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1 **Beyond reaction norms: the temporal dynamics of phenotypic**
2 **plasticity**

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4

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11

12 **Abstract**

13 Phenotypic plasticity can allow organisms to cope with environmental changes. While reaction norms
14 are commonly used to quantify plasticity along gradients of environmental conditions, they often miss
15 on the temporal dynamics of phenotypic change, especially the speed at which it occurs. Here we argue
16 that studying the rate of phenotypic plasticity is a crucial step to quantify and understand its
17 adaptiveness. Iteratively measuring plastic traits allows to describe the actual dynamics of phenotypic
18 changes and avoid quantifying reaction norms at times that do not truly reflect the organism's capacity
19 for plasticity. Integrating the temporal component in how we describe, quantify and conceptualise
20 phenotypic plasticity can change our understanding of its diversity, evolution and consequences.

21

22 **Keywords:** phenotypic plasticity, plasticity rate, adaptiveness, reaction norm, reversible plasticity,
23 developmental plasticity.

24

25

26 **From reaction norms to the temporal dynamics of plasticity**

27 **Phenotypic plasticity** (see Glossary) can be defined as a genotype's ability to produce
28 different phenotypes across a range of environmental conditions [1]. Plastic organisms are expected to
29 transduce meaningful environmental cues into interpreted, internal signals that can ultimately lead to
30 directional changes in their phenotype. In the case of **adaptive plasticity**, their ability to produce
31 environmental-dependent phenotypic traits is thought to buffer some fitness costs resulting from
32 environmental changes [2] and allow them to thrive in spatially heterogeneous and temporally
33 fluctuating environments [3,4]. Within populations, these plastic changes in trait distributions have been
34 identified as potential pivots for eco-evolutionary dynamics (e.g., [2,5–7]). We typically distinguish
35 between **reversible plasticity** and **irreversible plasticity**; the former is thought to provide benefits
36 under rapid fluctuations relative to generation time [3], while the latter refers to irreversible
37 developmental switches within the timeframe of ontogeny [3,8]. In order to quantify phenotypic plasticity
38 in either of these cases, trait values are commonly measured along gradients of environmental
39 conditions to derive **reaction norms**. When reaction norms take the form of a linear relationship
40 between phenotype and environment, the steepness of the slope represents the **plastic capacity** and
41 the direction of phenotypic changes [9].

42

43 Despite being widely used to quantify phenotypic plasticity, reaction norms usually miss out on
44 a key aspect of plastic responses: the speed at which phenotypic changes occur [10]. When organisms
45 are faced with changes of their local environment, informative cues concerning the environmental
46 change have to be detected and transduced, before eventually leading to phenotypic changes.
47 Developmental plasticity in Crustaceans from the *Daphnia* genus, which includes the production of
48 cuticular expansions when growing in the presence of a predator [1,11], can be used to illustrate this
49 chain of events. *Daphnia* individuals first detect dissolved kairomones produced by fish predators
50 through chemoreceptors of olfactory neurons [1,11,12], with a possibly evolved detection threshold or
51 cue specificity [13]. Information is then transduced through neurohormonal communication and
52 transcriptional changes, ultimately leading to the production of helmets or spines [12,14]. Each of these
53 steps takes an incompressible amount of time, which forcibly translates into a lag between the onset of
54 the environmental change and that of the plastic response.

55

56 So far, most studies have concentrated on characterising the plastic capacity and its variation
57 within or between species, at a fixed time after the onset of the perturbation, thereby assuming that it
58 should be representative of the global plastic response [1,4,15]. However, the speed at which plasticity
59 occurs, referred to as the **rate of plasticity**, is rarely considered [10]. This omission could be due to a
60 usual reasoning behind reaction norms assuming that phenotypic changes reach asymptotic values
61 specific to each novel environment. In this setting, sampling the temporal shape of plastic changes may
62 seem unnecessary provided that experimenters wait sufficiently long to extract the new phenotypic
63 values expressed in the alternative conditions. It may also seem unnecessary if the question is only to
64 ask if a trait is plastic at all, especially given the technical cost of implementing protocols allowing to
65 measure the dynamics of plasticity through time.

