Addressing multi-generational non-genetic inheritance in experimental studies of evolution

Giacomo Zilio[1], Stéphanie Bedhomme[1], Emanuel A. Fronhofer[2], Staffan Jacob[3], Delphine Legrand[3], Hervé Philippe[3], Luis-Miguel Chevin[1]

[1] CEFE, University de Montpellier, CNRS, EPHE, IRD, Montpellier, France
[2] ISEM, University of Montpellier, CNRS, EPHE, IRD, Montpellier, France.
[3] Station d'Ecologie Théorique et Expérimentale, CNRS, UAR 2029, Moulis 09200, France

Giacomo Zilio[1], giacomo.zilio@cefe.cnrs.fr, gcm.zilio@gmail.com ORCID ID: https://orcid.org/0000-0002-4448-3118

Stéphanie Bedhomme[1], stephanie.bedhomme@cefe.cnrs.fr, ORCID ID: https://orcid.org/0000-0001-8075-0968

Emanuel A. Fronhofer[2], emanuel.fronhofer@umontpellier.fr, ORCID ID: https://orcid.org/0000-0002-2219-11 784X

Staffan Jacob[3], staffan.jacob@sete.cnrs.fr, ORCID: https://orcid.org/0000-0003-1956-9646

Delphine Legrand[3], delphine.legrand@sete.cnrs.fr, ORCID ID: https://orcid.org/0000-0002-5186-2909

Hervé Philippe[3], herve.philippe@sete.cnrs.fr, ORCID ID: https://orcid.org/0000-0002-1335-8015

Luis-Miguel Chevin[1], luis-miguel.chevin@cefe.cnrs.fr, ORCID ID: https://orcid.org/0000-0003-4188-4618

Abstract

Populations that face environmental change reducing their fitness can recover by adaptive genetic evolution over multiple generations, but their immediate responses often involve non-genetic mechanisms. When such non-genetic responses have dynamics that span multiple generations, their effects at the population level can be difficult to distinguish from those of evolution by selection of genetic variants. While the existence of non-genetic inheritance is no longer controversial, we here argue that its potential contribution to observed patterns in evolutionary studies is still largely overlooked, especially regarding processes that unfold over multiple generations, which we call multigenerational non-genetic inheritance (MUNGI). We highlight three major forms of MUNGI that should be particularly problematic if not properly accounted for: delayed impact of stress, transgenerational plasticity, and priming. We summarize how each may impact the dynamics of observed phenotypic change across generations in concrete experimental contexts (*e.g.*, experimental evolution, common gardens, ecotoxicological experiments, dose-response assays). We propose that analysing the dynamic properties (rate, stability, reversibility, etc.) of MUNGI processes, as well as their relative contributions to overall phenotypic responses, and how they interact with genetic changes, should help build a more comprehensive understanding of evolutionary responses to novel or changing environments.

Keywords

Adaptive evolution, Environmental stress, Epigenetics, Experimental evolution, Transgenerational plasticity

1) Population responses to stressful environments

Understanding how populations respond to harmful environmental changes reducing their fitness is a central goal of basic research in ecology and evolution (Côté *et al.*, 2016; Orr *et al.*, 2020; Taborsky *et al.*, 2021), with important applied consequences for conservation, global change research, human health and agriculture (Urban *et al.*, 2023). The two main processes allowing organisms to cope with environmental challenges *in situ* (*i.e.*, without dispersing) are phenotypic plasticity, the expression by one genotype of different phenotypes in different environments (Pigliucci, 2005), and adaptive genetic evolution, the increase in frequency of beneficial alleles in a population through natural selection. These processes are traditionally described as occurring over different timescales, with plasticity taking place mostly within generation, while genetic evolution unfolds across generations. However non-genetic inheritance (NGI), defined as any form of inheritance that is not directly mediated by genetic variation (Bonduriansky *et al.*, 2012; Bonduriansky & Day, 2018), can blur this separation line, by allowing phenotypic variation (including that induced by the environment) to spill-over from one generation to the next.

The potential importance of NGI for evolutionary processes has been discussed since the late 1980s (Jablonka & Lamb, 1989, 1995; Kirkpatrick & Lande, 1989; Mousseau & Fox, 1998; Wolf *et al.*, 1998; Herman *et al.*, 2014; Laland *et al.*, 2015; Burggren, 2016; Deichmann, 2016; Charlesworth *et al.*, 2017; Futuyma, 2017; Loison, 2021), and the advance of molecular and sequencing techniques over the last 20 years has created additional momentum (Allis & Jenuwein, 2016; Verhoeven *et al.*, 2016; Richards *et al.*, 2017; Lind & Spagopoulou, 2018; Ashe *et al.*, 2021). However, despite NGI being well established today, we here argue that its implications for experimental studies of evolution remain under-appreciated, especially when its dynamics span multiple generations.

