Improving our understanding of adaptation to environmental change by addressing multi-generational non-genetic inheritance

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Running title

Adaptation and multigenerational non-genetic inheritance

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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 multigenerational non-genetic inheritance (MUNGI) should be crucial to better understand and predict eco-evolutionary responses to environmental stress. We survey the most salient forms of MUNGI (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 in concrete experimental contexts (experimental evolution, common gardens, ecotoxicological experiments, dose-response assays). Analysing the rate, stability, and reversibility of MUNGI, 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.

Teaser Text

Although the existence of non-genetic inheritance is now well accepted, the consequences of their dynamics over generations for the study of evolution are still largely underappreciated. Neglecting or discarding such non-genetic responses can lead to wrong inference and prediction of population responses to environmental change. We propose making multigenerational non-genetic inheritance (MUNGI) an object of study in and by themselves, and flesh out our argument by delineating 3 main categories of responses: delayed effects of stress, transgenerational plasticity, and priming. We believe that our concern is likely to hold for a broad diversity of organisms and categories of experiments, from experimental evolution and field approaches to eco-toxicological studies.

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.*, 2023). The two main processes allowing organisms to cope with environmental challenges *in situ* (*i.e.*, without dispersing) are phenotypic plasticity, the expression by one genotype of different phenotypes in different environments (Pigliucci, 2005), and adaptive genetic evolution, the increase in frequency of beneficial mutations in a population through natural selection. Although not mutually exclusive, these processes are usually thought to occur over clearly distinguishable timescales, with plasticity taking place mostly within generations, while genetic evolution unfolds across generations. This assumed timescale separation has consequences for predicting responses to environmental stress at the phenotypic and demographic levels. An immediate plastic response, if adaptive, can limit the initial reduction of fitness - and potentially population size - in a novel, stressful environment. On the longer term, natural selection acting on new mutations, standing genetic variation, or a mixture of both (Barrett & Schluter, 2008), leads to gradual evolutionary changes in traits and fitness that accrue over generations, and might even prevent extinction of a declining population if it happens sufficiently fast (Gonzalez *et al.*, 2013), a phenomenon known as evolutionary rescue (Bell, 2017).

Nevertheless, this timescale separation between plasticity and genetic evolution, although conceptually useful, is an oversimplified 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, may blur 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 increasingly recognized as important mechanism of response to environmental change. Bell & Hellmann (2019) recently proposed a useful framework to study such responses, reporting evidence for six different patterns, including bounce-back (visible only in the first generation) or persistent effects (still visible several generations after stress exposure). 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 (for a more adaptive perspective see (Herman *et al.*, 2014)).

While TGP and other forms of NGI are now well-accepted phenomena, their prevalence, duration, and cumulative aspect remain underappreciated. There is accumulating evidence that a diversity of mechanisms can transmit effects of environmental stress over times spanning multiple generations in most organisms (Quadrana & Colot, 2016; Pilling *et al.*, 2017; Sengupta *et al.*, 2023). Some of the well-elucidated mechanisms, such as transmission of non-coding RNAs, patterns of DNA methylation, and histone modification, have durations spanning at least ~3, 8, and 10 generations, respectively (Jablonka & Raz, 2009; Bošković & Rando, 2018; Tikhodeyev, 2018; Adrian-Kalchhauser *et al.*, 2020; Fitz-James & Cavalli, 2022). These effects may be even more prevalent in unicellular organism, where the lack of a soma/germline divide (with the notable exception of ciliates, see below) means that many aspects of their phenotype, including proteins, gene regulatory factors and epigenetic modifications, are 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 in natural conditions as well (Gibson *et al.*, 2018)). Similarly, the half-life of mRNAs in bacteria is often of a similar order of magnitude as the generation time in ideal

117 conditions (Mohanty & Kushner, 2016). Gene overexpression in yeast occurs at least 1h after
118 a heat shock event, which overlaps with its doubling time of approximately 90 minutes
119 (Mühlhofer *et al.*, 2019). In the green microalga *Chlamydomonas reinhardtii*, synthesis of new
120 proteins and lipids in response to shifting temperature can take 24 hours (Tanaka *et al.*, 2000),
121 for a generation time of 14h-36h depending on light and temperature conditions (Vítová *et al.*,
122 2011).

123 It is thus clear that non-genetic responses to environmental stress can span multiple
124 generations, during which they can accumulate gradually, or decay/revert, depending on the
125 mechanism. To emphasize this long-term aspect, we describe such non-genetic processes with
126 dynamics that unfold over multiple generations as multigenerational non-genetic inheritance
127 (MUNGI). As MUNGI and rapid genetic evolution may have similar timeframes (Hairston *et*
128 *al.*, 2005), the phenotypic changes they produce at the population level may be difficult to
129 distinguish, despite having a completely different origin (Figure 1). While evolution by natural
130 selection involves repeated spreads of lineages with a different genotype, but fixed phenotype
131 within lineage (top panel in Fig. 1), MUNGI involves gradual phenotypic change within
132 lineages (bottom panel in Fig. 1). We argue below that ignoring the temporal dynamics of
133 MUNGI and its contribution to fitness, and not clearly distinguishing these effects from those
134 of genetic changes, is likely to limit our ability to infer and predict population responses to
135 changing environments, especially in long-term experimental evolution and common garden
136 experiments. We suggest that making MUNGI a study object of its own, by considering it
137 explicitly while designing experiments, deciphering how it interacts with adaptive genetic
138 evolution, and how it evolves itself, will improve our understanding of eco-evolutionary
139 dynamics in changing environments.

140

2) Major types of multigenerational non-genetic inheritance

We are interested in population responses to an environmental change that initially causes substantial maladaptation, manifested by a decrease in mean 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 because of environmental stress, then slowly increases again (blue line in Figure 2) as beneficial alleles rise in frequency. However, different forms of MUNGI can alter this pattern. 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 not be immediately observable, but instead could be delayed, and only manifest itself some generations after exposure to the stressor(s). The dynamics of this form of MUNGI will resemble baseline expectations for a fitness decline and rebound following environmental changes, but the manifestation of detrimental effects (and rebound) will only start sometime after the stressor exposure (Fig. 2A, yellow line). Stress-induced reductions in fitness may have a number of causes, all of which can have dynamics that span multiple generations. This can be for purely mechanical or physical reasons that do not involve any specifically evolved mechanism. For example, toxic or harmful molecules (*e.g.*, pesticides) can accumulate passively by slowly permeating into cells, but only start to have measurable detrimental impacts once their concentrations cross a tolerance threshold, beyond which they impair some cellular function. For instance, nanoparticle exposure of *Caenorhabditis elegans* impaired germ cells can lead to reproductive abnormalities and fecundity reduction only in the second 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).