66 Here, we argue that neglecting the temporal dynamics of plastic responses may lead to a
67 truncated comprehension of phenotypic plasticity, by preventing us from assessing if it is adaptive and
68 from accurately estimating plastic capacities. We especially discuss how the rate of plasticity directly
69 relates to the adaptiveness of plastic responses and why retracing their temporal dynamics is a crucial
70 step to obtain informative reaction norms. We bring forward a series of questions which could represent
71 key prospects for future investigation in the field of phenotypic plasticity.

72

73 **The rate of plasticity and the adaptiveness of plastic responses**

74 It is generally expected that both reversible and irreversible phenotypic plasticity are positively
75 selected under conditions of relatively fast and predictable fluctuations [3,16,17]. However, the
76 adaptiveness of plastic traits appears inconsistent across empirical studies, questioning the conditions
77 favouring the evolution of plasticity or its consequences [18–21]. These inconsistencies have been often
78 – but not only – attributed to differences in characteristics of environmental fluctuations between studies
79 [20]. The crucial aspect of the temporal dynamics of plasticity may be involved in these discrepancies
80 as well, but has for now been relatively overlooked [10,22–25]. This is especially the case in empirical
81 studies, despite exceptions (*e.g.* [26] and others listed in [10]) which often concern traits that intrinsically
82 include time (*e.g.*, growth rate, metabolic rate [27–29]) or have historically acknowledged a temporal
83 component (*e.g.*, changes in gene expression, both because of transcription initiation or RNA lifetime
84 [30]). Below we propose that some of the apparent contradictions surrounding the adaptiveness of

85 phenotypic plasticity might result from the reaction norm approach, in which the temporal dynamics of
86 plastic responses and their interaction with the characteristics of environmental fluctuations are ignored.
87

88 Investigating whether phenotypic plasticity is adaptive has been a recurrent focus, yet
89 compelling evidence is often hard to gather: either because costs are neglected [22], or because
90 benefits are only assessed in a single, constant environmental condition and conceal possible adaptive
91 trade-offs in other contexts [19,31]. For instance, reaction norms and **tolerance curves** in constant
92 environments have sometimes turned out to be poor predictors of performance in fluctuating
93 environments [28,32–34]. A key element that may improve predictive power to explain fitness under
94 fluctuating conditions could be to consider non-zero lag times in adaptive phenotypic plasticity
95 [27,28,35]. Although the plastic capacity is key when facing environmental changes, it is probably not
96 sufficient if plasticity is too slow to reach the optimal phenotype in time, and depends on associated
97 costs, as hinted at by models confronting reversible and irreversible plasticity (e.g., [3,22,23]). For a
98 given distance between the average fitness of a plastic population and its closest local adaptive peak
99 (see “*lag-load*” in [20]), the *kinetics of plasticity* will define *how much time* the organisms spend in a
100 novel environment with a suboptimal phenotype, while the *capacity* will constrain the *maximal distance*
101 *travelled* in fitness space. A necessary condition for phenotypic plasticity to be adaptive is for a sufficient
102 portion of the capacity to be mounted for some benefits to be obtained within the timeframe of
103 environmental change [10,23,24]. Therefore, the rate and the capacity are interlocked properties of
104 phenotypic plasticity. The adaptiveness of a plastic response can be expected to result from their
105 combined interaction with the environmental context (Box 1).

106

107 Given the possible role of the plasticity rate in the adaptiveness of a plastic response, it is likely
108 to evolve together with the plastic capacity. For instance, relatively fast fluctuations (*i.e.*, shorter than
109 the generation time) are classically expected to select for reversible phenotypic plasticity [3]. However,
110 this prediction assumes an almost immediate plastic response. If phenotypic plasticity incurs some
111 delay, its adaptiveness and resulting probability to be positively selected with a given capacity will
112 depend on the match between the plasticity rate and that of fluctuations. This match need not be perfect,
113 as even partial phenotypic plasticity (e.g., moderate amounts of HSP proteins due to too-slow a
114 plasticity rate) could still provide sufficient fitness benefits for plastic genotypes to outperform non-

115 plastic strategies. Importantly, whether plastic responses can be fast enough to be adaptive will depend
116 on constraints or costs specific to the mechanisms that underlie plasticity for the trait of interest. Most
117 of the phenotypic traits we look at are underlaid by a series of lower-scale phenomena, which are
118 themselves kinetically limited and potentially costly. In a case of thermal stress for instance, the speed
119 of heat-shock protein (HSP) production after thermal transduction will never exceed that of the
120 preceding transcription of hsp mRNAs (of the order of 30min for the Killifish (*Fundulus heteroclitus*) in
121 [36]). Knowing the maximum speed of phenotypic changes and how much it differs between traits and
122 species are therefore important but usually underrated questions.

