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### ► To cite this version:

Giacomo Zilio, Stéphanie Bedhomme, Emanuel Fronhofer, Staffan Jacob, Delphine Legrand, et al.. Addressing multi-generational non-genetic inheritance in experimental studies of evolution. 2025. <hal-05392935>

**HAL Id: hal-05392935**

**<https://hal.science/hal-05392935v1>**

Preprint submitted on 2 Dec 2025

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

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## Running head

MUNGI processes and adaptation

34 **Data availability**

35 Does not apply, Perspective article.

36

37 **Author contributions**

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

40

41 **Funding**

42 This work was funded by the Occitanie Regional Council's program "Key challenge  
43 BiodivOc", grant ComplexAdapt. The studies of D.L. and S.J. in the context of the Agence  
44 Nationale de la Recherche (ANR) projects POLLUCLIM (ANR-19-CE02-0021-01) and  
45 CHOOSE (ANR-19-CE02-0016) respectively contributed to this work. This is publication  
46 ISEM YYYY-XXX of the Institut des Sciences de l'Evolution - Montpellier. D.L., S.J. and  
47 H.P. are part of TULIP (Laboratory of Excellence Grant ANR-10 LABX-41) including a  
48 senior package attributed to H.P. (ANR-11-IDEX-0002-02).

49

50 **Acknowledgments**

51 We thank members of the ExpEvolOcc network for discussion at the early stages of this  
52 project, and Nicholas Planidin for suggestions.

53

54 **Conflict of interest**

55 The authors declare no conflict of interest.

56

57 **Abstract**

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

75 **Keywords**

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

78

## 79 **1) Population responses to stressful environments**

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

94 The potential importance of NGI for evolutionary processes has been discussed since the late  
95 1980s (Jablonka & Lamb, 1989, 1995; Kirkpatrick & Lande, 1989; Mousseau & Fox, 1998;  
96 Wolf *et al.*, 1998; Herman *et al.*, 2014; Laland *et al.*, 2015; Burggren, 2016; Deichmann,  
97 2016; Charlesworth *et al.*, 2017; Futuyma, 2017; Loison, 2021), and the advance of  
98 molecular and sequencing techniques over the last 20 years has created additional momentum  
99 (Allis & Jenuwein, 2016; Verhoeven *et al.*, 2016; Richards *et al.*, 2017; Lind & Spagopoulou,  
100 2018; Ashe *et al.*, 2021). However, despite NGI being well established today, we here argue  
101 that its implications for experimental studies of evolution remain under-appreciated,  
102 especially when its dynamics span multiple generations.

103 There is accumulating evidence that the effects of environmental stress can be transmitted  
104 over more than a few generations in many organisms, through a diversity of mechanisms  
105 (Quadrana & Colot, 2016; Pilling *et al.*, 2017; Sengupta *et al.*, 2023). For example,  
106 transmission of non-coding RNAs, patterns of DNA methylation, and histone modification,  
107 can last up to 10 generations (Jablonka & Raz, 2009; Bošković & Rando, 2018; Tikhodeyev,  
108 2018; Adrian-Kalchhauser *et al.*, 2020; Fitz-James & Cavalli, 2022). These effects are likely  
109 prevalent in many unicellular organisms, which are widely used in laboratory studies of  
110 evolution, notably to measure distributions of fitness effects of mutations (Gordo *et al.*,  
111 2011), or perform long-term experimental evolution (Elena & Lenski, 2003). In microbes, the  
112 lack of a soma-germline divide means that many aspects of their phenotype, including  
113 proteins, gene-regulatory factors and epigenetic modifications, are directly transmitted to  
114 their descendants (Sengupta *et al.*, 2023). In *Escherichia coli*, the average protein's half-life  
115 (~20 hours) is much longer than its generation time of ~20 minutes (Moran *et al.*, 2013;  
116 Gibson *et al.*, 2018). The half-life of mRNAs in bacteria is often of similar order of  
117 magnitude as generation time (Mohanty & Kushner, 2016). Gene overexpression in yeast  
118 occurs at least 1h after a heat shock event, which overlaps with its doubling time of  
119 approximately 90 minutes (Mühlhofer *et al.*, 2019). In the green microalga *Chlamydomonas*  
120 *reinhardtii*, synthesis of new proteins and lipids in response to shifting temperature can take  
121 24 hours, thereby overlapping with its generation time of 14-36h (Tanaka *et al.*, 2000).

