Inferring the dynamics of selective constraints across complex ontogenies

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Abstract

Most organisms undergo a series of complex phenotypic changes throughout their life cycles that allow them to meet the demands of different niches throughout ontogeny. Theory suggests the significant coordination required to undergo such ontogenetic transitions can impose evolutionary constraints on variation to developmental programs. This produces patterns known as developmental hourglasses, which are periods during development where phenotypic and/or genetic variation is constrained between taxa relative to other points throughout ontogeny. Although empirical support for developmental hourglasses has been well established in animal embryonic development, it remains unclear if other non-animal or non-embryonic development programs exhibit such patterns. Furthermore, more recent investigations of developmental hourglasses have largely relied on relating gene age and sequence divergence to their temporal expression profiles across development, an approach highly susceptible to noise due to historical contingency and developmental system drift. Likewise, more recent investigations have described more complex fluctuations in the strength of selective constraints across ontogeny, suggesting our understanding may be improved by more nuanced and flexible approaches for quantifying developmental constraints across ontogeny. To this end, here I present a theoretical and empirical framework for leveraging population-level variation in developmental gene/trait expression dynamics to infer the strength of selective constraints across an ontogeny. I first provide theoretical precedence for this approach using an extended geometric model, which suggests that patterns of variation in gene/trait expression within populations are stable and recapitulate the latent ontogenetic selective dynamics. I then describe an empirical approach for inferring these latent ontogenetic selective dynamics and use a simple simulation to illustrate its utility. Finally, I utilize this approach to infer the dynamics of selective constraints from population-level transcriptional data of various stages across the monarch butterfly *Danaus plexippus* metamorphosis, which suggests both the transitions from larva to pupa and from pupa to adult constitute developmental hourglasses.

Keywords: Developmental constraints, geometric model, metamorphosis

Introduction

Most organisms undergo some degree of morphological, physiological, and/or behavior transition throughout their life cycles. These dynamics are often complex and abrupt, resulting in the expression of disparate phenotypes across ontogeny that facilitate organismal development and activity in different niches. Biologists have long been interested in explaining the evolution of ontogenetic complexity, as changes in life cycle dynamics are often associated with patterns of diversification throughout natural history (Haldane 1932, Moran 1994, Wheeler et al. n.d., Reiss 2002). Although the interplay between ontogenetic and evolutionary dynamics is well appreciated, explaining prominent patterns in the diversification of developmental programs and assessing their generality has been more challenging (Drost et al. 2017).

Developmental hourglasses are among the most striking patterns in the diversification of many ancient ontogenetic programs. In a general sense, developmental hourglasses are marked by periods towards the middle of a developmental stage where phenotypic and/or genetic diversification is constrained between taxa (Baer 1828, Duboule 1994, Raff 1996). This pattern has been mostly defined and explored in animal embryonic development, where it is thought to be driven by selective constraints imposed by development of the basic body plan (Irie et al. 2014). More recently, studies have shifted towards describing developmental hourglasses by examining the interplay between gene divergence/age and their patterns of expression across development. This has provided evidence for hourglass patterns during the embryonic development of major animal groups, including Chordates, Arthropods, and Nematodes (Cruickshank et al. 2008, Yanai et al. 2011, Drost et al. 2015, Drost et al. 2017, Ma et al. 2023). While previous studies have largely focused on animal embryonic development, there is also some evidence of a developmental hourglass during plant embryogenesis (Drost et al. 2015, Drost et al. 2017).

Despite the growing body of evidence for hourglass patterns being a general feature embryonic development particularly in animals, there are several areas in which expanding our approach to studying the dynamics of selective constraint across development may improve our understanding of ontogenetic evolution. First, developmental hourglasses have been largely defined phenomenologically in relatively few taxa and ontogenetic programs. Therefore, it remains unclear how general these patterns are to a broad array of taxa and to non-embryonic development. For example, hourglass patterns of selective constraint have been documented in fungal fruiting body development, and it is hypothesized that insect metamorphosis and angiosperm flower development my also exhibit hourglass patterns of selective constraint (Cheng et al. 2015, Drost et al. 2017). However, there are relatively few studies on these kinds of non-animal or non-embryonic developmental programs, thus limiting our understanding of how ontogenies have evolved throughout natural history. Furthermore, more recent investigations of developmental hourglasses have been largely based on examining gene ages or degrees of sequence divergence in relation to their temporal expression profiles across development. However, historical contingency and developmental system drift stand to introduce a (high) degree of noise when examining such patterns across macroevolutionary scales, which may impede quantification of selective constraints (Haag et al. 2021). Similarly, the dynamics of selective constraints can be more complex than simple hourglasses, as more recent studies have found irregular shapes and fluctuations across ontogeny (Wu et al. 2019, Cordero et al. 2020, Aleksandra M. Ozerova et al. 2025). Taken together, previous studies suggest more rigorous quantifications of how selective constraints acts across diverse developmental programs may improve our general understanding of the evolution of complex ontogenies.