There is accumulating evidence that the effects of environmental stress can be transmitted over more than a few generations in many organisms, through a diversity of mechanisms (Quadrana & Colot, 2016; Pilling *et al.*, 2017; Sengupta *et al.*, 2023). For example, transmission of non-coding RNAs, patterns of DNA methylation, and histone modification, can last up to 10 generations (Jablonka & Raz, 2009; Bošković & Rando, 2018; Tikhodeyev, 2018; Adrian-Kalchhauser *et al.*, 2020; Fitz-James & Cavalli, 2022). These effects are likely prevalent in many unicellular organisms, which are widely used in laboratory studies of evolution, notably to measure distributions of fitness effects of mutations (Gordo *et al.*, 2011), or perform long-term experimental evolution (Elena & Lenski, 2003). In microbes, the lack of a soma-germline divide means that many aspects of their phenotype, including proteins, gene-regulatory factors and epigenetic modifications, are directly transmitted to their descendants (Sengupta *et al.*, 2023). In *Escherichia coli*, the average protein's half-life (~20 hours) is much longer than its generation time of ~20 minutes (Moran *et al.*, 2013; Gibson *et al.*, 2018). The half-life of mRNAs in bacteria is often of similar order of magnitude as generation time (Mohanty & Kushner, 2016). Gene overexpression in yeast occurs at least 1h after a heat shock event, which overlaps with its doubling time of approximately 90 minutes (Mühlhofer *et al.*, 2019). In the green microalga *Chlamydomonas reinhardtii*, synthesis of new proteins and lipids in response to shifting temperature can take 24 hours, thereby overlapping with its generation time of 14-36h (Tanaka *et al.*, 2000).

Non-genetic responses to environmental stress can thus span multiple generations, during which they can accumulate gradually or decay/revert. To emphasize the long-term aspect of such non-genetic processes that unfold over multiple generations, we call them multigenerational non-genetic inheritance (MUNGI). As MUNGI and rapid genetic evolution may occur over similar time scales, the phenotypic changes they induce at the population level may be hard to distinguish, despite having a completely different origin (Figure 1): evolution

by natural selection occurs by successive replacements of lineages with fixed genotypes (top panel in Fig. 1), while MUNGI involves gradual phenotypic change within lineages (bottom panel in Fig. 1). We argue below that ignoring the temporal dynamics of MUNGI and its contributions to fitness across generations, and not clearly distinguishing its effects from those of genetic changes, is likely to limit our ability to infer and predict population responses to changing environments from experimental approaches such experimental evolution or common garden experiments. Considering MUNGI explicitly while designing experiments, deciphering how it interacts with adaptive genetic evolution, and how it evolves, will be necessary for experimental studies to yield more useful insights into eco-evolutionary dynamics in changing environments.

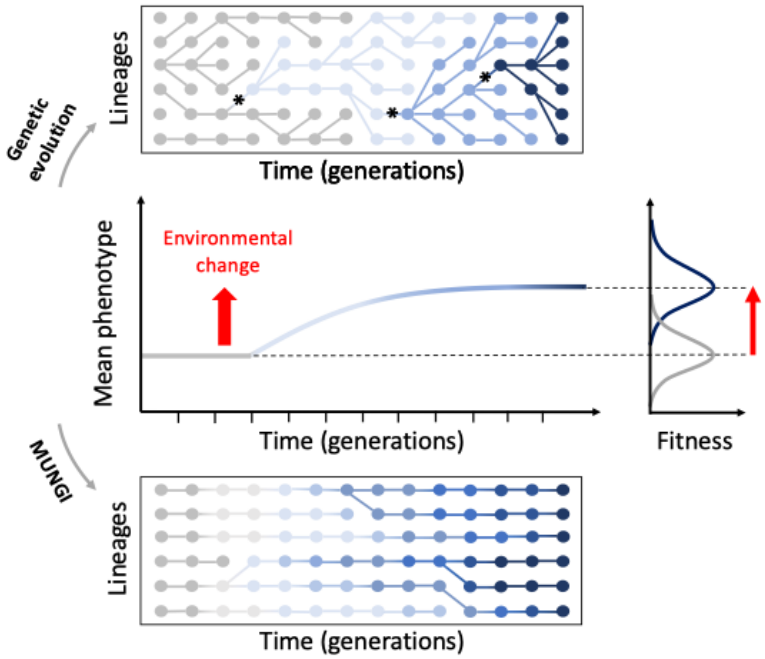

Figure 1 Alternative modes of response to environmental change. The line in the middle panel represents the dynamics of the mean phenotypic trait in a population (value along the y-axis and colour), following an abrupt environmental change (red arrow). The genealogy plots in the top and bottom panels illustrate two alternative explanations for this population-level response. The top genealogy represents adaptive evolution, illustrated in the case of *de novo* mutations. Here, environmental change is assumed to have caused an upward shift in the optimum

