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Heterokairic Genes and the Eco-Evo-Devo of Timing

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ABSTRACT

Concepts of developmental timing have traditionally been framed under heterochrony as evolved (genetically based) differences in timing, while environmentally induced shifts in timing within genotypes have been treated more loosely. In this article, heterokairy is presented as plasticity in the timing of developmental events, and the term "heterokairic genes" is proposed for environmentally modulated heterochronic genes that underlie this plasticity. Evidence from nematodes, insects, plants, and vertebrates is assembled, with emphasis placed on systems where environmental cues are relayed through endocrine or metabolic pathways to known timing modules/genes. On this basis, a distinction is drawn between validated heterokairic genes, supported by direct mechanistic data, and a broader set of candidates inferred from gene–environment interactions in developmental timing. The eco-evolutionary consequences of such genes are considered, and experimental and genomic strategies for their identification are outlined. It is argued that heterokairic genes provide a useful bridge between environmental variation, developmental mechanisms, and evolutionary change in timing.

1 | Introduction

Changes in the timing of developmental events have long been recognized as a major route by which morphology and life histories evolve. The classical concept of heterochrony describes shifts in the onset, rate, or duration of developmental processes relative to an ancestral state, and has been used to interpret a wide range of evolutionary transformations in body size, shape, and life-cycle structure (Keyte and Smith 2014). Mechanistic work has shown that such timing shifts are not merely descriptive patterns but arise from alterations in cellular and molecular "timekeeping" mechanisms that operate during embryogenesis (Rougvie 2005; Moss 2007; Monsalve and Frand 2012; Keyte and Smith 2014). Within this framework, heterochronic genes have been defined as genes whose perturbation alters the temporal component of cell fate or developmental programs, thereby shifting when specific events occur (Moss 2007). These genes, first characterized in *Caenorhabditis elegans* and subsequently in other animals and plants, are now viewed as components of conserved timing networks that integrate intrinsic developmental schedules with other patterning information (Rougvie 2005; Moss 2007; Monsalve and Frand 2012). Classical treatments have emphasized intrinsic timekeeping, yet it has been repeatedly noted that these mechanisms operate in environments that fluctuate in temperature, nutrition, oxygen, density, and other factors, and that extrinsic cues can influence the tempo of development (Rougvie 2005; Monsalve and Frand 2012).

A complementary concept, heterokairy, was introduced to capture environmentally induced shifts in developmental timing within a species. In the original formulation, heterokairy was defined as plasticity in the timing of physiological development in an individual of a given genotype, in response to environmental influences (Spicer and Rundle 2007). Subsequent work generalized this definition to include changes in the timing of morphological or physiological landmarks within a population, treating heterokairy as a specific form of developmental plasticity in ontogenetic schedules rather than as an evolutionary comparison between lineages (Rundle and Spicer 2016; Spicer

Summary

- Defines heterokairic genes as environmentally modulated heterochronic regulators of developmental timing.
- Surveys cross-taxon mechanisms and eco-evo-devo roles of timing plasticity.

et al. 2018). This framing has been used to organize trait-based studies in comparative developmental physiology and developmental plasticity, and to argue that altered developmental timing should be considered alongside changes in trait magnitude or form when phenotypic plasticity is analyzed (Spicer and Rundle 2007; Rundle and Spicer 2016; Spicer et al. 2018; Burggren 2021).

Empirical examples indicate that heterokairy is common. Shifts in the timing of physiological or behavioral milestones, such as the onset of ventilation, osmoregulatory performance, or hatching, have been documented across invertebrates and vertebrates in response to temperature, salinity, hypoxia, and other stressors (Spicer and Rundle 2007; Tills et al. 2010; Spicer et al. 2018). In amphibians, insects, and nematodes, plastic variation in the age or size at metamorphosis or in the number and duration of larval instars has been interpreted as heterokairy at key ontogenetic switch points (Rundle and Spicer 2016; Burggren 2021; Edwards and Smallegange 2025). Plastic shifts in the timing of ornament initiation, such as social-environment–dependent onset of egg-spot formation in cichlids, extend heterokairy to sexually selected pigmentation traits as well as life-history transitions (Clark et al. 2024). Recent eco-evolutionary work has suggested that such plastic shifts in developmental rate and schedule can feed back on population dynamics, for example, when density-dependent growth interacts with heterokairy in the expression of alternative morphs or life histories (Edwards and Smallegange 2025). In parallel, developmental physiologists have called for heterokairy to be treated explicitly as a core concept, arguing that intraspecific changes in the timing of developmental landmarks can illuminate how plasticity is generated and how early-life environments shape fitness (Spicer et al. 2018; Burggren 2021).

Despite these advances at the trait level, the gene-level basis of heterokairy has remained comparatively underexplored. It has been widely appreciated that extrinsic inputs such as nutrition and temperature can impinge on developmental timing pathways—for example, through insulin/insulin-like growth factor (IGF) signaling, steroid hormones, or other endocrine and metabolic mediators—but most mechanistic studies have been framed in terms of heterochrony or generic environmental modulation of development rather than in terms of heterokairy (Rougvie 2005; Monsalve and Frand 2012). In plants, work on vegetative phase change has shown that the decline of the heterochronic regulator miR156 and the rise of its SPL transcription factor targets are not purely autonomous but are modulated by sugars and multiple phytohormones, linking resource status and hormonal environment to phase-transition timing (Poethig and Fouracre 2024). Comparable principles are increasingly being recognized in animals, where endocrine axes and metabolic state govern the timing of puberty, metamorphosis, and other transitions that have historically been described in heterochronic terms (Rougvie 2005; Monsalve and Frand 2012).

At the same time, studies of "heterochronic genes" have expanded beyond their original worm and fly context. Heterochronic gene expression and regulatory interactions are now being examined in systems ranging from polyphenic marine annelids to vertebrate life-history polymorphisms, highlighting that timing mechanisms can be redeployed and modified in a broad range of developmental trajectories (Moss 2007; Harry and Zakas 2024). In fishes, for instance, the transcriptional cofactor vgll3, a component of the Hippo pathway, has emerged as a major determinant of age at sexual maturation in Atlantic salmon, and recent work has linked allelic differences at this locus to differences in testicular developmental schedules and to seasonal patterns of lipid storage and utilization (Ahi et al. 2024, 2025c). These findings have positioned vgll3 as a heterochronic gene whose effects are intertwined with environmental signals related to energy balance and seasonality, and they illustrate how genetic and environmental effects on timing can be tightly coupled (Ahi et al. 2024, 2025c). The term "heterokairic genes" has begun to appear only sporadically and without a unified definition, typically to describe developmental timing genes whose effects are thought to depend on environmental context. Yet the theoretical appeal of such genes is clear: if heterokairy is to be understood mechanistically, the environmentally responsive components of timing networks must be identified, and their roles in generating plastic developmental schedules must be characterized (Spicer et al. 2018; Burggren 2021).