To contribute to this goal, here I present a theoretical and empirical framework for leveraging population-level variation in developmental dynamics to infer the dynamics of selective constraints across ontogenies. This framework shifts the focus from testing for developmental hourglasses to the more general task of inferring the strength of stabilizing selection as a function of ontogenetic time. First, I use an extended geometric model to show that variation in the strength of stabilizing selection across ontogeny gives rise to patterns of variation in gene expression within populations that are stable and recapitulate the latent selective dynamics. I then describe an empirical approach for inferring how the strength of stabilizing selection varies across ontogeny using population-level gene expression profiles, and use a simple simulation to illustrate its efficacy. Finally, I employ this approach to infer the dynamics of selective constraints using population-level transcriptional data from various life stages across the monarch butterfly (*Danaus plexippus*) metamorphosis.

Methods

A extended geometric model for ontogenetic dynamics

Consider an organism with G genes that may vary in their expression throughout the course of ontogeny. Let $x^g(t)$ represent the expression of gene g at time t, where $t \in [0, T]$ and T is the total organism's life span. The transcriptional state at time t can then be written as

$$x(t) = \begin{bmatrix} x^1(t) \\ x^2(t) \\ \vdots \\ x^G(t) \end{bmatrix} \in \mathbb{R}^G \tag{1}$$

Likewise, the optimal transcriptional state at time t is

$$o(t) = \begin{bmatrix} o^1(t) \\ o^2(t) \\ \vdots \\ o^G(t) \end{bmatrix} \in \mathbb{R}^G \tag{2}$$

Fitness declines with the squared distance from the optimum, which is defined at time t as

$$d^2(t) = \sum_{g=1}^{G} (x^g(t) - o^g(t))^2 \tag{3}$$

In a traditional geometric model, fitness is determined by the distance from the optimum and the (constant) width of the fitness peak σ. This assumes that the fitness landscape is uniform throughout ontogeny. To allow for varying degrees of selective constraint at different points throughout ontogeny, I instead define a fitness ridge $\sigma(t)$ whose width can change over the course of development. Let m be the number of major transitions, $\vec{S} = (S_1, \ldots, S_m) \in [0, T]^m$ be the midpoints of each transition, $\vec{\epsilon} = (\epsilon_1, \ldots, \epsilon_m)$ be the transition durations, and $\vec{\Delta} = (\Delta_1, \ldots, \Delta_m)$ be the associated changes in width to the fitness ridge. The fitness ridge function is then

$$\sigma(t) = \sigma_0 + \sum_{i=1}^{m} \Delta_i \cdot \exp\left(-\frac{(t - S_i)^2}{2\epsilon_i^2}\right) \tag{4}$$

where $\sigma_0 > 0$ is the baseline ridge width. Using a Gaussian fitness function, fitness W is defined with respect to distance from the optimum as

$$W = \exp\left(-\int_0^T \frac{d^2(t)}{2\sigma(t)^2}\, dt\right) \tag{5}$$

Temporal gene expression patters $x^g(t)$ are mutated through the addition of Gaussian pulses. Letting μ_a be the average pulse amplitude and τ_a be the standard deviation in pulse amplitude, pulse amplitudes are drawn from a Gaussian distribution: $a \sim \mathcal{N}(\mu_a = 0, \tau_a)$. Likewise, letting μ_l and τ_l be the mean and standard deviation in pulse widths, pulse widths are $l \sim |\mathcal{N}(\mu_l, \tau_l)|$. Finally, let s be the time point on which the pulse is centered, where $s \sim \mathcal{U}(1, 2, \ldots, T)$. To prevent systematic inflation at the beginning or end of ontogeny due to pulse truncation, pulses are normalized in discrete time so that their contribution to expression variance is independent of where s is positioned along the interval. Let

$$\tilde{p}(t; s, l) = \exp(-\frac{(t-s)^2}{2l^2}) \tag{6}$$

be the raw discrete Gaussian pulse and $\tilde{p}_{\text{ref}}(t; l)$ be the same pulse centered at the midpoint of the time interval. The normalized pulse is then

$$p(t; s, l) = \tilde{p}(t; s, l) \cdot \frac{\Sigma_t \tilde{p}_{\text{ref}}(t; l)^2}{\Sigma_t \tilde{p}(t; l)^2} \tag{7}$$

Finally, temporal expression patterns are mutated as

$$x'(t) = x(t) + a \cdot p(t; s, l), \tag{8}$$

Evolutionary simulations

To examine how selective constraint acting at different points across ontogeny shapes patterns of within-population transcriptional variance, I used the previously described model to conduct individual-based evolutionary simulations. For simplicity, I considered the ontogenetic and evolutionary dynamics of a single gene ($G = 1$). To highlight selective effects on variation, I defined a simple linear ontogeny with uniform selective dynamics and contrasted it with that of a more complex ontogeny marked by a period of selective constraint, both of which lasted 50 time points. For the simple linear ontogeny, I defined the stabilizing selection function $\sigma(t) = 0.9$. For the complex ontogeny, I defined a developmental hourglass by specifying $m = 1$ (one transition), $S_1 = 25$ (occurs during the middle of the 50 time point ontogeny), $\epsilon_1 = 5$ (concentrated over five time points), and $\Delta_1 = 0.6$ (increase in the strength of stabilizing selection) for Equation 4. I conducted both sets of simulations using a standard Wright-Fisher approach and population sizes of 1000. At each generation, I recorded the population variance in expression at each point in ontogeny t. To decrease numerical noise for better pattern visualization, I used a Gaussian filter to smooth variance data using the *gaussian_filter* function from the *SciPy* Python library (v.1.13.1) (Virtanen et al. 2020).

