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

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Abstract

Populations that face environmental change reducing their mean fitness can recover by adaptive genetic evolution over multiple generations, but their immediate responses may also involve non-genetic mechanisms, though the latter can be difficult to demonstrate. When the dynamics of such non-genetic changes in mean phenotype and fitness span multiple generations, their effects at the population level can be difficult to distinguish from those of natural selection on genetic variants. While the existence of non-genetic inheritance is no longer controversial, we argue that its potential contribution to observed patterns in evolutionary studies remains overlooked, especially for processes leading to phenotypic change that unfolds over multiple generations, which we call multigenerational non-genetic responses (MUNGER). We highlight three major forms of MUNGER that, if not properly accounted for, could confound inference about genetic changes: delayed impact of stress, transgenerational plasticity, and priming. We summarize how each may impact the dynamics of phenotypic change across generations in concrete experimental contexts (*e.g.*, experimental evolution, common gardens, ecotoxicological experiments). We propose that analysing the dynamic properties of MUNGER, 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 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 populations 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 or decay/revert. To emphasize the long-term aspect of such non-genetic responses that unfold over multiple generations, as opposed to short-term non-genetic responses over one or a few generations, or highly heritable epimutations causing the phenotype to change in the first generation but later remain constant, we describe them as multigenerational non-genetic responses (MUNGER). As MUNGER and rapid genetic

evolution may occur over similar time scales, the phenotypic changes that they induce at the population level may be hard to distinguish, despite having a completely different origin (Fig. 1). Indeed, while genetic evolution by natural selection results from changes in the relative frequencies of genotypes that differ in phenotype, but retain a constant phenotype within lineage (except for the effect of mutation, top panel in Fig. 1), MUNGER instead involve cumulative phenotypic modifications *within* lineages, leading to changes in the population mean phenotype even when all lineages have similar phenotypes at all times (bottom panel in Fig. 1). Changes in the frequencies of lineages with diverse MUNGER is also possible (but not illustrated in Fig. 1 for simplicity). We argue below that ignoring the temporal dynamics of MUNGER and their contributions to phenotypes and fitness across generations, and not clearly distinguishing its effects from those of genetic changes, is likely to limit our ability to analyse and predict evolutionary responses to changing environments from experimental approaches such as experimental evolution or common garden experiments, as well as to confound inference from quantitative-genetic designs. Considering MUNGER explicitly while designing experiments, deciphering how they interact with adaptive genetic evolution, and how they evolve, will be necessary for experimental studies to yield more useful insights into eco-evolutionary dynamics in changing environments.

