A selection-based framework for evaluating transgenerational phenotypic plasticity under environmental change

Amanda K. Pettersen[1,2*] & Frank Seebacher[1]

1. School of Life and Environmental Sciences, The University of Sydney, New South Wales, 2006, Australia
2. Institute for Marine and Antarctic Studies, University of Tasmania, Tasmania, 7004, Australia

*Corresponding author: amanda.pettersen@sydney.edu.au. School of Life and Environmental Sciences, The University of Sydney. Science Road, Camperdown, New South Wales, 2006, Australia.

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Abstract

Rapid environmental change has intensified interest in inter- and transgenerational phenotypic plasticity (TGP) as a potential mechanism by which populations may buffer the fitness consequences of climate change. Parental environments frequently induce shifts in offspring phenotypes, and such responses are often interpreted as adaptive, implicitly assumed to enhance offspring fitness and thus population persistence. However, evidence for phenotypic change alone is insufficient to demonstrate adaptive value, particularly when fitness is inferred from performance proxies rather than survival or reproduction. Here, we argue that a more agnostic null hypothesis is required when evaluating the fitness consequences of TGP: that intergenerational phenotypic shifts may be neutral, maladaptive, or context-dependent, rather than inherently beneficial. We propose selection analysis as a general, quantitative framework for assessing whether phenotypic variation associated with parental environments align with fitness variation in offspring. This approach does not seek to identify the mechanistic basis of TGP, nor to disentangle plasticity from genetic or epigenetic causation, but instead provides a diagnostic test of whether observed phenotypic shifts are associated with positive, negative, or absent selection. Using worked examples across taxa and environmental contexts, we demonstrate that apparent TGP frequently fails to align with selection, despite pronounced changes in offspring phenotype. These findings highlight the risk of over-interpreting intergenerational phenotypic responses as evidence of resilience to environmental change. Integrating selection analysis into studies of TGP provides a tractable and comparable framework for evaluating when intergenerational responses are likely to enhance, constrain, or have no effect on population persistence under environmental change.

Introduction

Climate variability is one of the most ubiquitous forces shaping ecosystems. Natural environments change at different time scales, from variation at geological scales to the immediate impacts of weather events. These different rates and magnitudes of change impact the physiology that underlies reproduction, growth, and dispersal of individuals (Baduel et al., 2024; Lema, 2020; Schulte et al., 2011), and thereby affect population dynamics and communities (Amarasekare, 2024; Lema et al., 2024). For example, the composition of communities changes in response to current climate warming as more susceptible species are replaced by species more tolerant to warmer temperature (Khaliq et al., 2024). Similarly, drought and storm activity were associated with species turn-over in a subtropical forest (Wu et al., 2024). Environmental variation, and anthropogenic climate change in particular, is causing population declines in some species. For example, declines in arctic auks (*Alle alle*) are associated with climate change-induced increases in sea surface temperatures and decreases in sea ice (Jakubas et al., 2024). Population declines may be associated with loss of genetic diversity and thereby further reduce the potential for genetic adaptation to climate change (Aagaard et al., 2022; Zhou et al., 2024). Understanding how organisms respond across generations to such rapid environmental change is therefore central to predicting population persistence under global change.

Many species have evolved mechanisms to respond to environmental change and to reduce or alleviate its potentially negative impacts (McGaughran et al., 2021; Seebacher et al., 2015). Darwinian selection drives genetic adaptation to favour phenotypes that perform best under prevailing environmental conditions and thereby alters population allele frequencies. However, genetic adaptation is slow relative to the potential pace of environmental change. Even rapid evolution takes tens of generations before there are significant phenotypic effects that can render a population more resistant to environmental change (Lescak et al., 2015). The pace of adaptation is therefore too slow to mount effective responses to climate change, except for species with very short generation times.

In addition to genetic adaptation, most if not all organisms have evolved the capacity for phenotypic plasticity, that is the expression of different phenotypes from a single genotype (Aliaga et al., 2019; DeWitt & Scheiner, 2004). Plastic responses are much faster than genetic adaptation, and therefore can be more effective in buffering individuals and populations from rapid environmental variability. Inter- and trans-generational phenotypic plasticity describes cases where parental environments influence offspring phenotypes, regardless of the underlying regulatory mechanism (Brass et al., 2021; Chapelle & Silvestre, 2022; Klughammer et al., 2023; Lynch & Kemp, 2014; Stajic & Jansen, 2021). Such effects are often associated with epigenetic regulation, including DNA methylation and histone modification (Best et al., 2018; Fellous et al., 2022; Heckwolf et al., 2020, 2020; Loughland et al., 2021; Sävilammi et al., 2021; Skvortsova et al., 2018; Venney et al., 2022), but their mechanistic basis is frequently unresolved, particularly in non-model organisms.