Furthermore, delayed detrimental impacts of stress could occur because specific coping mechanisms have fitness costs that accumulate over time. For instance, exposure to silver nanoparticles induced reproductive costs in *Drosophila melanogaster* only from generation F2 onwards, with increasing effects in the following generations (Panacek *et al.*, 2011). This occurred because the accumulation of oxidative stress led to the upregulation of heat shock protein 70, which reduced investment in reproduction. In addition, specific response molecules, from heat shock proteins to molecular pumps, have intrinsic limits, beyond which they cannot operate optimally (Knapp & Huang, 2022), and the coping machinery needs resources for its maintenance and functioning (DeWitt *et al.*, 1998; Hoffmann & Bridle, 2022).

2.2 Speed and reversibility of transgenerational plasticity

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) - such as transgenerational acclimation responses to changing temperatures observed in fish (Munday, 2014) and microalgae (Kremer *et al.*, 2018) - and biotic (Tariel *et al.*, 2020; Shahmohamadloo *et al.*, 2025). 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 comprehensively quantify the temporal dynamics of TGP across generations.

An important temporal aspect to consider is the speed at which phenotypic traits change over generations after exposure to environmental change, that is, the rate of TGP. The rate of TGP change is critical to understand and predict when it is likely to be confounded with adaptation

189 by genetic evolution (Fig. 2B orange line). In a simple illustrative scenario of abrupt
190 environmental change, phenotypic traits start changing in the first generations following
191 exposure to the new stressor, until they may reach a plateau of stationary expression (Fig.3A-
192 B grey line). If this TGP is beneficial, then faster initial rates of change should lead to faster
193 initial increases in fitness, without any genetic evolution. Subsequently, the overall increase in
194 fitness caused by beneficial TGP will also depend on the TGP capacity, that is, the height of
195 the phenotypic plateau. If this plateau is stable over many generations, then TGP capacity
196 determines how much genetic evolution is needed at all for adaptation. Such stable TGP was
197 found in the pea aphid *Acyrthosiphon pisum*, which increases the production of winged morphs
198 in their progeny from ~25% to ~45% when exposed to the predator ladybird (*Harmonia*
199 *axyridis*), sustained over 25 generations (Sentis *et al.*, 2018). Specifically, after a first high
200 increase in frequency (~75%) at the second generation, winged morphs production then
201 stabilised to a ~45% frequency over 25 generations.

202 However, the phenotypic response can also be transient, with the phenotype eventually
203 reverting back towards its initial value after some generations of exposure. Such reversals may
204 occur for several reasons. First, responses might be costly to sustain because they require
205 important metabolic investments that trade off against other functions of the organism, and/or
206 lead to accumulating metabolic defects (such as free radicals) over generations (which may
207 contribute to the delayed impacts of stress described above). Second, some responses may
208 involve emergency mechanisms, such as heat shock response to thermal stress (Richter *et al.*,
209 2010), which last for a few generations before they are replaced by more specific and durable
210 physiological adjustments.

211 In scenarios where the environment changes again after a few generations, for instance going
212 back to its pre-stress value, the crucial question becomes whether - and how fast - the phenotype

213 can go back to its initial state, *i.e.* how reversible it is (Fig.3B). This was empirically
214 investigated in the yellow monkeyflower plant (*Mimulus guttatus*), where trichome production
215 increased in response to wounding that simulated insect damage. Such response was stable for
216 3 generations in the absence of subsequent damage, before starting to reverse, and eventually
217 decreasing to the level of control unwounded plants after generation 4 (Akkerman *et al.*, 2016).
218 Interestingly, both parents seem to contribute to the TGP responses additively, but via different
219 mechanism (maternal methylation *vs.* paternal histone modifications). In the aphid study above
220 (Sentis *et al.*, 2018), reversibility of TGP responses was studied by establishing parallel aphid
221 lines at generation 3, 13, and 22 of the main experiment, to follow the production of winged
222 morphs once the ladybird predator was removed. During one generation, winged morph
223 frequency remained high, but then dropped below, and finally converged to the same frequency
224 of the control lines. In contrast, a study on *Daphnia ambigua* showed the absence of
225 reversibility for TGP responses in life-history traits (Walsh *et al.*, 2015). Similarly,
226 temperature- (Klosin *et al.*, 2017) and olfactory-induced (Remy, 2010) TGP responses in *C.*
227 *elegans* were stable for tens of generations after the environmental stimulus was removed.
228 Differences in the speed of induction *vs.* reversibility of TGP for osmotolerance were found in
229 the unicellular alga *Dunaliella salina* (Rescan *et al.*, 2020). Transferring populations across
230 salinity levels showed that increasing glycerol content (when going from low to high salinity)
231 was much slower than decreasing it (from high to low salinity), because the mechanisms
232 involved are completely different: increasing glycerol level requires synthesizing it, whereas
233 decreasing it can be simply achieved by excretion. Environmental perturbations can also cause
234 non-genetic changes in gene regulatory networks that are poorly reversible, as shown in *E. coli*
235 (Zhao *et al.*, 2024). Beyond these examples, little is known about how reversibility unfolds
236 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 (Hoffmann & Bridle, 2022).

2.3 Trans-generational priming: memory of past responses

A third major class of MUNGI 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 *E. coli* cells primed with antimicrobial peptides (AMP) exhibited increased 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).