In this article, heterokairic genes are considered to lie at the interface of environment, development, and evolution when they satisfy two conditions: (i) they contribute to the control of developmental timing, and (ii) they are modulated, directly or indirectly, by environmental variation during the relevant developmental window. Building on existing work on heterochrony, heterokairy and developmental timing networks, this review aims to (i) clarify the relationship between heterochrony and heterokairy, (ii) propose a working definition and classification for heterokairic genes as environmentally modulated heterochronic genes, (iii) synthesize direct and emerging evidence for such genes across taxa, and (iv) outline how these genes may shape reaction norms for developmental time and contribute to adaptive evolution.

2 | From Heterochrony to Heterokairy: Definitions and Levels of Analysis

Heterochrony has classically been defined as an evolutionary change in the timing or rate of development, measured relative to an inferred ancestor or to other taxa within a phylogeny (McNamara 2012; Keyte and Smith 2014) (Figure 1A). In this sense, heterochrony is anchored at the macro-evolutionary scale: paedomorphosis and peramorphosis are described as outcomes of altered onset, offset, or rate of growth, and these shifts are used to explain morphological divergence among lineages (McNamara 2012; Keyte and Smith 2014). Methods for detecting such changes, including event-pairing and subsequent parsimony-based approaches, were developed explicitly to infer heterochrony from comparative developmental sequences mapped onto phylogenies (McNamara 2012; Keyte and Smith 2014). This comparative emphasis has ensured that heterochrony

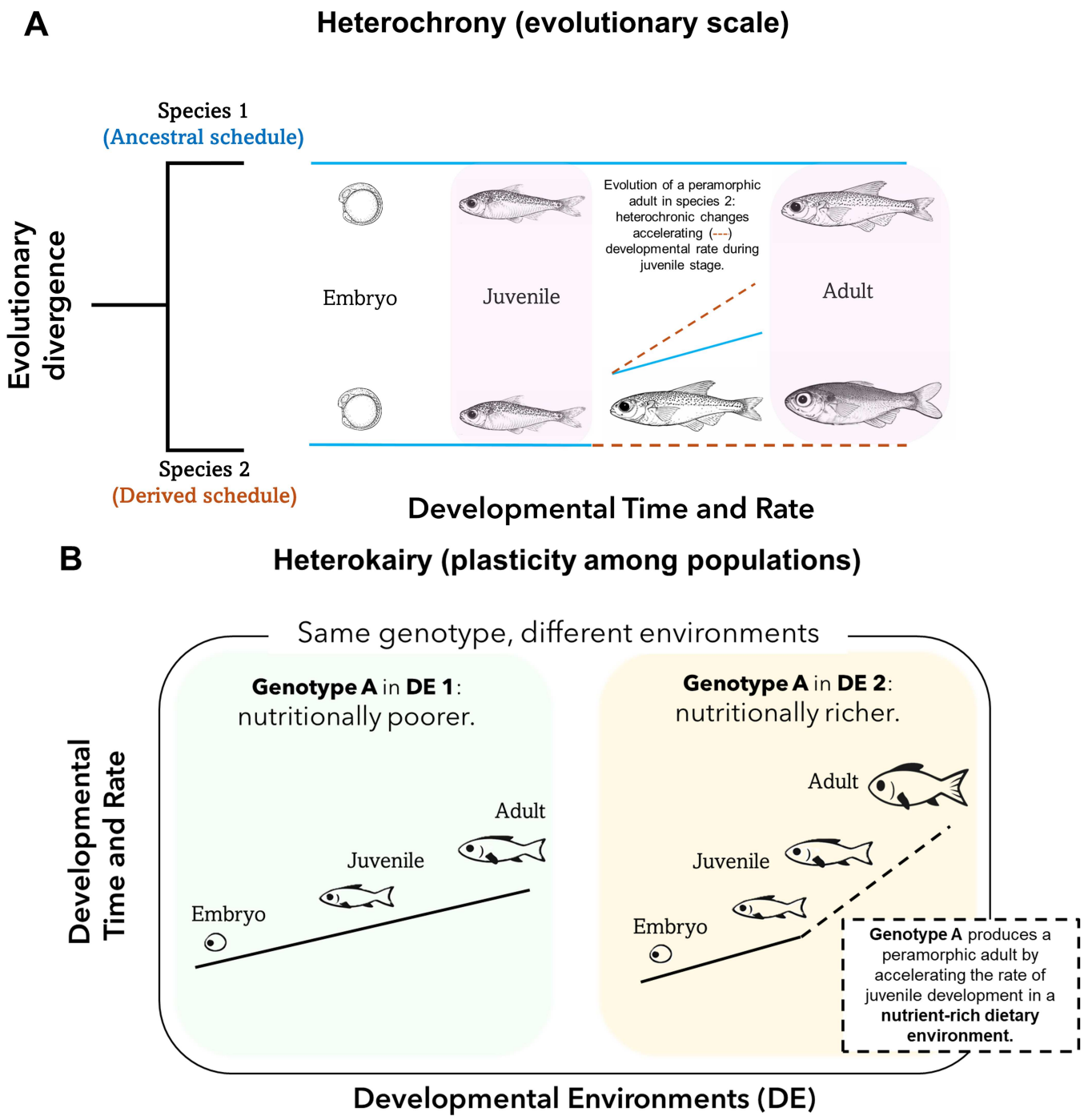

FIGURE 1 | From heterochrony to heterokairy and heterokairic genes. (A) Heterochrony evolutionary scale: Heterochrony is defined as fixed, evolved differences in developmental timing and rate between lineages. Here, we illustrate this phenomenon by showing the evolution of a peramorphic adult in Species 2 resulting from the acceleration of its juvenile developmental rate (derived schedule) when compared with Species 1, keeping the ancestral schedule. Ancestral development timeline: blue arrow; Derived development timeline: orange dashed arrow; The pink bar shows the ontogenetic window where heterochronic changes occur between two species: juvenile and adult. In this example, the time remains the same, but the transition from juvenile to adult accelerates in Species 2 (shown by the slopes of the orange dashed and blue lines). (B) Heterokairy (plasticity among populations). Heterokairy is the plasticity of development in time and rate among populations. Here, we illustrate this phenomenon by showing the reaction norms of genotype A in two developmental environments (DEs), one nutritionally poorer (DE 1, light green) and the other richer (DE 2, light orange). Reaction norms are shown by straight (DE 1) and dashed (DE 2) lines and represent the rate of development. In DE 2, genotype A induces accelerated juvenile development (dashed line), resulting in a peramorphic adult when compared to genotype A individuals growing in DE 1.

remains a central concept in evolutionary developmental biology, but it has also encouraged a focus on between-lineage differences rather than within-genotype plasticity (McNamara 2012; Keyte and Smith 2014; de Abreu e Melo-Moreira et al. 2025). By contrast, heterokairy was proposed to describe plastic shifts in developmental timing and rate within individuals or populations of a species (Spicer and Burggren 2003; Spicer and Rundle 2007) (Figure 1B). In the original physiological formulation, heterokairy

was defined as plasticity in the timing of the onset of a developmental event, especially the onset of a physiological regulatory system, during an individual's ontogeny (Spicer and Burggren 2003). This definition was later broadened to encompass morphological and behavioral events and to emphasize that heterokairy concerns changes in event timing or sequence expressed by a given genotype in different environments, rather than evolutionary changes between ancestral and descendant ontogenies (Spicer and Rundle 2007; Rundle and Spicer 2016). Heterokairy has thus been framed explicitly as a form of developmental plasticity in ontogenetic schedules (Spicer and Rundle 2007; Rundle and Spicer 2016; Spicer et al. 2018).

