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Review Article

Phenotypic plasticity as a facilitator of microbial evolution

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Abstract

Tossed about by the tides of history, the inheritance of acquired characteristics has found a safe harbor at last in the rapidly expanding field of epigenetics. The slow pace of genetic variation and high opportunity cost associated with maintaining a diverse genetic pool are well-matched by the flexibility of epigenetic traits, which can enable low-cost exploration of phenotypic space and reactive tuning to environmental pressures. Aiding in the generation of a phenotypically plastic population, epigenetic mechanisms often provide a hotbed of innovation for countering environmental pressures, while the potential for genetic fixation can lead to strong epigenetic–genetic evolutionary synergy. At the level of cells and cellular populations, we begin this review by exploring the breadth of mechanisms for the storage and intergenerational transmission of epigenetic information, followed by a brief review of common and exotic epigenetically regulated phenotypes. We conclude by offering an in-depth coverage of recent papers centered around two critical issues: the evolvability of epigenetic traits through Baldwinian adaptive phenotypic plasticity and the potential for synergy between epigenetic and genetic evolution.

Key words: phenotypic plasticity; noise; Baldwin effect; epigenetics; inheritance; evolution

Introduction

Broadly defined as environment-induced changes in gene expression or physiological characteristics of cells and animals, phenotypic plasticity is an adaptive capacity that confers fitness advantage to the host organisms [1–3]. Stochasticity is an inherent facilitator of phenotypic plasticity because it is a driver of noise or variability in gene expression and in broader cellular activities [4, 5]; stochasticity is naturally involved in the progression of biochemical and biomechanical processes.

The key role played by phenotypic plasticity in adaptation to new environments has historically been championed by James Mark Baldwin. Collectively defined as the Baldwin effect [6, 7], the concept of "organic selection" refers to the ability of phenotypic plasticity to increase survival, while "orthoplasy" refers to the directional influence of organic selection on evolution. Baldwin proposed that plasticity was a positive driving force of evolution, which separates it from both the Darwinian and Lamarckian theories of evolution.

Gene expression variability in genetically identical cells is considered to be an evolutionary consequence of variable environments, as a constant environment would lead to the selection of a gene expression profile with no variance. Since natural environments can fluctuate at rates that are frequently too high for beneficial genetic mutations to accumulate for adaptive evolution, faster response and adaptation strategies are often needed to rescue populations from extinction [8–11]. In this context, non-genetic or epigenetic mechanisms can facilitate fast response strategies that either actively or passively cope with the challenges of dynamically changing environments. For example, multistable gene expression states can easily be formed on purely non-genetic grounds when there is positive feedback and sufficient non-linearity among the system components; with each gene expression state being optimal for a different environment, multistability can constitute a passive strategy to protect cellular populations from extinction [12]. As another example, when activated by environmental cues, epigenetic mechanisms not only increase expression plasticity for the environments to select on, but they can also guide the gene expression profiles to more optimal regimes dictated by the current environment [13].

During cell division, in addition to the partitioning of cytoplasmic and genetic content between two cells, epigenetic states established in the ancestral cell can be passed on to the next generation with varying degrees of stability, which depends on the cell type and epigenetic marks [14]. Effectively corresponding to an intergenerational "memory," the inheritance of epigenetic states has recently been suggested to act as an enhancer of genetic evolutionary potential and even as an independent target of evolution [15–18]. The slow pace of genetic variation and the high

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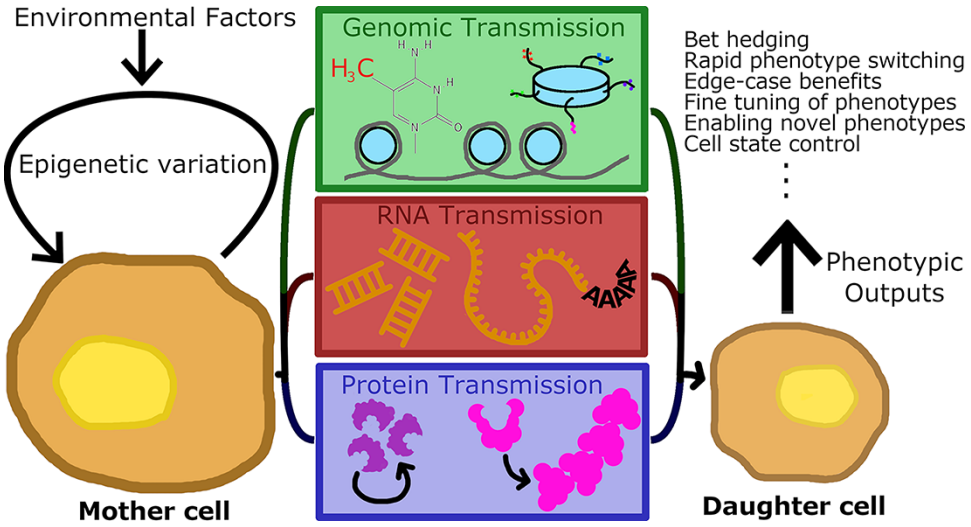