Inter- and trans-generational transmissions of environmentally-induced information are often proposed to enable phenotypes to better match prevailing environmental conditions across single or multiple generations, and may influence population dynamics and responses to environmental change (Brass et al., 2021; Chapelle & Silvestre, 2022; Klughammer et al., 2023; Lynch & Kemp, 2014; Stajic & Jansen, 2021). As such, trans-generational plasticity has received increasing attention as a potential mechanism by which populations may persist under rapid climate change. However, the presence of intergenerational phenotypic responses alone does not provide evidence that such responses enhance fitness or population persistence. Despite their potential to improve resistance and resilience to environmental change, intergenerational phenotypic effects not necessarily beneficial. Parental environments may induce offspring phenotypes that are neutral or detrimental if they do not match the environment experienced by offspring (Beaman et al., 2016; DeWitt & Scheiner, 2004; Gibert et al., 2019; Schwanz et al., 2020). Moreover, changes in regulatory states can arise stochastically, independent of environmental cues. For example, spontaneous epimutations and age-related changes in DNA methylation can accumulate over time (Bertucci-Richter et al., 2024; Bogan & Yi, 2024; Han, 2024; Johannes & Schmitz, 2019), generating phenotypic variation that may have neutral or negative fitness consequences.

In a now seminal paper, Gould and Lewontin (1979) argued against the "adaptationist program" that accepted the "near omnipotence of natural selection in forging organic design and fashioning the best among possible worlds". In other words, they observed that studies often implicitly assumed that observed biological traits were the result of selection optimising fitness. However, as Gould and Lewontin (1979) point out, there are a myriad stochastic processes, constraints, and historical contingencies that can produce patterns that are not adaptive (Nielsen, 2009). A similar adaptationist bias can arise in studies of trans-generational plasticity. Environmentally-induced intergenerational phenotypic shifts are frequently interpreted as adaptive responses to environmental change without explicit tests of fitness consequences. Empirical evidence that such responses enhance fitness under climate change remains limited and context dependent (Byrne et al., 2020; Gilbert & Warner, 2026).

Here we propose selection analysis as a quantitative framework to evaluate whether phenotypic variation associated with inter- and trans-generational plasticity (hereon referred to collectively as 'TGP') aligns with variation in offspring fitness. These methods, grounded in quantitative genetics, quantify the direction and strength of selection acting on phenotypic traits, independent of their genetic or epigenetic basis. Rather than testing whether TGP evolved adaptively or identifying its mechanistic origins, selection analysis provides a diagnostic tool to assess whether intergenerational phenotypic shifts are beneficial, netural, or detrimental with respect to offspring fitness. The goal of incorporating this approach is to generate formal, comparable estimates of selection across systems, thereby improving inference about the fitness consequences of TGP under environmental change.

Evaluating the fitness consequences of intergenerational phenotypic plasticity

There is a growing focus on the potential benefits of "adaptive" TGP as a mechanism by which populations may persist in the face of rapid environmental change (Galloway & Etterson, 2007; Harmon & Pfennig, 2021; Via et al., 1995). Rather than relying on selection on standing genetic variation, which typically produces population-level responses only after many generations, parental environments may directly influence offspring phenotypes via regulatory mechanisms that operate across generations (Burggren, 2015; Skvortsova et al., 2018). Such responses are often interpreted as adaptive because they may increase offspring performance under environmental conditions similar to those experienced by parents, and buy time for evolution via genetic adaptation to take place (Chevin et al., 2010). While the utility of TGP is appealing particularly in the face of anthropogenic effects, this interpretation rests on the assumption that environmentally-induced phenotypic shifts are aligned with fitness benefits, an assumption that is rarely explicitly tested (Byrne et al., 2020; Gilbert & Warner, 2026; Sánchez-Tójar et al., 2020).

We suggest that a more agnostic approach is required to evaluate the fitness implications of TGP. Importantly, intergenerational phenotypic effects are not always beneficial or "adaptive" (DeWitt et al., 1998). For example, moderate heat stress experienced by parents can induce offspring phenotypes that confer increased tolerance to elevated temperatures, whereas more severe or prolonged stress can disrupt cellular function and induce DNA methylation patterns that ultimately reduce offspring performance and survival (Murray et al., 2022). In such cases, the direction and magnitude of parental environmental effects determine whether offspring phenotypes are beneficial, netural, or detrimental with respect to fitness.