123

124 Non-plastic strategies might be favoured at the expense of plastic ones in cases where the
125 mechanisms underlying plastic responses are too slow compared with the rapidity of environmental
126 fluctuations, making the balance between the costs and benefits of plasticity unfavourable. Whether
127 fast plastic responses incur higher costs compared to slower ones, and how these costs balance with
128 the effect of fluctuations on fitness are key, unsolved questions (see Outstanding Questions). We should
129 also keep in mind that even plasticity rates matching the speed of environmental change may still be of
130 limited adaptiveness. Under rapidly-fluctuating conditions, phenomena such as cue-response
131 mismatches may emerge (*i.e.*, increasing environmental noise, resulting in fast and costly plasticity in
132 the wrong direction [3,37,38]) and lead to maladaptive plasticity, despite its sufficient speed and
133 capacity. Overall, better estimating the contextual adaptiveness of phenotypic plasticity will require to
134 (i) effectively measure rates and plastic capacities across traits and organisms, (ii) compare them to
135 the rapidity of environmental fluctuations organisms are facing [20,39] while (iii) accounting for their
136 potential costs [22,37,38,40] and (iv) for the organism's performance in fluctuating conditions [32,34]
137 (Box 1).

138

139 **Accurately describing the temporal dynamics of phenotypic plasticity**

140 Measuring the speed at which phenotypic plasticity occurs requires datasets in which traits are
141 measured iteratively through time after the onset of an environmental change. Below we expose how
142 this objective should allow to quantify a set of kinetic parameters that all contribute to gradual phenotypic
143 plasticity. We explain how this should in turn help derive more compelling reaction norms by adjusting
144 the timing of samplings and revise some expectations about the shape of plastic responses throughout

145 their time course. As stated above, accurately describing the temporal dynamics of plasticity is a pivotal
146 step to understand its adaptiveness.

147 Going back to the underlying mechanisms of plastic responses, the temporal dynamics of
148 phenotypic plasticity and its resulting rate may be decomposed into three key parameters (Figure 1).
149 First, a minimum amount of environmental change, *i.e.*, an **activation threshold** as described in cases
150 of developmental plasticity [8], may play an important role in the kinetics of reversible plasticity.
151 Thresholds would result in slower plastic responses when facing gradual environmental changes,
152 increasing the measured lag time. Activation thresholds are likely to result from the balance between
153 the fitness costs of fluctuations an organism is exposed to, the costs of activating a plastic pathway
154 (*e.g.*, polymerising more chitin and precipitating CaCO₃ to form cuticular expansions in *Daphnia*), and
155 mechanistic constraints in the detectability of environmental changes (*e.g.*, the affinity constant of
156 kairomone receptors in *Daphnia*).

157

158 Plastic changes can then be characterised by a **lag time** before phenotypic changes even take
159 place (t_{lag} in Figure 1). This lag may result from lower-scale mechanisms that underlie changes in the
160 trait of interest, like transcriptional activity leading to behavioural changes [41]. We can expect strong
161 variance in the duration of this lag depending on the mechanisms underlying the plastic response for
162 the chosen trait, which may actually be an information of interest [34]. In particular, **low-level**
163 **phenotypic traits** such as gene transcription levels may only depend on a few transduction steps and
164 transcription factors, which activity can quickly be modified covalently. In these cases, the lag time (t_{lag})
165 is likely to be relatively low (*e.g.*, order of the hour for transcription). But these low-level plastic cogs
166 may themselves contribute to plasticity of **high-level phenotypic traits**, spanning higher biological
167 scales in both time and space (*i.e.*, within the organism). In this regard, the accumulation of low-level
168 time delays could lead to increased high-level lag times before the onset of plasticity. The interweaving
169 of mechanisms at different scales results in some high-level phenotypic changes that are almost
170 instantaneous (*e.g.*, no delay for a fight-or-flight behavioural response, directly based on endocrine
171 adrenaline secretion; [42]), while others are much slower (*e.g.*, spine production in response to
172 predators in *Daphnia*, which necessitates cuticular polymerisation [1,11,12]) or even involve
173 mechanisms that play across multiple generations (*i.e.*, transgenerational plasticity, from one
174 generation in [43–45] to tens of generations in [46]).