The distinction between heterochrony and heterokairy has been stressed repeatedly, because both concepts involve developmental timing but operate at different comparative scales (Spicer and Burggren 2003; Denoël and Ficetola 2014; Spicer et al. 2018). Heterochrony is typically diagnosed from fixed genetic differences in timing among lineages or populations, whereas heterokairy is inferred when individuals from a genotype or population exhibit environment-dependent variation in developmental timing (i.e., different timing reaction norms) across conditions (Spicer and Burggren 2003; Spicer and Rundle 2007; Rundle and Spicer 2016) (Figure 1A,B). In practice, intraspecific variation in timing can reflect both genetically based heterochronic differences among genotypes or populations and environmentally induced (heterokairic) variation within genotypes. Because these distinct sources of variation have sometimes been collectively labeled "heterochrony," this has contributed to terminological ambiguity (Spicer and Burggren 2003; Denoël and Ficetola 2014; Spicer et al. 2018). The introduction of heterokairy was intended to reserve heterochrony for evolutionary comparisons and to provide a term for plastic temporal shifts that occur within individuals or populations (Spicer and Burggren 2003; Denoël and Ficetola 2014; Spicer et al. 2018).

Quantitatively, heterochrony and heterokairy can both be characterized using similar tools—such as event-pairing or continuous analyses of developmental sequences—but the reference frame differs (McNamara 2012; Lamsdell 2021). In comparative heterochrony studies, event orders or normalized ages are compared among species and reconstructed at ancestral nodes (McNamara 2012). In heterokairy studies, the same event sequence or developmental trajectory is followed across environments, and environmental differences are treated as the independent axis along which reaction norms of event timing are measured (Spicer and Rundle 2007; Tills et al. 2013; Rundle and Spicer 2016; Spicer et al. 2018; Lofeu et al. 2025). This perspective has led to the notion of "developmental timing reaction norms," in which heterokairy is captured by changes in the slope or shape of the relationship between an environmental variable and the timing of a focal event (Rundle and Spicer 2016; Edwards and Smallegange 2025) (Figure 1B).

Because heterokairy is explicitly a plastic phenomenon, it has been linked to broader frameworks of phenotypic plasticity and eco-evolutionary dynamics (Moczek et al. 2011; Hendry 2016; Rundle and Spicer 2016; Burggren 2021; Smallegange 2022; Edwards and Smallegange 2025). Developmental plasticity has been argued to facilitate evolutionary innovation through genetic accommodation of environmentally induced phenotypes (Moczek et al. 2011), and heterokairy provides a specific case in which the plastic trait is the schedule of developmental events rather than their endpoint morphology or size (Moczek et al. 2011; Rundle and Spicer 2016; Smallegange 2022; Edwards and Smallegange 2025). Modeling work on gene regulatory networks further suggests that plastic responses themselves can bias the direction of evolutionary change, making environmentally induced states more likely evolutionary outcomes when selection repeatedly acts on them (Espinosa-Soto 2025). Conceptual work on eco-evolutionary dynamics has also emphasized that plasticity in traits affecting demography—such as age and size at maturation—can generate feedbacks between population dynamics and evolutionary change (Moczek et al. 2011; Hendry 2016; Smallegange 2022). Recent eco-phenotypic models have extended this logic to heterokairy, showing that density-dependent shifts in development rate can produce distinct eco-phenotypic pathways and "heterokairic polyphenisms" in which alternative morphs differ in development time (Smallegange 2022; Edwards and Smallegange 2025).

These conceptual developments motivate a closer examination of how heterokairy should be linked to underlying mechanisms. Timing differences can arise at multiple levels of organization. Work on developmental timing networks has emphasized that temporal control is distributed among interacting "clocks" or timers at cellular, tissue, and organismal scales, rather than being governed by a single master clock (Rougvie 2005; Moss 2007; Monsalve and Frand 2012). Timing information can be encoded in gene regulatory networks, oscillatory signaling pathways, hormonal cycles, chromatin dynamics, and cell-cycle or growth controls (Rougvie 2005; Moss 2007; Monsalve and Frand 2012; Harry and Zakas 2024; Poethig and Fouracre 2024). Some of these systems appear largely intrinsic, whereas others are known to be sensitive to nutritional, thermal, or other environmental signals (Rougvie 2005; Monsalve and Frand 2012; Smallegange 2022; Poethig and Fouracre 2024). This multilevel structure implies that heterokairy can, in principle, be generated by environmental modulation at any level, from gene expression kinetics to systemic endocrine axes.

Against this backdrop, we define heterokairic genes as genes that participate in developmental timing and whose activity is modulated by environmental context in ways that plausibly generate heterokairy. Conceptually, such genes can be viewed as environmentally responsive components of heterochronic pathways: they contribute to when developmental events occur, rather than only to whether they occur or what form they take, and their timing-related effects vary among environments (Spicer and Burggren 2003; Rougvie 2005; Moss 2007; Spicer et al. 2018; Harry and Zakas 2024; Poethig and Fouracre 2024). Two categories are distinguished for clarity. In this review, "validated" is used strictly in an evidential sense and indicates direct mechanistic support linking environmental modulation to shifts in developmental timing. It does not imply network centrality or special importance. Validated heterokairic genes are those for which direct evidence has been obtained that environmental variation alters gene activity or expression in a defined developmental window and that this modulation changes the timing of a developmental event. Candidate heterokairic genes are those that are known heterochronic regulators, or are strongly associated with timing variation, and for which environmental sensitivity has been documented, but where the causal chain from environmental cue to altered event timing has not yet been fully resolved (Spicer and Burggren 2003;

Rougvie 2005; Moss 2007; Spicer et al. 2018; Harry and Zakas 2024; Poethig and Fouracre 2024).

This working definition is intended to delimit the focus of the review. Genes that influence only trait magnitude or morphology without clear links to developmental timing are not considered heterokairic, nor are genes that respond to environmental cues outside the developmental windows in which timing decisions are made. By treating heterokairy and heterokairic genes as linked concepts—one phenotypic, one mechanistic—developmental plasticity in timing can be discussed in a way that remains consistent with classical heterochrony while highlighting the specific routes through which environmental variation enters developmental timing networks.

3 | Mechanistic Routes From Environment to Developmental Timing Networks

Environmental conditions are sensed and translated into changes in developmental timing through a series of intermediate regulatory layers rather than by direct action on most heterochronic genes. A recurring pattern across animals and plants is that external cues are encoded by metabolic state and endocrine signals, which then act on gene regulatory networks that control the onset, rate, or duration of key developmental programs (Rougvie 2005; Moss 2007; Monsalve and Frand 2012; Aguilaniu et al. 2016; Poethig and Fouracre 2024) (Figure 1C). In this way, environmental information is fed into timing circuits via a limited set of "relay nodes," while the downstream architecture of heterochronic pathways remains partly conserved (Rougvie 2005; Moss 2007; Monsalve and Frand 2012; Aguilaniu et al. 2016; Poethig and Fouracre 2024).