phenotype favoured by natural selection (as depicted by the fitness landscape on the right in the middle panel), favouring "darker blue" phenotypes. When a mutation (black star) generates a more adapted phenotype (symbolized by its colour), the corresponding lineage progressively replaces the less fit ones, leading to the observed gradual change in mean phenotype. The bottom genealogy illustrates MUNGI. Here, the environment induces non-genetic phenotypic changes that accumulate gradually across generations within each lineage (from light grey to dark blue). The lineages may vary to some extent in their responses (as illustrated by the small heterogeneity in colour gradients among lineages), but the mean phenotypic change in the population is no longer mainly driven by the replacement of lineages. These alternative explanations for phenotypic change are difficult to distinguish based only on population phenotypic data, even though the outcome is likely to be more variable for MUNGI owing to the diversity of mechanisms (illustrated in Fig. 2).

2) Major types of multigenerational non-genetic inheritance

Consider an environmental change that initially causes substantial maladaptation. Following this initial decrease, fitness is expected to slowly increase again through adaptive genetic evolution (blue line in Fig. 2), as beneficial alleles rise in frequency. However, different forms of MUNGI, which we summarize below in three main categories, can modify this picture.

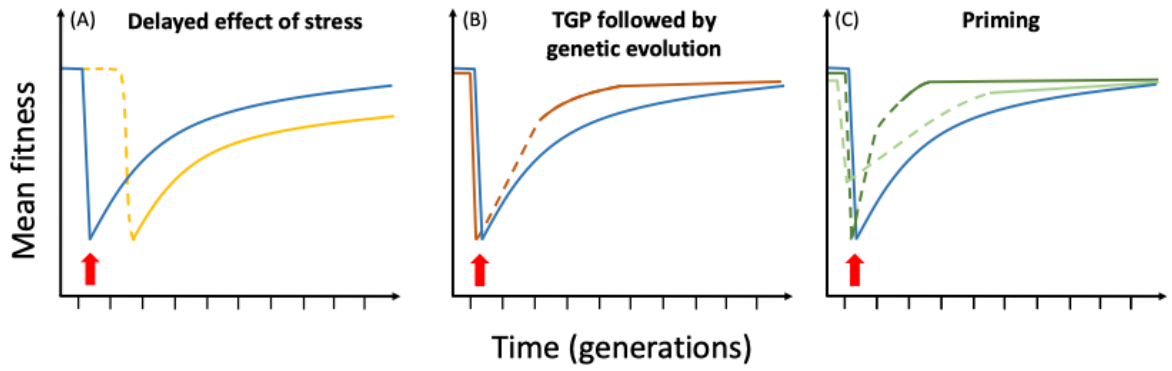

Figure 2 Three broad categories of MUNGI. Dynamics of mean population fitness over time following an abrupt environmental change (bottom red arrow), under different mechanisms of MUNGI. The blue line in all panels illustrates the baseline scenario, with an instantaneous effect of stress reducing mean fitness, followed by adaptation via genetic evolution. The coloured lines illustrate different forms of MUNGI. Their effects are shown with dashed lines, and are followed by genetic evolution in full lines. (A) Delayed effect of stress (yellow). (B)

Dynamic TGP (orange). (C) Priming effect of previous stress exposure on initial fitness drop (light green), or on rate of fitness recovery by dynamic TGP (dark green).

2.1 Delayed impact of stress: time-to-response

The detrimental impacts of environmental change may not be immediately observable, but instead could be delayed, and only manifest some generations after exposure to the stressor(s) (Fig. 2A, yellow line). This can occur for purely mechanical or physical reasons that do not involve any specifically evolved mechanism. For example, toxic or harmful molecules can accumulate passively by slowly permeating into cells, but only start to have measurable detrimental impacts once their concentrations cross a threshold, beyond which they impair cellular function.

Alternatively, the detrimental impacts of stress could be delayed because specific coping mechanisms need resource for their maintenance and functioning (DeWitt *et al.*, 1998; Hoffmann & Bridle, 2022), leading to fitness costs that accumulate across generations. For instance, exposure to silver nanoparticles induced reproductive costs in *Drosophila melanogaster* only from generation F2 onwards (Panacek *et al.*, 2011), because the accumulation of oxidative stress led to the upregulation of heat shock protein 70, which reduced investment in reproduction. However, distinguishing "active" from "passive" causes for delayed impact of stress is sometimes difficult, for instance when thresholds are passed once stress-response get overwhelmed (Kassahn *et al.*, 2009).