Variance dynamics in ontogenetic expression patterns under stabilizing selection

For population-level variation in ontogenetic patterns of expression to be useful for inferring how stabilizing selection acts at different points across ontogeny, it is important to first define the dynamics of variance stabilization within a population. First, consider the discrete time log fitness function, which from Equation 5, is

$$\log W \propto -\sum_t \frac{d^2(t)}{2\sigma(t)^2} \tag{9}$$

Selection at each time point t multiplies the distribution of $x^g(t) - o^g(t)$ by a Gaussian distribution with a variance of $\sigma(t)^2$. Let $V(t)$ be the variance of this distribution before selection. The post-selection variance is then given by the precision additivity rule for the products of Gaussians:

$$\frac{1}{V_{\text{select(t)}}} = \frac{1}{V(t)} + \frac{1}{\sigma(t)^2} \implies V_{\text{select(t)}} = \frac{V(t)\sigma(t)^2}{V(t) + \sigma(t)^2} \tag{10}$$

To add mutational input from pulses, let $M(t) = \lambda\tau_a^2\mathbb{E}_{s,l}[p(t;s,l)^2]$ be the per-generation mutational variance input at time t, where λ is expected population mutation rate (number of pulses) per generation. Since $p(t;s,l)$ is normalized to be independent of s, $\mathbb{E}_{s,l}[p(t;s,l)^2$ is constant in t for any fixed l. Therefore, $M(t)$ is constant over time, leaving us with the mutational input M. Combining the mutational input with Equation 10 gives the per-generation recursion

$$V_{gen+1}(t) = \underbrace{\frac{V(t)\sigma(t)^2}{V(t) + \sigma(t)^2}}_{\text{selection}} + \underbrace{M}_{\text{mutation}} \tag{11}$$

For tractability, this recursion can be substituted in the continuous time approximation (assuming small changes per generation) $\frac{dV}{dg} \approx V_{g+1} - V_g$, which gives

$$\frac{dV}{dg} \approx M - \frac{V^2}{V + \sigma^2} \tag{12}$$

Estimating selective constraints across ontogeny using sampled transcriptional variation in a multi-dimensional space

As previously described, transcriptional variation should be constrained at points during ontogeny where the strength of stabilizing selection is higher. At each time point, this constraint can be empirically estimated by calculating the average squared distance from each transcriptional profile in a multivariate Euclidean space to the centroid (population mean). Let N_t be the number of individuals sampled, and the centroid at time t be

$$\mu_t = \frac{1}{N_t}\sum_{i=1}^{N_t} x_i(t) \tag{13}$$

Therefore, the mean squared distance to the centroid is

$$\delta^2(t) = \frac{1}{N_t} \sum_{i=1}^{N_t} \|x_i(t) - \mu_t\|^2 \tag{14}$$

This empirical dispersion corresponds to the population-level variance in transcriptional states at time t. From theoretical expectations described in Equation 5, if individuals are distributed around the optimum $o(t)$ with a covariance $\Sigma_t \approx \sigma^2(t)I$, then the expected squared distance is the trace of the covariance matrix

$$\mathbb{E}[d^2(t)] = \text{trace}(\Sigma_t) = G\sigma^2(t) \tag{15}$$

Substituting the sample centroid μ_t for the unobserved $o(t)$ shows that the estimated strength of stabilizing selection is proportion to the square root of the mean squared distance to the centroid divided by the number of genes:

$$\widehat{\sigma}(t) \propto \sqrt{\frac{\delta^2(t)}{G}} \tag{16}$$

Overall, this plug-in estimator shows how the strength of stabilizing selection can theoretically be estimated from a population sample of transcriptional profiles. However, there are practical considerations when directly applying Euclidean geometry for these purposes, which are discussed below.

Empirically inferring the dynamics of selective constraint across the monarch butterfly metamorphosis

To empirically demonstrate the utility of using inter-individual variation in expression to infer the dynamics of selective constraints across ontogeny, I analyzed existing transcriptional data from various life stages across the monarch butterfly *D. plexippus* metamorphosis. In addition to an empirical demonstration, this also provides inference into the dynamics of selective constraints across the holometabolous development, a developmental program that been less studied than the common embryonic development. The data comes two mRNA sequencing studies from third instar larvae, fifth instar larvae, early pupae (the day following pupation), late pupae (six to eight days following pupation), and newly eclosed adults (the day of eclosion) (DuBose et al. 2024, DuBose et al. 2025). Each life stage is represented by 18-20 replicate individuals, resulting in a total of $n = 96$ samples for modeling fitting. Raw mRNA sequence data can be accessed using the NCBI GEO accession number GSE253389 or the BioProject accession number PRJNA1065445, and I processed said data into a transcripts/million matrix as described in the associated references.

The first step for empirically inferring selective dynamics is to calculate the pairwise dissimilarity matrix as described in Equation 18, which I used the *cor* R function for (R Core Team 2022). I then used the *pcoa* function from the *stats.ordination* module within the scikit-bio Python library (v.0.7.0) for principal coordinate analysis and pairwise dissimilarity projection into a reduced multidimensional space. I calculated the distance from each sample to its corresponding life stage centroid as described in Equation 20, and fit a generalized additive model (GAM) to these distances to estimate a smooth function of how transcriptional dispersion changes across ontogenetic time. Here, I encoded ontogenetic time as the order of life stages (e.g., third instar = 1, fifth instar = 2, etc.) and used the *pygam* Python library (v.0.10.1) for model fitting (Servén et al. 2018).