175

176 Finally, the time course of plastic changes can be described by a characteristic time, the
177 reciprocal of the **plasticity rate per se** ($\tau_{\text{forward/backward}}$ in Figure 1), which relates to an acclimation delay
178 [23,27,28,47,48]. Such acclimation time for temperature tolerance may range from less than a day (20h
179 for Amphibians) to almost a week (6 days for Crustaceans) among ectothermic animals [49]. Both the
180 lag and rate of plasticity are probably simultaneously at play in most cases of phenotypic plasticity and
181 contribute to an overall delay, leading to more or less gradual plastic responses. Deciphering the relative
182 importance of lag and rate in the dynamics of plastic responses, and whether their importance differs
183 between traits and organisms are important questions to answer.

184

185 Overall, the kinetics of plasticity probably results from a combination of different phenotypic
186 traits, playing distinct roles in its temporal dynamics. The activation threshold and the lag may stem
187 from limiting cellular transduction steps, whereas the rate of plasticity should be related to the
188 mechanisms of ongoing phenotypic change, including other low-level phenotypic traits that contribute
189 to the high-level phenotypic trait plasticity of interest. Being able to quantify these parameters will
190 require experimental designs with iterative and frequent-enough phenotypic sampling (Box 2). Beyond
191 the importance of adequately quantifying plastic changes, knowing whether the kinetics underlying the
192 capacity are fast or slow is a crucial step to better understand plasticity and its consequences.
193 Determining if the combination of plasticity's kinetics and capacity is adaptive will require links with the
194 organism's fitness in fluctuating environments (Box 1, [32]).

195

196 Experiments with increased temporal resolution will also allow to depart from the widespread
197 but simplified scheme of linear or logistic phenotypic changes up to an asymptotic state (Figure 1B).
198 Although this may be valid for many plastic traits (e.g., "helmet length" in the *Daphnia* genus, [1,11,12]),
199 we can rarely assert that the phenotypic value measured at a chosen time to describe the capacity for
200 plasticity was indeed that of the *plateau* (Figure 1). In cases of reversible plasticity, the duration of the
201 plateau (Δt) could be variable, and reverse dynamics may occur even when the new environmental
202 conditions remain unchanged (Figure 1A). This is for instance the case of HSP expression [30,36,50],
203 where the phenotypic response transitorily peaks (Figure 1A).

204

205 This refined knowledge of the temporal dynamics of plasticity should furthermore help answer
206 the question of “when” measuring the plastic capacity. As exposed above, without a precise
207 understanding of *if* and *when* traits reach their asymptotic value, we are exposed to the risk of deriving
208 unsteady reaction norms. This may not be an issue if rankings between conditions (*e.g.*, genotypes,
209 organisms, treatments) stay the same throughout the experiment (as in Box 3), but such temporal
210 correlations can rarely be asserted *a priori*. For instance, characterising the dynamics of phenotypic
211 plasticity in a ciliate revealed that the shape of reaction norms can change through time across
212 environmental conditions, even for a trait that follows an asymptotic trajectory (Box 3). If the aim of a
213 study is to determine the capacity of plasticity, then it requires combining reaction norms and iterative
214 phenotypic measurements through time to assess *when* traits stabilize following a change in the
215 environment (Figure 1 and Box 3). In addition to expanding our description of phenotypic plasticity
216 beyond reaction norms to their temporal dynamics, which we believe is key to understanding its
217 adaptiveness (Box 1), approaching plasticity in the proposed mindset can bring higher confidence in
218 the quantification of the plastic capacity.