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

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

## 138 **2) Major types of multigenerational non-genetic inheritance**

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

### 144 **2.1 Delayed impact of stress: time-to-response**

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

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

## 161 **2.2 Speed and reversibility of transgenerational plasticity**

162 Transgenerational plasticity (hereafter TGP), wherein the expression of phenotypic traits  
163 depends on the abiotic (Donelson *et al.*, 2012; Kremer *et al.*, 2018; Donelan *et al.*, 2020;  
164 Castano-Sanz *et al.*, 2022) or biotic (Tariel *et al.*, 2020; Shahmohamadloo *et al.*, 2025)  
165 environments experienced by previous generations, is receiving increasing attention from  
166 evolutionary biologists (Bell & Hellmann, 2019), but its dynamics across generations remain  
167 understudied (as argued for within-generation plasticity by Burton *et al.*, 2022; Dupont *et al.*,  
168 2024)).

169 An important aspect to consider is the speed at which phenotypic traits change, that is, the  
170 rate of TGP. This rate determines how likely it is for TGP to be confounded with adaptation  
171 by genetic evolution (Fig. 2B orange line). Following exposure to environmental stress,  
172 phenotypic traits may typically change rapidly in the first few generations, until expression of  
173 the trait becomes stationary (Fig. 3A grey line). If TGP is beneficial, faster rates of change  
174 should lead to faster increases in fitness, without requiring any genetic evolution.  
175 Subsequently, the overall increase in fitness caused by beneficial TGP will also depend on

176 TGP capacity, that is, the height of the phenotypic plateau. If this plateau is stable over many  
177 generations, then TGP capacity determines how much genetic evolution is additionally  
178 needed for complete fitness recovery. Such stable TGP was found in the pea aphid  
179 *Acyrtosiphon pisum*, where exposure of adults to the predator ladybird (*Harmonia axyridis*)  
180 increased the production of winged morphs in their progeny from ~25% to ~45%, sustained  
181 over 25 generations of exposure (Sentis *et al.*, 2018).

182 However, not all traits reach a stable plateau, and the phenotypic response can instead be  
183 transient, even under constant exposure to the inducing environment (Fig. 3A). The reason  
184 may be that phenotypic responses require important metabolic investments that trade off  
185 against other functions, and/or may lead to accumulation of metabolic defects over  
186 generations. Another possible cause of transient responses is that generic emergency  
187 mechanisms, such as heat shock response triggered by a variety of stresses (Richter *et al.*,  
188 2010), only last for a few hours or generations, before they are replaced by more specific and  
189 durable physiological adjustments.

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

### 199 **2.3 Trans-generational priming: memory of past responses**

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

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

### 221 **3) Impacts of MUNGI in experimental studies of evolution**

222 Because they lead to changes in fitness that unfold over multiple generations, the MUNGI  
223 phenomena described above can phenomenologically resemble genetically-based evolution

224 (Figure 1). To avoid reaching misleading conclusions about eco-evolutionary processes, we  
225 suggest below some approaches and ideas to measure MUNGI while conducting stress  
226 response experiments over few generations, experimental evolution, and common garden  
227 experiments.

### 228 **3.1 Stress-response experiments**

229 Experiments on stress responses, or dose-response curves, aim at identifying stressor levels  
230 (dose concentrations, or exposure time) that cause sufficiently strong detrimental effects to be  
231 clearly detectable in the short term, without leading to rapid population extinction. Evidence  
232 suggests that MUNGI is potentially an important contributor to major classes of such  
233 experiments, from (eco)toxicological stress-response studies to antibiotic resistance assays  
234 (Gouin *et al.*, 2023), and responses to climate change (McGuigan *et al.*, 2021, see Table 1  
235 therein). For example, several heat-stress experiments in microbes (Andrade-Linares *et al.*,  
236 2016), plants (Zhong *et al.*, 2013; Wang *et al.*, 2016; Liu *et al.*, 2019; Louis *et al.*, 2023) and  
237 animals (Adrian-Kalchhauser *et al.*, 2020; Walzer *et al.*, 2020; Reshma *et al.*, 2023) have  
238 shown transgenerational heat-stress effects, with changes in transcriptomics, physiology and  
239 life-history traits lasting for many generations. Interestingly, a recent study on different  
240 phytoplankton species across generations suggested a general mechanism of  
241 transgenerational response acting via temperature-dependent changes in uptake and  
242 assimilation of resources (Anderson *et al.*, 2025).