Results

Ontogenetic constraints give rise stable patterns of transcriptional variation within populations that recapitulate latent selective dynamics

To infer patterns of selective constraint across ontogeny from population samples of transcriptional profiles, said constraints must give rise to distinct patterns of transcriptional variation that are stable over time. To establish theoretical precedence for such patterns, I used the previously described geometric model to conduct individual-based evolutionary simulations of two different ontogenetic programs and examined patterns of within-population transcriptional variation over ontogenetic and evolutionary time. First, I considered a linear ontogeny in which the effect of stabilizing selection is uniform across development. I then considered an ontogenetic program that involves a life stage transition accompanied by greater selective constraint (decrease in $\sigma(t)$). As shown in Figure 1A, patterns of variance in gene expression for a linear ontogeny remain relatively uniform across developmental and evolutionary time. In contrast, the increase in selective constraint associated with a significant life stage transition results in significantly reduced expression variance during said transition (Figure 1B).

To discern the stability of these patterns for a given selective regime, I examined the behavior of the expected variance dynamics over generations (Equation 12). This showed that variance in expression rapidly saturates, where the apparent plateau is determined by the strength of selection (inversely related to σ) (Figure 1C). Solving (Equation 12) for $\frac{dV}{dg} = 0$ gives the equilibrium variance V^*

$$V^* = \frac{M + \sqrt{M^2 + 4M\sigma^2}}{2} \tag{17}$$

which scales approximately linearly with σ (Figure 1D). This shows that within-population variance in expression rapidly stabilizes, suggesting such patterns can be used to infer ontogenetic selective dynamics.

A framework for empirically inferring the dynamics of ontogenetic selective constraints

As described by Equation 16, the strength of stabilizing selection acting at different points across across ontogeny can be estimated using expression variance in a multivariate space. In practice however, estimating variance with direct Euclidean geometry is difficult due noise and high-dimensionality (organisms usually have thousands of genes). Instead, $\sigma(t)$ can be estimated by calculating the sample dispersion around their centroid after using correlation-based distances and dimensionality reduction to project individuals into a reduced multidimensional space. Let $D(t)$ be a transcriptional dissimilarity matrix computed between all individuals at each time point, where

$$D_{ij}(t) = \frac{1 - \rho(x_i(t), x_j(t))}{2}. \tag{18}$$

and ρ is the Pearson correlation coefficient. In this, z-scoring each individual's expression vector within time t and calculating the Euclidean distance between said standardized vectors satisfies $\|z_i(t) - z_j(t)\|^2 = 2G(1 - \rho_{ij})$. Therefore, multidimensional scaling/principal coordinate analysis on $D_ij \propto (1 - \rho)$ is equivalent (up to a constant scale) to principal component analysis on the