Figure 1: cartoon depiction of mechanisms for epigenetic inheritance and common phenotypic outputs from epigenetic information

opportunity cost of maintaining a diverse genetic pool make a strict reliance on genetically derived phenotypes disadvantageous under certain environmental conditions. Epigenetic inheritance can alleviate some of these difficulties by enabling lower-cost exploration of novel phenotypes and flexible tuning to environmental pressures [16, 19–21]. The evolution of epigenetic mechanisms, combined with genetic fixation of epigenetically acquired phenotypes, expands the tools available for organisms to adapt to environmental pressures [20, 22]. In this review, we explore the breadth of epigenetic mechanisms, epigenetic evolution through phenotypic plasticity, and the synergy between epigenetic and genetic evolution.

Breadth of Epigenetic Mechanisms

Mechanisms for Epigenetic Inheritance

Although occasionally confined to the realm of histone modification and DNA methylation due to their experimental tractability and importance to human disease, mechanisms of epigenetic inheritance are as varied as the phenotypes they produce. Broadly, epigenetic information can be transmitted via the genome, RNA, and proteins (Fig. 1).

"Genomic transmission" of epigenetic information can occur via a multitude of DNA and histone modifications. DNA modifications usually consists of cytosine methylation [23] and rarely hydroxymethylation [24], adenine methylation in bacteria [25], and uracil glycosylation in the protozoan kinetoplastids [26], all of which are generally used to repress gene transcription [27, 28], although DNA methylation can also be used for self/target differentiation by bacterial anti-phage systems [29, 30]. Histone modifications include methylation and acetylation [23, 24], while chromatin remodeling can lead to heritable shifts in nucleosome packing density, both of which tend to alter gene transcription rates [25–27]. Chromatin silencing tends to produce a binary response (e.g. DNA regions are either transcribed or not) [14]. DNA methylation and chromatin structure over transposable elements can also alter the frequency of transposon activation and propagation, corresponding to another mechanism for epigenetics-based inheritance [28, 29]. Different chromatin alterations tend to be retained over different timescales (ranging from hours to indefinite silencing), enabling flexibility in the storage-time for epigenetic information [14]. Defects in epigenetic gene silencing are associated with a multitude of human diseases including cancer [30] and male infertility [31].

"RNA transmission" of epigenetic information primarily occurs via the transfer of a plethora of non-coding RNAs, with a wide range of downstream effects. A traditional example is heritable post-transcriptional gene silencing via small-interfering RNA (siRNA) [32, 33]. In a particularly unusual example of small RNA epigenetic inheritance, recent work found that *Caenorhabditis elegans* can detect small RNAs from a pathogenic bacteria, leading to an avoidance phenotype that is heritable for four generations and depends on the function of RNA interference pathway components and two small RNA transporters [34]. In mammals, sperm RNAs include a mixture of coding mRNA (whose role is largely unknown) and small RNAs (such as transfer-RNA derived small RNAs) that may be responsible for the heritability of certain metabolic disorders [35–38]. Recent work in *C. elegans* has found that exposure to heat stress induces production of heritable small RNAs that transgenerationally bolster early sexual attraction, male production, and consequently the rate of genetic outcrosses [39]. This mechanism both increases genetic diversity in the population in response to stress and is itself positively selected for by increasing carrier-progeny reproductive rates.

"Protein transmission" of epigenetic information depends primarily on two mechanisms, gene expression feedback and prion propagation. Certain relatively stable proteins can enhance their own expression either directly or indirectly [40]. These proteins can be found as independent sensors or regulators of a biological state, or as part of a more complicated network, such as the galactose (GAL) network in yeast [41, 42]. Inheritance of these stable proteins during cell division imparts the parental state to progeny. Prion propagation presents a more "purely epigenetic" mechanism for protein-mediated transmission of epigenetic information. Prions are stable, alternate foldings of cellular proteins that are capable of self-propagating by imposing their conformation on natively folded counterparts. Prions are frequently associated with deleterious phenotypic effects, such as transmissible spongiform encephalopathies. A notable human example is the lethal Kuru, transmitted by the prion–protein PrP^{Sc} [43]. Beneficial prions do exist; one interesting example is the [PSI^{+}] prion in *Saccharomyces cerevisiae*. [PSI^{+}] preferentially appears

under stress conditions [44] and can occasionally improve stress resistance [45].