2.2 Speed and reversibility of transgenerational plasticity

Transgenerational plasticity (hereafter TGP), wherein the expression of phenotypic traits depends on the abiotic (Donelson *et al.*, 2012; Kremer *et al.*, 2018; Donelan *et al.*, 2020; Castano-Sanz *et al.*, 2022) or biotic (Tariel *et al.*, 2020; Shahmohamadloo *et al.*, 2025) environments experienced by previous generations, is receiving increasing attention from

evolutionary biologists (Bell & Hellmann, 2019), but its dynamics across generations remain understudied (as argued for within-generation plasticity by Burton *et al.*, 2022; Dupont *et al.*, 2024)).

An important aspect to consider is the speed at which phenotypic traits change, that is, the rate of TGP. This rate determines how likely it is for TGP to be confounded with adaptation by genetic evolution (Fig. 2B orange line). Following exposure to environmental stress, phenotypic traits may typically change rapidly in the first few generations, until expression of the trait becomes stationary (Fig. 3A grey line). If TGP is beneficial, faster rates of change should lead to faster increases in fitness, without requiring any genetic evolution. Subsequently, the overall increase in fitness caused by beneficial TGP will also depend on TGP capacity, that is, the height of the phenotypic plateau. If this plateau is stable over many generations, then TGP capacity determines how much genetic evolution is additionally needed for complete fitness recovery. Such stable TGP was found in the pea aphid *Acyrthosiphon pisum*, where exposure of adults to the predator ladybird (*Harmonia axyridis*) increased the production of winged morphs in their progeny from ~25% to ~45%, sustained over 25 generations of exposure (Sentis *et al.*, 2018).

However, not all traits reach a stable plateau, and the phenotypic response can instead be transient, even under constant exposure to the inducing environment (Fig. 3A). The reason may be that phenotypic responses require important metabolic investments that trade off against other functions, and/or may lead to accumulation of metabolic defects over generations. Another possible cause of transient responses is that generic emergency mechanisms, such as heat shock response triggered by a variety of stresses (Richter *et al.*, 2010), only last for a few hours or generations, before they are replaced by more specific and durable physiological adjustments.

When environments revert to their pre-stress value, the crucial question becomes how reversible the phenotype is (Fig. 3B). In the yellow monkeyflower (*Mimulus guttatus*), trichome production triggered by insect damage was stable for 3 generations without insect damage, before reverting to initial levels (Akkerman *et al.*, 2016). Differences in the speed of induction *vs.* reversibility of TGP for osmotolerance were found in the unicellular alga *Dunaliella salina* (Rescan *et al.*, 2020), where intracelullar glycerol decreased faster (when going from high to low salinity) than it increased (from low to high salinity), because the former involves excretion – a rapid process – whereas the latter requires synthesis – a slower process. Yet, little is known about how reversibility unfolds across generations.

2.3 Trans-generational priming: memory of past responses

A third major class of MUNGI is trans-generational priming, which occurs when prior exposure of an organism to a stressor (priming) prepares its descendants to better respond to the same – or different – stressors upon later exposure (triggering). Trans-generational priming therefore occurs across cycles of stress/non-stress. For instance, the descendants of *E. coli* cells primed with antimicrobial peptides (AMP) exhibited increased persistence when re-exposed to AMP after some generations (Rodríguez-Rojas *et al.*, 2021). The fourth- and fifth-generation descendants of *Saccharomyces cerevisiae* originally primed with salt exhibited increased resistance to hydrogen peroxide, and faster gene expression due to the activation of the long-lived cytosolic catalase Ctt1p, which was then propagated across generations by NGI (Guan *et al.*, 2012). Exposure of *Arabidopsis thaliana* to caterpillar herbivory primed the descendants for enhanced insect resistance for two generations, due to the production of interfering RNAs (Rasmann *et al.*, 2012). Similarly, parental exposure to parasite infections can alter offspring's immune responses across generations, a phenomenon known as transgenerational immune priming (reviewed by Roth *et al.*, 2018).

Nonetheless, empirical studies are scarce, especially those with an evolutionary perspective. In particular, we need to understand whether the effect of trans-generational priming is mostly immediate, reducing the impact of stress immediately upon re-exposure (light green line in Fig. 2C), or also more durable, influencing the rate of TGP in subsequent generations (dark green line in Fig. 2C) (Hilker *et al.*, 2016; Wesener & Tietjen, 2019). In addition, it would be necessary to measure for how many generations trans-generational priming could be effective in the absence of re-exposure to stress.

