop) have consistently been raised in highly standardized settings (Fig. 8C) and were fed throughout life and over generations with a single pellet food (Tetra WaferMix). A 3-year-old individual of the Petshop lineage was used for sequencing of the reference genome. Wild invasive populations are known from Europe (Fig. 8B) and Madagascar. They have established themselves since 2003 as the result of introductions [131, 163–167].

Apparently, all invasive populations originate from the same source as our laboratory populations, namely an individual or small isogenic group that first appeared in the German aquarium trade in about 1995 [155, 157]. Comparison of the complete mitochondrial genomes, nuclear microsatellites, and larger segments of genomic DNA between members of the two reference lineages and two wild populations from Lake Moosweiher (Germany) and Madagascar revealed complete genetic identity (Fig. 8F and G), demonstrating that all populations are of single origin and monoclonal [76, 153]. The wild populations have evolved independently from our laboratory lineages for some 10–20 generations and have adapted to different habitats such as creeks, rivers, oligotrophic to eutrophic lakes (Fig. 8D), thermal and ice-covered lakes, acidic and polluted ponds, and swamps and rice fields. Adaptation to these highly diverse ecosystems cannot be explained by genetic variation and may have been achieved by epigenetic variation instead. The first comparison of global DNA methylation between Heidelberg and Moosweiher specimens revealed a higher value in the former (Fig. 8E). However, meaningful information on the involvement of DNA methylation in environmental adaptation is only expected from the ongoing comparison of genome-wide methylomes.

**Obligately Parthenogenetic American Water Flea Clone Invasive in Africa**

The second monoclonal invader that could contribute significantly to ecological and evolutionary epigenetics is an obligately parthenogenetic American *Daphnia pulex* clone (Fig. 9A) that invaded Africa about 90 years ago [128]. Genotyping of dormant eggs sampled from sediment layers of known age allowed the reconstruction of the dynamics of this invasion in Lake Naivasha, Kenya. In about 1930, the new genotype appeared first in the lake and increased then steadily in relative frequency until 1955 when it accounted for about 60% of the total water flea population (Fig. 9B). After a 30-year period of relative stability the invasive clone expanded further until 1998 when it had completely displaced the native *D. pulex* genotypes. Genetic

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**Figure 9**: Invasive American clone of water flea *Daphnia pulex* in Africa as promising model to study epigenotype, genotype, and phenotype diversification across space and time. (A) Obligately parthenogenetic female carrying ephippium with dormant eggs (arrow) in brood chamber (from [128], photograph by Joachim Mergeay). (B) The American clone invaded Lake Naivasha in Kenya in about 1927. It successively replaced native *D. pulex* as revealed by microsatellite analysis of dormant eggs from dated sediments since the year 1920. Stippled line shows gradual decrease of genetic diversity over time (bars indicate 95% confidence). (C) The analysis of 12S rRNA genes of dormant eggs from sediments of Lake Naivasha (LN) dated to the years 1925, 1955 and 2003 demonstrates close genetic relationship of the pre-invasive *D. pulex* to European strains and identity of the invasive clone with a Canadian strain. (D) The invasive clone has meanwhile spread across the cooler regions of Eastern and Southern Africa and replaced the native populations. Arrow indicates Lake Naivasha (graphs modified after [128])
analysis of dormant eggs from different decades (Fig. 9C) and laboratory rearing of resured specimens identified the invading clone as an obligately parthenogenetic D. pulex × D. pulicaria hybrid, which is common in North America [128, 168]. The displaced native water flea was geneti~ically more closely related to European representatives of the D. pulex species complex (Fig. 9C). The absence of variation in the mitochondrial NDH dehydrogenase subunit 5 gene and 10 hypervariable nuclear markers of several other African populations of the invasive clone strongly suggest single origin and monoclona~ity. Most likely, this clone was accidentally introduced into Lake Naivasha in the years 1927–29 during stocking of largemouth bass from the USA [128].

