Improving our understanding of adaptive evolution by addressing multi-generational non-genetic responses

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, 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 abrupt environmental change reducing their fitness can recover by adaptive genetic evolution over tens to hundreds of generations, but their immediate responses often involve non-genetic mechanisms. When such non-genetic responses span multiple generations, their dynamics may be difficult to distinguish from those of genetic evolution. We here argue that focusing research on such multi-generational non-genetic responses (MGNGR) should be crucial to better understand and predict eco-evolutionary responses to environmental stress. We survey the most salient forms of MGNGR (delayed impact of stress, transgenerational plasticity, and priming), with a focus on how they may impact the dynamics of observed phenotypic change across multiple generations. Analysing the rate, stability, and reversibility of MGNGR, as well as their relative contributions to overall phenotypic responses, and their interactions with genetic changes, should be particularly fruitful towards a more comprehensive deciphering of evolutionary responses to novel or changing environments.

1) Population responses to stressful environments

Understanding how populations respond to environmental change with detrimental impacts on biological function and fitness is a critical 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.*, 2024). 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 genetic evolution, the increase in frequency of beneficial mutations in a population through natural selection. These processes are usually thought to occur over clearly distinguishable timescales, with plasticity taking place within generations, while genetic evolution unfolds across generations. This assumed timescale separation has consequences for predicted population responses to environmental stress at the phenotypic and demographic levels. On the one hand, an immediate plastic response, if adaptive, can limit the initial reduction of fitness - and potentially population size - in a novel, stressful environment. On the other hand, adaptive genetic evolution acting on new mutations, standing genetic variation, or a mixture of both (Orr & Unckless, 2008, 2014), leads to gradual change in traits and fitness that accrues over generations, and might even prevent extinction of a declining population if sufficiently fast (Gonzalez *et al.*, 2013), a phenomenon known as evolutionary rescue (reviewed by Bell, 2017). Such a clear-cut timescale difference between plasticity and evolution, if true, would not preclude interactions among them. For instance, adaptive plasticity may shield phenotypes from selection, reducing the efficiency of the following adaptive genetic evolution (Ghalambor *et al.*, 2007). Conversely, non-adaptive or maladaptive plasticity can be the main driver of compensatory evolutionary changes (Grether, 2005; Ghalambor *et al.*, 2015). Other effects involve interactions of evolution with demography. For instance, adaptive plasticity can allow populations to persist over the first few generations of exposure to environmental stress, until natural selection “takes over” to rescue the population, a phenomenon often described as the Baldwin effect (Simpson, 1953; Crispo, 2007).

Nevertheless, this timescale separation between plasticity and genetic evolution, although conceptually useful, remains a simplified representation of an organism's set of responses to environmental changes. Non-genetic inheritance (NGI, Bonduriansky *et al.*, 2012; Bonduriansky & Day, 2018), defined as any form of inheritance that does not rely on DNA, blurs this separation line, by allowing phenotypic variation (including that induced by the environment) to spill-over from one generation to the next. Transgenerational plasticity (TGP), whereby trait expression depends on the environments experienced by the previous generations, is also increasingly recognized as an important biological phenomenon. Bell & Hellmann (2019) recently proposed a useful framework to study such responses, reporting evidence for six different patterns, including bounce-back (visible only in F1 generations) or persistent effects (still visible several generations after stress exposure; Table 1 in Bell & Hellman 2019). Using a more systematic approach, (Yin *et al.*, 2019) conducted a meta-

analysis on TGP, showing their potentially fundamental role in responses to changing environmental conditions across a diversity of taxa (see also Herman *et al.* 2014 for a more adaptive perspective).

While NGI and TGP are now well-accepted phenomena, their prevalence and contribution to the dynamics of environmental stress responses remain underappreciated. There is accumulating evidence that a diversity of mechanisms can transmit effects of environmental stress across generations in plants, unicellular eukaryotes, and animals (Quadrana & Colot, 2016; Pilling *et al.*, 2017; Sengupta *et al.*, 2023). Histone modification, patterns of DNA methylation, and the transmission of non-coding RNAs, are some of the well-elucidated mechanisms (Bošković & Rando, 2018; Adrian-Kalchhauser *et al.*, 2020). But NGI may be even more diverse for unicellular organism, where the lack of a soma/germline divide (at the notable exception of ciliates, see below) means that many aspects of their phenotype, including proteins, gene regulatory factors and epigenetic modifications, are indeed directly transmitted to their descendants over multiple generations (Sengupta *et al.*, 2023). In *Escherichia coli* for instance, the average protein's half-life (~20 hours) is much longer than its generation time of ~20 minutes under ideal conditions (Moran *et al.*, 2013), and even that estimated under natural conditions (Gibson *et al.*, 2018). Similarly, the half-life of mRNAs is often on a similar order of magnitude as the generation time in ideal conditions (Mohanty & Kushner, 2016).

Non-genetic responses to environmental stress can thus span multiple generations, during which they can accumulate gradually, or decay/revert, depending on their mechanism. In other words, they can exhibit dynamics similar to those of rapid genetic evolution (Fig. 1), making these alternatives difficult to distinguish based solely on observations of changes in phenotypes and fitness. To emphasize this similarity of timescale with evolutionary change, we describe as multi-generational non-genetic responses (MGNGR) any form of NGI and TGP with dynamics that span multiple generations. We argue that dismissing the temporal dynamics of such MGNGR (whether adaptive or not) and their contribution to fitness, and not clearly distinguishing them from genetic change, is likely to limit our ability to infer and predict population responses to changing environments, especially in long-term experimental evolution and common garden experiments. We therefore suggest that making MGNGR a study object of their own, by considering them explicitly while designing experiments, deciphering how they interact with adaptive genetic evolution, and how they themselves evolve, will improve our understanding of eco-evolutionary dynamics in changing environments.

2) Major types of multi-generational non-genetic responses (MGNGR)

We are interested in population responses to an environmental change that initial causes substantial maladaptation, manifested by a decrease in fitness. Unless explicitly stated, we are considering constant conditions following this initial environmental change. The standard

scenario for adaptive genetic evolution to such abrupt environmental changes posits that fitness first declines sharply, then slowly increases again (Fig. 1 middle), through the establishment and rise in frequency of beneficial mutations (Fig. 1 top). However, different forms of MGNGR might alter the dynamics of adaptive evolution while closely resembling the genetic mechanisms. We focus below on three main categories that are particularly relevant in this context.