In an extensive review on both within- and trans-generational priming, Hilker et al. (2016) briefly proposed some temporal scenarios for primed stress responses. 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) further 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. In particular, we need to understand

whether trans-generational priming mostly has an immediate effect of reducing the direct impact of stress just upon exposure (light green line in Fig 2C), or also has a more prolonged effect by leading to faster rate (or capacity) of TGP in subsequent generations (dark green line in Fig 2C). 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 approaches to disentangle MUNGI from genetic responses

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

3.1 Stress-response experiments

Experiments on stress responses, or dose-response curves, often use stressor levels (*e.g.*, dose concentration or exposure time) causing sufficiently strong detrimental effects to be clearly detectable in the short run, without leading to rapid extinction of the population. However, this approach may lead to discarding or biasing relevant biological information and variation, especially if used to calibrate longer experiments (such as experimental evolution, discussed below). For instance, a treatment level leading to rapid population decline might be discarded as too stressful, while longer observations over multiple generations might show that fitness recovers through MUNGI (Fig. 2B-C). Conversely, a presumably permissive treatment during short-term experiments could turn out to represent stressful conditions over the longer run, because of delayed impacts of the stressor (Fig. 2A). In both cases, performing experiments for a longer time (*i.e.*, over several cycles of batch culture, or more generations), could allow

thoroughly describing a stressor effect on fitness. Such considerations are particularly relevant for (eco)toxicological studies and antibiotic resistance testing (Oziolor *et al.*, 2017; Zhao *et al.*, 2023). Typically, most of these experiments 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). Descendants from primed populations might have stronger or faster responses upon exposure to the same or another stressor. Additional risks of misleading inference could arise because MUNGI (*e.g.,* increased lipid content and fecundity after exposure to insecticide (Piiroinen *et al.*, 2014)), may not necessarily be stable on the long run. Overall, *(i)* implementing longer assays (spanning across generations), *(ii)* sampling at regular intervals to tackle short-term dynamics, and *(iii)* measuring additional traits, from growth to survival and fecundity, should help separate genetic and non-genetic causes, and directly improve risk assessment and policy making in such eco-toxicological studies.

3.2 Experimental evolution

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

More insights on the role of MUNGI in experimental evolution can be achieved by performing more complete assays, *i.e.*, beyond the conditions from the evolution experiment. In particular, sampling evolved lines (populations) at several points during experimental evolution is likely the simplest and most powerful complementary approach, allowing to obtain precise temporal profiles (phenotypic, genetic, non-genetic). Transferring evolved populations back to the

ancestral environment, and then moving them again from ancestral to the stressful experimental evolution treatment, would help identify whether the initial response in the experiment was mediated by genetic or non-genetic mechanisms, and measure the repeatability of phenotypic and fitness changes. Further, re-exposing them to their ancestral environments may allow identifying whether any putative responses to stress reverse too quickly to be explained by genetic evolution (Zilio *et al.*, 2023). Nonetheless, this approach would already require somewhat precise knowledge about the rate of TGP, and its degree of reversibility (see above).

Simple order-of-magnitude estimations can help assess whether the observed changes are consistent with the expected timescales of genetic evolution in the considered biological system. Expectations will of course differ depend on model organisms and specificities of the experiment, for instance whether genetic evolution has to involve *de novo* mutations (when starting from isogenic or inbred populations), or can occur via standing genetic variation. Very rapid phenotypic dynamics taking place over a few generations are more likely to involve MUNGI than genetic evolution, unless selection is extremely strong and acts on genetically diverse populations, or on mutations of very large effects (including transposable elements, structural variants as chromosomal rearrangements, or genetic switching (Yau *et al.*, 2016)). Making these arguments more quantitative requires reliable knowledge about mutation rates, levels of standing genetic variation, and distribution of fitness effects in the studied organism in response to the investigated stressor.

The relative contributions of MUNGI *vs.* allele frequency changes to phenotypic changes can also be investigated by using a combination of omics analyses (*e.g.*, transcriptomics and epigenomics *vs.* genomics) with phenotypic assays over time. In practice, this requires assessing the genetic and epigenetic composition of the population at several timesteps, to track detailed changes of genomes, epigenomes and transcriptomes, together with phenotypic traits

and/or fitness. But even when simultaneous genomic and phenotypic change is observed, showing that the former explains the latter can be challenging when only using population-based measurements. Progress can be achieved by isolating genotypes, for instance by creating clonal populations for microbes, before phenotyping and sequencing them. Deciphering the genotype-phenotype map is however difficult (Wagner & Zhang, 2011; Aguilar-Rodríguez *et al.*, 2018), as it requires more resolution (*e.g.* low linkage disequilibrium) than is typically available in experimental evolution designs. Using functional (epi)genetics to validate candidates or introducing (epi)mutations of interest in the ancestral background to isolate effect (reverse (epi)genetics) are useful approaches with model organisms.

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. Typically, this procedure allows controlling for the potential misleading outcomes due to parental effects (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 an overestimation of the genetic components of trait variation. In principle, the number of generations of common garden should account for the possibility of long MUNGI when it is suspected to exist, while limiting the opportunity for *de novo* mutations to arise (but as above, depending on the order of magnitude).

A productive way forward would be to systematically measure the dynamics of traits variation 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 MUNGI. 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 MUNGI in this context, as it could lead to phenotypic effects in responses to environments that differ from those where populations have been sampled. One option to overcome this problem is to transfer samples from different natural environments (or evolutionary treatments) to control condition / common garden, and then back from control to treatment (see 3.2 above). Additionally, changing the environment gradually *vs*. abruptly (or modifying stress intensity and duration) 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 to identify specific triggers or transgenerational responses (*e.g.*, priming). Similarly, sampling over different time periods and/or using sliding windows approaches might improve inferences of these priming effects, for populations sampled and assayed along a known gradient.

4) The evolution of transgenerational effects

Beyond potentially blurring the detection of adaptive genetic evolution, MUNGI can produce variation on which selection may act, and its underlying mechanisms may themselves vary genetically, and thus evolve (Bonduriansky & Day, 2018; Yin *et al.*, 2019). We propose some promising basic research questions on the evolutionary consequences of MUNGI, to hopefully motivate more theoretical and empirical studies on this topic.