Nutritional status is one of the best-characterized inputs into developmental timing networks. In many animals, nutrient availability is translated into circulating insulin or IGF signals, which act through conserved intracellular cascades to regulate growth rate, developmental progression, and body size (Rougvie 2005; Suzawa and Bland 2023). Work in *Drosophila* has shown that reduced insulin signaling slows larval growth and extends total developmental time, whereas increased signaling accelerates growth and advancement through larval stages (Koyama et al. 2014). These effects have been linked to insulin/IGF control of ecdysone synthesis and release, with nutrient-sensitive TOR signaling in the prothoracic gland and fat body modulating ecdysone production and thereby the timing of metamorphic hormone pulses (Rewitz et al. 2013; Yamanaka et al. 2013; Koyama et al. 2014). Similar logic applies in vertebrates, where variation in food intake and temperature is reflected in changes in growth hormone–IGF signaling that affect growth trajectories and the timing of maturation (Suzawa and Bland 2023). Nutritional modulation of insulin/IGF pathways, therefore, represents a major route by which environmental conditions are coupled to developmental clocks.

Temperature provides a second pervasive environmental input. Basic kinetic effects on biochemical reactions are expected, but specific regulatory mechanisms have also been identified. In invertebrates and vertebrates, changes in ambient temperature can alter insulin signaling, TOR activity, and endocrine outputs independently of nutritional status, yielding temperature-dependent shifts in growth rate and developmental duration (Rougvie 2005; Mortzfeld et al. 2019; Suzawa and Bland 2023). In Hydra, for example, temperature and insulin/FoxO signaling have been shown to converge on Wnt and TGF-β pathways that jointly control the timing of a developmental switch between growth and asexual reproduction, thereby linking environmental temperature to life-history timing through a defined signaling module (Mortzfeld et al. 2019). Temperature effects on growth-hormone and reproductive endocrine axes have similarly been documented in other taxa, indicating that thermal cues can reshape developmental schedules through modulation of hormone systems already involved in growth and maturation (Mortzfeld et al. 2019; Suzawa and Bland 2023).

Steroid hormones occupy a central position in timing networks in many animals. In insects, pulses of the steroid hormone ecdysone coordinate molting and metamorphosis, and the temporal pattern of ecdysone production has been shown to depend on both internal checkpoints and environmental inputs (Rewitz et al. 2013; Yamanaka et al. 2013; Koyama et al. 2014). Nutritional state influences ecdysone synthesis via insulin and TOR signaling in the prothoracic gland, creating nutritional checkpoints that delay or advance the attainment of a critical weight and the subsequent ecdysone pulses (Layalle et al. 2008; Koyama et al. 2014). Photoperiod and temperature can modulate neuropeptides such as prothoracicotropic hormone, which, in turn, regulate ecdysone production and thereby adjust metamorphic timing to seasonal conditions (Rewitz et al. 2013; Yamanaka et al. 2013). Together, these pathways form a layered system in which information on body size, energy reserves, and environmental context is integrated to time the transition between larval and pupal or adult stages (Rewitz et al. 2013; Yamanaka et al. 2013; Koyama et al. 2014).

An analogous role is played by nuclear hormone receptors and their ligands in nematodes. In *Caenorhabditis elegans*, the nuclear receptor *daf-12* and its bile acid–like ligands, the dafachronic acids, govern the decision between continued reproductive development and entry into the dauer diapause, as well as the timing of later developmental events (Rougvie 2005; Fielenbach and Antebi 2008; Aguilaniu et al. 2016). Production of dafachronic acids depends on environmental conditions, including food availability, pheromone levels, and temperature, via upstream sensory, insulin/IGF, and TGF-β pathways (Fielenbach and Antebi 2008; Schaedel et al. 2012; Aguilaniu et al. 2016). *daf-12* activity has been shown to modulate expression of *let-7* family microRNAs in a feedback circuit that integrates environmental signals into the heterochronic pathway, thereby linking steroid signaling to the rate at which developmental timing genes are activated (Hammell et al. 2009; Schaedel et al. 2012). This system exemplifies how hormonal relays can couple environmental information to the timing of developmental events via defined heterochronic modules.

In plants, developmental phase transitions are also linked to resource status and environmental signals through a combination of metabolic and hormonal pathways. The juvenile-to-adult vegetative transition is controlled by the miR156–SPL module, in which high levels of miR156 maintain juvenile identity and decreasing miR156 allows SPL transcription factors to promote adult traits (Poethig and Fouracre 2024; Yang et al. 2013). Experimental manipulations and genetic studies have shown that exogenous sugar application reduces miR156 abundance, whereas impaired photosynthesis or reduced carbohydrate

availability increases it, thereby delaying phase change (Yang et al. 2013). These sugar effects are mediated in part by trehalose-6-phosphate signaling, which connects carbohydrate status to miR156 expression and SPL activity. Several phytohormones and epigenetic regulators have been found to modulate the same pathway, suggesting that multiple environmental inputs are funneled into a central timing module that controls the schedule of vegetative development (Yang et al. 2013; Poethig and Fouracre 2024).

Other environmental factors, including hypoxia, salinity, and population density, can influence developmental timing through less well-characterized routes. Hypoxia has been reported to alter the timing of hatching and the onset of cardiorespiratory function in aquatic embryos, often via changes in endocrine and metabolic status rather than direct effects on heterochronic genes (Spicer and Rundle 2007; Tills et al. 2013; Spicer et al. 2018). Salinity and osmotic stress can similarly shift the timing of osmoregulatory maturation in developing aquatic organisms, again implicating hormonal and transcriptional adjustments in endocrine organs (Spicer and Rundle 2007; Tills et al. 2013; Spicer et al. 2018). Population density has been shown to affect growth rates and developmental schedules in a range of taxa, and modeling work has suggested that density-dependent feedbacks acting on endocrine or metabolic pathways can generate heterokairy and even distinct alternative developmental trajectories (Suzuki and Toh 2021; Smallegange 2022; Edwards and Smallegange 2025). Although the molecular details of these pathways are less well resolved than for nutrition and temperature, they are likely to converge on the same classes of timing modules: gene regulatory networks, hormonal cycles, and growth regulators that determine when key thresholds are reached.

Across these examples, a common structure can be discerned. Environmental cues are detected by sensory systems or tissues (e.g., chemosensory neurons, photoreceptors or leaves), converted into changes in metabolic state and endocrine output (such as insulin/IGF, steroid hormones or plant hormones), and then integrated by gene regulatory networks that include recognized heterochronic components (microRNAs, transcription factors, chromatin regulators) (Rougvie 2005; Moss 2007; Fielenbach and Antebi 2008; Monsalve and Frand 2012; Yamanaka et al. 2013; Yang et al. 2013; Aguilaniu et al. 2016; Poethig and Fouracre 2024) (Figure 1C). Developmental progression is often organized around checkpoints that require specific physiological or morphological criteria to be fulfilled before a transition can proceed, such as attainment of a critical weight or a threshold hormone concentration (Layalle et al. 2008; Rewitz et al. 2013; Suzuki and Toh 2021). Environmental modulation of growth rate, hormone synthesis, or signal sensitivity can shift the timing at which these thresholds are reached or reset, thereby altering the schedule of developmental events without necessarily changing the underlying sequence.