Common Uses for Epigenetic Information

It is useful to review some of the typical uses for epigenetic information. Note that the same end is frequently achieved by a myriad of means; our interest here lies more in highlighting common outputs than in fully exploring mechanistic details. Broadly, a few common uses for epigenetic information are enhancing phenotypic diversity, improving a particular phenotype, and enabling novel cellular states.

Enhance Diversity to Survive Unexpected Adverse Conditions

Epigenetics are often utilized as part of bet-hedging strategies against adverse environmental variation [46] and stress conditions [47]. These strategies can utilize bistable or multistable systems (e.g. heritable protein expression state of the GAL network), generate novel phenotypes in the face of adverse conditions (e.g. [*PSI*$^+$] prion in yeast), or generate noise in a particular phenotype (e.g. stochastic allocation of massive Hsp104 aggregates in *S. pombe*). All of these epigenetic mechanisms increase phenotypic variability, which can positively interact with evolution under strong selective pressure [48]. Epigenetic bet-hedging strategies often interact with genetic elements, allowing a cell to rapidly enact genetically derived phenotypic change without waiting for genetic "timescales" of variation. For example, the presence of the aforementioned [*PSI*$^+$] prion in yeast enables sporadic stop codon read-through, unveiling previously hidden genetic variation that, while often deleterious, can occasionally generate beneficial effects (e.g. enabling survival on galactose or lactate, lithium resistance, and bleomycin resistance in certain yeast strains [45]).

Enhance Diversity to Derive Edge-Case Benefits in Static Conditions

Outside of variable adverse environments, phenotypic diversity can be beneficial by enabling the abuse of certain edge cases. Exponentially growing single-celled organisms benefit from a variable (and heritable) generational doubling time. In a population with a homogenous doubling time $d = 1$, the population size at time t can be defined as $p(t) = 2^t$. In a population with a heterogenous doubling time $d = [0.5, 1.5]$, where d switches between the two values for each cell at each generation, the population size at time t can be defined (on average) as $p(t) \approx e^{(0.43t+0.12)}$, which rapidly outgrows the homogenous population. The faster growing proportion of the population generates an additional benefit with each reproductive event (e.g. more cells that in turn have the potential to replicate quickly), leading to an exponential growth benefit. Thus, a seemingly net-neutral increase in the variability of a phenotype (mean doubling time is 1 for both cases outlined above) can lead to a positive fitness outcome. Cerulus *et al*. demonstrated this directly through a combination of experimental data and modeling of *S. cerevisiae* doubling time [21]. They found that for a given mean doubling time, increasing the rate of deviations from the mean could increase net population doubling time if these deviations were epigenetically heritable.

Enhance or Enable a Directly Favorable Phenotype

Acting in a more traditional evolutionary manner, epigenetic information is frequently used to optimize an existing phenotype or to enable a novel beneficial phenotype. This benefit is typically present in all members of a population and tends to be direct and stable rather than indirect and variable (e.g. phenotypic diversity from bet-hedging is present in all members, but the resultant phenotype tends to be variable and the benefit is dependent on fluctuating selection pressures). A classical example would be the restriction–modification systems present in over 74% of ~2210 bacterial and archaeal genomes [49]. These systems utilize DNA methylation to differentiate self-DNA from phage-DNA, allowing for the safe expression of a restriction endonuclease that cleaves at a target unmethylated DNA sequence. Thus, epigenetic information storage, paired with the appropriate genetically encoded mechanism, gives rise to a net benefit in phage resistance [50].