A first critical aspect for understanding the evolution of MUNGI is establishing its genetic basis and heritability. This is a challenging task due to the diversity of processes that may underlie MUNGI, from the perception of the environmental "signal", to its transmission through the cellular environment or via the endocrine system (in multicellular organisms), and

381 the effector mechanism (*e.g.,* cis- or trans-acting genetic variants, spontaneous *vs*. random).
382 Nonetheless, there is growing evidence that epigenetic variation, for instance, is genotype
383 dependent. In *Arabidopsis thaliana*, the disruption of methylation-sensing gene regulatory
384 circuit in engineered mutant plants caused genome-wide methylation losses, which ultimately
385 led to abnormal phenotypes that worsened across generations (Williams & Gehring, 2017).
386 These results highlight the presence of a genetic basis for stable and long-term epigenetic
387 inheritance, and confirm previous findings in the same plant species suggesting the presence
388 of genetic control on epigenetic marks (Dubin *et al.*, 2015). Analysis from two publicly
389 available data sets in humans (247 sequenced) further determined that their methylation
390 patterns were likely under genetic control too (Liu *et al.*, 2014). Laboratory experiments also
391 demonstrated genetic variation for TGP. For instance, genotypic-specific TGP responses to
392 temperature were found for several phenotypic traits in *A. thaliana* (Alvarez *et al.*, 2021), as
393 well as genotype-specific TGP response of dispersal-related traits in the ciliate *Tetrahymena*
394 *thermophila* (Cayuela *et al.*, 2022).

395 The next necessary aspect (beyond genetic variation) is to elucidate how selection operates on
396 MUNGI. Selection on phenotypic plasticity is mediated by environmental variation within and
397 across generations, but we still know little about which pattern of environmental change
398 favours each type of response, and why. Fortunately, theory has started exploring this problem
399 (Bonduriansky & Day, 2009, 2018). Furrow & Feldman (2014) found that slow temporal
400 environmental fluctuations can lead to the evolution of more faithfully transmitted
401 transgenerational effects, providing that their underlying mechanisms entail little costs (see
402 also Rivoire & Leibler, 2014). Similarly, other mathematical models showed that
403 transgenerational effects can rapidly evolve, depending on the accuracy of the environmental
404 stressor as a predictor of future (strong) selective pressures (Leimar & McNamara, 2015; Uller
405 *et al.*, 2015). More recently, a population-genetic model of two habitats interconnected by

406 dispersal found that adaptive transgenerational effects were likely to evolve under moderate
407 dispersal, and when the direction of selection differed between habitats (Greenspoon &
408 Spencer, 2018). However, to our knowledge little attention has still been given to the evolution
409 of dynamic aspects of MUNGI, such as the rate of TGP, the stability and reversibility of
410 responses across generations, or the duration of priming.

411 Beyond the evolution of transgenerational effects mediated by MUNGI, a key question is how
412 they influence adaptive genetic evolution. Firstly, heritable but non-genetic phenotypic
413 changes can mask genotypic variation from selection, thereby modifying evolutionary
414 trajectories (Sengupta *et al.*, 2023). Secondly, some MUNGI mechanisms can interact with the
415 origination of genetic variation. In particular DNA methylation, by influencing mutation rate
416 and transposon insertion, can affect genome stability, and therefore directly contribute to DNA
417 sequence evolution (Ashe *et al.*, 2021; Yi & Goodisman, 2021). These combined influences of
418 epigenetics on selection and mutation could lead to potentially strong positive effects on
419 adaptive evolution, opposite to the abovementioned buffering hypothesis.

420 Such interactions between transgenerational effects and genetic evolution have been
421 investigated in a few experimental studies. An evolutionary experiment with an engineered
422 strain of *Saccharomyces cerevisiae* showed that MUNGI can modify rates of evolutionary
423 adaptation (Stajic *et al.*, 2019). This occurred because transgenerational silencing of a gene
424 responsible for cell growth increased the effective population size, thereby facilitating the
425 appearance of new mutational targets and alleles that could accelerate adaptation. Furthermore,
426 the transgenerational gene silencing was rendered more stable and strongly heritable by the
427 novel alleles introduced by mutation. In another study, populations of the unicellular green alga
428 *Chlamydomonas reinhardtii* were evolved under high salt, low phosphate, and high CO_2 for
429 200 asexual generations (Kronholm *et al.*, 2017). The populations genetically adapted in all

environments, and their fitness increased. The same treatment was applied to algal populations in which the authors 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, but had little impact on adaptation to low phosphate. The consequences of MUNGI may even scale 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).

Interestingly, differences between MUNGI and genetic adaptation might sometimes not be fundamentally clear-cut even conceptually, and cases exist where mechanisms of MUNGI and genetic adaptation overlap. Ciliate are an extraordinary example in which TGP might actually occur through genetic mechanisms. These unicellular eukaryotes have two nuclei (one acting as germline and the other as soma), with genetic mutations occur in both, 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 MUNGI influence its effects on genetic evolution. We may predict that MUNGI mechanisms that are both rapidly induced and stable through time are likely have more long-lasting influences on genetic evolution. This could be investigated by manipulating the dynamics of MUNGI through engineering where feasible (Kronholm *et al.*, 2017), combined with mathematical modelling (McNamara *et al.*, 2016; Fey *et al.*, 2021). 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). The simulations showed that when such epigenetic mechanisms are genetically encoded, they can favour the evolution of phenotypic plasticity, particularly under periodically changing environments. Extending similar models to allow for epigenetic inheritance would allow investigating how transgenerational effects, possibly accumulating over generations, evolve and interact with evolution of purely genetic effects. The development of such theory would guide future empirical work on these questions, by suggesting experimental design strategies, and informing on specific ecological conditions favouring each of these phenomena. Lastly, the development of single-cell sequencing, and analyses of epigenetic quantitative trait loci (epiQTLs), are promising ways forward in linking the genetic to epigenetic basis and phenotypes. For instance, in *A. thaliana* it was shown that epiQTLs contributed to the heritability of complex traits such as flowering time and root length (Cortijo *et al.*, 2014).

5) Concluding remarks

Although its relevance for adaptation is still being debated (Charlesworth *et al.*, 2017), non-genetic inheritance is 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 also can span multiple generations, and thus take place over similar timescales as rapid genetic evolutionary responses. Techniques allowing for precise in-depth investigations of the underlying mechanisms of MUNGI are now available to go a step further in their comprehension, provided we make them an object of study rather than a mere nuisance parameter. Here, we highlighted some major types of MUNGI, 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 (de Villemereuil *et al.*, 2016), evolutionary experiments, or natural populations. This would provide valuable information on the extent of these effects and their relative contributions to short- and long-term responses to environmental change. In the current context of global change, explicitly considering the contribution of MUNGI to population responses to environmental changes, and potentially of adaptation, should prove particularly important.