Even when TGP is associated with changes in offspring performance, such measures are often insufficient to conclude that intergenerational responses are adaptive. Logistical constraints often prevent direct measurement of survival or reproductive output, particularly in long-lived or field-based systems. As a result, the literature is dominated by proxies of fitness, including offspring size, growth rate, metabolic capacity, and locomotor performance (Donelson et al., 2012; Le Roy et al., 2017; Pettersen et al., 2019). While such traits are biologically meaningful, they may be weakly correlated with fitness, or even trade off against fitness components such as survival or reproduction (**Figure 1A**). For example, trade-offs between reproduction and aging or lifespan (Cohen et al., 2020), growth (Larue et al., 2021), resistance to parasitism (Ives et al., 2020), and survival (Cox et al., 2010) are well documented. Consequently, shifts in offspring phenotype alone cannot be interpreted as evidence that TGP enhances fitness or populations persistence. Rigorous tests of the adaptive nature of TGP should include estimates of offspring fitness (lifetime reproductive output) or at least major components of fitness (survival, mating success [fertility], or reproductive output) (Burgess & Marshall, 2014; Matesanz et al., 2022).

Conversely, TGP can act as a conduit for transmitting environmental stress across generations (Marshall & Uller, 2007). Challenging or damaging parental environments may induce offspring phenotypes that reduce fitness rather than enhance it. For example, chronic paternal stress in mice alters sperm microRNA composition and is associated with altered hypothalamic-pituitary-adrenal axis function in offspring (Rodgers *et al.* 2015). However, as with putatively adaptive responses, evidence for maladaptive TGP requires explicit demonstration that such intergenerational effects reduce offspring fitness, rather than simply altering offspring phenotype or physiology.

Here, we argue that an a priori assumption of neutral intergenerational phenotypic effects provides a more conservative and unbiased starting point for studies of TGP. In this context, we use the term “epigenetic drift” to describe intergenerational phenotypic variation that arises independently of selection and does not necessarily confer fitness benefits, analogous to genetic drift. Such variation may result from stochastic changes in regulatory states, incomplete transmission of environmental information, or mismatches between parental and offspring environments (Bertucci-Richter et al., 2024; Bogan & Yi, 2024; Han, 2024; Johannes & Schmitz, 2019). Importantly, this concept is not intended to imply a specific molecular mechanism, nor to suggest that all intergenerational effects are epigenetic in origin (McGuigan et al., 2021). Rather, it provides a parsimonious null hypothesis against which adaptive or maladaptive fitness consequences of TGP can be tested.

From this perspective, the adaptive, neutral, or detrimental nature of TGP cannot be inferred from phenotypic change alone and instead must be evaluated experimentally by quantifying fitness consequences. Below, we outline how selection analysis can be integrated into studies of inter- and trans-generational plasticity to assess whether phenotypic variation associated with parental environments aligns with selection on offspring fitness. This approach does not seek to disentangle the mechanistic origins of TGP or to test whether such responses evolved via selection but rather provides a general diagnostic framework for evaluating fitness alignment under environmental change.

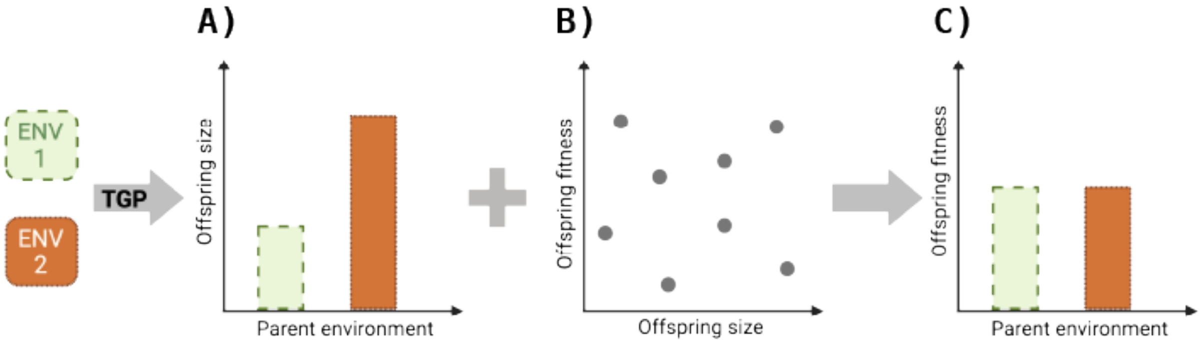

Figure 1. Measures of parental performance, such as offspring size or aerobic scope may not be correlated, or may even trade-off, with actual fitness (that has not been measured). In this hypothetical example, there appears to be adaptive inter-generational plasticity in offspring size: parents reared in Environment 2 (‘ENV 2’, orange shading) produce larger offspring than parents exposed to Environment 1 (‘ENV 1’, green shading); **A)**. However, if there is no relationship between offspring fitness and size (**B**) then there will be no fitness benefit of the inter-generational plasticity (**C**). It is therefore essential to link measures of offspring fitness (survival and reproductive output) to plastic traits (size in this case) to avoid misinterpreting the adaptive nature of TGP.