Enable Novel Cellular States

Instead of acting to alter particular phenotypes, epigenetic information can be used to maintain novel states. This use of epigenetic information can overlap heavily with its role in enhancing phenotypic diversity; however, here, the emphasis is on processes that reshape the entire cell or organism instead of only a handful of phenotypic outputs. This process is commonly studied in the field of cellular differentiation, although other phenotypes can fit into this category (e.g. in *S. cerevisiae*, heat shock can trigger a metastable prion that in turn can induce the formation of other yeast prions [51, 52]). A classic example is the epigenetic differentiation of pluripotent stem cells [53]; this differentiation is reversible via epigenetic reprogramming, although interestingly, some epigenetic marks can escape this process [54]. Exotic examples of this process include epigenetically driven temperature-dependent sex determination in a variety of reptiles [55, 56]. More controversially, cellular and organismal aging could be viewed as an extreme example of an epigenetically encoded state, as there are distinct chromatin availability features in aged cells [57]. However, for daughter cells born from aged mothers with a reset lifespan, the epigenetic state would be expected to be erased at the time of its transmission to progeny so that the daughter cell can live a full lifespan. In yeast and mice, the natural rate of mutation is far below the threshold required to alter mortality [58–60], making aging a largely non-genomic process. Aging can generate significant phenotypic variability, such as young and old *Cryptococcus neoformans*, which present unique pathogenic properties with substantially different drug resistance profiles [61]. Similarly, mid-life *S. cerevisiae* exhibit increased resistance to UV light [62], whereas aged *S. cerevisiae* are resistant to mating pheromone [63] and outcompete young cells on non-glucose carbon sources [64]. Young *S. cerevisiae* mother cells typically divide faster and are more resistant to acetic acid and heat shock than virgin or old mother cells [65]. Although aging may be unavoidable in higher organisms, the existence of non-aging in bacteria and some yeast (achieved in *S. pombe* through uniform cell division [66]) suggests that aging could represent an epigenetic–genetic strategy for survival.

Microbial Evolution Driven by Phenotypic Plasticity of Cells in an Epigenetic Landscape

The term epigenetics was initially coined by C. H. Waddington to describe the developmental processes that control the relationship between genotype and phenotype as part of the study of epigenesis (organismal growth and cellular differentiation). Waddington proposed that these epigenetic processes could be viewed as part of an epigenetic landscape akin to a potential energy landscape. Organisms move through this landscape toward the lowest local point. Patterns in epigenetic processes create troughs that organisms can be trapped in, and developmental pathways are represented by troughs that lead to a final, inescapable deep point [67–69]. Interestingly, bistable systems

such as the GAL network in yeast [41, 42, 70, 71] can be easily adapted to Waddington's epigenetic landscape theory, by viewing each of the two states as distinct yet adjacent troughs, where the switching rate is determined by the depth of the respective troughs and the height of the intermediary space. Thus, the evolution of a bistable system could be reduced to changes in the properties of the resultant epigenetic landscape.

Although Waddington and others have generated a rich theory of epigenetic evolution, experimental investigations have proven more challenging to generate. A recent expansion of epigenetic evolution research has emphasized the role of non-genetic elements in regulating phenotypic outcomes from the GAL network including lasting alterations in gene-localization as a form of transcription-activity memory [72] and direct protein-transfers (Gal1p) to progeny as a mechanism for preserving gene network state [73]. In turn, Luo *et al.* (2020) offer a comprehensive exploration of epigenetic evolution in the GAL network in *S. cerevisiae* [74].

The GAL network is a bistable system controlling the expression of regulatory and metabolic genes involved in galactose metabolism. In brief, constitutively expressed Gal4p drives expression of various GAL network genes, including GAL80 (which represses Gal4p) and GAL3 (which sequesters Gal80p in the presence of galactose, in turn derepressing Gal4p). Thus, in a galactose environment, GAL3 is stably expressed and galactose metabolism is active, whereas in a galactose-free environment, galactose metabolism is dormant. The ability of *S. cerevisiae* cells to switch between the active and dormant states of the GAL network enables the cells to remain galactose-competent when necessary without constantly maintaining the galactose metabolic machinery. It was previously shown that the activity of the GAL network is heritable across several generations [75].

For their initial exploration of epigenetic evolution in the GAL network, Luo *et al.* placed the yellow fluorescent protein (YFP) under the control of the GAL1 promoter, enabling direct readout of GAL network activity. Then, they grew these cells under GAL-ON conditions and sorted them daily based on the intensity of YFP expression (lowest 5%, middle 5%, and highest 5%) repeatedly for ~100 generations over 7 days (Fig. 2). While the 7th-day middle and highest sorting groups did not differ significantly from the starting population, the lowest 5% group exhibited a significantly lower average YFP signal. Further exploration revealed that this reduction in protein expression was localized specifically to the GAL network, suggesting that the targeted sorting process (selection during evolution) was successful. However, the relatively complex feedback architecture within the wild-type GAL network made it difficult to determine which components of the network had changed and through which mechanism [74].