219

220 **Concluding remarks**

221 Despite extended knowledge on the amount of phenotypic plasticity organisms can express throughout
222 a gradient of constant conditions, a fundamental aspect of plastic responses has been largely
223 understudied: the speed at which organisms can change their phenotype. In this Opinion piece, we
224 point at the necessity to go beyond reaction norms alone, as their explanatory and predictive power in
225 fluctuating conditions may be limited. Although putting the temporal dynamics of plasticity in the
226 spotlight may not always be an easy task from a methodological viewpoint (Box 2), it should enable to
227 refine expectations regarding the shape of plastic responses through time (Box 3), with expectedly
228 major implications for our understanding of the adaptiveness (Box 1), evolution and consequences of
229 phenotypic plasticity, including in eco-evolutionary dynamics (see Outstanding Questions). Previous
230 works had emphasised the need to test the adaptiveness of plasticity in fluctuating environments [32]
231 or showed that the properties of environmental change were a determining factor in plasticity’s role for
232 adaptive evolution [20], especially given its limits and costs [22]. Measuring the temporal dynamics of
233 plasticity may help to fill a long-standing, yet discrete, gap by shedding light on key parameters which
234 interact and evolve with the properties of environmental change.

235

236

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240 41).

241

242 **Figure 1: beyond reaction norms, the temporal dynamics of phenotypic plasticity can be**
243 **characterised through a series of kinetic parameters.** Following an acute environmental change
244 happening at $t=0$, the temporal dynamics of plastic traits may follow a transient response (upper panel;
245 e.g., HSP response, [36]) or a stationary plastic response (lower panel; e.g., developmental plasticity
246 in *Daphnia*, cell velocity in *Box 3*). A lag time (t_{lag}) may precede any change in phenotypic traits and
247 could result from both activation thresholds and lower-level plastic mechanisms (e.g., transduction
248 steps, early small-scale responses such as protein (de)phosphorylation, etc.). In both the transient and
249 the stationary scenarios, a forward rate ($\tau_{forward}$) describes the speed at which the phenotype shifts from
250 the initial state (P1) towards a new value (P2) throughout a transitory phase. For the stationary plastic
251 response, the phenotype is stable once the plateau (yellow area) is reached. For the transient plastic
252 response, a plateau can be derived but is transitory (duration = Δt): the trait eventually reverses back
253 to its initial state following a backward rate of plasticity ($\tau_{backward}$). In the stationary case, this reversibility
254 rate can be experimentally accessed by shifting the environment back to the initial conditions (see
255 Outstanding Questions). Note that in the transient case, the trait could also reverse to a value differing
256 from the initial state. In both scenarios, it is critical to consider acute sampling time when aiming at
257 estimating the plastic capacity in order to capture the plateau (*Box 3*). Interestingly, a transient dynamic
258 may wrongly be interpreted as a case of stationary response if the duration of the experiment is smaller
259 than Δt .

260 Box 1: Exploring the adaptiveness of plasticity by linking the rate of plasticity to changes in performance
261 under fluctuating conditions.