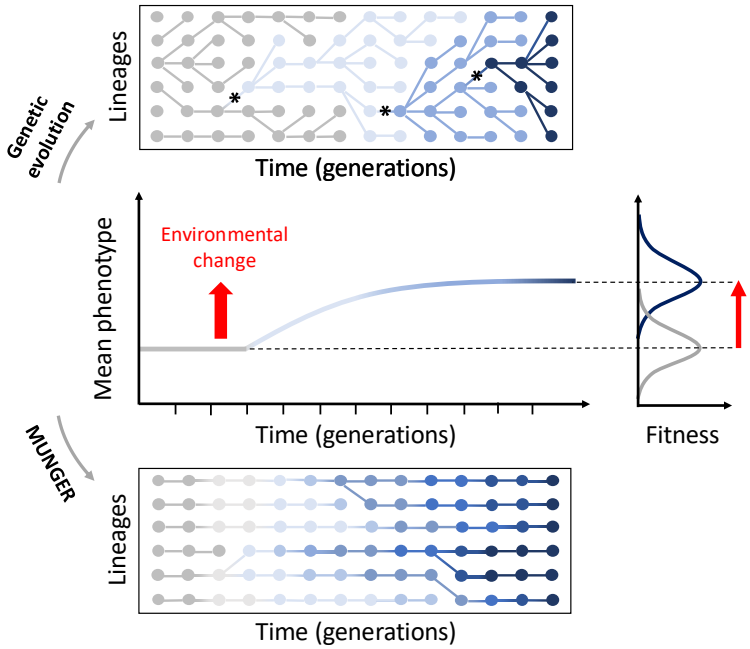

Figure 1 Alternative sources of phenotypic responses to environmental change. The line in the middle panel represents the dynamics of the mean phenotypic trait in a population (value along the y-axis and colour), following an environmental change (red arrow). The genealogy plots in the top and bottom panels illustrate two alternative explanations for this population-level response. The top genealogy represents adaptive genetic evolution, illustrated in the case of *de novo* mutations (ignoring any non-genetic mechanism for simplicity). 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 MUNGER (without genetic change). Here, the environment induces non-genetic phenotypic change that accumulates across generations within each lineage (from light grey to dark blue). The lineages may vary to some extent in their phenotypic 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. Note that we do not consider as MUNGER the types of non-genetic mechanisms leading to phenotypic change that does not accumulates gradually over multiple generations, such as highly heritable major-effect epimutations. These alternative explanations for phenotypic change (genetic evolution vs MUNGER) are difficult to distinguish based only on population phenotypic data. The outcome is likely to be more variable across systems for MUNGER owing to the diversity of mechanisms (illustrated in Fig. 2), but perhaps more repeatable within system if the same mechanism is repeatedly involved.

2) Major types of multigenerational non-genetic responses

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 MUNGER, which we summarize below in three main categories, can modify this picture.

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.

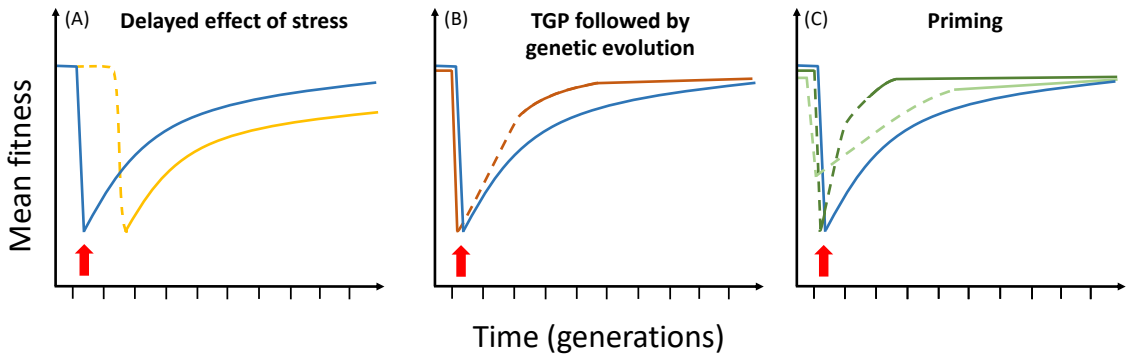

Figure 2 Three broad categories of MUNGER. Dynamics of mean population fitness over time following an environmental change (bottom red arrow), under different types of MUNGER. The blue line in all panels illustrates the baseline scenario, with an instantaneous effect of stress reducing mean fitness,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

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 change occurs across generations, 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 coloured lines). 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 intracellular 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 MUNGER 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.

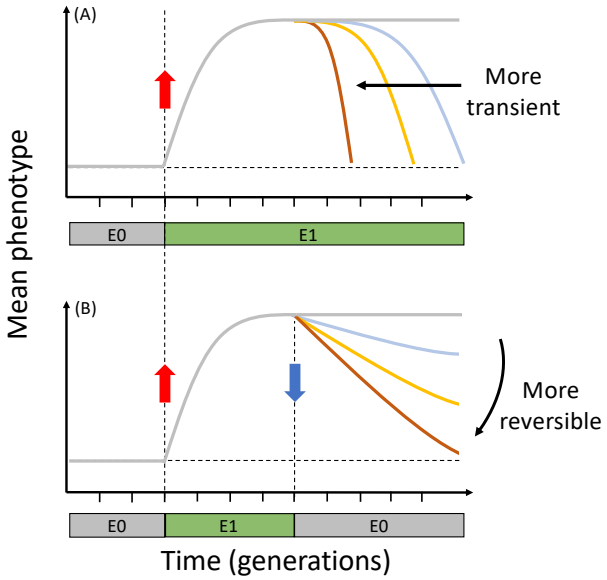

Figure 3 Stability and reversibility of phenotypic responses across generations. (A) Transient dynamics occur when the phenotypic trait goes back to its initial state, even though the environment has remained unchanged following the initial environmental shift (from E0 to E1), indicated by the red arrow. 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 MUNGER in experimental studies of evolution