Applying a quantitative genetics framework to TGP

To evaluate whether TGP contributes to resilience to environmental change, it is necessary to quantify whether phenotypic shifts induced by parental environments are associated with fitness consequences in offspring. Specifically, TGP can be considered beneficial when it aligns with selection (positive covariation with survival or reproductive output), detrimental when it is misaligned, and neutral when there is no detectable association between phenotype and fitness (Pettersen et al., 2024). Importantly, we use "selection" in the Lande-Arnold sense as differential fitness among phenotypically different individuals (Lande & Stevan J. Arnold, 1983), without implying any particular genotype-phenotype mapping. Selection therefore manifests as non-random differences in survival or reproductive output across phenotypes (Falconer &

Mackay, 1996; Fisher, 1930; Haldane, 1954). While selection acts on phenotypes, its longer-term evolutionary consequences depend on inheritance and constraints (Arnold & Wade, 1984). Here, our focus is on the immediate inferential step that is often missing in studies of TGP: whether observed intergenerational phenotypic responses align with fitness variation in offspring under environmental change.

Phenotypic plasticity is sometimes viewed as reducing the efficacy of selection by increasing phenotypic variation without corresponding heritable variation, thereby weakening evolutionary responses (Chevin et al., 2010; Gienapp et al., 2007; Merilä & Hendry, 2014; Oostra et al., 2018). Yet, selection acts on phenotypes regardless of their underlying causes (Lande & Stevan J. Arnold, 1983). Selection analysis therefore provides a tractable and comparable approach for estimating the direction, form, and strength of associations between phenotypic variation and fitness across environmental contexts (**Box 1**). In the context of TGP, this shifts interpretation away from assuming that intergenerational traits changes indicate adaptive “buffering”, and towards explicitly testing whether those changes predict higher (or lower) fitness.

Selection analysis is a simple, yet powerful framework for producing standardised, comparable estimates for the relationship between phenotypes and fitness. Although it cannot distinguish phenotypic selection from an evolutionary (genetic) response to selection (Haldane, 1954), it is well suited as a diagnostic tool for evaluating whether intergenerational phenotypic shifts are associated with positive, negative, or absent fitness consequences in offspring among parental treatments (**Box 1**). Related approaches have been applied to study within-generation phenotypic plasticity, for example by regressing plasticity on individual fitness (DeWitt, 1998) or genotype-mean fitness (Arnold et al., 2019). However, equivalent worked examples for evaluating TGP in explicitly intergenerational designs are rare, despite the central role such studies play in climate-change biology. Here, we provide practical guidance and worked examples to show how selection analysis can be incorporated into common parent x offspring experimental designs to generate comparable estimates of fitness alignment across environmental treatments and taxa. Selection analysis can thereby improve inference about when TGP is likely to enhance, constrain, or have no effect on population persistence under environmental change.

Selection analysis methods

Selection analysis required paired measurements of individual phenotypes and fitness within a population (Lande & Stevan J. Arnold, 1983). Fitness components such as survival, reproductive success, or reproductive output can therefore be used to quantify viability, fertility, or fecundity selection, respectively. Data collected in inter- and transgenerational studies are particularly well suited to this framework. In many experience, parents are exposed to contrasting environmental treatments and offspring are then tracked to measure variation in traits and performance. This experimental design can produce the phenotype-fitness data needed to estimate selection gradients within each combination of parental and offspring environments. However, several methodological considerations need to be addressed to isolate the effects of parental and offspring environments (see below). Once phenotypic and fitness data have been collected for each environmental combination, the selection landscape can be quantified using the following steps.

1. **Check for multicollinearity among traits.**
   Before including multiple traits in a selection analysis, it is important to assess whether these traits are too strongly correlated with one another. If traits are highly correlated, it can be difficult to determine statistically how selection acts on each trait independently. To evaluate this, variance inflation factor (VIF) values should be calculated (Zuur et al., 2010). VIF values provide a measure of how much the variance of one trait is inflated by its relationship with other traits.
   - A VIF value < 5 generally indicates low multicollinearity, meaning the traits are not overly correlated and can be analysed together.
   - A VIF value > 5 suggests high multicollinearity, meaning it may be difficult to estimate independent selection gradients for each trait.