To render further investigation of the GAL network tractable, Luo *et al.* deleted the GAL80 gene (responsible for the negative feedback loop), constitutively locking the network into the GAL-ON state. Then, in a repeat of the sorting experiment using the GAL1 promoter driving YFP, almost all biological replicates exhibited significant decreases (~15% to 70%) in mean YFP expression. Five of the replicates were further grown continuously without selection and found to retain the decreased YFP expression over ~115 generations, demonstrating a sustained, heritable behavior. Interestingly, an increase in the variability or noise of single-cell YFP expression was observed for several of the replicates, empirically demonstrating an increase in phenotypic plasticity as a result of microevolution. This also offers a clear-cut demonstration of the principle outlined in the section "Enhance diversity to derive edge-case benefits in static conditions": Higher variability in YFP expression increases the odds of a particular cell falling into the bottom 5% of YFP expression and thus passing the selection event [74].

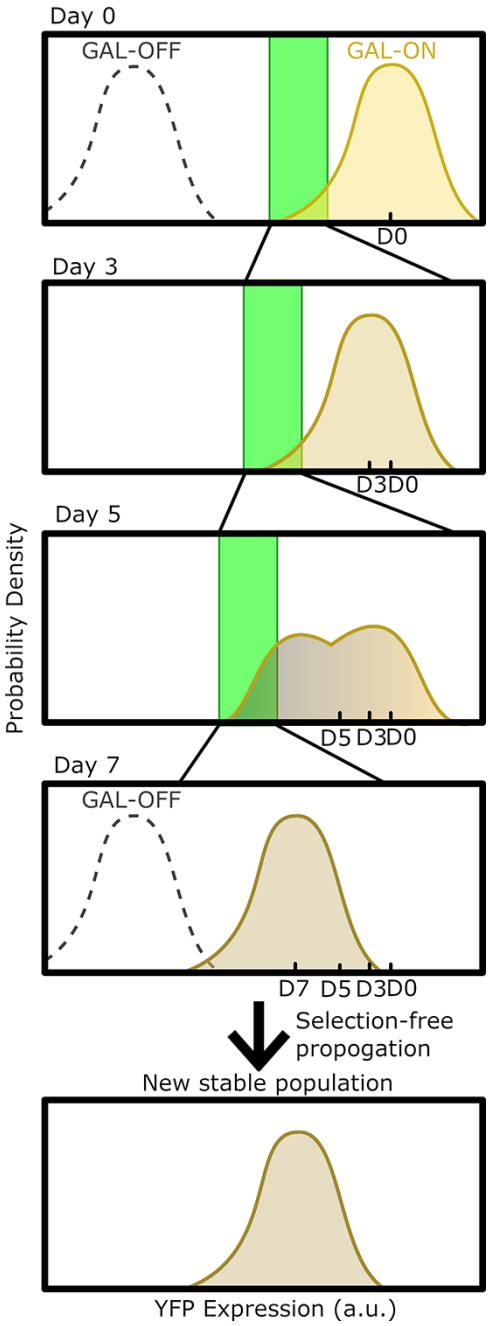

Figure 2: cartoon depiction of epigenetic evolution experiment from [56]

Based on whole genome sequencing, targeted Chromatin Immunoprecipitation coupled with quantitative Polymerase Chain Reaction of selected GAL network genes and the reporter, and sporulation experiments between selected strains and WT to check for Mendelian inheritance, Luo *et al.* confirmed that changes in certain heritable epigenetic marks, such as loss of transcription enhancing H3K4me3 and H3K27Ac, and gain of repressing H3K36me3, contributed to the observed decrease in reporter expression. A mutation reducing the efficacy of RNA Pol

II was found alongside the aforementioned epigenetic changes in a subset of evolved strains [74].

Thus, microevolution of a complex bistable system yielded synergistic changes in epigenetic and genetic state. Selection for certain epigenetically malleable traits led to a rapid phenotypic shift motivated by changes in histone markers at key GAL network elements. In some strains, these epigenetic changes were complemented by a mutation that reduces the performance of RNA Pol II, demonstrating at the molecular level the involvement of epigenetic and genetic mechanisms in evolution [74]. The study by Luo *et al.* provides an ideal empirical example to the textbook definition of the Baldwin effect [7]: phenotypic plasticity is achieved by stochasticity-induced variation in gene expression; mean phenotypic value in the inducing environment changes as a result of directional selection; and plasticity either increases or stays the same in evolving populations.

Impact of Epigenetic–Genetic Synergy in Evolutionary Dynamics

Waddington used his epigenetic landscape model to explain his work on genetic assimilation of the crossveinless phenotype in flies, by viewing the transformation of a phenotype from environmentally dependent to static as equivalent to the carving out of a novel canal in the epigenetic landscape. Waddington observed that heat-shocked larval flies occasionally display a novel phenotype, crossveinless. By repeatedly selecting for this phenotype, he was able to eventually secure it in untreated progeny. Thus, an epigenetic reaction to environmental stimuli could eventually be cemented as a genetic trait.