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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). *Environmental Epigenetics*, **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.

Barrett, R.D.H. & Schluter, D. (2008) Adaptation from standing genetic variation. *Trends in Ecology & Evolution*, **23**, 38–44.

Bell, A.M. & Hellmann, J.K. (2019) An Integrative Framework for Understanding the Mechanisms and Multigenerational Consequences of Transgenerational Plasticity. *Annual Review of Ecology, Evolution, and Systematics*, **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. *Evolutionary Applications*, **5**, 192–201.

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

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

Bošković, A. & Rando, O.J. (2018) Transgenerational Epigenetic Inheritance. *Annual Review of Genetics*, **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*, Ecology • Parasites/Parasitoids/Biological control, **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.

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.

Cortijo, S., Wardenaar, R., Colomé-Tatché, M., Gilly, A., Etcheverry, M., Labadie, K., *et al.* (2014) Mapping the Epigenetic Basis of Complex Traits. *Science*, **343**, 1145–1148.

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.

DeWitt, T.J., Sih, A. & Wilson, D.S. (1998) Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution*, **13**, 77–81.

568 Donelan, S.C., Hellmann, J.K., Bell, A.M., Luttbeg, B., Orrock, J.L., Sheriff, M.J., *et al.*
569 (2020) Transgenerational Plasticity in Human-Altered Environments. *Trends in Ecology &*
570 *Evolution*, **35**, 115–124.
571
572 Donelson, J.M., Salinas, S., Munday, P.L. & Shama, L.N.S. (2018) Transgenerational
573 plasticity and climate change experiments: Where do we go from here? *Global Change*
574 *Biology*, **24**, 13–34.
575
576 Dubin, M.J., Zhang, P., Meng, D., Remigereau, M.-S., Osborne, E.J., Paolo Casale, F., *et al.*
577 (2015) DNA methylation in Arabidopsis has a genetic basis and shows evidence of local
578 adaptation. *eLife*, **4**, e05255.
579
580 Dupont, L., Thierry, M., Zinger, L., Legrand, D. & Jacob, S. (2024) Beyond reaction norms:
581 the temporal dynamics of phenotypic plasticity. *Trends in Ecology & Evolution*, **39**, 41–51.
582
583 Fey, S.B., Kremer, C.T., Layden, T.J. & Vasseur, D.A. (2021) Resolving the consequences of
584 gradual phenotypic plasticity for populations in variable environments. *Ecological*
585 *Monographs*, **91**, e01478.
586 Fitz-James, M.H. & Cavalli, G. (2022) Molecular mechanisms of transgenerational
587 epigenetic inheritance. *Nature Reviews Genetics*, **23**, 325–341.
588
589 Furrow, R.E. & Feldman, M.W. (2014) Genetic variation and the evolution of epigenetic
590 regulation. *Evolution; International Journal of Organic Evolution*, **68**, 673–683.
591

592 Ghalambor, C.K., Hoke, K.L., Ruell, E.W., Fischer, E.K., Reznick, D.N. & Hughes, K.A.
593 (2015) Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in
594 nature. *Nature*, **525**, 372–375.
595
596 Ghalambor, C.K., McKAY, J.K., Carroll, S.P. & Reznick, D.N. (2007) Adaptive versus non-
597 adaptive phenotypic plasticity and the potential for contemporary adaptation in new
598 environments. *Functional Ecology*, **21**, 394–407.
599
600 Gibson, B., Wilson, D.J., Feil, E. & Eyre-Walker, A. (2018) The distribution of bacterial
601 doubling times in the wild. *Proceedings of the Royal Society B: Biological Sciences*, **285**,
602 20180789.
603
604 Gonzalez, A., Ronce, O., Ferriere, R. & Hochberg, M.E. (2013) Evolutionary rescue: an
605 emerging focus at the intersection between ecology and evolution. *Philosophical*
606 *Transactions of the Royal Society B: Biological Sciences*, **368**, 20120404.
607
608 Greenspoon, P.B. & Spencer, H.G. (2018) The evolution of epigenetically mediated adaptive
609 transgenerational plasticity in a subdivided population. *Evolution*, **72**, 2773–2780.
610
611 Greenspoon, P.B., Spencer, H.G. & M'Gonigle, L.K. (2022) Epigenetic induction may speed
612 up or slow down speciation with gene flow. *Evolution*, **76**, 1170–1182.
613
614 Grether, G.F. (2005) Environmental change, phenotypic plasticity, and genetic compensation.
615 *The American Naturalist*, **166**, E115-123.
616

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

Hairston, N.G., Ellner, S.P., Geber, M.A., Yoshida, T. & Fox, J.A. (2005) Rapid evolution and the convergence of ecological and evolutionary time. *Ecology Letters*, **8**, 1114–1127.

Herman, J.J., Spencer, H.G., Donohue, K. & Sultan, S.E. (2014) HOW STABLE 'SHOULD' EPIGENETIC MODIFICATIONS BE? INSIGHTS FROM ADAPTIVE PLASTICITY AND BET HEDGING: SPECIAL SECTION. *Evolution*, **68**, 632–643.

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

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

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

Jablonka, E. & Raz, G. (2009) Transgenerational Epigenetic Inheritance: Prevalence, Mechanisms, and Implications for the Study of Heredity and Evolution. *The Quarterly Review of Biology*, **84**, 131–176.

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

642

643 Kim, S.W., Kwak, J.I. & An, Y.-J. (2013) Multigenerational study of gold nanoparticles in
644 Caenorhabditis elegans: transgenerational effect of maternal exposure. *Environmental*
645 *Science & Technology*, **47**, 5393–5399.

646

647 Klosin, A., Casas, E., Hidalgo-Carcedo, C., Vavouri, T. & Lehner, B. (2017)
648 Transgenerational transmission of environmental information in C. elegans. *Science*.

649

650 Knapp, B.D. & Huang, K.C. (2022) The Effects of Temperature on Cellular Physiology.
651 *Annual Review of Biophysics*, **51**, 499–526.

652

653 Kohanski, M.A., Dwyer, D.J. & Collins, J.J. (2010) How antibiotics kill bacteria: from
654 targets to networks. *Nature Reviews Microbiology*, **8**, 423–435.