262

263 Phenotypic plasticity is expected to underlie some degree of ecological generalism by broadening the
264 range of environmental conditions under which the organism's performance is maintained (e.g., [51–
265 54]). Under environmental fluctuations, this adaptiveness can only emerge if the plastic capacity is
266 mounted fast enough relative to environmental change (e.g., [17,23,24,48,55]) and if the resulting
267 benefits compensate plasticity costs ([22], see Main text). Hereunder, we develop why focusing on the
268 temporal dynamics of plasticity is a crucial lever to understand tolerance curves and their underlying
269 adaptive plasticity in fluctuating environments [32]. We take a simple framework where plasticity of a
270 phenotypic trait contributes to the breadth of environmental tolerance (Figure I). Let τ be the rate of
271 adaptive plasticity associated to the implementation of the plastic capacity (e.g., τ_{forward} , Figure 1, main
272 text), represented in frequency space in Figure I (red dot, dashed line). In **(1)**, we consider a classical
273 tolerance curve, describing changes in performance throughout a gradient of constant conditions ($f_1 =$
274 0). This tolerance curve is described by a breadth (**(1)**, grey area below the curve), from which
275 generalism is usually defined. In **(2)**, the mean values of the environmental conditions are the same as
276 previously but the environment fluctuates at a slow frequency (f_2) around these means. The fluctuating
277 environment may lead to a slight decrease in performance at the optimum, but since $f_2 < \tau$, the speed
278 of environmental change is low enough for plasticity to be fully implemented. Therefore, the plastic
279 generalist is able to buffer environmental changes and maintain the breadth of its environmental
280 tolerance by matching its phenotype to the conditions *in time* ($f < \tau$). As fluctuations become faster than
281 the rate of adaptive plasticity (e.g., $f_3 > \tau$), traits change too slowly for the plastic capacity to be wholly
282 implemented. The expected benefits of plasticity are mitigated and so is the associated tolerance
283 breadth and performance **(3)**. We may expect the decrease in environmental generalism to vary in
284 amplitude, as plasticity for the trait of interest may underlie a large degree (**3a, green path**) or a more
285 limited degree of the observed generalism in constant conditions (**3b, orange path**). We can especially
286 expect **3b** to emerge if several traits, each with a different rate, contribute to generalism along the
287 environmental axis of interest. This reasoning (Figure I) could be used to design protocols comparing
288 threshold frequency values (i.e., breaking points in the breadth of tolerance) to values of plasticity rates

289 acquired within other experiments [32]. We would expect adaptive plasticity to result in a correlation
290 between the rate of plasticity and tipping points in achieved generalism across the fluctuation gradient.
291

292 **Figure 1: the interaction between the rate of adaptive plasticity and the rate of environmental**
293 **change can result in changes of the achieved degree of generalism.**
294

295 Box 2: Experiments to measure the temporal dynamics of plastic responses

296 Designing experiments aiming at decrypting the temporal dynamics of various plastic responses is key
297 to investigate the adaptiveness of plasticity (Box 1), its consequences and evolution (Outstanding
298 questions). Hereunder, we highlight crucial points to consider when designing protocols targeting the
299 kinetics of plasticity.

300 • **Technical and logistic limitations.** Iteratively sampling the phenotype to retrace the temporal
301 dynamics of plasticity trades off with other dimensions. The number of experimental replicates, the
302 number of environmental conditions or the number of studied traits will all compromise with the
303 experimental sampling frequency.

304 • **Constraints due to sampling speed.** Information theory [56] states that only plasticity rates that are
305 at least two times slower than the sampling frequency will be correctly estimated. This limitation may not
306 be a major issue for high-level phenotypes (e.g., morphological traits), for which temporal dynamics are
307 usually long enough to allow for multiple measurements through the time course of plasticity. However,
308 it might be more problematic for low-level traits (e.g., reflex behavioural responses, molecular
309 processes). For instance, Bukhari and colleagues [41] examined the plasticity of gene expression
310 underlying a fixed-action pattern behaviour in the three-spined stickleback (*Gasterosteus aculeatus*).
311 Despite pinpointing a strong temporal structure of transcriptional responses, their sampling rate was
312 too low to derive plasticity rates.

313 • **Choosing the sampling speed.** Although sufficient knowledge of the life history traits of the studied
314 species will be key to design such experiments, the specificities of the investigated traits and
315 environments should be considered to properly choose the sampling frequency. Although one would
316 not define fluctuation predictability at the same temporal scale for a mouse compared to an elephant,
317 both exhibit rapid metabolic plasticity in response to brutal heat or drought stress, through highly-
318 conserved mammalian pathway [57].

319 • **Constraints due to methodology.** Our ability to measure plasticity rates is likely to be trait-
320 dependent. Destructive or invasive measurements (e.g., RNAseq of a brain region or retro-orbital blood
321 collection, respectively) are technically more difficult to implement at high temporal resolutions than
322 non-invasive phenotypic samplings (e.g., video recordings of behaviour). In this sense, targeting the
323 kinetics of plasticity is likely to favour the development of less invasive techniques that can be repeated
324 in time more easily (e.g., using images to estimate melanin levels instead of clipping skin samples;

325 using portable thermal loggers instead of cloacal probe measurements, etc.). These trends may
326 themselves lead to a bias in *how* we measure phenotypic traits and, more importantly, *which* phenotypic
327 traits we choose to study.