Although the use of epigenetics for cheaply generating (i.e. with low evolutionary cost) phenotypic heterogeneity is well established, it is difficult to establish the ideal range of epigenetic effects to encourage both survival and eventually genetic adaptation. Too weak of an effect might not be advantageous, while overly strong epigenetic effects can preclude eventual genetic integration (a strong epigenetic phenotype carrier could outcompete weak genetic phenotype carriers akin to clonal interference). This can be viewed as equivalent to determining the optimal phenotypic plasticity capable of driving adaptive evolution (Baldwin expediting effect), in which a delicate balance between selective pressure and degree of plasticity determines whether the adaptive speed is enhanced by phenotypic plasticity [76]. Phenotypic plasticity is also capable of benefiting population survival under changing selective pressures, although again this is sensitive to the degree of plasticity and the heritability of this plasticity [8]. Baldwinian theory frequently identifies phenotypic plasticity with behavioral plasticity ("learning"), which in turn drives genetic selection to cement the phenotype that is approximated by the "learned" behavior [1]. However, some sources of phenotypic plasticity can be identified directly at the molecular level via epigenetic mechanisms, increasing experimental tractability and enabling a clearer view of the relationship between phenotypic plasticity and genetic fixation during microevolution.

Mechanisms generating phenotypic plasticity are needed in moderate amounts in order to guarantee survival in changing environments, particularly if the new environment is not radically different from the previous one [1]. Population genetics modeling has shown that the existence of such mechanisms are beneficial to increase the odds of evolutionary rescue after environmental change if the population has access to an allele that can facilitate increased and partly heritable phenotypic variation [8]. However, it is important to note that other studies modeling the impact of the Baldwin effect and phenotypic plasticity in evolution have concluded that the importance of phenotypic plasticity for evolutionary adaptation was very much context dependent [76]. Nonetheless, given the spectrum of existing examples for heritable phenotypic variability across the tree of life [18, 48, 74, 77], it is highly likely that mechanisms generating phenotypic plasticity play an important role in the evolution of biological systems.

As a recent example showing the impact of epigenetic effect sizes on adaptation and comparative effects of epigenetic–genetic mechanisms, Kronholm and Collins [16] treated epigenetic–genetic evolution as an adaptive walk through an n-dimensional (n = No. of "phenotypes" being simultaneously optimized) hypersphere to test the varying benefits of epigenetic mutations based on their stability and effect size. The hypersphere here represents the possible phenotypic space. One point within this n-dimensional space is selected as representing the "optimal" combination of phenotypes. Fitness is directly proportional to the difference between an individual's combination of phenotypes and the "optimal" combination. Thus, environmental selection is determined by the distance between the point in space that an individual's combination of phenotypes maps to and the "optimal" point in space. It is important to note that the targeted "optimal" phenotype combination was a static point; their model does not account for the potential impact of a dynamic environment. They found that intermediate epigenetic effects (somewhat smaller than the range of genetic effects) with moderate stability greatly increased the initial rate of adaptation over genetic-only controls at the cost of sub-optimal end fitness. Weak epigenetic effect sizes (much smaller than genetic effects) slowed the initial rate of adaptation but improved end fitness relative to genetic-only controls. Extreme epigenetic phenotypes (larger than genetic effects) both slowed adaptation and reduced end fitness, for reasons that can be understood as akin to attempting numerical integration with an over-large step-size [16].

Stajic *et al.* (2019) [18] set out to experimentally explore epigenetic–genetic synergy during evolution and to test whether epigenetic effect size actually impacts survival and rate of genetic adaptation. By integrating a uracil selection marker (URA3) at varying distances from a known silenced region of yeast chromosome XI, the authors were able to control how easily the selection marker was turned on/off. Yeast containing this silencable marker was initially grown under -URA conditions, priming the cells to maintain the epigenetic-ON (unsilenced) URA3 state. Then, yeast was grown in media containing 5-Fluoroorotic Acid (5-FOA), which is processed by URA3 to form the toxic 5-fluorouracil; this in turn strongly favored epigenetic silencing of 5-FOA expression. Based on the URA3-silencing region distance, the authors identified three URA3 strains containing high, moderate, and low levels of silencing capability. These strains were then grown under 5-FOA conditions for ~90 generations, with daily plating tests for 5-FOA resistance and survival on -URA media; loss of -URA survival was assumed to be caused by genetic silencing of URA3 (Fig. 3).