2.1 Delayed impact of stress: time-to-response

The detrimental impact of environmental change on fitness may be delayed, and only manifest some generations after exposure to the stressor(s). Delayed impact of stress may be largely passive, due to purely mechanical or physical reasons that do not involve any specifically evolved response; or in contrast, an organism may have evolved specific mechanisms for coping with the stressor. An example of essentially passive delayed impact is the accumulation of toxic or harmful molecules (*e.g.*, pesticides) that slowly permeate into cells, and only start to have measurable detrimental impacts once a tolerance threshold is crossed for the affected cellular functions (*e.g.*, DNA replication, protein synthesis/folding, cell metabolism or structure). For instance, exposing *Drosophila melanogaster* to silver nanoparticles induced reproductive costs only from generation F2 onwards, and these costs increased in the following generations (Panacek *et al.*, 2011). This was due to the accumulation of oxidative stress and the upregulation of heat shock protein 70, which reduced flies' investment in reproduction, and thereby fecundity. Similarly, nanoparticle exposure of *Caenorhabditis elegans* impaired germ cells, leading to reproductive abnormalities and fecundity reduction only in the F2 generation (Kim *et al.*, 2013). Another possible cause of passive delay is when a stressor only acts at a specific stage in the life cycle. For instance, some antibiotics only target newly formed cell membranes (Kohanski *et al.*, 2010), such that they cannot express their detrimental effect until cell division occurs.

On the more active side, delayed detrimental impacts of stress could also occur because the mechanisms for coping against stressors cannot be sustained for long, eventually affecting an organism survival or reproduction. For instance, specific enzymes, from heat shock proteins to molecular pumps, have intrinsic biophysical and biochemical limits, beyond which they cannot operate. This could also be mediated by the resources needed to maintain the coping machinery. In other words, delays in the onset of stress impacts may be tightly related to the permanency and stability of immediate stress response mechanisms (which we address below). The dynamics of this form of MGNGR will resemble baseline expectations for a fitness decline following environmental changes, but the manifestation of detrimental effects will only start sometime after the stressor exposure (Fig. 2A, yellow line).

2.2 Speed and reversibility of TGP

There is increasing evidence for the importance of TGP in the context of environmental change (Donelson *et al.*, 2018), both abiotic (Donelan *et al.*, 2020; Castano-Sanz *et al.*, 2022) and biotic (Tariel *et al.*, 2020; Shahmohamadloo *et al.*, 2024). Despite attempts to

conceptualise TGP and its underlying mechanisms (Bell & Hellmann, 2019), a key aspect that remains understudied is its dynamics over multiple generations. Extending recent arguments about within-generation plasticity (Burton *et al.*, 2022; Dupont *et al.*, 2024), we suggest that it would be useful to quantify the whole temporal dynamics of TGP, both within the first F1 generation and in the following ones.

An important temporal aspect to consider is the speed of trait change over generations after exposure to environmental change, that is, the rate of TGP. This rate is likely to affect short- and long-term responses to stress (fitness), and is thus critical to understand and predict when the dynamics of MGNGR is likely to mimic those of adaptation through genetic evolution (Fig. 2B orange line). In a simple scenario, we can envisage that following environmental change, the trait changes over generations, until reaching a plateau of stationary trait expression (Fig.3A-B grey line). Populations with a faster rate of TGP (before the plateau) should have faster fitness increase in face of the stressors, and recover more quickly from the initial detrimental impacts of environmental stress, without any genetic evolution. The maximum magnitude of transgenerational plasticity (at the phenotypic plateau), or TGP capacity, in turn determines to what extent genetic evolution is needed at all for adaptation.

Another important aspect of TGP is the stability (or stationarity) and reversibility of responses. Most work to date assumes a permanent stress exposure in all generations following the initial environmental change, as illustrated in our baseline scenario in Fig. 3A (E1). In this context, a critical question is whether the phenotypic response can be sustained at a phenotypic plateau (stable response), or instead is only transient, and eventually reverts back towards its initial value after some generations of exposure. There are several reasons for which TGP may not be sustained after many generations of exposure. First, responses might be costly to sustain, for instance because they lead to accumulating defects over generations (as discussed for the delayed impacts of stress), or because they require important metabolic investments that trade off against other functions of the organism. Alternatively, TGP could act as a more emergency mechanism lasting a few generations, such as for heat shock response to thermal stress (Richter *et al.*, 2010), before it is replaced by more specific and durable physiological responses. The stability of TGP was empirically investigated in a plant system. The yellow monkeyflower plant (*Mimulus guttatus*) increased trichome production in response to wounding that simulated insect damage. This response was stable for 3 generations in the absence of subsequent damage, before starting to decrease to the level of control unwounded plants in generation 4 and 5 (Akkerman *et al.*, 2016). Interestingly, the results also suggested that both parents contributed to the TGP responses additively, but via different mechanism (maternal methylation *vs.* paternal histone modifications).

In scenarios where the environment is changed again after a few generations, such as going back to its pre-stress value, the crucial question becomes whether - and how fast - the phenotype can go back to its initial state, *i.e.* how reversible it is (Fig.3B). Differences in the

speed of induction *vs.* reversibility for TGP of osmotolerance were found in the unicellular alga *Dunaliella salina* (Rescan *et al.*, 2020). Transferring populations across salinity levels showed that increasing glycerol content (when going from low to high salinity) was much slower than decreasing it (from high to low salinity), because the mechanisms involved are completely different: increasing glycerol level requires synthesizing it through metabolism, whereas decreasing it can be simply achieved by excretion. The asymmetry between synthesis of molecule and excretion could be a rather common mechanism. Environmental perturbations can also cause non-genetic changes in gene regulatory networks that are poorly reversible, as shown in *Escherichia coli* by Zhao *et al.* (2024). Beyond these examples, little is still known about how reversibility unfolds across generations. Maintaining the machinery to sense the environment and revert the phenotype is likely to have significant costs, as emphasized for within-generation plasticity by Hoffmann & Bridle (2022), but multigenerational reversibility also depends on how the mechanisms of NGI are affected by patterns of environmental fluctuations among generations.