655

656 Kremer, C.T., Fey, S.B., Arellano, A.A. & Vasseur, D.A. (2018) Gradual plasticity alters
657 population dynamics in variable environments: thermal acclimation in the green alga
658 Chlamydomonas reinhartdii. *Proceedings of the Royal Society B: Biological Sciences*, **285**,
659 20171942.

660

661 Kronholm, I., Bassett, A., Baulcombe, D. & Collins, S. (2017) Epigenetic and Genetic
662 Contributions to Adaptation in Chlamydomonas. *Molecular Biology and Evolution*, **34**,
663 2285–2306.

664

665 Leimar, O. & McNamara, J.M. (2015) The evolution of transgenerational integration of
666 information in heterogeneous environments. *The American Naturalist*, **185**, E55-69.

667

668 Liu, Y., Li, X., Aryee, M.J., Ekström, T.J., Padyukov, L., Klareskog, L., *et al.* (2014) GeMes,
669 Clusters of DNA Methylation under Genetic Control, Can Inform Genetic and Epigenetic
670 Analysis of Disease. *The American Journal of Human Genetics*, **94**, 485–495.

671

672 Lovell, R.S.L., Collins, S., Martin, S.H., Pigot, A.L. & Phillimore, A.B. (2023) Space-for-
673 time substitutions in climate change ecology and evolution. *Biological Reviews*, **98**, 2243–
674 2270.

675

676 Marshall, D.J. (2024) Principles of experimental design for ecology and evolution. *Ecology*
677 *Letters*, **27**, e14400.

678

679 McNamara, J.M., Dall, S.R.X., Hammerstein, P. & Leimar, O. (2016) Detection vs. selection:
680 integration of genetic, epigenetic and environmental cues in fluctuating environments.
681 *Ecology Letters*, **19**, 1267–1276.

682

683 Mohanty, B.K. & Kushner, S.R. (2016) Regulation of mRNA Decay in Bacteria. *Annual*
684 *Review of Microbiology*, **70**, 25–44.

685

686 Moran, M.A., Satinsky, B., Gifford, S.M., Luo, H., Rivers, A., Chan, L.-K., *et al.* (2013)
687 Sizing up metatranscriptomics. *The ISME journal*, **7**, 237–243.

688

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

Mühlhofer, M., Berchtold, E., Stratil, C.G., Csaba, G., Kunold, E., Bach, N.C., *et al.* (2019) The Heat Shock Response in Yeast Maintains Protein Homeostasis by Chaperoning and Replenishing Proteins. *Cell Reports*, **29**, 4593-4607.e8.

Munday, P.L. (2014) Transgenerational acclimation of fishes to climate change and ocean acidification. *F1000Prime Reports*, **6**.

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

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

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

Panacek, A., Prucek, R., Safarova, D., Dittrich, M., Richtrova, J., Benickova, K., *et al.* (2011) Acute and chronic toxicity effects of silver nanoparticles (NPs) on Drosophila melanogaster. *Environmental Science & Technology*, **45**, 4974–4979.

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

Piiroinen, S., Boman, S., Lyytinen, A., Mappes, J. & Lindström, L. (2014) Sublethal effects of deltamethrin exposure of parental generations on physiological traits and overwintering in Leptinotarsa decemlineata. *Journal of Applied Entomology*, **138**, 149–158.

Pilling, O.A., Rogers, A.J., Gulla-Devaney, B. & Katz, L.A. (2017) Insights into transgenerational epigenetics from studies of ciliates. *European Journal of Protistology*, Integrating the three dimensions of ciliate diversity: function, taxonomy, and genetics, **61**, 366–375.

Planidin, N.P., Carvalho, C.F. de, Feder, J.L., Gompert, Z. & Nosil, P. (2022) Epigenetics and reproductive isolation: a commentary on Westram et al., 2022. *Journal of Evolutionary Biology*, **35**, 1188–1194.

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

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

Remy, J.-J. (2010) Stable inheritance of an acquired behavior in Caenorhabditis elegans. *Current Biology*, **20**, R877–R878.

740 Rescan, M., Grulois, D., Ortega-Aboud, E. & Chevin, L.-M. (2020) Phenotypic memory
741 drives population growth and extinction risk in a noisy environment. *Nature Ecology &*
742 *Evolution*, **4**, 193–201.
743
744 Richter, K., Haslbeck, M. & Buchner, J. (2010) The Heat Shock Response: Life on the Verge
745 of Death. *Molecular Cell*, **40**, 253–266.
746
747 Rivoire, O. & Leibler, S. (2014) A model for the generation and transmission of variations in
748 evolution. *Proceedings of the National Academy of Sciences*, **111**, E1940–E1949.
749
750 Rodríguez-Rojas, A., Baeder, D.Y., Johnston, P., Regoes, R.R. & Rolff, J. (2021) Bacteria
751 primed by antimicrobial peptides develop tolerance and persist. *PLOS Pathogens*, **17**,
752 e1009443.
753
754 Romero-Mujalli, D., Fuchs, L.I.R., Haase, M., Hildebrandt, J.-P., Weissing, F.J. & Revilla,
755 T.A. (2024) Emergence of phenotypic plasticity through epigenetic mechanisms. *Evolution*
756 *Letters*, **8**, 561–574.
757
758 Sengupta, T., Kaletsky, R. & Murphy, C.T. (2023) The Logic of Transgenerational
759 Inheritance: Timescales of Adaptation. *Annual Review of Cell and Developmental Biology*,
760 **39**, 45–65.
761
762 Sentis, A., Bertram, R., Dardenne, N., Ramon-Portugal, F., Espinasse, G., Louit, I., *et al.*
763 (2018) Evolution without standing genetic variation: change in transgenerational plastic
764 response under persistent predation pressure. *Heredity*, **121**, 266–281.