The high-silencing URA3 strain was, perhaps predictably, the fastest to adapt to the 5-FOA media but was also the last to acquire genetic 5-FOA resistance as measured by loss of URA- survival, with a median time to initial mutant detection of 72 h. The high-silencing strain also exhibited inconsistent 5-FOA resistance at experiment end (~60% of the population was 5-FOA resistant via silencing and/or mutation). Note that this result matches most of the theoretical predictions of Kronholm and Collins for epigenetic–genetic "clonal" interference with overly strong epigenetic effects. The moderate-silencing URA3 strain paid an initial survival penalty relative to the high-silencing strain but was much faster to acquire genetic 5-FOA resistance (median time 48 h) and

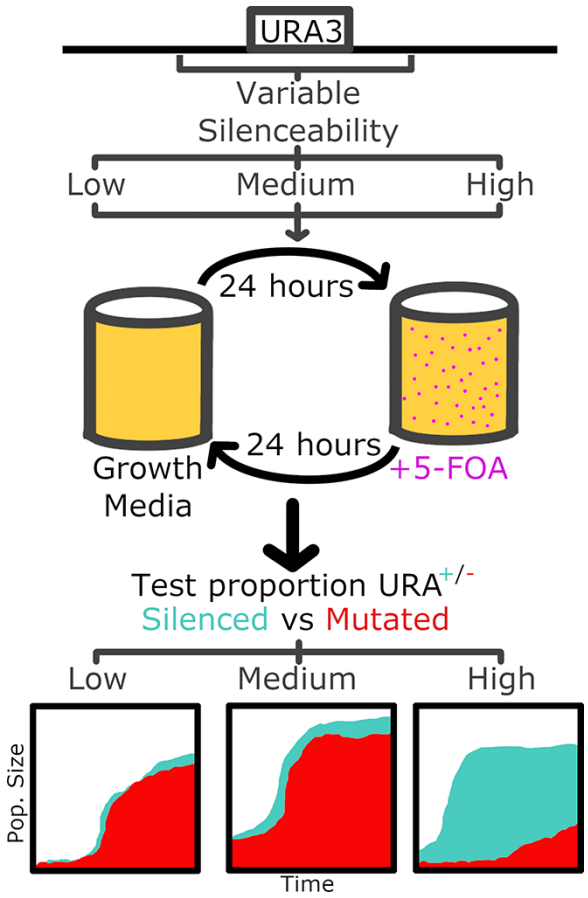

Figure 3: cartoon depiction of epigenetic evolution experiment in [59]

exhibited a more consistent 5-FOA resistance phenotype at experiment end as a result (~90% of the population was 5-FOA resistant via silencing and/or mutation). The low-silencing URA3 strain was similar to the moderate-silencing, except it was slower to acquire genetic 5-FOA resistance (likely due to reduced population size). In addition, four out of twenty-one low-silencing URA3 replicates went extinct during the experiment [18].

Interestingly, differences in epigenetic effect size were associated with variations in the genetic mutations developed. While high-silencing strains were found to almost exclusively exhibit deletion of URA3 (URA3 deletion was also predominantly observed in strains without the capability of silencing URA3) or no identifiable mutation, moderate and low-silencing strains frequently exhibited mutations targeted to loci other than URA3. Mutations at PPR1, RPD3, and YEF3 isolated from moderate and low-silencing strains were found to greatly increase the URA3 ON → OFF switching rate, presenting an obvious survival benefit during 5-FOA selection. Thus, intermediate epigenetic effect sizes enhance not only the rate of genetic evolution but also the exploration of novel genetic space by expanding population size without directly outcompeting weak beneficial genetic mutations [18].

Transgenerational Epigenetics in Complex Organisms

Although the main goal of this review is to recapitulate the molecular mechanisms behind epigenetic inheritance, which have been mainly studied in unicellular organisms, there are a few well-established examples where transgenerational epigenetics play a role in the evolution of complex organisms, especially plants and simple metazoans [78]. One of the earliest examples of transmission of acquired traits, although controversial, is the midwife toad (*Alytes obstetricians*) experiment done by Paul Kammerer in the early twentieth century [79]; it is important to note that his conclusions were strongly criticized by his peers. In light of the current knowledge about epigenetics, more recent examinations of Kammerer's findings have questioned the original criticisms [80, 81]; the question remains open because those experiments have not been repeated in modern times.