These mechanistic routes from environment to timing networks define the context in which heterokairic genes operate. Genes that lie at the intersection of endocrine and metabolic relays with heterochronic pathways—such as nuclear hormone receptors, hormone biosynthetic enzymes, nutrient-sensing kinases, and environmentally responsive microRNAs—are positioned to mediate heterokairy by altering the timing of downstream developmental programs when external conditions change (Figure 1C).

4 | Heterokairic Genes Across Taxa

Heterokairic genes, as defined here, are heterochronic regulators whose activity is modulated by environmental context in ways that plausibly shift the timing of developmental events. On the basis of current evidence, a small set of genes can be treated as "validated" heterokairic genes, with direct mechanistic support for both timing and environmental sensitivity, while a broader set of "candidate" genes shows strong but incomplete evidence for this dual role (Figure 2 and Table 1).

4.1 | Nematodes: daf-12, daf-16 and Heterochronic MicroRNAs

In *C. elegans*, the nuclear hormone receptor *daf-12* is a central regulator of both developmental age and the decision between reproductive growth and dauer diapause. Loss- and gain-of-function analyses showed that *daf-12* controls stage-appropriate cell fates, dauer entry, and adult longevity, establishing it as a heterochronic gene that acts at the convergence of dauer and developmental timing pathways (Antebi et al. 2000; Fielenbach and Antebi 2008). Environmental cues such as food scarcity, crowding pheromone, and temperature are transduced into insulin/IGF and TGF-β signaling changes, which in turn alter dafachronic acid synthesis and thus the ligand state of *daf-12* (Fielenbach and Antebi 2008). A key advance was the discovery that *daf-12* regulates a network of *let-7* family microRNAs and their targets, forming a feedback circuit that integrates environmental signals with the heterochronic pathway (Hammell et al. 2009). *let-7* and related miRNAs had already been established as validated heterochronic regulators at that time, the transition to adulthood by controlling the terminal differentiation of seam cells (Reinhart et al. 2000; Großhans et al. 2005). The *daf-12–let-7* circuit links environmental inputs, via steroid hormone signaling, to the rate at which the heterochronic cascade proceeds, thereby altering the schedule of post-embryonic events without changing their order (Reinhart et al. 2000; Großhans et al. 2005; Fielenbach and Antebi 2008; Hammell et al. 2009). This combination of a clear timing role and direct environmental modulation justifies treating *daf-12* and its associated *let-7* family miRNAs as validated heterokairic components.

The FOXO transcription factor *daf-16* provides a complementary route by which the environment influences timing. DAF-16 is activated under low insulin/IGF and TGF-β signaling, conditions that promote dauer formation in adverse environments (Fielenbach and Antebi 2008). Recent work has shown that in dauer larvae, DAF-16 prevents precocious adoption of adult cell fates by upregulating the heterochronic gene *lin-41*, thereby maintaining stem-cell-like properties of seam cells until favorable conditions resume (Wirick et al. 2021). The developmental timing regulator hbl-1, a transcription factor that specifies L2 versus L3 cell fates, also modulates the dauer formation decision through interactions with dauer-promoting pathways (Karp and Ambros 2011). Together, these studies indicate that heterochronic genes such as lin-41 and hbl-1 sit downstream of environmentally responsive factors (*daf-12, daf-16*) and are redeployed when larvae take the dauer route. The *daf-12–let-7–lin-41* module in particular exemplifies a validated heterokairic system: environmental information is captured by steroid

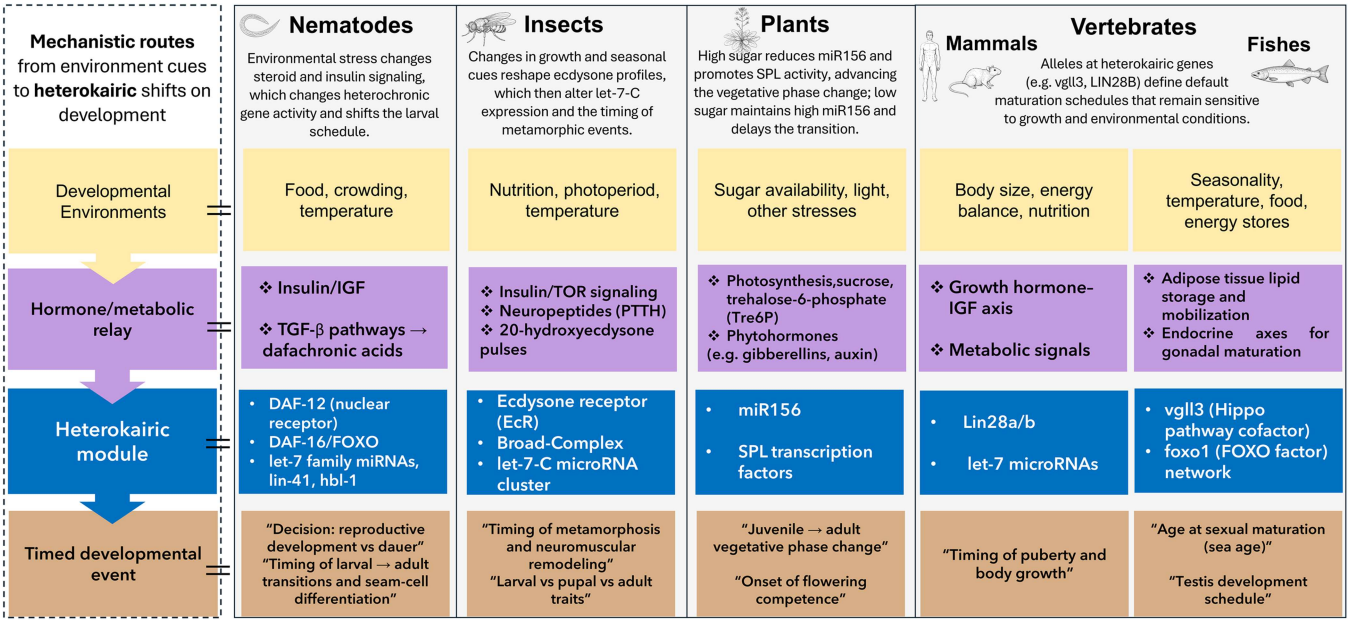

FIGURE 2 | Mechanistic routes from environment to heterokairic genes across taxa. Each column illustrates a simplified pathway by which external conditions are relayed to developmental timing genes (detailed in the first column). Cues from Developmental Environments (top yellow row) are converted into endocrine and metabolic signals (second purple row), which act on heterochronic regulators whose activity depends on the environment (third blue row). These heterokairic modules then control the timing of key developmental transitions (bottom brown row). In *Caenorhabditis elegans* (second column), insulin/IGF and TGF-β pathways regulate dafachronic acids and the nuclear receptor DAF-12, together with DAF-16/FOXO and let-7 family microRNAs, to adjust dauer entry and larval timing. In insects (third column), nutrient- and season-dependent changes in insulin/TOR and neuropeptides shape ecdysone pulses, which activate the let-7-C microRNA cluster and thereby control the timing of metamorphic remodeling. In plants (fourth column), sugar and hormone signals converge on the miR156–SPL module to regulate the juvenile-to-adult vegetative phase change and flowering competence. In vertebrates (fifth column), the Lin28/let-7 axis (mammals) and the Hippo cofactor vgll3 (fishes) respond to growth and energy balance, influencing puberty onset and age at maturation. Together, these examples illustrate conserved architectures in which endocrine and metabolic relays feed into heterokairic genes that shape developmental schedules.

and insulin signaling and passed to heterochronic genes that adjust the timing of developmental progression.