328 Overall, having these points in mind should help designing efficient protocols to quantify the temporal
329 dynamics of plasticity. The output parameters may be further used in experiments aimed at
330 understanding the adaptiveness of plastic changes (Box 1).

331

332 Box 3: measuring the temporal dynamics of phenotypic plasticity can help us ascertain the right time to
333 derive reaction norms

334

335 Measuring the temporal dynamics of phenotypic plasticity is not only of interest to derive kinetic
336 *parameters*, it also helps to estimate the variance in reaction norms through time, and therefore to rightly
337 choose the time to measure the phenotypic response. As an illustration, the temporal dynamics of a
338 phenotypic trait (cell velocity) in an isogenic population of the ciliate *Tetrahymena thermophila* exposed
339 to a thermal gradient (T=19, 23, 27, 31 and 35°C) are presented hereunder (Figure II; main graph). For
340 each temperature, an exponential-decay model was used to fit the phenotypic changes as a function of
341 time. Although the trait of interest followed a classic logistic dynamic, the rate of plasticity revealed to
342 be temperature dependent, leading to reaction norms which shape change through time (**a-d**). In this
343 example, reaction norms are presented at 0, 15, 30 and 120 minutes. Initially (**a**, t=0), no plasticity was
344 observed, as expected from the common-garden conditions at 23°C. After 15 minutes (**b**), a linear-
345 shaped reaction norm was observed due to faster rates at T=19°C and T=35°C. Thirty minutes after
346 exposition (**c**), the reaction norm was log-shaped. It then stabilised from two hours onwards into a
347 sigmoid (**d**, t=120min; transparent datapoints are all mean velocity values between t = 2h and t = 4h).
348 Such insights reveal the importance of knowing the dynamics of plasticity to extract a reaction norm
349 that is representative of the stable state. In this example case, the ranking of plastic capacities between
350 conditions did not change, but such trends can rarely be asserted before retracing the temporal
351 dynamics. This may be even more essential when plasticity is transient and when the “correct”
352 measurement window has a lower (*i.e.*, plasticity has not happened yet) and an upper (*i.e.*, plasticity is
353 not expressed anymore) time margin (*e.g.*, transient plastic response in Figure 1), which may not be
354 known *a priori*. This reasoning applies both to intra- or transgenerational reversible plasticity and to
355 irreversible plasticity. Sampling the trait iteratively, at a sensible frequency given the trait identity and
356 timescale (Box 2), is a robust way to ascertain when to extract the capacity and to further question how
357 the temporal dynamics of plasticity interact with environmental fluctuations (Box 1). Notice that here,
358 we can assert that the response is stationary within a 4-hour period, but ruling out that cell-speed
359 plasticity is a transient response over a longer timescale is not possible.

360 **Figure II: the context-dependency of plasticity’s temporal dynamics can result in unstable**
361 **reaction norms.**

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Glossary

• **Activation threshold:** refers here to the minimum amount of environmental change needed for a plastic pathway to be activated. This threshold can itself contribute to a time lag in the plastic response.

• **Adaptive plasticity:** phenotypic plasticity is considered adaptive when the fitness benefits drawn from this strategy compensate its maintenance and production costs. Costs set aside however, all plastic changes do not forcibly bring the organism closer to a new phenotypic optimum; plasticity can be neutral or maladaptive.

• **Irreversible plasticity:** some plastic responses occur during ontogeny (e.g., winged offspring in Aphids, cuticular spines in *Daphnia*) and are irreversible within the lifetime of individuals. In this sense, **irreversible plasticity** refers to a type of **developmental plasticity** acting through evolutionary switches.

• **Low-level / high-level phenotypic traits:** sometimes referred to as primary and secondary phenotypic traits. High-level phenotypic traits are plastic traits (e.g., behavioural change) resulting from plasticity happening at lower spatial and temporal scales within the individual (e.g., transcription of new genes). Performance proxies are closest to the highest phenotypic level, contrary to molecular responses.

• **Phenotypic plasticity:** the ability of a genotype to produce a variety of phenotypes depending on environmental conditions.