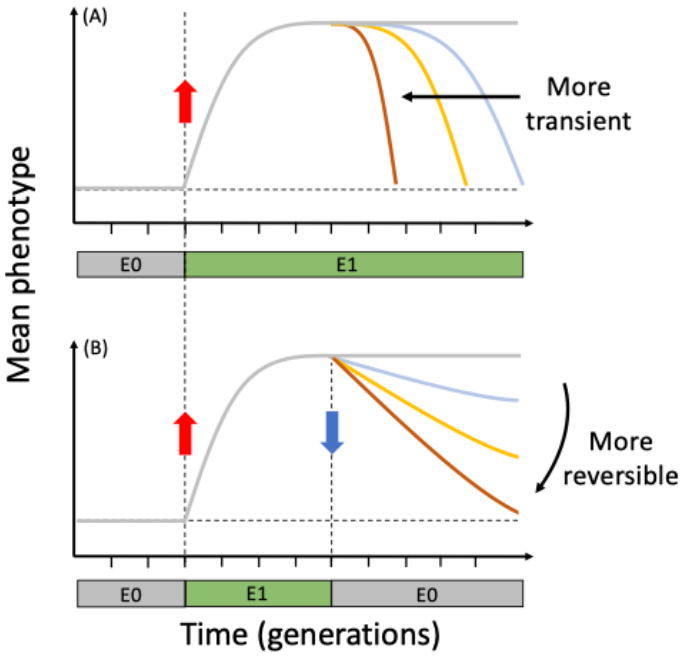

Figure 3 Stability and reversibility of phenotypic responses across generations. (A) Transient dynamics occur when the phenotypic trait goes back to its initial state, even though the environment has remained unchanged following the initial environmental shift (from E0 to E1), indicated by the red arrow. The grey line is a non-transient phenotypic response, while coloured lines show increasingly transient responses, from light blue to brown. (B) Reversibility is the ability of a phenotype to go back to its initial state after the environment has changed back (from E1 to E0), as indicated by the blue arrow. The grey line shows an irreversible phenotype, while coloured lines show increasingly reversible responses, from light blue to brown.

3) Impacts of MUNGI in experimental studies of evolution

Because they lead to changes in fitness that unfold over multiple generations, the MUNGI phenomena described above can phenomenologically resemble genetically-based evolution (Figure 1). To avoid reaching misleading conclusions about eco-evolutionary processes, we suggest below some approaches and ideas to measure MUNGI while conducting stress response experiments over few generations, experimental evolution, and common garden experiments.

3.1 Stress-response experiments

Experiments on stress responses, or dose-response curves, aim at identifying stressor levels (dose concentrations, or exposure time) that cause sufficiently strong detrimental effects to be clearly detectable in the short term, without leading to rapid population extinction. Evidence suggests that MUNGI is potentially an important contributor to major classes of such experiments, from (eco)toxicological stress-response studies to antibiotic resistance assays (Gouin *et al.*, 2023), and responses to climate change (McGuigan *et al.*, 2021, see Table 1 therein). For example, several heat-stress experiments in microbes (Andrade-Linares *et al.*, 2016), plants (Zhong *et al.*, 2013; Wang *et al.*, 2016; Liu *et al.*, 2019; Louis *et al.*, 2023) and animals (Adrian-Kalchhauser *et al.*, 2020; Walzer *et al.*, 2020; Reshma *et al.*, 2023) have shown transgenerational heat-stress effects, with changes in transcriptomics, physiology and life-history traits lasting for many generations. Interestingly, a recent study on different phytoplankton species across generations suggested a general mechanism of transgenerational response acting via temperature-dependent changes in uptake and assimilation of resources (Anderson *et al.*, 2025).

Importantly, stress response experiments often serve as first steps towards identifying selective treatments for experimental evolution (discussed below), yet misleading conclusions could be

drawn if MUNGI are ignored. For instance, a treatment level that initially leads to rapid population decline will generally be discarded as too stressful for experimental evolution, even though fitness might recover through MUNGI over the longer run (Fig. 2B-C), making the initial response misleading about the severity of stress and strength of selective pressure. Conversely, a permissive treatment during short-term assays could turn out to represent stressful conditions over the longer run, because of delayed detrimental impacts of the stressor (Fig. 2A). While examples thereof are difficult to pinpoint in the literature – as they mostly pertain to unpublished preliminary designs and assays – we have experienced such effects repeatedly in our own experimental practice.

We propose that *(i)* performing longer stress-response assays (*i.e.*, over several cycles of batch culture, or more generations), *(ii)* sampling at regular intervals to tackle short-term dynamics, and *(iii)* measuring additional traits, from growth to survival and fecundity, should identify sources of stress responses, towards improving risk assessment and policy making from eco-toxicological studies, as well as the design of future experimental evolution.