765

766 Shahmohamadloo, R.S., Fryxell, J.M. & Rudman, S.M. (2025) Transgenerational epigenetic
767 inheritance increases trait variation but is not adaptive. *Evolution*, qpaf050.
768
769 Smith, G. & Ritchie, M.G. (2013) How might epigenetics contribute to ecological speciation?
770 *Current Zoology*, **59**, 686–696.
771
772 Smith, T.A., Martin, M.D., Nguyen, M. & Mendelson, T.C. (2016) Epigenetic divergence as
773 a potential first step in darter speciation. *Molecular Ecology*, **25**, 1883–1894.
774 Stajic, D., Perfeito, L. & Jansen, L.E.T. (2019) Epigenetic gene silencing alters the
775 mechanisms and rate of evolutionary adaptation. *Nature Ecology & Evolution*, **3**, 491–498.
776
777 Straub, L., Strobl, V. & Neumann, P. (2020) The need for an evolutionary approach to
778 ecotoxicology. *Nature Ecology & Evolution*, **4**, 895–895.
779
780 Taborsky, B., English, S., Fawcett, T.W., Kuijper, B., Leimar, O., McNamara, J.M., *et al.*
781 (2021) Towards an Evolutionary Theory of Stress Responses. *Trends in Ecology &*
782 *Evolution*, **36**, 39–48.
783
784 Tanaka, Y., Nishiyama, Y. & Murata, N. (2000) Acclimation of the Photosynthetic
785 Machinery to High Temperature in Chlamydomonas reinhardtii Requires Synthesis de Novo
786 of Proteins Encoded by the Nuclear and Chloroplast Genomes1. *Plant Physiology*, **124**, 441–
787 450.
788

789 Tariel, J., Plénet, S. & Luquet, É. (2020) Transgenerational plasticity of inducible defences:
790 Combined effects of grand-parental, parental and current environments. *Ecology and*
791 *Evolution*, **10**, 2367–2376.
792
793 Tikhodeyev, O.N. (2018) The mechanisms of epigenetic inheritance: how diverse are they?
794 *Biological Reviews*, **93**, 1987–2005.
795
796 Uller, T., English, S. & Pen, I. (2015) When is incomplete epigenetic resetting in germ cells
797 favoured by natural selection? *Proceedings of the Royal Society B: Biological Sciences*, **282**,
798 20150682.
799
800 Urban, M.C., Swaegers, J., Stoks, R., Snook, R.R., Otto, S.P., Noble, D.W.A., *et al.* (2023)
801 When and how can we predict adaptive responses to climate change? *Evolution Letters*,
802 qrad038.
803
804 Verdonck, R., Legrand, D., Jacob, S. & Philippe, H. (2021) Phenotypic plasticity through
805 disposable genetic adaptation in ciliates. *Trends in Microbiology*, S0966842X21001396.
806 Villemereuil, P. de, Gaggiotti, O.E., Mouterde, M. & Till-Bottraud, I. (2016) Common
807 garden experiments in the genomic era: new perspectives and opportunities. *Heredity*, **116**,
808 249–254.
809
810 Vítová, M., Bišová, K., Hlavová, M., Kawano, S., Zachleder, V. & Čížková, M. (2011)
811 Chlamydomonas reinhardtii: duration of its cell cycle and phases at growth rates affected by
812 temperature. *Planta*, **234**, 599–608.
813

814 Wagner, G.P. & Zhang, J. (2011) The pleiotropic structure of the genotype–phenotype map:
815 the evolvability of complex organisms. *Nature Reviews Genetics*, **12**, 204–213.
816
817 Walsh, M.R., Cooley, F., Biles, K. & Munch, S.B. (2015) Predator-induced phenotypic
818 plasticity within- and across-generations: a challenge for theory? *Proceedings of the Royal*
819 *Society B: Biological Sciences*, **282**, 20142205.
820
821 Wesener, F. & Tietjen, B. (2019) Primed to be strong, primed to be fast: modeling benefits of
822 microbial stress responses. *FEMS Microbiology Ecology*, **95**, fiz114.
823
824 Williams, B.P. & Gehring, M. (2017) Stable transgenerational epigenetic inheritance requires
825 a DNA methylation-sensing circuit. *Nature Communications*, **8**, 2124.
826
827 Yau, S., Hemon, C., Derelle, E., Moreau, H., Piganeau, G. & Grimsley, N. (2016) A Viral
828 Immunity Chromosome in the Marine Picoeukaryote, Ostreococcus tauri. *PLOS Pathogens*,
829 **12**, e1005965.
830
831 Yi, S.V. & Goodisman, M.A.D. (2021) The impact of epigenetic information on genome
832 evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **376**,
833 20200114.
834
835 Yin, J., Zhou, M., Lin, Z., Li, Q.Q. & Zhang, Y.-Y. (2019) Transgenerational effects benefit
836 offspring across diverse environments: a meta-analysis in plants and animals. *Ecology*
837 *Letters*, **22**, 1976–1986.
838

839 Zhao, Y., Chen, J., Wang, R., Pu, X. & Wang, D. (2023) A review of transgenerational and
840 multigenerational toxicology in the in vivo model animal. *Journal of Applied Toxicology*, **43**,
841 122–145.
842
843 Zhao, Y., Wytock, T.P., Reynolds, K.A. & Motter, A.E. (2024) Irreversibility in bacterial
844 regulatory networks. *Science Advances*, **10**, eado3232.
845
846 Zilio, G., Krenek, S., Gougat-Barbera, C., Fronhofer, E.A. & Kaltz, O. (2023) Predicting
847 evolution in experimental range expansions of an aquatic model system. *Evolution Letters*,
848 qrad010.
849