• **Plastic capacity** (or capacity for plasticity): for a trait in a given organism, describes the absolute amount of phenotypic change that is observed between two environmental conditions.

• **Rate of plasticity:** following an abrupt environmental change, the plasticity rate is the speed at which a plastic trait changes towards its new value. The overall **temporal dynamics** of plasticity is the combination of the activation threshold, the lag time and the rate.

396 • **Reaction norm:** a continuous function describing changes in trait values along an environmental
397 gradient. Most of the time, a linear function is fitted to a series of phenotypic values sampled in distinct
398 environmental conditions.

399

400 • **Reversible plasticity** (also known as 'phenotypic flexibility'): refers to phenotypic changes that can
401 be reverted back and forth if the environment shifts back to the initial state within the lifetime of the
402 individual.

403

404 • **Tolerance curve:** a particular case of reaction norm for a trait considered as a proxy of performance
405 (typically: growth rate for microorganisms, maximum speed in a sprint test for lizards, etc.). If the
406 environmental gradient is made of temperature values, we may find the "Thermal Performance Curve"
407 denomination. Most tolerance curves are derived from trait values measured across a gradient of
408 constant conditions.

409

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Outstanding questions about the temporal dynamics of plasticity

1. The rate of plasticity combines the ability to detect environmental changes and the rapidity of mechanisms underlying phenotypic changes. Both could incur costs, constraining the optimal rate expressed in a given environmental condition. **Do the temporal dynamics of plasticity depend on the environmental conditions, either within an environmental gradient or between different environmental axes?**

2. The rate of plasticity will determine the duration of the phenotype-environment mismatch and the subsequent fitness costs. Fluctuations of high amplitude may hence require both strong and fast plastic responses. **Should we expect the rate of plasticity and the plastic capacity to be positively correlated or do interactions with costs bend this relationship?**

3. Determining if plasticity is reversible is often a key question, experimentally tested for by placing the organisms back into their original environment. **Is the speed at which these changes occur the same forward and backward** (relationship between τ_{forward} and τ_{backward} , Figure 1) **and how can this inform on the mechanisms underlying plasticity?**

4. **How variable is the temporal dynamics of plasticity intra- and inter-specifically for a given trait?** Measuring how variable rates are between organisms may be a good lead to explore the interactions between life history traits, phenotypic plasticity and environmental fluctuations.

5. Limiting factors surround the idyll of high plastic capacity occurring quickly *e.g.*, maintenance and production costs for both the rate and the capacity, environmental noise leading to cue-response mismatches. **How do these constraints interact and how may they shape the evolution of plasticity under environmental fluctuations of varying characteristics?**

6. Phenotypic plasticity is sometimes considered to play a key role in colonisation dynamics or adaptation to new environmental conditions. **Can the rate of plasticity provide explanations to the unfolding of key eco-evolutionary processes relative to environmental change?** The rate at which

organisms react to spatial heterogeneity may play an important role in range expansion, especially depending on the interaction between the rates of plasticity and dispersal.

7. The capacity of phenotypic plasticity is known to influence ecological and evolutionary dynamics.

How could the rate of plasticity itself or its interaction with the plastic capacity may affect eco-evolutionary processes such as metapopulation dynamics, local adaptation or even speciation?

For instance, the adaptiveness of phenotypic plasticity should depend on the match between the rate of plasticity and the rapidity of environmental fluctuations, leading the buffering effect of plasticity on selection (e.g., Bogert effect) to vary accordingly.

Highlights

Changes in environmental conditions can lead to variation in the traits expressed by a given genotype within the lifetime of an individual. This phenomenon, referred to as phenotypic plasticity, has been extensively studied in the past decades.

The majority of plasticity studies rely solely on reaction norms, describing the amplitude of trait changes across gradients of environmental conditions. Reaction norms however miss out on the temporal dynamics of plasticity and especially the speed at which it occurs.

We highlight why iteratively sampling phenotypic traits through time can help us understand the adaptiveness of plasticity relative to environmental change. We illustrate how this endeavour complements the reaction-norm approach and triggers a series of unanswered questions of high interest.

Temporal dynamics of plasticity