3.2 Experimental evolution

Experimental evolution is a powerful and versatile approach to test (eco-)evolutionary predictions under controlled conditions (Kawecki *et al.*, 2012). Phenotypic assays and measurements over generations allow tracking the dynamics of change in fitness and other traits of interest. However, how MUNGI might act during these long-term experiments and influence their outcomes is still too rarely considered.

The relative contributions of MUNGI *vs.* allele frequency changes to phenotypic changes can be investigated using a combination of omics analyses (*e.g.*, transcriptomics and epigenomics *vs.* genomics) and phenotypic assays. In practice, this requires tracking the genetic – and possibly also epigenetic and/or transcriptomic – composition of the population over time,

together with phenotypic traits and/or fitness. Such integrative approaches provided useful insights into the contribution of NGI to adaptation to novel environments in microbial species (Walworth *et al.*, 2021; Gopalan-Nair *et al.*, 2024, see also the review by Stajic & Jansen, 2021). Similarly, DNA methylation was shown to lead to the rapid adaptation of the bacterial pathogen *Ralstonia pseudosolanacearum* to different host plant species, but was likely not the only driver, and the concurrent additive genetic and other undescribed non-genetic modifications also contributed to the total fitness gain (Gopalan-Nair *et al.*, 2024). Nevertheless, even when simultaneous genomic and phenotypic change is observed, showing that the former explains the latter can be challenging when only using population-based measurements.

Progress can be achieved by isolating genotypes, for instance by creating clonal populations for microbes, before phenotyping and sequencing. Deciphering the genotype-phenotype map is nevertheless difficult (Wagner & Zhang, 2011; Aguilar-Rodríguez *et al.*, 2018), as it requires more resolution (*e.g.*, low linkage disequilibrium) than is typically available in experimental evolution designs. Using functional (epi)genetics to validate candidates, or introducing (epi)mutations of interest in the ancestral background to isolate effect (reverse (epi)genetics), are useful approaches with model organisms. More insights into the role of MUNGI in experimental evolution can be achieved by performing more extensive assays. For instance, re-exposing evolved populations to their ancestral environments may allow identifying whether any putative responses to stress reverse too quickly to be explained by genetic evolution (Zilio *et al.*, 2023). Transferring evolved populations back to the ancestral environment, and then again from ancestral to the stressful treatment, can help identify whether the initial response during experimental evolution was mediated by genetic or non-genetic mechanisms. Nonetheless, this approach already requires knowledge about the rate of TGP, and its degree of reversibility.

Simple order-of-magnitude estimations can also help assess whether the observed changes are consistent with the expected timescales of genetic evolution, either from *de novo* mutations or via standing genetic variation. Very rapid phenotypic dynamics taking place over few generations are more likely to involve MUNGI than genetic evolution, unless selection is extremely strong and acts on genetically diverse populations, or on mutations of very large effects (including transposable elements, structural variants as chromosomal rearrangements, or genetic switching (Yau *et al.*, 2016)). Making these arguments more quantitative requires knowledge about mutation rates, levels of standing genetic variation, and distribution of fitness effects in the focal organism in response to the investigated stressor. For example, Denman (2017) reanalysed experimental evolution data of the alga *Emiliania huxleyi* and found that the timescale of fitness gain was better explained by a combination of non-genetic and genetic changes.

3.3 Common-garden experiments

Common garden and transplant experiments, where individuals from different origins are placed in the same environmental condition(s), are routinely used to partition genetic from environmental/non-genetic components of trait variation (de Villemereuil *et al.*, 2016). However if not properly accounted for, NGI can bias their results by being wrongly interpreted as genetic variance (V_G), or by influencing its additive (V_A) and epistatic (V_I) components (Banta & Richards, 2018; Thomson *et al.*, 2018). It is common practice to use 2 generations of common garden to remove potentially misleading interpretations due to parental effects (Mousseau & Dingle, 1991), but in principle, the number of generations of common garden should account for the possibility of long MUNGI when it is suspected to exist, while limiting the opportunity for *de novo* mutations to arise. A productive way forward would be to systematically measure the dynamics of phenotypic variation during the generations of

common garden, together with assessing genomic and epigenomic variation where feasible (Leung *et al.*, 2016; Gao *et al.*, 2017; Groot *et al.*, 2018; Sammarco *et al.*, 2024).