4.2 | Insects: Ecdysone-Regulated let-7-C and Metamorphic Timing

In insects, heterochronic functions have been assigned to the let-7-C microRNA cluster, which is expressed in a pulse around the onset of metamorphosis and regulates late larval and pupal events. Temporal expression profiling revealed that *Drosophila* let-7 is absent during early larval stages and induced sharply at the larval–pupal transition, in concert with the major ecdysone pulse (Sempere et al. 2002). Functional analyses have shown that let-7 is required for correct timing of neuromuscular remodeling and neuronal specification during metamorphosis, and that its misexpression leads to inappropriate persistence of larval traits or premature adult differentiation (Sempere et al. 2002; Kucherenko et al. 2012). Mechanistically, the steroid hormone 20-hydroxyecdysone (ecdysone) directly activates the let-7-C locus via the ecdysone receptor (EcR) and the Broad-Complex, establishing a hormone–miRNA cascade that links endocrine timing cues to developmental events (Chawla et al. 2016). Culture experiments in S2 cells demonstrated that ecdysone treatment is sufficient to induce let-7 expression in an EcR- and Broad-Complex-dependent manner, while genetic perturbations of EcR or its cofactors reduce or abolish let-7 induction in vivo (Chawla et al. 2016). Because ecdysone production is itself controlled by nutrient-dependent insulin/TOR signaling and by photoperiod and temperature via neuroendocrine inputs (Yamanaka et al. 2013), let-7-C sits at the interface between environmental conditions and the temporal control of metamorphic remodeling. This makes the ecdysone–let-7-C axis a strong candidate for inclusion among validated heterokairic genes.

4.3 | Plants: miR156–SPL as a Sugar- and Hormone-Responsive Timing Module

In flowering plants, the miR156–SPL module is a well-characterized heterochronic system controlling the juvenile-to-adult vegetative phase change. High levels of miR156 maintain juvenile traits, while declining miR156 allows SPL transcription factors to promote adult leaf morphology and competence to flower (Poethig and Fouracre 2024). Genetic and transgenic studies in *Arabidopsis* and other species have established that altering *miR-156* or *SPL* expression changes the timing of phase change and flowering, without fundamentally altering the sequence of developmental stages (Poethig and Fouracre 2024). Crucially, miR156 is not purely autonomously regulated. Exogenous sucrose reduces the abundance of pri-MIR156A/C transcripts, while reduced photosynthesis or sugar deprivation increases miR156 levels and delays phase change (Yang et al. 2013). These sugar effects are mediated in part by the trehalose-6-phosphate pathway, which connects carbohydrate

TABLE 1 | Examples of heterokairic genes and gene modules across taxa.

Taxon/system	Gene/module	Type	Env. cue(s)	Timing process	References
Caenorhabditis elegans	daf-12 + let-7 + lin-41	Nuclear receptor + miRNAs	Food, pheromone, temp.	Dauer versus growth; larval–adult timing	Antebi et al. (2000); Fielenbach and Antebi (2008); Hammell et al. (2009); Reinhart et al. (2000); Großhans et al. (2005)
C. elegans	daf-16 → lin-41, hbl-1	FOXO + timing TFs	Low insulin/TGF-β	Block adult fates in dauer	Fielenbach and Antebi (2008); Karp and Ambros (2011); Wirick et al. (2021)
Drosophila	20E–EcR/Broad → let-7-C	Steroid hormone, receptor, miRNAs	Nutrition, photoperiod, temp.	Metamorphosis onset; remodeling	Sempere et al. (2002); Chawla et al. (2016); Yamanaka et al. (2013); Rewitz et al. (2013)
Flowering plants	miR156–SPL	miRNA + TFs	Sugar, Tre6P, hormones	Juvenile → adult phase change; flowering competence	Poethig and Fouracre (2024); Yang et al. (2013); Fichtner and Lunn (2021)
Flowering plants	FLC–FT	Floral TFs	Vernalization, photoperiod	Floral transition timing	Takagi et al. (2023); Huang et al. (2024)
Mammals	Lin28/let-7 axis	RNA-binding + miRNAs	Body size, nutrition, energy	Puberty and growth timing	Ong et al. (2009); Sangiao-Alvarellos et al. (2013); Cao et al. (2020); Robinton et al. (2019); Thevarajan et al. (2025)
Atlantic salmon	vgll3 + Hippo/FOXO network	Cofactor + TF network	Season, temp., food	Age at maturation; testis schedule	Barson et al. (2015); Ahi et al. (2022, 2025a–d)
Amphibians	TH receptors, deiodinases	Hormone receptors/ enzymes	Pond drying, crowding, stress	Onset and speed of metamorphosis	Denver (2009); Holzer and Laudet (2015); Thambirajah et al. (2019); Laslo et al. (2019)
Insects (various)	JH enzymes, JH receptor, diapause hormone receptor	Endocrine enzymes/ receptors	Photoperiod, temp., nutrition	Diapause onset and duration	Schiesari and O'Connor (2013); Li et al. (2022); Hafeez et al. (2025)
C. elegans and relatives	lin-28, lin-42, elt-1	Heterochronic regulators	Conditions affecting dauer/growth	Larval stage timing; link to dauer	Großhans et al. (2005); Hammell et al. (2009); Karp and Ambros (2011)
Vertebrate cell systems	Menin, SUZ12	Chromatin timing regulators	Culture conditions	Pace of differentiation programs	Xu et al. (2025)

status to *miR-156* expression and SPL activity (Fichtner and Lunn 2021). Additional work has shown that multiple phytohormones and chromatin regulators modulate the same module, suggesting that resource status and environmental signals converge on *miR-156–SPL* to control the schedule of vegetative development (Fichtner and Lunn 2021; Poethig and Fouracre 2024). Because *miR-156* is both a central heterochronic regulator and a direct target of environmentally sensitive metabolic signaling, it is well supported as a validated heterokairic gene in plants.

4.4 | Vertebrates: The lin28/let-7 Axis and Puberty Timing

The lin-28/let-7 axis, first discovered as a heterochronic system in *C. elegans*, has conserved roles in vertebrates in controlling the timing of developmental transitions. In mammals, Lin28a/b maintain stem and progenitor cell states, and rising let-7 levels promote differentiation and restrict growth, providing a molecular timing mechanism for tissue development (Robinton et al. 2019; Thevarajan et al. 2025). Genome-wide association studies identified common variants near *LIN28B* as major determinants of age at menarche and other aspects of pubertal timing in humans, indicating that genetic variation in this axis contributes to heterochronic variation in reproductive maturation (Ong et al. 2009). Experimental and expression studies have linked hypothalamic and gonadal changes in *Lin28/let-7* expression to pubertal onset. In rodents, hypothalamic *Lin28* expression declines, and *let-7* increases over the peri-pubertal period, and manipulations of *Lin28* or *let-7* levels alter the timing of puberty and body growth (Sangiao-Alvarellos et al. 2013; Cao et al. 2020). Nutritional and metabolic cues, including body weight and energy balance, have been shown to influence this axis, suggesting that environmental variation in growth conditions is translated into altered timing of puberty through Lin28/let-7-mediated control of neuroendocrine circuits (Sangiao-Alvarellos et al. 2013; Cao et al. 2020). Although the mechanistic chain from specific environmental variables to Lin28/let-7 activity is still being clarified, the combination of a timing role and clear links to growth and metabolic state supports classification of the Lin28/let-7 axis as a strong candidate and, in some contexts, a validated heterokairic system.