Because they lead to changes in fitness that unfold over multiple generations, the MUNGER described above can phenomenologically resemble genetically-based evolution (Fig. 1). To avoid reaching misleading conclusions about eco-evolutionary processes, we suggest below some approaches and ideas to measure MUNGER 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 MUNGER are potentially important contributors 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 MUNGER 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 MUNGER 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), by tracking the dynamics of changes in fitness and other traits of interest over generations. However, how MUNGER might contribute to the outcome of these long-term experiments is still too rarely considered.

A first indication of a contribution from MUNGER can be provided by simple order-of-magnitude computations assessing whether the observed rates of changes are consistent with the expected timescales of genetic evolution, either from *de novo* mutations or via standing genetic variation (Ament-Velásquez *et al.*, 2022). Very rapid phenotypic dynamics taking place over few generations are more likely to involve MUNGER 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 algae *Emiliania huxleyi* and found that the timescale of fitness gain was better explained by a combination of non-genetic and genetic changes.

More progress can be achieved by relying on isogenic clonal populations, as can be established with most microorganisms (bacteria, protists, yeasts, microalgae), as well as some animals (*e.g., Daphnia*, *Artemia*, *Caenorhabditis elegans*, rotifers) and plants. Comparing rates of

phenotypic change per generation in single isogenic lines (caused only by MUNGER as in Fig 1, bottom) versus mixtures thereof (combination of MUNGER and evolution) can help quantify the relative contributions of MUNGER versus genetic evolution. For instance, Leung et al. (2020) showed that the trajectories of cell morphology over 10 days (and about as many generations) following a salinity change in the microalga *D. salina* were similar between genetically variable and isogenic populations, indicating that transgenerational plasticity was the main contributor to their dynamics. While useful, isogenic lines are not without limitations over longer timescales, as *de novo* mutations are likely to arise and segregate at low frequency. This is especially the case in microbes, owing to their large population sizes (a single bacterial colony in a petri dish easily containing several million cells), which can lead to a significant contribution of mutations to rapid evolution if selection is strong.

To circumvent this problem, the genetic composition of the population can be tracked over time together with phenotypic traits and/or fitness. Quantifying the part of the observed phenotypic change that result from underlying genetic changes can then help distinguishing it from the variance caused by non-genetic effects. Nevertheless, even when simultaneous genomic and phenotypic changes are observed, showing that the former explains the latter can be challenging based on population measurements only. Deciphering the genotype-phenotype map is notoriously 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. Combining genomics with more functional omics analyses (*e.g.*, transcriptomics, epigenomics) can be a productive way forward, as it may allow detecting the mechanisms underlying MUNGER. Observed phenotypic change across generations can be correlated to changes in mean gene expression or regulatory mechanisms (*e.g.*, small RNAs or DNA methylations) on one hand, and frequency change of genetic variants on the other hand. Provided sufficient temporal resolution for this omics data, the dynamics of MUNGER

process might be disentangled from those of genetic evolutionary responses, and their interactions could even perhaps be revealed (see section below on evolution of MUNGER). Such integrative approaches have 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). 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.

Lastly, more insights into the role of MUNGER in experimental evolution can be achieved by performing more extensive experimental assays. For instance, re-exposing evolved populations to their ancestral environments may allow identifying whether any putative response to stress reverses (Zilio *et al.*, 2023), and whether this reversal is too quick to be explained by genetic evolution (provided the timescales of both processes do not overlap much). 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.

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 MUNGER when they are 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), as suggested above for experimental evolution (but subject to the same limits).