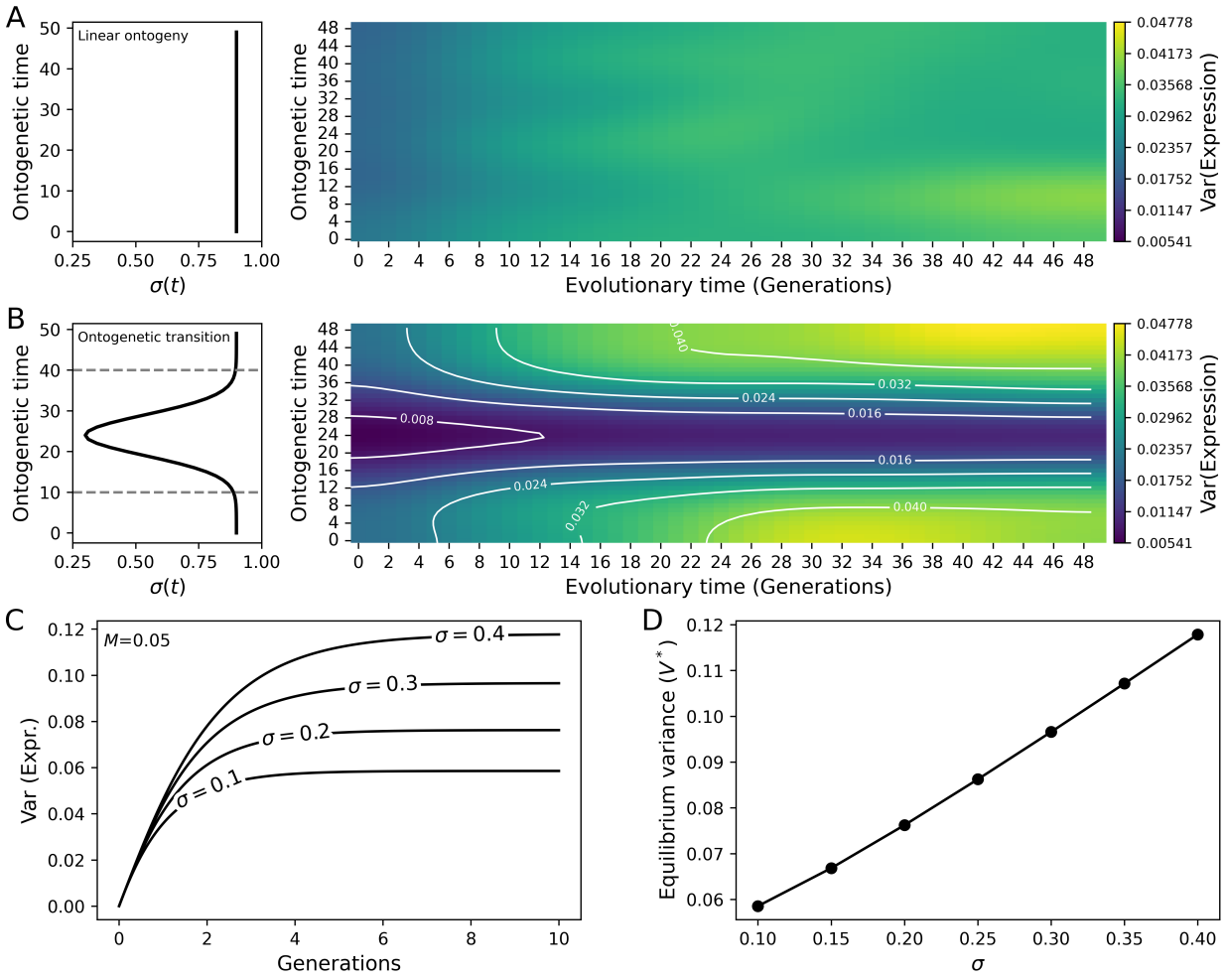

Figure 1: Patterns of transcriptional variance associated with dynamic selective constraints across ontogeny recapitulate the latent selective dynamics and are stable over evolutionary time. A) Individual-based evolutionary simulations based on a linear ontogeny in which the strength of stabilizing selection (x-axis) is uniform across ontogeny (y-axis). The left panel shows the latent selective dynamics and the right panel shows the dynamics of within-population transcriptional variance across ontogenetic time (y-axis) and evolutionary time (x-axis), where darker colors indicate less variance. B) Individual-based evolutionary simulations based on an ontogeny that includes a significant transition accompanied by period of greater stabilizing selection (lower $\sigma(t)$). C) Expected dynamics of variance in expression over generations, where different lines correspond to different strengths of stabilizing selection (σ). D) Equilibrium variances as a function of the strength of stabilizing selecting (σ). Points correspond to where variance functions in C plateau at 0. Taken together, these findings illustrate how dynamic stabilizing selection across ontogeny produces patterns in transcriptional variation that are stable and recapitulate the latent selective dynamics.

z-scored features. Furthermore, this Euclidean link is approximated if the Spearman rank correlation coefficient is used in place of the Pearson correlation coefficient. Multidimensional scaling (MDS) or principal coordinate analysis (PCoA) can then be applied to project individuals into a multidimensional space $z_i(t)$ that preserves pairwise dissimilarities. The centroid for this multidimensional space is

$$\bar{z}(t) = \frac{1}{N_t} \sum_{i=1}^{N_t} z_i(t) \tag{19}$$

The mean squared distance to the centroid then provides an empirical estimate of transcriptional dispersion, which is

$$\delta^2_{\text{emp}}(t) = \frac{1}{N_t} \sum_{i=1}^{N_t} \|z_i(t) - \bar{z}(t)\|^2 \tag{20}$$

Following Equation 16, this can be transformed into an estimator of the ridge width $\sigma(t)$.