Meanwhile, the invasive clone has spread from Lake Naivasha throughout Eastern and Southern Africa and displaced native D. pulex populations (Fig. 9D). The invader was found in different types of freshwater habitat including small temporary ponds, eutrophic sewage ponds, shallow turbid lakes, reservoirs, and clear lakes rich in aquatic macrophytes [128]. The absence of genetic variation did not hamper its continent-wide establishment or the effective displacement of the well adapted and genetically diverse native sibling species, which reproduced by cyclic parthenogenesis (alternation of sexual and asexual reproduction). Cyclic parthenogenetic Daphnia populations have been shown to genetically adapt to changing environmental conditions within a few years [169]. The invasive D. pulex clone may also have used epigenetic mechanisms instead.

The main advantages of the invasive water flea clone for research on environmental and evolutionary epigenetics are short generation time, good knowledge of its genetics and epigenetics, and the production of dormant eggs. In natural habitats, D. pulex reproduce several times per year suggesting an African invasion history of a few hundred generations. Under optimal laboratory conditions, these water fleas have generation times of only 10 days allowing monitoring of epigenetic and genetic signatures over many generations in reasonable time. The genome of the species is fully sequenced and annotated [170] and analysis of the methylome was recently performed [171], albeit not in the invasive clone. The DNA methylation data suggest that epigenetic modifications in D. pulex are involved in regulation of gene expression and may thus contribute to the generation of phenotypic variation and environmental adaptation [171, 172]. An outstanding feature of D. pulex is the regular production of dormant eggs, which accumulate in the sediment and remain viable for up to 700 years [173]. DNA from these dormant eggs enables the investigation of epigenetic and genetic diversification of the invader across time and space. Resured individuals from dormant eggs additionally allow the identification of corresponding morphological and physiological trait alterations [173]. A disadvantage of the water flea model system is the small body size, which currently allows whole genome and methylome analyses only for pooled samples and not for individuals or single organs as in marbled crayfish.

Conclusions

Phenotypes are determined by the genome and the epigenome. Since phenotypes provide the raw material for adaptation and natural selection, epigenetic phenotype variation may play an important yet overlooked role in ecology and evolution. There is evidence from experiments with genetically identical organisms that epigenetic phenotype variation can be generated either by developmental stochasticity or environmental induction. SPV contributes to bet-hedging and may be regarded as a Darwinian mechanism whereas EPV serves for directed phenotype optimization and may be regarded as Lamarckian. Usually, both mechanisms act conjointly but their relative contribution to epigenetic phenotype variation depends on species, environment, and trait and is apparently subject to evolutionary change.

The production of epigenetically diverse phenotypes from the same genome is assumed to contribute significantly to environmental adaptation because it broadens the range of phenotypes and thus enhances the chance to stay in the game of life when the environment changes. The advantages of epigenetic phenotype variation are particularly obvious in asexually reproducing species, bottlenecked populations, and genetically uniform invasive groups, providing a mechanistic explanation for the ‘invasion paradox’ and the ‘general-purpose-genotype’. However, epigenetic phenotype variation is probably also important in sexually reproducing species and may help populations to cope with environmental changes in the short term. In times of global warming, it may become particularly relevant.

There is no doubt that epigenetic patterns change during speciation but it is not yet clear whether they passively follow genetic changes and help to consolidate the new genome or whether they can also trigger speciation, or in other words, whether they can be leaders rather than followers in evolution. Epigenetic mechanisms can contribute to reproductive isolation, chromatin reconfiguration, and alteration of gene expression in the neo-species. The possibility of a triggering role of epigenetic mechanisms in speciation would require trans- generational inheritance of phenotype-determining epigenetic patterns and final genetic fixation of the novelties. There are hypotheses and models on these issues but empirical evidence is scarce and contradictory.

Verhoeven et al. [18] have recently discussed what we know and what we need to know about the role of epigenetics in ecological adaptation and evolution. The authors emphasized that assessing the importance of epigenetic effects to ecology and evolution requires the surveillance of epigenetic variation in natural populations and a mechanistic understanding of the relation of epigenotypes to the underlying genotype, the linked phenotypes, and environmental signals. The authors further stressed that high-resolution genome-wide screening of genetic and epigenetic variation combined with expression analysis will be necessary to pinpoint the contributions of epigenetic variation to ecology and evolution. Combined long-term laboratory studies on genomics, epigenomics, transcriptomics, and phenomics of clonal lineages exposed to different environmental conditions and analyses of natural monoclonal invasions seem particularly suitable to address controversial key issues of ecological and evolutionary epigenetics.

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