Figures

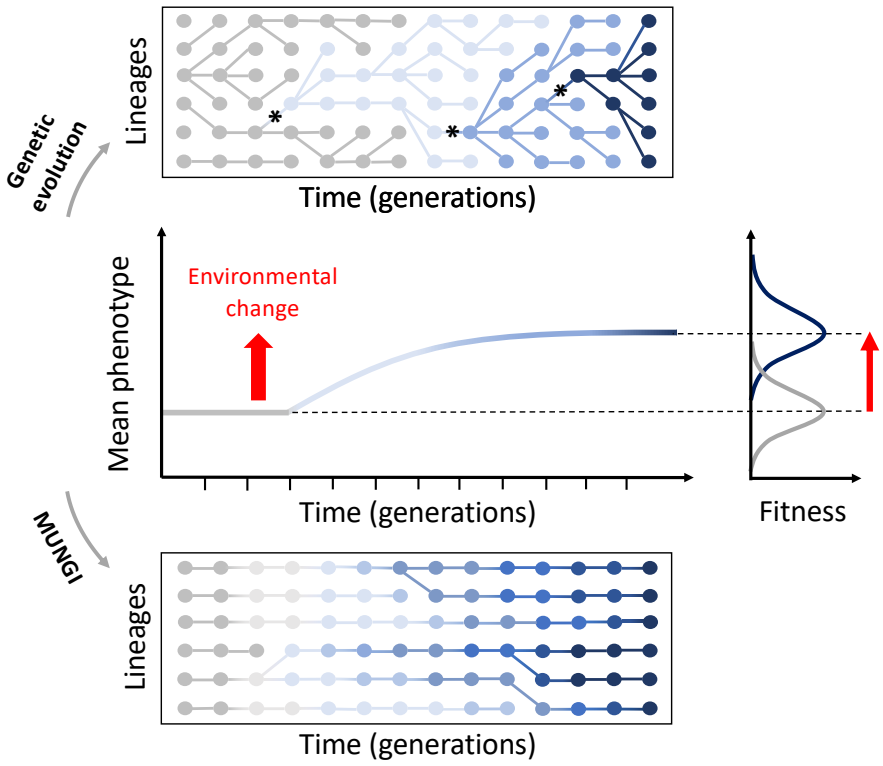
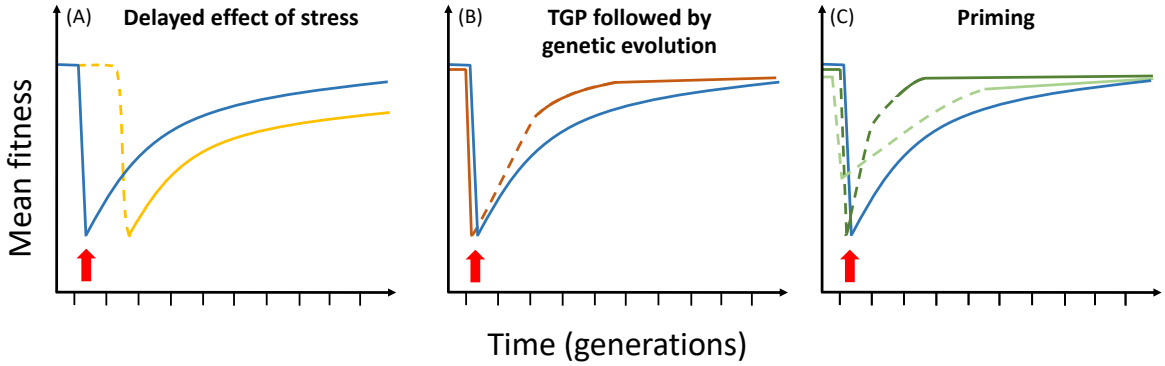


Figure 1 Alternative modes of response to environmental change. The line in the middle panel represents the dynamics of the mean phenotypic trait in a population (value along the y-axis and colour), following an abrupt environmental change (red arrow). The genealogy plots in the top and bottom panels illustrate two alternative explanations for this population-level response. The top genealogy represents adaptive evolution, illustrated in the case of de novo mutations. Here, environmental change is assumed to have caused an upward shift in the optimum phenotype favoured by natural selection (as depicted by the fitness landscape on the right in the middle panel), favouring "darker blue" phenotypes. When a mutation (black star) introduces a more adaptive phenotype (symbolized by its colour), the corresponding lineage progressively replaces the less fit ones, leading to the observed gradual change in mean phenotype. The bottom genealogy illustrates MUNGI. Here, the environment induces non-genetic phenotypic changes that accumulate gradually across generations within each lineage (from light grey to dark blue). The lineages may vary to some extent in their responses (as

865 illustrated by the small heterogeneity in colour gradients among lineages), but the mean
866 phenotypic change in the population is no longer driven by the replacement of lineages. These
867 alternative explanations of phenotypic change are difficult to distinguish based only on
868 population data.
869



870

871 **Figure 2 Three broad categories of MUNGI**. Dynamics of mean population fitness over time
872 following an abrupt environmental change (bottom red arrow), under different mechanisms of
873 MUNGI. The blue line in all panels illustrates the baseline scenario, with an instantaneous
874 effect of stress reducing mean fitness, followed by adaptation via genetic evolution. The
875 coloured lines illustrate different forms of MUNGI. Their effects are shown with dashed lines,
876 and are followed by genetic evolution in full lines. (A) Delayed effect of stress (yellow). (B)
877 Dynamic TGP (orange). (C) Priming effect of previous stress exposure on initial fitness drop
878 (light green), or on rate of fitness recovery by dynamic TGP (dark green).

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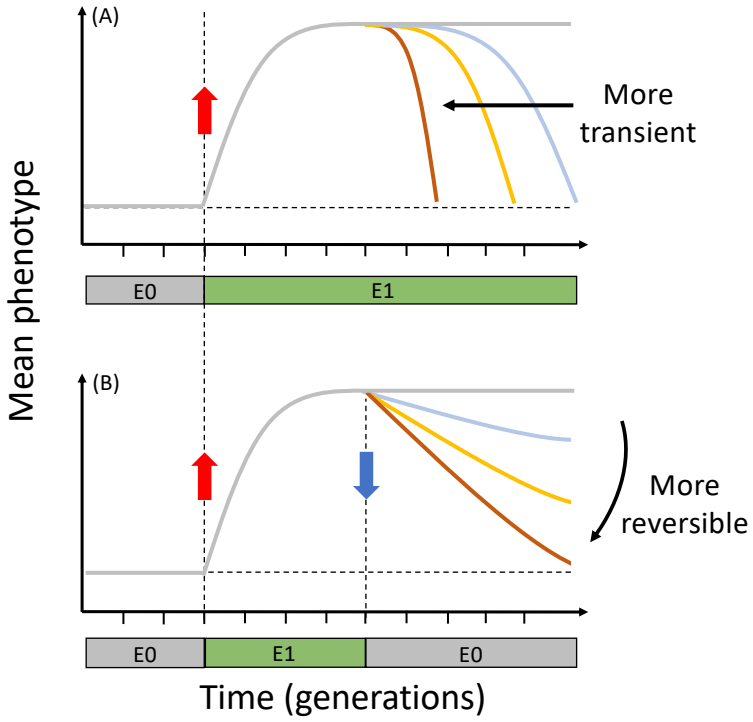

880

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