Importantly, the control conditions used in the common garden might represent a complex novel environment *per se* for the organisms, which could trigger MUNGER. 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). In this context, priming and TGP might 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 MUNGER

Beyond potentially blurring the detection of adaptive genetic evolution in experiments, MUNGER can produce phenotypic variation on which selection may act (Zhang *et al.*, 2018; Yin *et al.*, 2019; Baltazar-Soares *et al.*, 2024) , and its underlying mechanisms may themselves vary genetically, and thus evolve (Bonduriansky & Day, 2018).

A first critical aspect for understanding the evolution of MUNGER is establishing to what extent their variation, including for multigenerational dynamics and responses to the environment, is genetically inherited, *i.e.*, underlain by variation in the DNA sequence. This is a challenging task due to the diversity of processes behind MUNGER, from the perception of the environmental "signal", to its transmission through the cellular environment (or via the endocrine system in animals), and the effector mechanism (*e.g.,* cis- or trans-acting genetic variants). Nevertheless, there is growing evidence that epigenetic variation, for instance, is genotype-dependent. In *A. 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).

393 Experiments on the ciliate *Tetrahymena thermophila* showed genotype-specific TGP of
394 dispersal-related traits with dynamics that spanned ~35 generations (Cayuela *et al.*, 2022).
395 Nonetheless, evidence for genetic variation in epigenetic (or other forms of non-genetic)
396 responses to environmental change are still rare. A study on *A. thaliana* found that, once
397 different genotypes collected across Sweden were exposed to higher temperature (16 °C *vs.* 10
398 °C), there was ample variation in their epigenetic responses, increasing on average DNA
399 methylation of the CHH context (C=cytosine, and H = nucleotide different from C) by 14%
400 (Dubin *et al.*, 2015). The authors could demonstrate that part of the epigenetic variation was
401 also associated with variation in a gene involved in DNA methylation. Still in *A. thaliana*,
402 genotypic-specific TGP responses to temperature were found in several phenotypic traits
403 (Alvarez *et al.*, 2021). Overall, these examples point to the existence of a genetic basis for
404 mechanisms of phenotypic change that unfold over multiple generations via non-genetic
405 inheritance, *i.e.*, of MUNGER.

406 Once genetic variation for MUNGER is established, we need to elucidate how selection
407 operates on it. Selection on (trans-generational) phenotypic plasticity is mediated by
408 environmental variation within and across generations, but we still know little about which
409 patterns of environmental change favours each type of response (TGP, priming, …), their
410 dynamics, and why. Fortunately, theory has started exploring this problem (Bonduriansky &
411 Day, 2009, 2018). Furrow & Feldman (2014) found that slow temporal environmental
412 fluctuations can lead to the evolution of more faithfully transmitted transgenerational effects,
413 providing that underlying mechanisms entail little costs (see also Rivoire & Leibler, 2014).
414 Similarly, other mathematical models showed that transgenerational effects can rapidly evolve,
415 depending on the accuracy of the environmental stressor as a predictor of future (strong)
416 selective pressures (Leimar & McNamara, 2015; Uller *et al.*, 2015). In line with these
417 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 MUNGER, such as the rate of TGP, the stability and reversibility of responses across generations, or the duration of priming memory.

Another key question is how MUNGER influence "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, including that triggered by environmental change, can mask genotypic variation from selection, thereby modifying evolutionary trajectories (Sengupta *et al.*, 2023). Secondly, some mechanisms of MUNGER 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 MUNGER and genetic evolution have also been investigated empirically (Stajic & Jansen, 2021). An evolutionary experiment with an engineered strain of *S. cerevisiae* highlighted that MUNGER 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 led to changes in histone marks at key elements of galactose regulatory network that lasted multiple generations. This MUNGER was followed by a (genetic) mutation reducing the performance of RNA Pol II. In the green alga *C. 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 MUNGER 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 MUNGER influence genetic evolution is needed. MUNGER that are both rapidly induced and stable through time should influence mutation and selection processes for longer, and are thus likely to have more impacts on genetic evolution. This could be investigated by manipulating the dynamics of MUNGER 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 can 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 MUNGER), they are likely to alter our interpretation of experimental studies of evolution. Here, we highlighted three major types of MUNGER, 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 MUNGER to population responses to environmental changes, and potentially to adaptation, should prove particularly important.

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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