2.3 Trans-generational priming: memory of past responses

Another major class of MGNGR is trans-generational priming, whereby prior exposure of an organism to a stressor (priming) can prepare 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 *Escherichia coli* cells primed with antimicrobial peptides (AMP) exhibited both increased tolerance and persistence when re-exposed to AMP after some generations (Rodríguez-Rojas *et al.*, 2021). Similarly, the four- and five-generation descendants of *Saccharomyces cerevisiae* originally primed with salt exhibited increased resistance to hydrogen peroxide and faster gene expression. The salt priming in the parental generation activated the synthesis of the long-lived cytosolic catalase Ctt1p, which was then propagated through 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). Stress exposure can also prime *E. coli* and/or rewire the regulatory network for several generations (Zhao *et al.*, 2024) without any immediate costs. In their extensive review, which included both within- and trans-generational priming, Hilker *et al.* (2016) briefly proposed some temporal scenarios for primed stress responses (Fig. 2 in Hilker et al. 2016). For example, the primed organisms can respond faster or earlier, or even produce a stronger response with higher amplitude than the non-primed counterparts upon triggering. Wesener & Tietjen (2019) explored some of these temporal aspects, by modelling population performance under different (trans-generational) priming and stress conditions associated with different costs. Faster and earlier responses were favoured under short and severe/lethal stress, whereas stronger responses were favoured for stress of longer durations. Nonetheless, empirical studies are still limited, especially those with an evolutionary perspective. Understanding whether trans-generational priming reduces the immediate impact of stress upon next exposure (light green line in Fig 2C), or instead leads to

faster TGP in subsequent generations (dark green line in Fig 2C), is of central importance to fully unravel the implications of this process for population dynamics, fitness and evolutionary trajectories. In addition, it would be necessary to measure for how many generations the trans-generational priming could last in the absence of re-exposure to stress.

3) Empirical approach to disentangle MGNGR from genetic responses

Because they lead to changes in fitness that unfold over multiple generations, the described MGNGR can phenomenologically resemble genetically-based evolution. To avoid reaching misleading conclusions about eco-evolutionary processes, it is therefore crucial to identify MGNGR and measure their dynamics. We suggest below some approaches and ideas to measure MGNGR while conducting preliminary assays, experimental evolution, and common garden experiments.

3.1 Establishment of experimental protocol

Preliminary assays and pilot experiments are fundamental steps to optimise protocols, laboratory conditions, and reduce undesirable technical noise or variation. In experiments on stress responses, preliminary tests often serve to identify the level of stressor (*e.g.*, dose concentration or exposure time) that yields sufficiently strong detrimental effects to be clearly detectable, without leading to rapid extinction of the population. Although it is of course part of the scientific approach, this step may in some cases lead to discarding (and potentially biasing) relevant biological information and variation. For instance, a treatment level leading to rapid population decline might be discarded because the stressor would be deemed too strong for the envisaged protocol. However, longer observations (*e.g.*, over more cycles of batch culture in a microbe) might show fitness recovery through MGNGR (Fig. 2B-C), such that the treatment is actually compatible with long-term evolution. Conversely, a presumably permissive treatment during a short preliminary test could turn out to represent stressful conditions over the longer run because of delayed impacts of the stressor (Fig. 2A). In both cases, performing preliminary assays for a longer time (*i.e.*, over several cycles of batch culture, or more generations), could allow thoroughly describing a stressor effect on fitness.

From a more applied perspective, these considerations are particularly relevant for (eco)toxicological studies. Although it is well-known that chemicals and synthetic components can cause transgenerational effects affecting the basal levels of a pathway and/or molecular modifications (Oziolor *et al.*, 2017), this is rarely considered in conventional ecotoxicology tests, which might produce misleading results. Typically, most assays are of short duration (often stopping when any visible effect is observed), and thereby ignore the temporal and transgenerational components of stress responses (Brevik *et al.*, 2018; Nilsson *et al.*, 2022). For instance, descendants from primed populations might have stronger or faster responses upon exposure to the same or another stressor. Another shortcoming is that these tests often focus on survival without measuring sublethal effects, or other fundamental fitness components such as growth and reproduction over generations (Straub et al. 2020). Overall,

considering non-genetic aspects by *(i)* implementing longer assays (including across generations), *(ii)* sampling at regular intervals to tackle short-term dynamics, and *(iii)* measuring additional traits, might directly improve risk assessment and policy making in such eco-toxicological studies.

3.2 Experimental evolution

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

A first step towards better inclusion of MGNGR involves using order of magnitude computations to assess whether the timescale of observed changes is consistent with genetic evolution in the considered biological system. Beyond differences between model systems, expectations will differ depending on specificities of the experiment, for instance whether genetic evolution has to involve *de novo* mutations (when starting from isogenic populations), or can occur via standing genetic variation. Very rapid fitness dynamics taking place over a few generations are more likely to involve MGNGR than genetic evolution, unless selection is extremely strong and acts on a genetically diverse population (for instance when the population of wild types is rapidly depleted and replaced by an initially rare mutant). Making these arguments more quantitative requires reliable knowledge about mutation rates, levels of standing genetic variation, and distribution of fitness effects in the studied organism in response to this particular stressor.

Another quantitative aspect to consider is the repeatability of observed responses. Because of stochasticity in the origin of mutations and random genetic drift, genetic responses to selection are not expected to occur exactly at the same time and with identical effects (*e.g.* speed of fitness recovery) in different replicated lines, such that high repeatability of responses is more consistent with MGNGR. However, this argument should be used with caution. Highly polygenic traits could have highly repeatable evolutionary responses at the phenotypic level, despite being underlaid by very unpredictable genetic changes because of high redundancy in their genetic architecture (Ridenhour & Nuismer, 2007; Barghi *et al.*, 2019). Furthermore, the (possibly transient) presence of hypermutator strains, mutation hotspots, or any other sources of mutation bias, could increase the repeatability of evolutionary outcomes even at the molecular level (Cano *et al.*, 2023; Sane *et al.*, 2023).

The relative contributions of MGNGR *vs.* allele frequency changes to phenotypic changes at different timescales can be investigated by using a combination of omics analyses (*e.g.*, transcriptomics *vs.* genomics) with phenotypic assays over time. In practice, this would require assessing the genetic and epigenetic composition of the population at many

timesteps, to track the relative frequencies of genomes and epigenomes, together with phenotypic traits and/or fitness. Nevertheless, even when simultaneous genomic and phenotypic change is observed, showing that the former explains the latter is challenging when only using population-based measurements. More progress can be achieved by isolating genotypes, for instance by creating clonal populations for microbes, before phenotyping them. However even when this is feasible, deciphering the genotype-phenotype map remains extremely difficult (Wagner & Zhang, 2011; Aguilar-Rodríguez *et al.*, 2018), as it requires more resolution (low linkage disequilibrium) than is typically available in experimental evolution designs. Introducing a mutation of interest in the ancestral background to isolate its effect (reverse genetics) is another possible approach with model bacteria. Yet, this remains labour intensive and the dynamics are untraceable without reducing and precisely controlling the number of mutations to test.