   In cases of high multicollinearity, it may be necessary to i) combine correlated traits into composite variables, ii) remove one of the correlated traits, or iii) apply methods designed to handle multicollinearity such as ridge regression or principal component analysis.
2. **Define the environments in which selection will be estimated.**
   Selection analyses are typically conducted within the ecological contexts in which individuals experience selection. These contexts may represent experimental treatments, environments, or combinations of both. For example, in the zebrafish case study presented here (Pettersen et al., 2024), selection gradients were estimated separately within each parental temperature × parental food × offspring temperature

combination. Estimating selection within environments allows the strength and direction of selection to be compared across ecological contexts.

3. **Choose an appropriate fitness measure.**
   Selection analyses require a measure of individual fitness or a proxy for fitness. Depending on the study system, this may include survival, reproductive output, fertility, or growth to a critical life-history stage. The statistical model used will depend on the distribution of the fitness variable. For example:
   - Gaussian models are appropriate for continuous fitness measures such as reproductive output.
   - Binomial or logistic models are appropriate for binary fitness measures such as survival or fertility.

   In the zebrafish case study (**Box 1**), offspring fitness was measured as survival to two weeks post-hatching, which was analysed using logistic regression.
4. **Standardise trait values within environments.**
   To ensure that selection coefficients are comparable across traits, phenotypic traits should be standardised to have a mean of zero and a standard deviation of one. This procedure places all traits on the same scale and allows the resulting coefficients to be interpreted as selection acting on one standard deviation change in the trait (Lande & Stevan J Arnold, 1983).
   When selection is estimated within treatments or environments, traits should be standardised within each environment, rather than across the entire dataset. This ensures that trait variation is evaluated relative to the conditions in which selection occurs.
5. **Estimate directional selection gradients using multiple regression.**
   Directional selection gradients can be estimated using multiple regression models in which fitness is regressed on the standardised traits of interest. Including multiple traits in the same model allows the selection gradient for each trait to be estimated while accounting for covariance among traits.
   For example, the following logistic regression model can be used when fitness is measured as survival: Fitness ~ $\text{Trait}_1 + \text{Trait}_2$
   In the zebrafish case study (**Box 1**), survival was modelled as a function of standardised development time and growth rate within each environment.
6. **Transform logistic regression slopes to selection gradients.**
   When fitness is measured as a binary variable (e.g., survival), logistic regression

coefficients are expressed on the logit scale and therefore cannot be interpreted directly as selection gradients. To obtain average directional selection gradients (β*), these slopes should be transformed using the method described by Janzen and Stern (1998). This transformation rescales logistic slopes to reflect the expected change in relative fitness associated with variation in the trait.

The transformation incorporates:

- the predicted survival probabilities from the fitted model, and
- the mean fitness within the environment.

Applying this transformation yields directional selection gradients that are directly comparable across traits and environments.

7. **Interpret the direction and strength of selection.**
   Directional selection gradients indicate whether higher or lower trait values are associated with increased fitness.
   - Positive β* indicates selection favouring higher trait values.
   - Negative β* indicates selection favouring lower trait values.
   - Values overlapping zero indicate weak or absent directional selection.

   Comparing these selection gradients with observed intergenerational shifts in trait values allows researchers to determine whether plastic changes align with, oppose, or are unrelated to the direction of selection.
8. **Visualise and interpret selection patterns.**
   Selection gradients can be visualised using forest plots or similar graphical approaches that display the magnitude and uncertainty of β* estimates across environments. These visualisations facilitate comparison of the strength and direction of selection across treatments and traits.When combined with estimates of intergenerational trait shifts, these plots provide a clear framework for evaluating whether intergenerational plasticity aligns with selection on fitness.

Selection on TGP is often context dependent

In natural populations, environments can be stressful, resources can be limited, or predation may be present, such that variation in phenotypes can pose important fitness consequences (Reznick et al., 2002). The selection analysis we describe is not explicit in identifying the mechanisms underlying changes in offspring phenotype and fitness, and nor was it designed to do so. TPG could be the result of genetic or epigenetic mechanisms. Genetic changes (i.e., changes in allele frequencies affecting phenotypes) are relatively unlikely across single or few generations unless selection pressures are very high, such as in antibiotic exposure of bacterial populations, for example (Bakkeren et al., 2020). As far as we know, the fastest explicitly demonstrated genetic adaptation to sudden environmental change in a vertebrate animal – the relatively short-lived stickleback fish (*Gastereosteus aculeatus*), took around 50 years (or ~25 generations) (Lescak et al., 2015). Hence, at least among vertebrates, epigenetic mechanisms are more likely to cause TPG. To test for particular mechanisms explicitly, candidate epigenetic or genetic mechanisms would need to be manipulated experimentally, such as in a CRISPR knock-out of DNA methyltransferase 3 - the principal enzyme laying down new methylation patterns in response to environmental inputs (Loughland et al., 2021). Fitness consequences of knock-out vs wild type individuals can then be measured (Seebacher & Bamford, 2024) and analysed with the selection analysis we propose here. Such an approach would yield interesting insights, but those would not be essential to realise the benefits of the selection analysis we propose.