4.5 | Vertebrates: *vgll3* and Maturation Timing in Salmon

In Atlantic salmon, a genomic region containing *vgll3*, encoding a cofactor of the Hippo pathway, was identified as a major locus controlling age at sexual maturity. The Hippo pathway is emerging as a key mediator of signaling between environmental cues and the pace of life and developmental timing across animals (Ahi et al. 2025a). Association analyses in wild populations showed that alternative *vgll3* alleles exhibit sex-dependent dominance and together account for a large fraction of variation in sea age at maturation, making *vgll3* a textbook example of a heterochronic gene affecting life-history timing (Barson et al. 2015). Subsequent work has demonstrated that the *vgll3* genotype is associated with differences in testicular developmental schedules and with transcriptional profiles in adipose tissue that relate to lipid storage and mobilization across seasons (Ahi et al. 2022, 2024, 2025c; House et al. 2025). Furthermore, recent studies show that *foxo1*, an ortholog of *daf-16* in *C. elegans* (see above), is a hub gene in the gene network mediating sex- and tissue-specific differences in *vgll3* in Atlantic salmon (Ahi et al. 2023, 2025b, 2025d). These studies suggest that *vgll3* not only affects the intrinsic program of gonadal development but also interacts with seasonal changes in energy balance, a key environmental dimension for maturation decisions in salmon. Because *vgll3* is deeply implicated in maturation timing and its phenotypic effects are modulated by environmental conditions via energy balance and seasonality, it represents a compelling vertebrate example of a validated heterokairic gene.

4.6 | Other Environmentally Regulated Heterochronic Genes: Candidate Heterokairic Genes

Beyond these validated examples, many genes occupy an intermediate category in which heterochronic function and environmental sensitivity are supported, but the causal links to heterokairy have not yet been fully traced. In *C. elegans*, additional components of the heterochronic network, including *lin-28*, *lin-42*, and *elt-1*, have been shown to regulate temporal cell fates and to interact genetically with dauer pathways, suggesting that they help coordinate stage-specific programs with diapause decisions (Großhans et al. 2005; Hammell et al. 2009; Karp and Ambros 2011). Because their regulation by specific environmental cues remains less clearly defined than for *daf-12* and *daf-16*, they are best regarded as candidate heterokairic genes pending more detailed mechanistic work.

In amphibians, thyroid hormone (TH) receptors and deiodinase enzymes are canonical determinants of metamorphic timing and morphology, and extensive experimental evidence shows that environmental stressors such as pond drying and crowding act via corticotropin-releasing factor and corticosteroids to modify TH production and action (Denver 2009; Holzer and Laudet 2015; Laslo et al. 2019; Thambirajah et al. 2019). Changes in expression of TH receptors and deiodinases have been documented under different environmental regimes, and these changes correlate with accelerated or delayed metamorphosis (Denver 2009; Holzer and Laudet 2015; Laslo et al. 2019; Thambirajah et al. 2019). Because these genes clearly govern metamorphic timing and respond to environmental manipulation, they form a promising set of candidate heterokairic genes, although explicit tests that link their environmentally induced expression changes to quantified heterokairy are still rare.

Insects provide another rich source of candidates. Entry into diapause, a developmental arrest that delays or suspends progression to later stages, is controlled by juvenile hormone (JH), ecdysone, and their downstream effectors (Schiesari and O'Connor 2013). Recent work has implicated JH degradation enzymes and JH receptor signaling in regulating diapause onset and duration, with experimental evidence that changes in JH titers and signaling mediate photoperiod- and temperature-dependent diapause induction (Li et al. 2022; Hafeez et al. 2025). Another recent work on stage-specifying transcription factors shows that *Chinmo*, an environmentally responsive gene, is

deployed in a JH- and ecdysteroid-sensitive window and that heterochronic shifts in its expression can generate alternative life stages (Erezyilmaz 2024), making it a promising heterokairic candidate at the intersection of endocrine timing and growth. These genes govern whether and when development pauses, and their activity is modulated by environmental cues that encode seasonal conditions, making them strong candidates for heterokairic status in insects.

In plants, genes controlling the floral transition, such as *FLC* and *FT*, exemplify environmentally regulated heterochronic genes whose timing roles are well established (Takagi et al. 2023; Huang et al. 2024). FLC delays flowering by repressing FT and other floral integrators, and vernalization and photoperiod act through changes in FLC and FT expression to shift flowering time reaction norms across genotypes and environments (Takagi et al. 2023; Huang et al. 2024). These genes clearly integrate temperature and day-length information into developmental timing of reproduction, and can thus be considered candidate heterokairic genes for flowering-time heterokairy, even though their roles have not yet been framed in those terms.

Overall, the examples above show that validated heterokairic genes have been firmly identified in only a small number of systems, but a much larger set of environmentally regulated heterochronic genes is already known. As mechanistic studies increasingly combine environmental manipulations with high-resolution analyses of timing and gene activity, many of these candidates are likely to be promoted to validated status, revealing how widely heterokairy is implemented at the gene-network level (see also Table 1).

5 | Eco-Evolutionary Roles of Heterokairic Genes

Heterokairic genes shape how developmental timing reacts to environmental variation and therefore influence several key dimensions of life-history evolution. When such genes alter the age or stage at which transitions occur, reaction norms for developmental time are modified, and the set of ontogenetic trajectories available to a genotype is redefined (Moss 2007; Keyte and Smith 2014). Because age and size at metamorphosis, maturation, or reproduction strongly affect survival and fecundity, even modest shifts in timing can change the trade-offs that underpin life-history strategies (Moss 2007; Keyte and Smith 2014; Barson et al. 2015; Ahi et al. 2024).

In systems where strong major-effect loci on timing have been identified, such as *vgll3* in Atlantic salmon or *LIN28B* in human puberty, genetic variants at heterokairic genes can be viewed as encoding alternative "default" schedules that remain open to environmental modulation (Ong et al. 2009; Sangiao-Alvarellos et al. 2013; Barson et al. 2015; Ahi et al. 2024). For salmon, allelic differences at vgll3 influence the propensity to mature after one or several years at sea, but maturation probabilities also depend on growth, temperature, and seasonality (Barson et al. 2015; Ahi et al. 2024). This structure allows balancing of early versus late maturation strategies across heterogeneous environments and contributes to the maintenance of genetic variation at life-history loci (Barson et al. 2015; Ahi et al. 2024). A similar logic has been proposed for puberty timing in humans, where LIN28B variants shift the distribution of age at menarche while nutritional and social factors continue to exert plastic effects (Ong et al. 2009; Sangiao-Alvarellos et al. 2013). In both cases, heterokairic genes define the genetic architecture on which plastic responses act.