In addition to jointly tracking genetic and phenotypic change over time, more insights on the role of MGNGR in experimental evolution can be achieved by performing more complete assays, beyond the conditions from the evolution experiment. In particular, sampling lines at several points during experimental evolution, and exposing them to transfers from control to stress conditions, would help identify whether the initial response in the experiment was mediated by genetic or non-genetic mechanisms. Nonetheless, this approach would already require somewhat precise knowledge about the speed of reversibility (see above) of the response of interest in the model system. This would also imply some underlying knowledge of how many generations the study organisms should be maintained in control before switching back to stress conditions.

3.3 Common-garden experiments

Common garden and transplant experiments are standard protocols to evaluate whether populations from different experimental or natural origins show patterns of local genetic adaptation. They consist of placing individuals from different origins in the same environmental condition(s), to remove environmental and non-genetic effects, and thus quantify the genetic basis and trait variation. Typically, this procedure allows controlling for the potential misleading outcomes due to parental effects (maternal and paternal), which have been recognised for a long time (Mousseau *et al.*, 2009; Marshall, 2024). However, controlling for one generation (parental) might not be enough. As emphasised above, transgenerational effects are common, and they might lead to wrong inference even for well-designed studies that only control for parental effects. The extent to which MGNGR have to be accounted for, and consequently the required number of generations of common garden, depend on the question asked and the organism. The duration of the common garden should account for the possibility of long MGNGR when these are suspected, while limiting the opportunity for *de novo* mutations to arise.

A productive way forward would be to systematically measure the dynamics of traits during the common garden phase. In particular, transferring an organism to standardised laboratory

conditions might represent a complex set of changing environmental conditions *per se*, which could trigger undesired MGNGR. In fact, due to historical contingency, individuals or lines from different localities or treatments might react differently to the common garden, leading to a confusion between genetic adaptation and transgenerational effects. Priming might be the most problematic MGNGR in this context, as it could lead to phenotypic effects in responses to environments that differ from those where populations have been sampled. Priming could be particularly difficult to distinguish from the consequences of recent evolutionary history of stress exposure, *i.e.*, the "ghost of selection past" (Connell, 1980; Samani & Bell, 2016), making the explicit study of the timing of adaptation through multiple generations particularly important in eco-evolutionary studies.

Here again, more progress can be made by explicitly addressing MGNGR. For instance, samples can be transferred from different natural environments (or evolutionary treatments) to control condition / common garden, and then back from control to treatment, to measure the rate of phenotypic change in response to these environmental changes. Additionally, changing the environment gradually *vs*. abruptly (or modifying stress intensity and duration) could highlight differences in transient dynamics and potential costs.

4) The evolution of transgenerational effects

Although the main accepted mechanism of adaptive evolution (at least in the long run) is change in allele frequency by natural selection (Charlesworth *et al.*, 2017), MGNGR can lead to dynamics of phenotypic change over generations that may mimic patters thereof. In addition, MGNGR can produce variation on which selection may act, and they may themselves vary genetically, and thus evolve. We propose some promising basic research questions on this topic, to hopefully motivate more theoretical and empirical studies and stimulate further discussion.

A first critical question towards understanding evolution of MGNGR is how selection operates on them. We still know little about which pattern of environmental change favours each type of response and why, but theory has started exploring this problem as reviewed by Bonduriansky & Day (2009) for NGI and recently discussed their book (Bonduriansky & Day, 2018). Furrow & Feldman (2014) found that slow temporal environmental fluctuations can lead to the evolution of more faithfully transmitted transgenerational effects (and conversely), providing that their underlying mechanisms entail little costs (see also Rivoire & Leibler, 2014). More recently, a population-genetic model of two habitats interconnected by dispersal found that adaptive transgenerational effects were likely to evolve under moderate dispersal, and when the direction of selection differed between habitats (Greenspoon & Spencer, 2018). However, to our knowledge little attention has still been given to the evolution of dynamic aspects of MGNGR, such as the rate of TGP, the stability and reversibility of responses across generations, or the duration of priming.

Another fundamental question is the genetic basis and heritability of these processes. This is mostly an empirical question, which likely depends on the mechanisms of non-genetic inheritance. Field studies have shown the contribution of transgenerational mechanisms in generating phenotypic variation in natural populations (Husby, 2022). Supporting the idea that transgenerational effects can evolve by natural selection, laboratory experiments demonstrated the presence of genetic variation for such processes. For instance, Alvarez *et al.* (2021) showed genotypic-specific TGP responses to temperature for several phenotypic traits in *Arabidopsis thaliana*. Similarly, Cayuela *et al.*, (2022) found that in the ciliate *Tetrahymena thermophila* the TGP of traits related to dispersal was determined by their genotypes.

Beyond the evolution of transgenerational effects, a key question is how they influence genetic evolution. First, heritable non-genetic phenotypic changes can mask genotypic variation from selection, thereby modifying evolutionary trajectories (Sengupta *et al.*, 2023). In addition, some mechanisms of non-genetic inheritance can 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, opposite to the abovementioned buffering hypothesis. Such interactions between transgenerational effects and genetic evolution have been investigated in a few experimental studies. An evolutionary experiment with an engineered strain of *Saccharomyces cerevisiae* showed that transgenerational effects can modify rates of evolutionary adaptation (Stajic *et al.*, 2019). Intermediate levels of transgenerational gene silencing of the URA3 gene locus, responsible for the production of uracil (an essential component for cell growth), enabled better survival and faster adaptation to a novel environment (Stajic *et al.*, 2019). This occurred because transgenerational silencing increased the effective population size, thereby facilitating the appearance of new mutational targets and alleles that could accelerate adaptation. Further, the transgenerational gene silencing interacted with the novel alleles, rendering the transgenerational gene silencing itself more stable and strongly heritable. Using the same strains, the authors additionally showed that transgenerational gene silencing provided an adaptive advantage under fluctuation regimes (Stajic *et al.*, 2022). In another study, Kronholm *et al.*, (2017) evolved populations of the unicellular green alga *Chlamydomonas reinhardtii* for two hundred asexual generations in three different environments (high salt, low phosphate, and high CO_2). The populations genetically adapted in all environments and increased their fitness. The authors additionally evolved algal populations in parallel treatments (same environmental conditions and time period), but they genetically and chemically reduced the amount of non-genetic variation produced and transmitted. Decreasing non-genetic variation reduced or impeded adaptation to the high salt and CO_2 environments. In contrast, lower levels of non-genetic variation had little role in adaptation to low phosphate. Overall, these results highlight the role of transgenerational

effects in adaptive evolution, and how this might depend on the environmental context. Smith *et al.* (2016) even found that transgenerational effects might explain behavioural isolation and divergence between fish of the genus *Etheostoma*, with potential consequences for speciation, thus scaling up to macro-evolutionary scale.