243 Importantly, stress response experiments often serve as first steps towards identifying  
244 selective treatments for experimental evolution (discussed below), yet misleading conclusions  
245 could be drawn if MUNGI are ignored. For instance, a treatment level that initially leads to  
246 rapid population decline will generally be discarded as too stressful for experimental  
247 evolution, even though fitness might recover through MUNGI over the longer run (Fig. 2B-

248 C), making the initial response misleading about the severity of stress and strength of  
249 selective pressure. Conversely, a permissive treatment during short-term assays could turn  
250 out to represent stressful conditions over the longer run, because of delayed detrimental  
251 impacts of the stressor (Fig. 2A). While examples thereof are difficult to pinpoint in the  
252 literature – as they mostly pertain to unpublished preliminary designs and assays – we have  
253 experienced such effects repeatedly in our own experimental practice.

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

### 259 **3.2 Experimental evolution**

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

265 The relative contributions of MUNGI *vs.* allele frequency changes to phenotypic changes can  
266 be investigated using a combination of omics analyses (*e.g.*, transcriptomics and epigenomics  
267 *vs.* genomics) and phenotypic assays. In practice, this requires tracking the genetic – and  
268 possibly also epigenetic and/or transcriptomic – composition of the population over time,  
269 together with phenotypic traits and/or fitness. Such integrative approaches provided useful  
270 insights into the contribution of NGI to adaptation to novel environments in microbial species  
271 (Walworth *et al.*, 2021; Gopalan-Nair *et al.*, 2024, see also the review by Stajic & Jansen,

272 2021). Similarly, DNA methylation was shown to lead to the rapid adaptation of the bacterial  
273 pathogen *Ralstonia pseudosolanacearum* to different host plant species, but was likely not  
274 the only driver, and the concurrent additive genetic and other undescribed non-genetic  
275 modifications also contributed to the total fitness gain (Gopalan-Nair *et al.*, 2024).  
276 Nevertheless, even when simultaneous genomic and phenotypic change is observed, showing  
277 that the former explains the latter can be challenging when only using population-based  
278 measurements.

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

294 Simple order-of-magnitude estimations can also help assess whether the observed changes  
295 are consistent with the expected timescales of genetic evolution, either from *de novo*

296 mutations or via standing genetic variation. Very rapid phenotypic dynamics taking place  
297 over few generations are more likely to involve MUNGI than genetic evolution, unless  
298 selection is extremely strong and acts on genetically diverse populations, or on mutations of  
299 very large effects (including transposable elements, structural variants as chromosomal  
300 rearrangements, or genetic switching (Yau *et al.*, 2016)). Making these arguments more  
301 quantitative requires knowledge about mutation rates, levels of standing genetic variation,  
302 and distribution of fitness effects in the focal organism in response to the investigated  
303 stressor. For example, Denman (2017) reanalysed experimental evolution data of the alga  
304 *Emiliana huxleyi* and found that the timescale of fitness gain was better explained by a  
305 combination of non-genetic and genetic changes.

### 306 **3.3 Common-garden experiments**

307 Common garden and transplant experiments, where individuals from different origins are  
308 placed in the same environmental condition(s), are routinely used to partition genetic from  
309 environmental/non-genetic components of trait variation (de Villemereuil *et al.*, 2016).  
310 However if not properly accounted for, NGI can bias their results by being wrongly  
311 interpreted as genetic variance ( $V_G$ ), or by influencing its additive ( $V_A$ ) and epistatic ( $V_I$ )  
312 components (Banta & Richards, 2018; Thomson *et al.*, 2018). It is common practice to use 2  
313 generations of common garden to remove potentially misleading interpretations due to  
314 parental effects (Mousseau & Dingle, 1991), but in principle, the number of generations of  
315 common garden should account for the possibility of long MUNGI when it is suspected to  
316 exist, while limiting the opportunity for *de novo* mutations to arise. A productive way  
317 forward would be to systematically measure the dynamics of phenotypic variation during the  
318 generations of common garden, together with assessing genomic and epigenomic variation  
319 where feasible (Leung *et al.*, 2016; Gao *et al.*, 2017; Groot *et al.*, 2018; Sammarco *et al.*,  
320 2024).