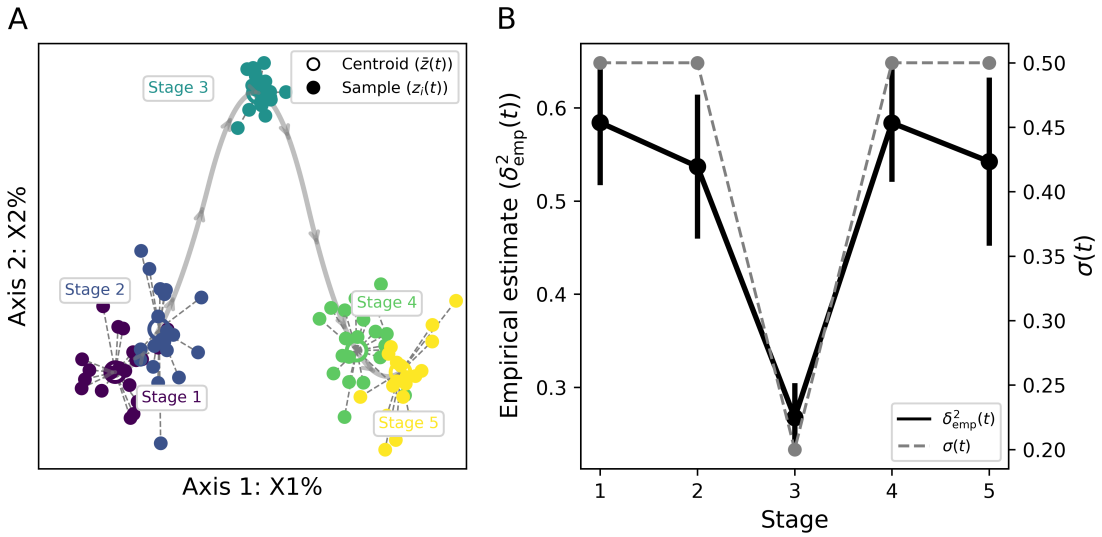

Figure 2: An illustration of how the dynamics of ontogenetic selective constraints can be empirically inferred from population sampling of transcriptional data. A) Simulated samples in a two-dimensional ordination space from five life stages across an ontogenic program. Each point represents the transcriptional state of an individual sample and dashed gray lines show the distance from each sample to the sample centroid. The thicker gray arrowed line depicts the latent ontogenetic program. B) The left y-axis and black line represents the empirical estimate of stabilizing selection for each life stage (x-axis), where error bars represent the standard error. The right y-axis shows the corresponding latent strength of stabilizing selection ($\sigma(t)$) for each life stage.

To illustrate the mechanics of this approach, I simulated population sampling of five life stages across an ontogeny with a distinct increase in selective constraint (decrease in $\sigma(t)$) during the third life stage. Here, I defined a centroid $\bar{z}(t)$ for each life stage and generated $N = 20$ individuals $z_i(t)$ by drawing coordinate variations (in a two-dimensional Euclidean/ordination space) from a normal distribution, such that each $z_i(t) \sim \mathcal{N}(\bar{z}(t), \sigma(t))$. Inspecting sample coordinates shows clearly reduced dispersion around the centroid for the more selectively constrained third life stage

(Figure 2A). Likewise, calculating the average distance from each life stage to its corresponding centroid shows that the empirical estimates $\delta^2_{\text{emp}}(t)$ closely align with the latent $\sigma(t)$ function (Figure 2B). For simplicity, this example considered time as discrete stages. However, inferring selective dynamics across ontogeny could also be accomplished by treating time continuously and fitting a generalized additive model to dispersion data (as done in the following section). Here, $\delta^2_{\text{emp}} \approx s(t)$ and $s(t)$ is a smooth function, which gives an estimate of how observed dispersion changes continuously across ontogeny.

Quantifying selective constraints across the monarch butterfly metamorphosis

Empirical investigations of selective constraints across ontogeny are largely limited to few representative developmental programs (e.g., embryonic development). Therefore, I used the previously described framework to infer patterns selective constraint across the metamorphosis of the monarch butterfly *D. plexippus*. This showed non-linear variation in dispersion across life stages (effective degrees of freedom = 4.98, $p < 0.001$), with the estimated smooth term $s(t)$ explaining 44% of the variance in sample distances to their stage centroid (Figure 3). Specifically, transcriptional dispersion slightly increased from the third instar larval stage to fifth instar larval stage, before sharply decreasing early in the pupal stage. Dispersion increased again throughout pupal development and decreased again in adults shortly after eclosion (Figure 3). Overall, this suggests the metamorphic transitions of pupation and eclosion are accompanied by significant selective constraint.

Discussion

To improve our understanding of how selective constraints have shaped the evolution of ontogenetic programs, quantification of said constraints from diverse systems and developmental programs, including embryonic and non-embryonic, are needed. Existing approaches for this task typically rely on examining the relationship between gene age/sequence divergence between taxa and their patterns of expression across development. However, historical contingency and developmental system drift across macro-evolutionary scales can add noise to extant signals of ontogenetic selective constraints (Haag et al. 2021). Likewise, the dynamics of selective constraints across ontogeny can have irregularities and fluctuations not accounted for by the simple hourglass model framework typically employed (Wu et al. 2019, Cordero et al. 2020, Aleksandra M. Ozerova et al. 2025). Here, I described a framework for inferring the dynamics of selective constraints acting across ontogeny based on within-population variation in developmental gene/trait expression. I first used a geometric model that considers expression values as functions of ontogenetic time to examine theoretical support for this concept. This showed that variation in the strength of stabilizing selection acting across ontogeny gives rise to stable patterns of expression variation that recapitulate the latent selective dynamics (Figure 1). I then describe a framework for leveraging this expression variation to empirically infer the strength of developmental selective constraints across ontogeny, and use a simple simulation to illustrate its mechanics (Figure 2). Finally, I employ this approach to describe the dynamics of selective constraints acting across the monarch butterfly metamorphosis. This showed evidence that the metamorphic transitions from larva to pupa and from pupa to adult were accompanied by significant developmental constraints, while within-stage development was subject to weaker constraints (Figure 3).