Interestingly, differences between MGNGR and genetic adaptation might sometimes not be fundamentally clearcut, even conceptually (not only experimentally), and cases exist where MGNGR and genetic adaptation mechanistically cross each other. Ciliates, unicellular eukaryotes characterised by nuclear dimorphism (germline and somatic), are an extraordinary example in which TGP might actually occur through genetic mechanisms. In brief, genetic mutations can occur in both their nuclei, but during sexual reproduction the somatic nucleus is lost, with (almost) no transfer to descendent cells. Thus, genetic evolution in the somatic nucleus can be considered a form of TGP at the scale of their sexual generations (Verdonck *et al.*, 2021).

We suggest that a productive future line of research would be to investigate how the dynamics of MGNGR influence their effects on genetic evolution. For instance, MGNGR that are both rapidly induced and stable through time could be expected to have more long-lasting influences on genetic evolution. This could be investigated by manipulating the dynamics of MGNGR though engineering where feasible, as done by (Kronholm *et al.*, 2017). The development of new theoretical work could help refine predictions and expectations to empirically demonstrate a direct relationship between non-genetic and genetic responses, suggest experimental design strategies, and inform on specific ecological conditions favouring each of these phenomena.

5) Concluding remarks

Although their relevance for adaptation is still being debated (Charlesworth *et al.*, 2017), NGI and TGP are an integral part of population responses to environmental change (Bonduriansky & Day, 2018; Donelson *et al.*, 2018; Sengupta *et al.*, 2023). Evidence is mounting that such responses are not only repeatable and widespread, but can also span multiple generations (which we describe as MGNGR), and thus take place over similar timescales as rapid genetic evolutionary responses. Modern techniques allowing for precise in-depth investigations of the underlying mechanisms are now available to go a step further in our comprehension of the many forms of MGNGR, provided we make them an object of study rather than a mere nuisance parameter. Here, we highlighted some major types of MGNGR, and proposed empirical assays that could help identify such effects and understand their consequences. Critical insights could be gained by jointly tracking changes in genotypes frequencies and within-genotypes phenotypic changes, in common gardens (see also de Villemereuil *et al.*, 2016), evolutionary experiments, or natural populations. This would provide precious information on the extent of these effects, and on their relative contributions to short- and long-term responses to environmental change. In the current context of global changes,

explicitly considering the contribution of MGNGR to population responses to environmental changes, and potentially of adaptation, may prove particularly important.

References

Adrian-Kalchhauser, I., Sultan, S.E., Shama, L.N.S., Spence-Jones, H., Tiso, S., Valsecchi, C.I.K., *et al.* 2020. Understanding "Non-genetic" Inheritance: Insights from Molecular-Evolutionary Crosstalk. *Trends in Ecology & Evolution* **35**: 1078–1089.

Aguilar-Rodríguez, J., Peel, L., Stella, M., Wagner, A. & Payne, J.L. 2018. The architecture of an empirical genotype-phenotype map. *Evolution* **72**: 1242–1260.

Akkerman, K.C., Sattarin, A., Kelly, J.K. & Scoville, A.G. 2016. Transgenerational plasticity is sex-dependent and persistent in yellow monkeyflower (Mimulus guttatus). *Environ Epigenet* **2**: dvw003.

Alvarez, M., Bleich, A. & Donohue, K. 2021. Genetic differences in the temporal and environmental stability of transgenerational environmental effects. *Evolution* **75**: 2773–2790.

Ashe, A., Colot, V. & Oldroyd, B.P. 2021. How does epigenetics influence the course of evolution? *Philosophical Transactions of the Royal Society B: Biological Sciences* **376**: 20200111.

Barghi, N., Tobler, R., Nolte, V., Jakšić, A.M., Mallard, F., Otte, K.A., *et al.* 2019. Genetic redundancy fuels polygenic adaptation in Drosophila. *PLOS Biology* **17**: e3000128.

Bell, A.M. & Hellmann, J.K. 2019. An Integrative Framework for Understanding the Mechanisms and Multigenerational Consequences of Transgenerational Plasticity. *Annu. Rev. Ecol. Evol. Syst.* **50**: 97–118.

Bell, G. 2017. Evolutionary Rescue. *Annual Review of Ecology, Evolution, and Systematics* **48**: 605–627.

Bonduriansky, R., Crean, A.J. & Day, T. 2012. The implications of nongenetic inheritance for evolution in changing environments. *Evol Appl* **5**: 192–201.

Bonduriansky, R. & Day, T. 2018. *Extended Heredity: A New Understanding of Inheritance and Evolution*. Princeton University Press, Princeton, New Jersey Oxford, United Kingdom.

Bonduriansky, R. & Day, T. 2009. Nongenetic Inheritance and Its Evolutionary Implications. *Annual Review of Ecology, Evolution, and Systematics* **40**: 103–125.

Bošković, A. & Rando, O.J. 2018. Transgenerational Epigenetic Inheritance. *Annu Rev Genet* **52**: 21–41.

Brevik, K., Lindström, L., McKay, S.D. & Chen, Y.H. 2018. Transgenerational effects of insecticides — implications for rapid pest evolution in agroecosystems. *Current Opinion in Insect Science* **26**: 34–40.

Burton, T., Ratikainen, I.I. & Einum, S. 2022. Environmental change and the rate of phenotypic plasticity. *Global Change Biology* **28**: 5337–5345.

Cano, A.V., Gitschlag, B.L., Rozhoňová, H., Stoltzfus, A., McCandlish, D.M. & Payne, J.L. 2023. Mutation bias and the predictability of evolution. *Phil. Trans. R. Soc. B* **378**: 20220055.

Castano-Sanz, V., Gomez-Mestre, I. & Garcia-Gonzalez, F. 2022. Evolutionary consequences of pesticide exposure include transgenerational plasticity and potential terminal investment transgenerational effects. *Evolution* **76**: 2649–2668.

Cayuela, H., Jacob, S., Schtickzelle, N., Verdonck, R., Philippe, H., Laporte, M., *et al.* 2022. Transgenerational plasticity of dispersal-related traits in a ciliate: genotype-dependency and fitness consequences. *Oikos* **2022**: e08846.