Since the discovery of transposable elements [82], the activity of which cycles over time due to changes in the methylation state of certain DNA segments, there have been plenty of plant-based examples to the transmission of complex traits (such as flowering time and primary root length) based on the epigenetic state of certain transposons [83]. Such transposable elements consist of differentially methylated regions (DMR) of DNA whose epigenetic state can be transmitted either mitotically or meiotically for at least eight generations in specifically engineered plants called "epi RILs" (epigenetic recombinant inbred lines) [84, 85]. The existence of DMRs acting as an epigenetic quantitative trait has also been found in natural plant populations [86]. In addition, plant-based studies also provided examples of other mechanisms of transgenerational epigenetics, such as transcriptional silencing mediated by RNA interference (RNAi) [87], which has also been found to work in metazoans including *C. elegans* and *Drosophila melanogaster* [88, 89], and there has even been a murine example [90]. We expect future investigations to uncover examples in mammals as well, because during germline biogenesis and fertilization, the epigenome is not fully reset; acting as a facilitator of epigenetic transmission of traits, certain segments of parental DNA preserve epigenetic information [91].

Discussion

Given the relative youth of epigenetics as a field, the potential future directions are staggering. Here, we outline a few particularly interesting pieces of recent work and the future directions they inspire.

Following up on the competition of epigenetic and genetic strategies under a static environment in Stajic *et al.* 2019, a recent study explores the relative value of epigenetic and genetic adaptation under fluctuating environmental conditions. Using similar experimental components (URA3-silenceable vs URA3-static strains in -URA or +5-FOA media), the authors directly competed these strains in environments with different switching rates (ranging from static -URA/+5-FOA to daily switching between these two conditions). As previously predicted [46], Stajic *et al.* found that epigenetic switching strains dominate in rapidly varying environments [17], highlighting an additional benefit of epigenetic switching beyond those outlined. Future work could explore the mechanistic basis for tunable silencing at the target locus, or the evolutionary tuning of switching rate over time (e.g. can a slow-switching strain evolve a higher switching rate when constrained to an environment that would render it favorable).

Another area of particular interest is the role of epigenetics in microbial community interactions. The [GAR^+] prion in *S. cerevisiae* offers a striking example of this; co-culturing with certain bacteria induces [GAR^+], which in turn inactivates glucose-mediated repression of various metabolic genes [92, 93]. [GAR^+] induction reduces ethanol production, potentially alleviating ethanol toxicity for both *S. cerevisiae* and their bacterial community. Further

exploration into [GAR^+] and other epigenetic regulators of microbial communities could yield valuable insight into both epigenetic co-evolution and the industrial production of fermented foods (alcohol, yogurt, cheese, etc.).

Synthetic prions or prion–protein hybrids could have valuable research applications for inducible isolation or elimination of a target protein. A synthetic prion-forming domain was used to mimic an age-dependent phenotype in young *S. cerevisiae* cells [94]. Synthetic prion–protein fusions also offer an unusual substrate for constructing functional nanomaterials—ideally the prion domain drives self-assembly and structural organization while leaving the functional domain intact [95, 96]. Additional research into the evolvability (difficulty in generating a novel prion-competent protein) and tunability (difficulty in controlling when a prion domain becomes active) of prion domains [97] would offer valuable insight into the flexibility of prions as tools for improving cell survival.

Clearly, the inheritance of acquired characteristics, at least when viewed through an epigenetic framework, is far from extinct. Epigenetic mechanisms appear in all three of the dominant biological mediums for information storage (DNA, RNA, and proteins). These mechanisms produce a wide range of effects, such as unveiling hidden genetic variation [44], altering carbon metabolism in response to bacterial competition [92, 93], controlling cancer rates and altering tumor growth [30], determining cell developmental fate [53], and enabling memory of prior metabolic responses within a changing environment [42, 46, 74]. Epigenetic mechanisms can be subject to selective pressure and can evolve in a heritable manner [18, 74]. Adjusting the parameters of epigenetic phenotypes can even alter which regions of genotypic space are accessible to genetic mutation and canalization. Although genetic explanations remain the foundation of evolutionary biology, epigenetic mechanisms also have their niche to fill, and their potential for synergistic interactions with traditional genetic systems suggests a bright future for "hybrid" inheritance contributed by both genetic and epigenetic factors.

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Author Contributions

E.S., D.F.M., and M.A. designed the content of the manuscript and identified the topics to include and papers to review. E.S. drafted the manuscript. D.F.M. and M.A. contributed to manuscript preparation by making further additions and editing. All authors read and approved the manuscript.

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