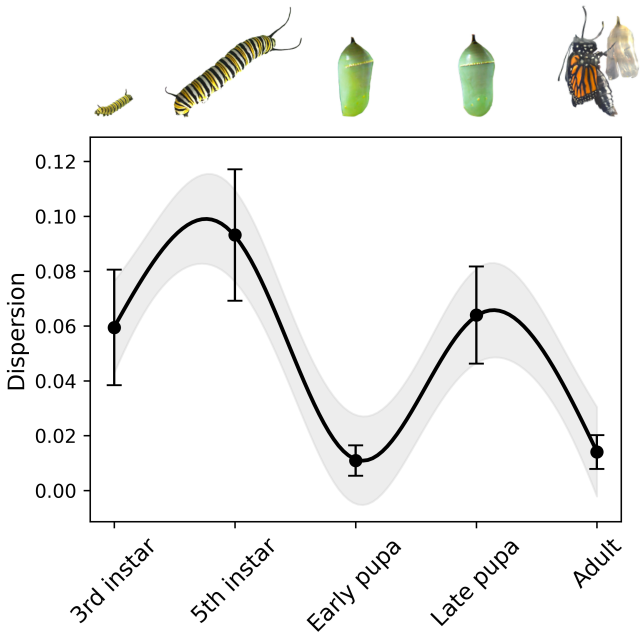

Figure 3: Evidence of selective constraint at life stage transitions across the monarch butterfly metamorphosis. Patterns of transcriptional dispersion (y-axis) across life stages (x-axis). The solid black line depicts the smooth function $s(t)$ estimated by fitting a generalized additive model ($R^2 = 0.44$), and the shaded area represents the 95% confidence interval. Each point represents the sample transcriptional dispersion, and error bars represent the standard error.

Generally speaking, the concepts I have described here are intuitive and by no means novel, as it is well established that stabilizing selection reduces variation. Furthermore, this approach is empirically justified, as inter-individual variation in developmental dynamics have been previously used, albeit to a lesser extent than macro-evolutionary focused approaches, to described developmental hourglasses (Cruickshank et al. 2008, Liu et al. 2020). However, macro-evolutionary approaches, most notably transcriptome age indexing, have become the primary way of studying ontogenetic evolutionary constraints (Domazet-Lošo et al. 2010). While this is not inherently problematic beyond possible noise (as previously described), our understanding of ontogenetic evolution could be improved by linking macro-evolutionary patterns with the underlying micro-evolutionary processes. To this end, I believe formalizing an approach for leveraging extant patterns of population variation in developmental dynamics may be useful for making such inferences, which is what this manuscript hopes to accomplish.

The majority of previous investigations into the evolutionary dynamics of developmental programs have focused on embryonic development, with an even more specific bias towards vertebrates. Therefore, here I focused my empirical case study on examining patterns of selective constraints across a the holometabolous development of the metamorphic insect *D. plexippus*. This showed that the transitions from larva to pupa and from pupa to adult were accompanied by significantly reduced transcriptional variation, while variation increased during development within larvae and pupae. This suggests that metamorphic transitions are accompanied by significant selective constraint, while development within a given life stage may be more free to vary. However,

this inference warrants caution, as it is possible that the decreases in transcriptional dispersion associated with metamorphic transitions were not driven by variation in transcriptional dynamics, but rather because the sampling of early pupae and newly eclosed adults was done with more precise relative times. In other words, the increased variation seen in later larval and pupal development may be attributed to differences in developmental rates that would have been removed by more precise sampling of early pupae and newly eclosed adults in relative ontogenetic time (day after pupation and day of eclosion). In this case, it may still be interesting if selective constraints are acting against heterochrony associated with metamorphic transitions, but more temporal resolution in expression profiles would be needed for further investigation. Nonetheless, these findings are consistent with previous studies, which have found evidence of increased regulatory conservation and decreased inter-individual variation early in pupal development (Artieri et al. 2010, Aleksandra M. Ozerova et al. 2025). Consistent with this decreased variation, there is also evidence that at least part of the embryonic developmental program is recapitulated in pupal development (Alexandra M. Ozerova et al. 2022, Aleksandra M. Ozerova et al. 2025). However, to my knowledge, the reduced transcriptional variation associated with eclosion in adults has yet to be described, as previous studies have typically focused on older adults. Therefore, future studies would be needed to discern the generality of this pattern.

Overall, the presented framework will hopefully facilitate future studies that focus on linking the well established macro-evolutionary patterns to micro-evolutionary processes across diverse taxa and developmental programs. In this, future focus on understanding selective constraints in non-embryonic development will improve our general understanding of developmental evolution throughout natural history.

Data and code availability

All code written for model simulations, output analysis, and visualization, are available on GitHub at `https://github.com/gabe-dubose/evontogeny/tree/main/scripts`. To aid in model exploration, I also wrote a small Python package for running simulations, which is available at `https://github.com/gabe-dubose/evontogeny`. All code is also archived via Zenodo at `https://doi.org/10.5281/zenodo.17107808`.

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References

Artieri, Carlo G. and Rama S. Singh (2010). "Molecular evidence for increased regulatory conservation during metamorphosis, and against deleterious cascading effects of hybrid breakdown in Drosophila". In: *BMC Biology* 8.1, p. 26. DOI: 10.1186/1741-7007-8-26.

Baer, Karl Ernst von (1828). *Über Entwickelungsgeschichte der Thiere: Beobachtung und Reflexion*. Königsberg: Bornträger.

Cheng, Xuanjin et al. (2015). "A "Developmental Hourglass" in Fungi". In: *Molecular Biology and Evolution* 32.6, pp. 1556–1566. DOI: 10.1093/molbev/msv047.

Cordero, Gerardo A., Marcelo R. Sánchez-Villagra, and Ingmar Werneburg (2020). "An irregular hourglass pattern describes the tempo of phenotypic development in placental mammal evolution". In: *Biology Letters* 16.5, p. 20200087. DOI: 10.1098/rsbl.2020.0087.