Importantly, the control conditions used in the common garden might represent a complex novel environment *per se* for the organisms, which could trigger MUNGI. For example, even simple changes in temperature or light conditions can induce a reprogramming of non-genetic mechanisms (Whittaker & Dean, 2017). These effects may even interact with the localities (or prior treatments) the individuals or lines came from, leading them to react differently to the common garden, *e.g.* by maintaining or losing their environmentally-acquired epigenetic marks (Groot *et al.*, 2018). For instance, alligator weeds (*Alternanthera philoxeroides*) sampled from different sites along southern China presented little genetic variation, but when reared in common garden, they showed significant phenotypic differences and genome-wide epigenomic changes via *de novo* methylation and demethylation (Gao *et al.*, 2010). Priming and TGP might be the most problematic in this context, as they could lead to phenotypic responses induced by common garden environment that differ from those where the populations were sampled (Agrelius & Dudycha, 2025). To investigate these effects in more details, one could transfer samples from different natural environments (or prior evolutionary treatments) to control condition / common garden, and then back from control to treatment (see 3.2 above, but also the design by Whipple & Holeski (2016)). Additionally, changing the environment gradually *vs*. abruptly, as typically done in acclimation studies (Donelson *et al.*, 2012; Parker *et al.*, 2012), could highlight differences in transient dynamics and potential costs. For natural populations, historical data could be used to identify recent environmental changes (Lovell *et al.*, 2023), and find which rearing conditions are best to assay the sampled populations, and identify specific triggers of transgenerational responses (*e.g.*, priming). Similarly, sampling over different time periods and/or using sliding windows approaches (see Huxman *et al.*, 2022)

might improve inferences of priming effects, for populations sampled and assayed along a known gradient.

4) The evolution of transgenerational effects

Beyond potentially blurring the detection of adaptive genetic evolution in experiments, MUNGI can produce phenotypic variation on which selection may act (Yin *et al.*, 2019; Zhang *et al.*, 2018), and its underlying mechanisms may themselves vary genetically, and thus evolve (Bonduriansky & Day, 2018).

A first critical aspect for understanding the evolution of MUNGI is establishing its genetic basis and heritability. This is a challenging task due to the diversity of processes that may underlie MUNGI, from the perception of the environmental "signal", to its transmission through the cellular environment (or via the endocrine system animals), and the effector mechanism (*e.g.,* cis- or trans-acting genetic variants). Nonetheless, there is growing evidence that epigenetic variation, for instance, is genotype-dependent. In *Arabidopsis thaliana*, the disruption of the methylation-sensing gene regulatory circuit in engineered mutant plants caused genome-wide methylation losses, which ultimately led to abnormal phenotypes that worsened across generations (Williams & Gehring, 2017). These results point to the existence of a genetic basis for stable and long-term epigenetic inheritance, and confirm previous findings suggesting genetic control on epigenetic marks (Liu *et al.*, 2014; Dubin *et al.*, 2015). Laboratory experiments also demonstrated genetic variation for TGP. For instance, genotypic-specific TGP responses to temperature were found for several phenotypic traits in *A. thaliana* (Alvarez *et al.*, 2021), as well as genotype-specific TGP response of dispersal-related traits in the ciliate *Tetrahymena thermophila* (Cayuela *et al.*, 2022).

Once genetic variation for MUNGI is established, we need to elucidate how selection operates on it. Selection on (trans-generational) phenotypic plasticity is mediated by environmental

variation within and across generations, but we still know little about which patterns of environmental change favours each type of response (TGP, priming, …), their dynamics, and why. Fortunately, theory has started exploring this problem (Bonduriansky & Day, 2009, 2018). Furrow & Feldman (2014) found that slow temporal environmental fluctuations can lead to the evolution of more faithfully transmitted transgenerational effects, providing that underlying mechanisms entail little costs (see also Rivoire & Leibler, 2014). Similarly, other mathematical models showed that transgenerational effects can rapidly evolve, depending on the accuracy of the environmental stressor as a predictor of future (strong) selective pressures (Leimar & McNamara, 2015; Uller *et al.*, 2015). In line with these theoretical expectations, *C. elegans* was shown to adapt to temporally predictable fluctuating environments by evolution of a transgenerational effect, namely maternal glycogen provisioning (Dey *et al.*, 2016; Proulx *et al.*, 2019). More recently, a population-genetic model of two interconnected habitats found that adaptive transgenerational effects were likely to evolve under moderate dispersal, and when the direction of selection differed between habitats (Greenspoon & Spencer, 2018; Planidin *et al.*, 2025). However, to our knowledge little attention has still been given to the evolution of dynamic aspects of MUNGI, such as the rate of TGP, the stability and reversibility of responses across generations, or the duration of priming.