321 Importantly, the control conditions used in the common garden might represent a complex  
322 novel environment *per se* for the organisms, which could trigger MUNGI. For example, even  
323 simple changes in temperature or light conditions can induce a reprogramming of non-genetic  
324 mechanisms (Whittaker & Dean, 2017). These effects may even interact with the localities  
325 (or prior treatments) the individuals or lines came from, leading them to react differently to  
326 the common garden, *e.g.* by maintaining or losing their environmentally-acquired epigenetic  
327 marks (Groot *et al.*, 2018). For instance, alligator weeds (*Alternanthera philoxeroides*)  
328 sampled from different sites along southern China presented little genetic variation, but when  
329 reared in common garden, they showed significant phenotypic differences and genome-wide  
330 epigenomic changes via *de novo* methylation and demethylation (Gao *et al.*, 2010). Priming  
331 and TGP might be the most problematic in this context, as they could lead to phenotypic  
332 responses induced by common garden environment that differ from those where the  
333 populations were sampled (Agrelius & Dudycha, 2025). To investigate these effects in more  
334 details, one could transfer samples from different natural environments (or prior evolutionary  
335 treatments) to control condition / common garden, and then back from control to treatment  
336 (see 3.2 above, but also the design by Whipple & Holeski (2016)). Additionally, changing the  
337 environment gradually *vs.* abruptly, as typically done in acclimation studies (Donelson *et al.*,  
338 2012; Parker *et al.*, 2012), could highlight differences in transient dynamics and potential  
339 costs. For natural populations, historical data could be used to identify recent environmental  
340 changes (Lovell *et al.*, 2023), and find which rearing conditions are best to assay the sampled  
341 populations, and identify specific triggers of transgenerational responses (*e.g.*, priming).  
342 Similarly, sampling over different time periods and/or using sliding windows approaches (see  
343 Huxman *et al.*, 2022) might improve inferences of priming effects, for populations sampled  
344 and assayed along a known gradient.

#### 345 **4) The evolution of transgenerational effects**

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

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

365 Once genetic variation for MUNGI is established, we need to elucidate how selection  
366 operates on it. Selection on (trans-generational) phenotypic plasticity is mediated by  
367 environmental variation within and across generations, but we still know little about which  
368 patterns of environmental change favours each type of response (TGP, priming, ...), their  
369 dynamics, and why. Fortunately, theory has started exploring this problem (Bonduriansky &

370 Day, 2009, 2018). Furrow & Feldman (2014) found that slow temporal environmental  
371 fluctuations can lead to the evolution of more faithfully transmitted transgenerational effects,  
372 providing that underlying mechanisms entail little costs (see also Rivoire & Leibler, 2014).  
373 Similarly, other mathematical models showed that transgenerational effects can rapidly  
374 evolve, depending on the accuracy of the environmental stressor as a predictor of future  
375 (strong) selective pressures (Leimar & McNamara, 2015; Uller *et al.*, 2015). In line with  
376 these theoretical expectations, *C. elegans* was shown to adapt to temporally predictable  
377 fluctuating environments by evolution of a transgenerational effect, namely maternal  
378 glycogen provisioning (Dey *et al.*, 2016; Proulx *et al.*, 2019). More recently, a population-  
379 genetic model of two interconnected habitats found that adaptive transgenerational effects  
380 were likely to evolve under moderate dispersal, and when the direction of selection differed  
381 between habitats (Greenspoon & Spencer, 2018; Planidin *et al.*, 2025). However, to our  
382 knowledge little attention has still been given to the evolution of dynamic aspects of MUNGI,  
383 such as the rate of TGP, the stability and reversibility of responses across generations, or the  
384 duration of priming.

385 Another key question is how MUNGI influences “standard” adaptive genetic evolution, some  
386 aspects of which were discussed in two special issues (Lind & Spagopoulou, 2018; Ashe *et*  
387 *al.*, 2021). Firstly, heritable but non-genetic phenotypic changes can mask genotypic  
388 variation from selection, thereby modifying evolutionary trajectories (Sengupta *et al.*, 2023).  
389 Secondly, some MUNGI mechanisms can directly interact with the origination of genetic  
390 variation. In particular DNA methylation, by influencing mutation rate and transposon  
391 insertion, can affect genome stability, and therefore directly contribute to DNA sequence  
392 evolution (Ashe *et al.*, 2021; Yi & Goodisman, 2021). These combined influences of  
393 epigenetics on selection and mutation could lead to potentially strong positive effects on  
394 adaptive evolution. Theory indeed suggests that populations can adapt faster when natural

395 selection acts on both non-genetic and genetic variation (Day & Bonduriansky, 2011;  
396 Geoghegan & Spencer, 2013; Klironomos *et al.*, 2013). When explicitly modelling adaptation  
397 from *de novo* mutations, it was additionally shown that epigenetic mutations can both  
398 accelerate or hinder the rate of adaptation, depending on their stability and impact on fitness  
399 compared to genetic mutations (Kronholm & Collins, 2016).