Key limitations and methodological considerations

Selection analysis requires trait and fitness data (with sufficient variation) for individuals within each treatment. It is often logistically difficult to measure relative fitness, hence many studies obtain proxies for fitness, such as body size, growth rate, or locomotor performance (Stinchcombe et al., 2017), which can also be used in selection analysis in place of measures of reproduction and survival. For example, it is possible to analyse TGP using morphometric parameters to assess the benefits or costs to locomotor performance and hence broaden the applicability of standardised measures of selection. However, caution should be exercised when analysing TGP using fitness proxies to infer its adaptive potential (Acasuso-Rivero et al., 2019). Franklin and Morrissey (2017) found that using performance measures in place of fitness often underestimates the actual strength of selection. Given the requirement of selection analyses for datasets containing measures of many individuals often tracked longitudinally, it might be assumed that selection analyses are constrained to model organisms measured in the laboratory. Yet, selection analyses have been performed on natural populations of plants (Munguía-Rosas et al., 2011), birds (Charmantier et al., 2004), lizards (Losos et al., 2004), fish (Brooks & Endler, 2001), and mammals (Boratyński & Koteja, 2010; Kruuk et al., 2002) for several decades. Further selection estimates, particularly on TGP, are crucially needed to build an understanding of the fitness consequences of trait variation under rapid environmental change (Arnold et al., 2019; Rivkin et al., 2019; Svensson, 2023). Here we outline some potential limitations and methodological considerations when utilising selection analysis for inferring the adaptive nature of TGP:

1. Parent and offspring treatments need to be applied independently wherever possible. Studying species with short brooding times, such as egg laying taxa, often allows for selection on offspring phenotypes to be analysed without the issue of parental environment as a confounding factor. For species with high parental care, however, it may be difficult to disentangle selection on offspring phenotype independently of parent environment or condition (Kielland et al., 2017).
2. Disentangling genetic versus environmental (epigenetic) effects on offspring phenotype and fitness. Most TGP studies (including the case studies examined here) test for adaptive TGP using polymorphic populations, where the underlying genetic basis of the phenotypic response to a parental stressor/environment is unknown. There is therefore a potential role for the genetic control of phenotypic plasticity, whereby differential mortality or reproductive output in offspring can be the result of the genotype in a given parental environment, to bias estimates of TGP (i.e., phenotypic change solely due to the parental environment). The conflation of within-generation selection with plasticity has been demonstrated by Santos et. al., (Santos et al., 2019) and may apply for TGP estimates. To clearly demonstrate that phenotypic changes occur in the absence of genetic differences, i.e., due to epigenetics, studies are needed that either use populations with a known genetic basis of the phenotypic response (e.g., offspring mass, metabolism, or immune response) such as via monitoring changes in allele frequencies across parental environment (e.g., temperature, drought, or parasite). Alternatively, this issue can be avoided altogether by using a protocol with either clonal replicates or isogenic lines (Signor, 2020).
3. Detecting selection requires that there is sufficient variation in both the traits of interest and fitness. One potential issue for using this type of analysis is that TGP might result in depletion of phenotypic variation in a given environment, such that even if TPG in response to a change in environment increases offspring fitness, it cannot be detected. For example, parents exposed to predation might increase offspring size, leading to relatively higher offspring survival in large versus small offspring, but selection (correlation between fitness and trait) cannot be detected statistically (Engqvist & Reinhold, 2016). In line with this, studies on populations already exposed to strong past selection, may not possess the available phenotypic variation required for selection to act on in the present generation.

4. Caution should be exercised when identifying the target of selection. For example, rather than acting upon the measured trait(s), selection may be targeting other unmeasured trait(s) that are correlated with trait(s) of interest, leading to a spurious correlation between fitness and the measured trait(s). It is important therefore to carefully select trait measurements, and to measure and test for selection on multiple traits.
5. It is important to apply a treatment independently from other confounding environmental effects (Rausher, 1992; Stinchcombe et al., 2002). For example, the spatial variation of treatments can be randomised to ensure that environmental conditions other than the treatment do not systematically alter phenotypic or fitness variation.
6. Measures of lifetime reproductive output in offspring and grand offspring from TGP studies are exceedingly rare. Hence, it is difficult to determine how long the benefits or costs of epigenetic effects can persist. Future studies measuring whether fitness consequences persist throughout the life history and across generations are needed to determine the temporal stability of selection acting on TGP (Siepielski et al., 2009). These studies could also shed light on the mechanisms underlying beneficial, detrimental, or neutral TGP.
7. It is important to consider that fitness benefits for individual offspring are not always beneficial for parents (Einum & Fleming, 2000; Stearns, 1992). Selection acting on offspring traits may not lead to adaptive evolution if there is antagonistic selection acting across generations (Wilson et al., 2005). To determine whether TGP is likely to evolve in response to selection, future studies that span generations and quantify phenotypic and fitness covariances between parents and offspring, are an important next step (Wolf & Wade, 2001).