Charlesworth, D., Barton, N.H. & Charlesworth, B. 2017. The sources of adaptive variation. *Proceedings of the Royal Society B: Biological Sciences* **284**: 20162864.

Connell, J.H. 1980. Diversity and the Coevolution of Competitors, or the Ghost of Competition Past. *Oikos* **35**: 131–138.

Côté, I.M., Darling, E.S. & Brown, C.J. 2016. Interactions among ecosystem stressors and their importance in conservation. *Proceedings of the Royal Society B: Biological Sciences* **283**: 20152592.

Crispo, E. 2007. The Baldwin effect and genetic assimilation: revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. *Evolution* **61**: 2469–2479.

de Villemereuil, P., Gaggiotti, O.E., Mouterde, M. & Till-Bottraud, I. 2016. Common garden experiments in the genomic era: new perspectives and opportunities. *Heredity* **116**: 249–254.

Donelan, S.C., Hellmann, J.K., Bell, A.M., Luttbeg, B., Orrock, J.L., Sheriff, M.J., *et al.* 2020. Transgenerational Plasticity in Human-Altered Environments. *Trends in Ecology & Evolution* **35**: 115–124.

Donelson, J.M., Salinas, S., Munday, P.L. & Shama, L.N.S. 2018. Transgenerational plasticity and climate change experiments: Where do we go from here? *Global Change Biology* **24**: 13–34.

Dupont, L., Thierry, M., Zinger, L., Legrand, D. & Jacob, S. 2024. Beyond reaction norms: the temporal dynamics of phenotypic plasticity. *Trends Ecol Evol* **39**: 41–51.

Furrow, R.E. & Feldman, M.W. 2014. Genetic variation and the evolution of epigenetic regulation. *Evolution* **68**: 673–683.

Ghalambor, C.K., Hoke, K.L., Ruell, E.W., Fischer, E.K., Reznick, D.N. & Hughes, K.A. 2015. Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature* **525**: 372–375.

555 Ghalambor, C.K., McKAY, J.K., Carroll, S.P. & Reznick, D.N. 2007. Adaptive versus non-
556 adaptive phenotypic plasticity and the potential for contemporary adaptation in new
557 environments. *Functional Ecology* **21**: 394–407.

558 Gibson, B., Wilson, D.J., Feil, E. & Eyre-Walker, A. 2018. The distribution of bacterial
559 doubling times in the wild. *Proc Biol Sci* **285**: 20180789.

560 Gonzalez, A., Ronce, O., Ferriere, R. & Hochberg, M.E. 2013. Evolutionary rescue: an
561 emerging focus at the intersection between ecology and evolution. *Philosophical*
562 *Transactions of the Royal Society B: Biological Sciences* **368**: 20120404.

563 Greenspoon, P.B. & Spencer, H.G. 2018. The evolution of epigenetically mediated adaptive
564 transgenerational plasticity in a subdivided population. *Evolution* **72**: 2773–2780.

565 Grether, G.F. 2005. Environmental change, phenotypic plasticity, and genetic compensation.
566 *Am Nat* **166**: E115-123.

567 Guan, Q., Haroon, S., Bravo, D.G., Will, J.L. & Gasch, A.P. 2012. Cellular Memory of Acquired
568 Stress Resistance in Saccharomyces cerevisiae. *Genetics* **192**: 495–505.

569 Herman, J.J., Spencer, H.G., Donohue, K. & Sultan, S.E. 2014. How stable 'should' epigenetic
570 modifications be? insights from adaptive plasticity and bet hedging: special section.
571 *Evolution* **68**: 632–643.

572 Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Bäurle, I., Geiselhardt, S., *et al.* 2016.
573 Priming and memory of stress responses in organisms lacking a nervous system.
574 *Biological Reviews* **91**: 1118–1133.

575 Hoffmann, A.A. & Bridle, J. 2022. The dangers of irreversibility in an age of increased
576 uncertainty: revisiting plasticity in invertebrates. *Oikos* **2022**: e08715.

577 Husby, A. 2022. Wild epigenetics: insights from epigenetic studies on natural populations.
578 *Proceedings of the Royal Society B: Biological Sciences* **289**: 20211633.

579 Kawecki, T.J., Lenski, R.E., Ebert, D., Hollis, B., Olivieri, I. & Whitlock, M.C. 2012.
580 Experimental evolution. *Trends in Ecology & Evolution* **27**: 547–560.

581 Kim, S.W., Kwak, J.I. & An, Y.-J. 2013. Multigenerational study of gold nanoparticles in
582 Caenorhabditis elegans: transgenerational effect of maternal exposure. *Environ Sci*
583 *Technol* **47**: 5393–5399.

584 Kohanski, M.A., Dwyer, D.J. & Collins, J.J. 2010. How antibiotics kill bacteria: from targets to
585 networks. *Nat Rev Microbiol* **8**: 423–435.

586 Kronholm, I., Bassett, A., Baulcombe, D. & Collins, S. 2017. Epigenetic and Genetic
587 Contributions to Adaptation in Chlamydomonas. *Molecular Biology and Evolution* **34**:
588 2285–2306.

589 Marshall, D.J. 2024. Principles of experimental design for ecology and evolution. *Ecology*
590 *Letters* **27**: e14400.

591 Mohanty, B.K. & Kushner, S.R. 2016. Regulation of mRNA Decay in Bacteria. *Annual Review*
592 *of Microbiology* **70**: 25–44.

593 Moran, M.A., Satinsky, B., Gifford, S.M., Luo, H., Rivers, A., Chan, L.-K., *et al.* 2013. Sizing up
594 metatranscriptomics. *ISME J* **7**: 237–243.

595 Mousseau, T.A., Uller, T., Wapstra, E. & Badyaev, A.V. 2009. Evolution of maternal effects:
596 past and present. *Philosophical Transactions of the Royal Society B: Biological*
597 *Sciences* **364**: 1035–1038.

598 Nilsson, E.E., Ben Maamar, M. & Skinner, M.K. 2022. Role of epigenetic transgenerational
599 inheritance in generational toxicology. *Environmental Epigenetics* **8**: dvac001.

600 Orr, H.A. & Unckless, R.L. 2008. Population extinction and the genetics of adaptation. *Am*
601 *Nat* **172**: 160–169.

602 Orr, H.A. & Unckless, R.L. 2014. The Population Genetics of Evolutionary Rescue. *PLOS*
603 *Genetics* **10**: e1004551.