Another key question is how MUNGI influences "standard" adaptive genetic evolution, some aspects of which were discussed in two special issues (Lind & Spagopoulou, 2018; Ashe *et al.*, 2021). Firstly, heritable but non-genetic phenotypic changes can mask genotypic variation from selection, thereby modifying evolutionary trajectories (Sengupta *et al.*, 2023). Secondly, some MUNGI mechanisms can directly interact with the origination of genetic variation. In particular DNA methylation, by influencing mutation rate and transposon insertion, can affect genome stability, and therefore directly contribute to DNA sequence evolution (Ashe *et al.*, 2021; Yi & Goodisman, 2021). These combined influences of epigenetics on selection and mutation could

lead to potentially strong positive effects on adaptive evolution. Theory indeed suggests that populations can adapt faster when natural selection acts on both non-genetic and genetic variation (Day & Bonduriansky, 2011; Geoghegan & Spencer, 2013; Klironomos *et al.*, 2013). When explicitly modelling adaptation from *de novo* mutations, it was additionally shown that epigenetic mutations can both accelerate or hinder the rate of adaptation, depending on their stability and impact on fitness compared to genetic mutations (Kronholm & Collins, 2016).

Such interactions between transgenerational effects and genetic evolution have also been investigated empirically (Stajic & Jansen, 2021). An evolutionary experiment with an engineered strain of *S. cerevisiae* showed that MUNGI can modify rates of evolutionary adaptation (Stajic *et al.*, 2019). This occurred because transgenerational silencing of a gene responsible for cell growth increased the effective population size, thereby facilitating the appearance of new mutational targets and alleles that could accelerate adaptation. Luo *et al.* (2020) demonstrated the key role of interacting non-genetic and genetic mechanisms in evolution of *S. cerevisiae*. Selection on the expression of a fluorescent protein produced changes in histone markers (non-genetic) at key elements of galactose regulatory network lasting generations, which was complemented by a (genetic) mutation reducing the performance of RNA Pol II. In the green alga *Chlamydomonas reinhardtii*, engineered reductions of non-genetic variation reduced or impeded genetic adaptation to high salt and CO_2 treatments, but not to low phosphate (Kronholm *et al.*, 2017). The consequences of MUNGI may even cascade up to the macro-evolutionary scale. For instance, epigenetic variation is a good predictor of behavioural isolation and divergence in the fish genus *Etheostoma* (Smith *et al.*, 2016), and may thus influence speciation, consistent with conceptual and theoretical findings (Smith & Ritchie, 2013; Greenspoon *et al.*, 2022; Planidin *et al.*, 2022).

More research on how the dynamics of MUNGI influence genetic evolution is clearly needed. MUNGI mechanisms that are both rapidly induced and stable through time are likely to have more long-lasting influences on genetic evolution. This could be investigated by manipulating the dynamics of MUNGI through engineering where feasible, in model (Bódi *et al.*, 2017; Kronholm *et al.*, 2017) and non-model species (Richards *et al.*, 2017), combined with computational and mathematical modelling (McNamara *et al.*, 2016; Fey *et al.*, 2021; Briffa *et al.*, 2024). The development of new theoretical work could help refine predictions and expectations, or even propose novel mechanisms. For instance, a recent model simulating gene silencing/activation *via* DNA-methylation and de-methylation demonstrated that epigenetic mutations could enable the evolution of phenotypic plasticity (Romero-Mujalli *et al.*, 2024). Extending similar models to include epigenetic inheritance would allow investigating how transgenerational effects, possibly accumulating over generations, evolve and interact with evolution of purely genetic effects. Lastly, promising ways forward in linking the genetic to epigenetic basis and phenotypes are cell-lineage tracking approaches and single-cell sequencing allowing to follow epigenetic dynamics (Bintu *et al.*, 2016; Chatterjee & Acar, 2018; Xue & Acar, 2018; Meir *et al.*, 2020), and analyses of epigenetic quantitative trait loci (epiQTLs), (Cortijo *et al.*, 2014).

5) Concluding remarks

Although its relevance for adaptation is still being debated (Charlesworth *et al.*, 2017), NGI is an integral part of population responses to environmental change (Bonduriansky & Day, 2018; Donelson *et al.*, 2018; McGuigan *et al.*, 2021; Sengupta *et al.*, 2023). When the dynamics of non-genetic responses unfold over multiple generations (which we describe as MUNGI), they are likely to alter our interpretation of experimental studies of evolution. Here, we highlighted some major types of MUNGI, and proposed a first set of empirical assays that could help identify such effects and understand their evolutionary consequences. In the current context of

global change, explicitly considering the contribution of MUNGI to population responses to environmental changes, and potentially to adaptation, should prove particularly important.

Data availability

Does not apply, Perspective article.

Author contributions

G.Z., S.B., E.A.F, S.J, D.L., H. P., and L.M.C. conceived the study. G. Z. and L.M.C. wrote the first version of the manuscript, and all authors commented on the draft.

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Conflict of interest

The authors declare no conflict of interest.

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