Conclusion

Inter- and transgenerational phenotypic plasticity is often invoked as a mechanism of resilience to rapid environmental change. Yet phenotypic shifts alone, especially when interpreted through performance proxies, do not demonstrate whether transgenerational responses enhance offspring fitness or population persistence. Here we propose selection analysis as a diagnostic framework for evaluating the adaptive value of transgenerational responses by testing whether parental-environment-associated trait variation aligns with selection to enhance survival or reproduction. Adopting fitness-based tests of alignment between plasticity and selection will sharpen prediction of population responses under environmental change, and clarify when transgenerational effects are likely to enhance, constrain, or have no effect on population persistence.

BOX 1. Testing whether intergenerational shifts in development and growth are adaptive

Inter- or trans-generational phenotypic plasticity (TGP) is frequently inferred to be adaptive when parental environments induce shifts in offspring traits linked to performance. In zebrafish (*Danio rerio*), development time and growth rate are commonly used as proxies for early-life success, and both traits are sensitive to parental thermal and nutritional environment (Pettersen et al., 2024). Here, we demonstrate how incorporating a simple selection analysis changes interpretation of TGP in these traits.

Intergenerational shifts in offspring traits

Across parental temperature treatments (24 °C and 30 °C), low parental food consistently produced offspring that developed more slowly (longer time to hatching) and exhibited reduced early growth rates, relative to offspring from high-food parents (**Table 1**). These shifts were observed at both offspring rearing temperatures (24 °C and 30 °C), indicating clear evidence of TGP. Without explicit fitness data, such shifts towards slower development and reduced growth might be interpreted as i) maladaptive consequences of parental stress, or ii) protective adjustments that enhance survival under stressful conditions. However, trait shifts alone cannot resolve this ambiguity.

Quantifying alignment of TGP with viability selection

To test whether these intergenerational shifts align with fitness, we quantified viability selection by regressing relative survival (to two weeks post-hatching) on standardised development time and growth rate within each parental x offspring environment combination. Logistic regression coefficients were transformed following Janzen and Stern (1998) to yield directional selection gradients (β) on a relative fitness scale. Selection gradients revealed that adaptive alignment was highly context-dependent (**Table 1**). Strong positive selection on slower development and negative selection on growth occurred only in one environmental combination (parents at 24 °C, low food; offspring at 30 °C). In this case, the direction of TGP (slower development and reduced growth under low parental food) matched the direction favoured by viability selection, consistent with beneficial IGP. In contrast, in several other environments selection was weak or absent, and in one combination (parents at 30 °C, high food; offspring at 24 °C), selection favoured faster development, opposite to the direction of the parental temperature-induced shift. Thus, intergenerational shifts that might be broadly described as adaptive based on trait patterns along were only beneficial under specific environmental conditions.

How inference changes

If analysis had stopped at documenting intergenerational trait shifts, we might conclude that parental thermal and nutritional stress modifies offspring development and growth in ways relevant to performance. However, incorporating explicit estimates of selection demonstrates that adaptive alignment is neither universal nor consistent across environments. Some trait shifts are strongly favoured by selection, others are effectively neutral, and some may oppose the direction favoured by viability (survival). This additional analytical step required only individual-level trait and survival data and does not depend on pedigree information or complex quantitative genetic modelling. Yet it fundamentally alters inference about whether TGP enhances, constrains, or has no effect on offspring fitness. Zebrafish are widely used in intergenerational and epigenetic studies, including investigations of DNA methylation dynamics (Loughland et al., 2021; Seebacher & Bamford, 2024). This case study illustrates how integrating selection analysis into such designs provides a direct and comparable test of adaptive alignment, strengthening inference about the evolutionary and ecological consequences of TGP under environmental change.