604 Orr, J.A., Vinebrooke, R.D., Jackson, M.C., Kroeker, K.J., Kordas, R.L., Mantyka-Pringle, C., *et*
605 *al.* 2020. Towards a unified study of multiple stressors: divisions and common goals
606 across research disciplines. *Proceedings of the Royal Society B: Biological Sciences*
607 **287**: 20200421.

608 Oziolor, E.M., Bickham, J.W. & Matson, C.W. 2017. Evolutionary toxicology in an omics
609 world. *Evolutionary Applications* **10**: 752–761.

610 Panacek, A., Prucek, R., Safarova, D., Dittrich, M., Richtrova, J., Benickova, K., *et al.* 2011.
611 Acute and chronic toxicity effects of silver nanoparticles (NPs) on Drosophila
612 melanogaster. *Environ Sci Technol* **45**: 4974–4979.

613 Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? *Trends in*
614 *Ecology & Evolution* **20**: 481–486.

615 Pilling, O.A., Rogers, A.J., Gulla-Devaney, B. & Katz, L.A. 2017. Insights into transgenerational
616 epigenetics from studies of ciliates. *European Journal of Protistology* **61**: 366–375.

617 Quadrana, L. & Colot, V. 2016. Plant Transgenerational Epigenetics. *Annual Review of*
618 *Genetics* **50**: 467–491.

619 Rasmann, S., De Vos, M., Casteel, C.L., Tian, D., Halitschke, R., Sun, J.Y., *et al.* 2012.
620 Herbivory in the Previous Generation Primes Plants for Enhanced Insect Resistance.
621 *Plant Physiology* **158**: 854–863.

622 Rescan, M., Grulois, D., Ortega-Aboud, E. & Chevin, L.-M. 2020. Phenotypic memory drives
623 population growth and extinction risk in a noisy environment. *Nat Ecol Evol* **4**: 193–
624 201.

625 Richter, K., Haslbeck, M. & Buchner, J. 2010. The Heat Shock Response: Life on the Verge of
626 Death. *Molecular Cell* **40**: 253–266.

Ridenhour, B.J. & Nuismer, S.L. 2007. Polygenic traits and parasite local adaptation. *Evolution* **61**: 368–376.

Rivoire, O. & Leibler, S. 2014. A model for the generation and transmission of variations in evolution. *Proceedings of the National Academy of Sciences* **111**: E1940–E1949.

Rodríguez-Rojas, A., Baeder, D.Y., Johnston, P., Regoes, R.R. & Rolff, J. 2021. Bacteria primed by antimicrobial peptides develop tolerance and persist. *PLOS Pathogens* **17**: e1009443.

Samani, P. & Bell, G. 2016. The ghosts of selection past reduces the probability of plastic rescue but increases the likelihood of evolutionary rescue to novel stressors in experimental populations of wild yeast. *Ecology Letters* **19**: 289–298.

Sane, M., Diwan, G.D., Bhat, B.A., Wahl, L.M. & Agashe, D. 2023. Shifts in mutation spectra enhance access to beneficial mutations. *Proceedings of the National Academy of Sciences* **120**: e2207355120.

Sengupta, T., Kaletsky, R. & Murphy, C.T. 2023. The Logic of Transgenerational Inheritance: Timescales of Adaptation. *Annual Review of Cell and Developmental Biology* **39**: 45–65.

Shahmohamadloo, R.S., Fryxell, J.M. & Rudman, S.M. 2024. Transgenerational epigenetic inheritance increases trait variation but is not adaptive. bioRxiv: https://doi.org/10.1101/2024.04.15.589575

Simpson, G.G. 1953. The Baldwin Effect. *Evolution* **7**: 110–117. Oxford University Press.

Smith, T.A., Martin, M.D., Nguyen, M. & Mendelson, T.C. 2016. Epigenetic divergence as a potential first step in darter speciation. *Molecular Ecology* **25**: 1883–1894.

Stajic, D., Bank, C. & Gordo, I. 2022. Adaptive Potential of Epigenetic Switching During Adaptation to Fluctuating Environments. *Genome Biology and Evolution* **14**: evac065.

Stajic, D., Perfeito, L. & Jansen, L.E.T. 2019. Epigenetic gene silencing alters the mechanisms and rate of evolutionary adaptation. *Nat Ecol Evol* **3**: 491–498.

Taborsky, B., English, S., Fawcett, T.W., Kuijper, B., Leimar, O., McNamara, J.M., *et al.* 2021. Towards an Evolutionary Theory of Stress Responses. *Trends in Ecology & Evolution* **36**: 39–48.

Tariel, J., Plénet, S. & Luquet, É. 2020. Transgenerational plasticity of inducible defences: Combined effects of grand-parental, parental and current environments. *Ecology and Evolution* **10**: 2367–2376.

Urban, M.C., Swaegers, J., Stoks, R., Snook, R.R., Otto, S.P., Noble, D.W.A., *et al.* 2024. When and how can we predict adaptive responses to climate change? *Evolution Letters* **8**: 172–187.

Verdonck, R., Legrand, D., Jacob, S. & Philippe, H. 2021. Phenotypic plasticity through disposable genetic adaptation in ciliates. *Trends in Microbiology* S0966842X21001396.

Wagner, G.P. & Zhang, J. 2011. The pleiotropic structure of the genotype–phenotype map: the evolvability of complex organisms. *Nat Rev Genet* **12**: 204–213.

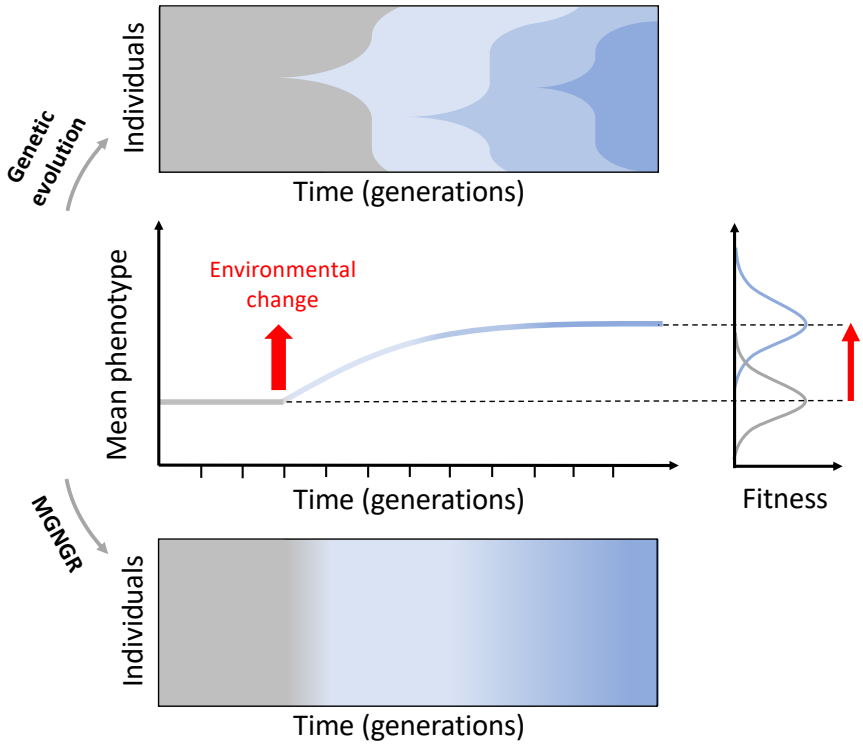
Wesener, F. & Tietjen, B. 2019. Primed to be strong, primed to be fast: modeling benefits of microbial stress responses. *FEMS Microbiology Ecology* **95**: fiz114.