Cruickshank, Tami and Michael J. Wade (2008). "Microevolutionary support for a developmental hourglass: gene expression patterns shape sequence variation and divergence in *Drosophila*". In: *Evolution & Development* 10.5, pp. 583–590. DOI: 10.1111/j.1525-142X.2008.00273.x.

Domazet-Lošo, Tomislav and Diethard Tautz (2010). "A Phylogenetically Based Transcriptome Age Index Mirrors Ontogenetic Divergence Patterns". In: *Nature* 468.7325, pp. 815–818. DOI: 10.1038/nature09632.

Drost, Hajk-Georg et al. (2015). "Evidence for Active Maintenance of Phylotranscriptomic Hourglass Patterns in Animal and Plant Embryogenesis". In: *Molecular Biology and Evolution* 32.5, pp. 1221–1231. DOI: 10.1093/molbev/msv012.

Drost, Hajk-Georg et al. (2017). "Cross-kingdom comparison of the developmental hourglass". In: *Current Opinion in Genetics & Development* 45, pp. 69–75. DOI: 10.1016/j.gde.2017.03.003.

DuBose, James G. and Jacobus C. de Roode (2024). "The link between gene duplication and divergent patterns of gene expression across a complex life cycle". In: *Evolution Letters* 8.5, pp. 726–734. DOI: 10.1093/evlett/qrae028.

— (2025). "Extensive transcriptional differentiation and specialization of a single-host parasite aligns with niche turnover generated by its host's metamorphosis". In: *International Journal for Parasitology* 55.6, pp. 273–279. DOI: 10.1016/j.ijpara.2025.01.006.

Duboule, Denis (1994). "Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony". In: *Development* 1994.Supplement, pp. 135–142. DOI: 10.1242/dev.1994.Supplement.135.

Haag, Eric S. and John R. True (2021). "Developmental System Drift". In: *Evolutionary Developmental Biology: A Reference Guide*. Ed. by Laura Nuño de la Rosa and Gerd B. Müller. Cham: Springer International Publishing, pp. 99–110. DOI: 10.1007/978-3-319-32979-6_83.

Haldane, J. B. S. (1932). "The time of action of genes, and its bearing on some evolutionary problems". In: *The American Naturalist* 66.702, pp. 5–24. DOI: 10.1086/280406.

Irie, Naoki and Shigeru Kuratani (2014). "The developmental hourglass model: a predictor of the basic body plan?" In: *Development* 141.24, pp. 4649–4655. DOI: 10.1242/dev.107318.

Liu, Jialin et al. (2020). "Inter-embryo gene expression variability recapitulates the hourglass pattern of evo-devo". In: *BMC Biology* 18.1, p. 129. DOI: 10.1186/s12915-020-00842-z.

Ma, Fuqiang and Chaogu Zheng (2023). "Transcriptome age of individual cell types in *Caenorhabditis elegans*". In: *Proceedings of the National Academy of Sciences* 120.9, e2216351120. DOI: 10.1073/pnas.2216351120.

Moran, Nancy A. (1994). "Adaptation and constraint in the complex life cycles of animals". In: *Annual Review of Ecology, Evolution, and Systematics* 25, pp. 573–600.

Ozerova, Aleksandra M. et al. (2025). "Temporal Dynamics of Gene Expression During Metamorphosis in Two Distant Drosophila Species". In: *Genome Biology and Evolution* 17.6, evaf100. DOI: 10.1093/gbe/evaf100.

Ozerova, Alexandra M. and Mikhail S. Gelfand (2022). "Recapitulation of the embryonic transcriptional program in holometabolous insect pupae". In: *Scientific Reports* 12.1, p. 17570. DOI: 10.1038/s41598-022-22188-y.

R Core Team (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Vienna, Austria. URL: https://www.R-project.org/.

Raff, Rudolf A. (1996). *The Shape of Life: Genes, Development, and the Evolution of Animal Form*. Chicago: University of Chicago Press.

Reiss, John O. (2002). "The phylogeny of amphibian metamorphosis". In: *Zoology* 105.2, pp. 85–96. DOI: 10.1078/0944-2006-00059.

Servén, D. and C. Brummitt (2018). *pyGAM: Generalized Additive Models in Python*. DOI: 10.5281/zenodo.1208723.

Virtanen, Pauli et al. (2020). "SciPy 1.0: Fundamental Algorithms for Scientific Computing in Python". In: *Nature Methods* 17, pp. 261–272. DOI: 10.1038/s41592-019-0686-2.

Wheeler, Ward C. et al. (n.d.). "The Phylogeny of the Extant Hexapod Orders". In: *Cladistics* 17.2 (), pp. 113–169. DOI: 10.1111/j.1096-0031.2001.tb00115.x.

Wu, Longjun, Kailey E Ferger, and J David Lambert (2019). "Gene Expression Does Not Support the Developmental Hourglass Model in Three Animals with Spiralian Development". In: *Molecular Biology and Evolution* 36.7, pp. 1373–1383. DOI: 10.1093/molbev/msz065.

Yanai, Itai et al. (2011). "Mapping Gene Expression in Two Xenopus Species: Evolutionary Constraints and Developmental Flexibility". In: *Developmental Cell* 20.4, pp. 483–496. DOI: 10.1016/j.devcel.2011.03.015.