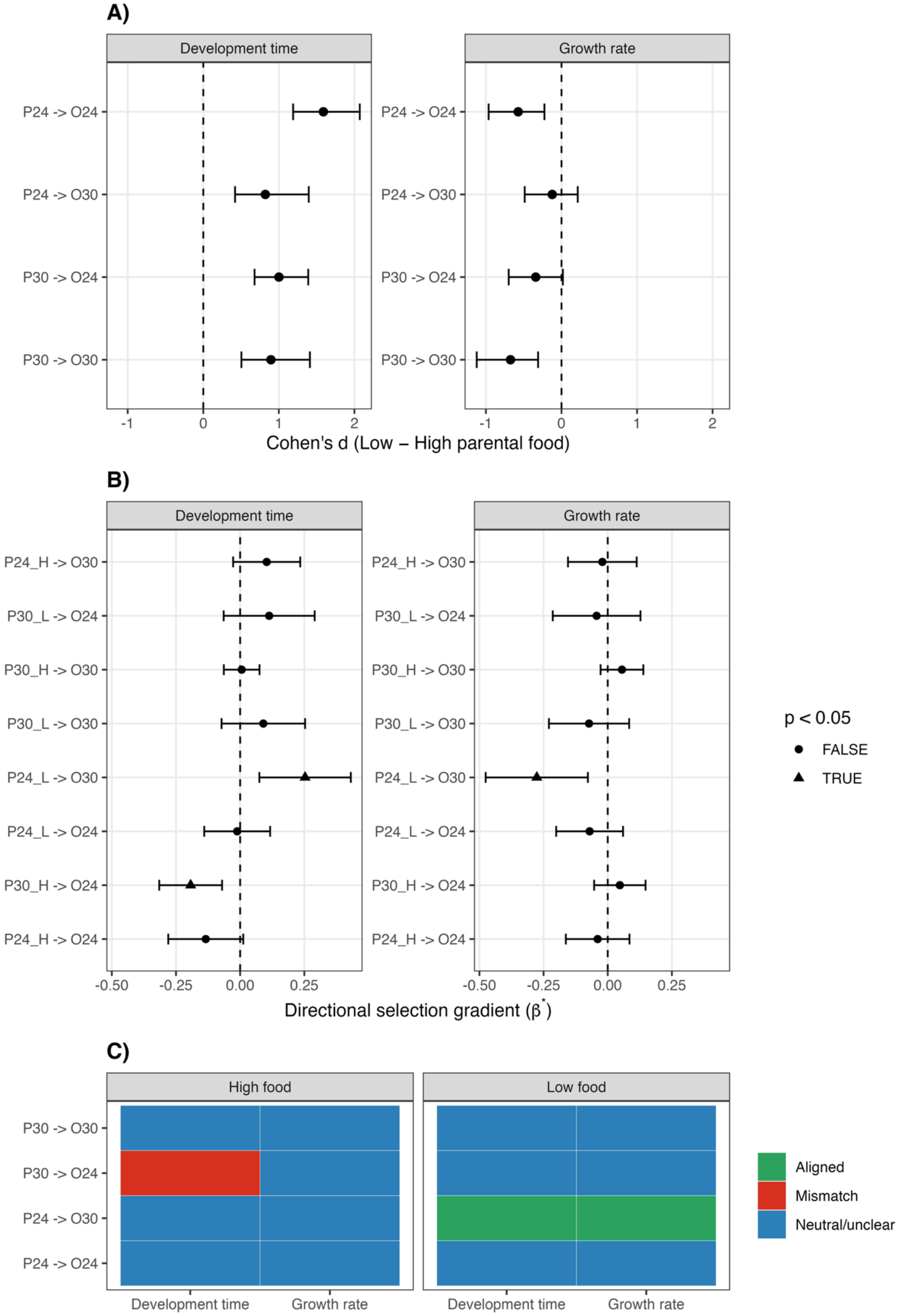

Figure (Box 1). Testing whether intergenerational phenotypic plasticity aligns with viability selection. A) Intergenerational shifts in offspring ("O") development time and growth rate induced by parental ("P") food environment, expressed as standardized mean differences (Cohen's *d*; low − high parental food) with bootstrapped 95% confidence intervals. Positive values indicate slower development or faster growth under low parental food; negative values indicate faster development or slower growth. **B)** Multivariate directional selection gradients ($\beta*$) estimated from logistic regressions of offspring ("O") survival on standardised development time and growth rate within each parental ("P") temperature (24 = 24 °C, 30 = 30 °C) × parental food (L = Low, H = High) × offspring temperature (24 = 24 °C, 30 = 30 °C) environment. Error bars show 95% confidence intervals. **C)** Alignment between the direction of intergenerational trait shifts and the direction of viability selection for each parental food environment. Green indicates alignment between plastic shifts and selection, red indicates mismatch, and blue indicates neutral or unclear outcomes where selection gradients overlap zero. Together, these panels illustrate how incorporating selection analysis can change inference about the adaptive value of intergenerational plasticity: although parental environment consistently altered offspring traits (panel A), only some shifts aligned with selection on survival (panels B–C). This workflow demonstrates a practical approach for testing whether intergenerational plasticity enhances offspring fitness under environmental change.

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