400 Such interactions between transgenerational effects and genetic evolution have also been  
401 investigated empirically (Stajic & Jansen, 2021). An evolutionary experiment with an  
402 engineered strain of *S. cerevisiae* showed that MUNGI can modify rates of evolutionary  
403 adaptation (Stajic *et al.*, 2019). This occurred because transgenerational silencing of a gene  
404 responsible for cell growth increased the effective population size, thereby facilitating the  
405 appearance of new mutational targets and alleles that could accelerate adaptation. Luo *et al.*  
406 (2020) demonstrated the key role of interacting non-genetic and genetic mechanisms in  
407 evolution of *S. cerevisiae*. Selection on the expression of a fluorescent protein produced  
408 changes in histone markers (non-genetic) at key elements of galactose regulatory network  
409 lasting generations, which was complemented by a (genetic) mutation reducing the  
410 performance of RNA Pol II. In the green alga *Chlamydomonas reinhardtii*, engineered  
411 reductions of non-genetic variation reduced or impeded genetic adaptation to high salt and  
412 CO<sub>2</sub> treatments, but not to low phosphate (Kronholm *et al.*, 2017). The consequences of  
413 MUNGI may even cascade up to the macro-evolutionary scale. For instance, epigenetic  
414 variation is a good predictor of behavioural isolation and divergence in the fish genus  
415 *Etheostoma* (Smith *et al.*, 2016), and may thus influence speciation, consistent with  
416 conceptual and theoretical findings (Smith & Ritchie, 2013; Greenspoon *et al.*, 2022;  
417 Planidin *et al.*, 2022).

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

## 435 **5) Concluding remarks**

436 Although its relevance for adaptation is still being debated (Charlesworth *et al.*, 2017), NGI  
437 is an integral part of population responses to environmental change (Bonduriansky & Day,  
438 2018; Donelson *et al.*, 2018; McGuigan *et al.*, 2021; Sengupta *et al.*, 2023). When the  
439 dynamics of non-genetic responses unfold over multiple generations (which we describe as  
440 MUNGI), they are likely to alter our interpretation of experimental studies of evolution. Here,  
441 we highlighted some major types of MUNGI, and proposed a first set of empirical assays that  
442 could help identify such effects and understand their evolutionary consequences. In the

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

446

447 **References**

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## 816 **Figures**

817 **Figure 1 Alternative modes of response to environmental change.** The line in the middle  
818 panel represents the dynamics of the mean phenotypic trait in a population (value along the y-  
819 axis and colour), following an abrupt environmental change (red arrow). The genealogy plots  
820 in the top and bottom panels illustrate two alternative explanations for this population-level  
821 response. The top genealogy represents adaptive evolution, illustrated in the case of *de novo*  
822 mutations. Here, environmental change is assumed to have caused an upward shift in the  
823 optimum phenotype favoured by natural selection (as depicted by the fitness landscape on the  
824 right in the middle panel), favouring “darker blue” phenotypes. When a mutation (black star)  
825 generates a more adapted phenotype (symbolized by its colour), the corresponding lineage  
826 progressively replaces the less fit ones, leading to the observed gradual change in mean  
827 phenotype. The bottom genealogy illustrates MUNGI. Here, the environment induces non-  
828 genetic phenotypic changes that accumulate gradually across generations within each lineage  
829 (from light grey to dark blue). The lineages may vary to some extent in their responses (as  
830 illustrated by the small heterogeneity in colour gradients among lineages), but the mean  
831 phenotypic change in the population is no longer mainly driven by the replacement of  
832 lineages. These alternative explanations for phenotypic change are difficult to distinguish  
833 based only on population phenotypic data, even though the outcome is likely to be more  
834 variable for MUNGI owing to the diversity of mechanisms (illustrated in Fig. 2).

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

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

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