Yi, S.V. & Goodisman, M.A.D. 2021. The impact of epigenetic information on genome evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences* **376**: 20200114.

Yin, J., Zhou, M., Lin, Z., Li, Q.Q. & Zhang, Y.-Y. 2019. Transgenerational effects benefit offspring across diverse environments: a meta-analysis in plants and animals. *Ecology Letters* **22**: 1976–1986.

Zhao, Y., Wytock, T.P., Reynolds, K.A. & Motter, A.E. 2024. Irreversibility in bacterial regulatory networks. *Science Advances* **10**: eado3232.

678 **Figures**



679
680 **Figure 1** Phenotypic response to environmental change can result from either genetic evolution (top panel) or
681 multi-generational non-genetic response (MGNGR, bottom panel). The line in the middle panel represents the
682 dynamics of the mean value of a phenotypic trait, following an environmental change (red arrow). The curves
683 on the right depict the fitness landscape before and after environmental change, with an optimum phenotype
684 shifted upwards (grey to blue fitness landscape). The Muller plot in the top panel represents the canonical case
685 of genetic evolution caused by changes in the genetic composition of the population, with an appearing colour
686 denoting a new mutation/genotype. The bottom panel illustrates MGNGR, in which the environment
687 experienced by one generation gradually impacts the phenotype of the following generations (from light grey
688 to dark blue), homogeneously in the population.
689

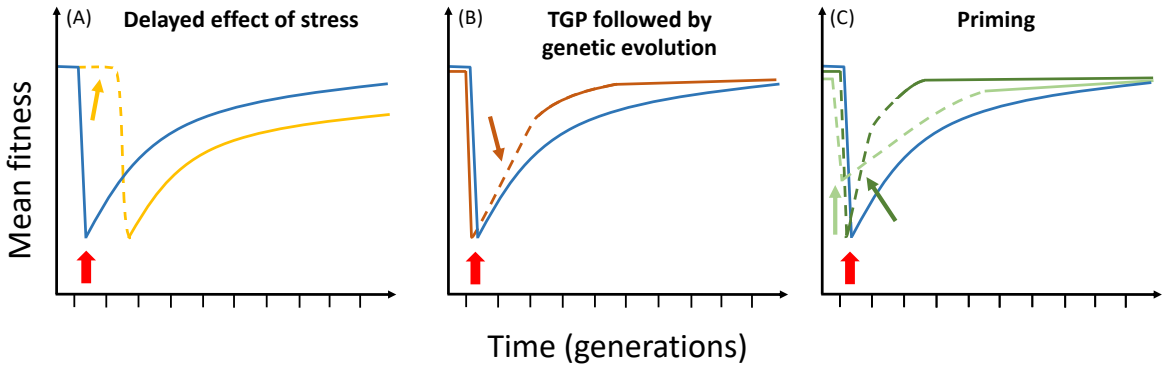


Figure 2 Examples of dynamics of mean population fitness over time after an environmental change (bottom red arrow), under different mechanisms of MGNGR. The blue line in all panels illustrates the baseline scenario, with an instantaneous effect of stress reducing mean fitness, followed by adaptation via genetic evolution. The coloured lines illustrate different forms of MGNGR mechanisms. 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).

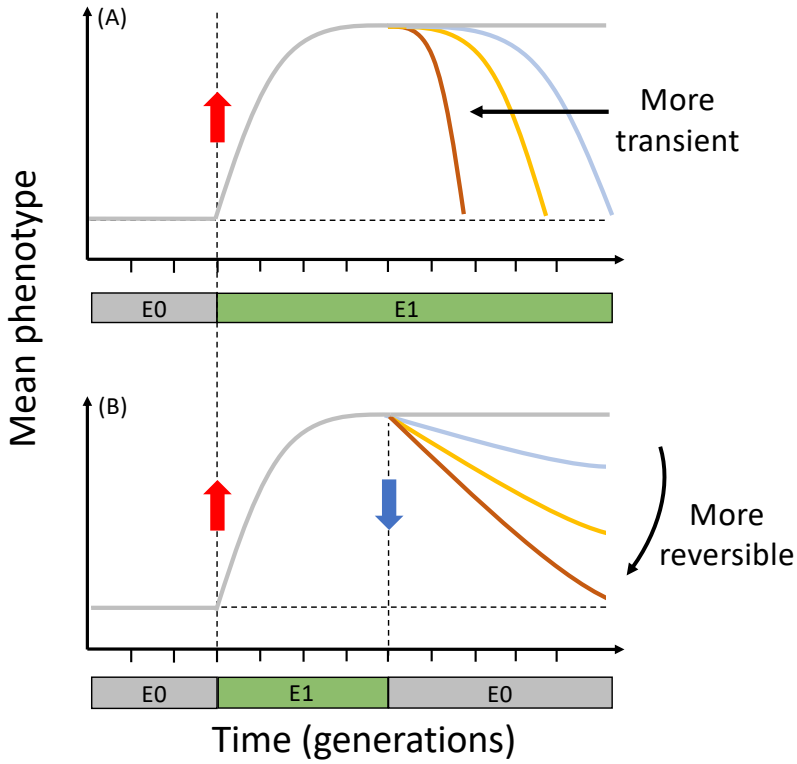


Figure 3. Stability and reversibility of phenotypic responses across generations. (A) Transient dynamics occur when the phenotypic trait goes back to its initial state, even though the environment has remained unchanged following the initial environmental shift (from E0 to E1), indicated by the red arrow.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