Heterokairic genes also provide a mechanistic substrate for genetic accommodation of timing plasticity. Theoretical and empirical work on developmental plasticity has proposed that environmentally induced phenotypes can become partially or fully genetically stabilized when selection consistently favors particular reaction norm shapes (Moczek et al. 2011). When the plastic trait is developmental timing, environmentally responsive heterochronic pathways offer obvious targets for such accommodation. Up- or down-regulation of heterokairic genes, changes in their sensitivity to endocrine or metabolic inputs, or alterations in their downstream targets can all modify how strongly developmental schedules respond to a given environmental gradient (Moss 2007; Moczek et al. 2011; Edwards and Smallegange 2025). Over evolutionary time, lineages may thus shift from broadly plastic heterokairy to more canalized heterochrony, or vice versa, by tuning the properties of heterokairic genes.

At the population level, heterokairy mediated by timing genes feeds back on ecological dynamics. Modeling studies have shown that plasticity in traits affecting demography, such as age at maturation, can alter population growth rates, age structure, and density dependence, thereby changing the selective environment experienced by subsequent generations (Hendry 2016; Smallegange 2022; Edwards and Smallegange 2025; Stewart and Smallegange 2025). When developmental time is shortened under high density or deteriorating conditions, for instance, heterokairic genes may promote earlier reproduction at smaller size, buffering populations against crashes but also shifting competitive and predation regimes (Hendry 2016; Smallegange 2022; Edwards and Smallegange 2025). Recent eco-phenotypic models that incorporate heterokairy explicitly have demonstrated that density-dependent modulation of development rate can generate distinct eco-phenotypic pathways and alternative morphs differing in development time, a phenomenon described as heterokairic polyphenism (Edwards and Smallegange 2025). In such scenarios, heterokairic genes are central to the strength and direction of eco-evolutionary feedbacks.

Differences among taxa in the organization of timing networks suggest that the eco-evolutionary impact of heterokairic genes will be system-specific. In amphibians, for example, stress-induced acceleration of metamorphosis is mediated by interactions between corticotropin-releasing factor, corticosteroids, and TH signaling (Denver 2009). Variation in receptors and enzymes within the thyroid axis can therefore alter how strongly hydroperiod or crowding affects metamorphic timing, with consequences for size at metamorphosis and terrestrial performance (Denver 2009; Holzer and Laudet 2015). In insects, JH and ecdysone signaling modules governing diapause and metamorphosis integrate photoperiod and nutritional cues; genes in these pathways have been repeatedly implicated in the evolution of seasonal life cycles and in responses to climate change (Schiesari and O'Connor 2013; Yamanaka et al. 2013). In plants, *FLC*, *FT*, and the *miR156–SPL* module link vernalization, photoperiod, and sugar status to the timing of phase change and flowering, thereby shaping how life cycles track growing seasons and resource pulses (Schiesari and O'Connor 2013; Yang et al. 2013; Poethig and Fouracre 2024). In each case, heterokairic genes determine how external seasonal structure is translated into internal developmental schedules.

Finally, heterokairic genes have implications for the robustness and evolvability of developmental systems. Because they sit at the interface between environmental inputs and timing modules, they can either buffer or amplify external variation. Increased canalization may arise if changes in heterokairic genes reduce the sensitivity of timing to noisy cues, whereas enhanced evolvability may follow from architectures in which modest genetic changes at such loci produce large shifts in reaction norms (Moss 2007; Moczek et al. 2011; Keyte and Smith 2014; Smallegange 2022). These properties are likely to influence how quickly populations can adjust developmental schedules under rapid environmental change, and whether adaptation proceeds through changes in timing genes themselves or through modifications of upstream sensory pathways and downstream effectors.

6 | Approaches for Identifying and Testing Heterokairic Genes

Heterokairic genes are, by definition, genes that link environmental variation to shifts in developmental timing. To identify them, experimental designs must combine variation in environment, genotype, and developmental time, and analytical methods must be able to resolve when regulatory changes occur and how they depend on external conditions. An overview of complementary study designs and analytical strategies is provided in Table 2. In practice, progress has come from three broad approaches: environmental transcriptomics and network inference, trajectory-based analyses at single-cell resolution, and functional genetic perturbation combined with environmental manipulations.

6.1 | Experimental Designs: Environment × Genotype × Time

A minimal design for discovering heterokairic genes requires that developmental trajectories are followed through time under at least two contrasting environments, ideally for multiple genotypes. This allows reaction norms of event timing to be quantified and makes it possible to associate shifts in developmental timing with changes in gene activity or expression across environments (Huang and Agrawal 2016; Lafuente and Beldade 2019). Time series in which environmental variables are switched at defined developmental stages are particularly informative, because they can reveal sensitive windows and distinguish genes that respond acutely to environmental change from those that track intrinsic developmental time (Huang and Agrawal 2016; Lafuente and Beldade 2019). When combined with measurements of developmental landmarks (e.g., onset of metamorphosis, phase change, or maturation), such designs make it possible to identify candidate genes whose expression profiles correlate with heterokairy rather than with chronological age alone.

6.2 | Time-Series Transcriptomics and Dynamic Regulatory Network Inference

Time-series transcriptomics across development has become a central tool for dissecting timing mechanisms. Dynamic gene regulatory networks can be inferred from bulk time-course RNA-seq by leveraging time-lagged correlations, regression models, and probabilistic graphical models that explicitly incorporate temporal ordering (Ding and Bar-Joseph 2020). These methods make it possible to identify regulators whose activity precedes and predicts changes in downstream gene sets or developmental landmarks, and to determine whether their activation time differs between environments. Reviews of time-series regulatory network inference emphasize that integrating multi-omics data (chromatin accessibility, transcription factor binding, proteomics) with gene expression improves the identification of key timing regulators and reveals how they respond to perturbations (Ding and Bar-Joseph 2020; Sun et al. 2025). Developmental time courses are increasingly being collected under multiple environmental conditions, making it possible to identify regulatory modules whose activation time is environment-dependent. Studies of developmental plasticity in animals have used transcriptomics to detect genes whose expression trajectories differ between environments, and genetic mapping to associate these expression plasticity patterns with specific loci (Moczek et al. 2011; Lafuente and Beldade 2019). More recent work has applied network inference to time-series datasets from developing seeds, embryos, and plastic phenotypes, showing that genes occupying central positions in dynamic networks often correspond to known developmental regulators and revealing modules whose temporal activity shifts under different environmental regimes (Tucci et al. 2024; Sun et al. 2025). Such analyses can be adapted to search specifically for heterokairic genes by asking which regulators show environment-dependent changes in their inferred activation time or in the timing of their downstream effects.

6.3 | Single-Cell Trajectory and Pseudotime Analyses

Bulk time series provide population averages, but developmental timing decisions are often made at the level of individual cells. Single-cell RNA-seq and related methods have therefore become important for reconstructing developmental trajectories and identifying genes whose expression changes mark progression along these trajectories. Trajectory inference and pseudotime methods order cells along inferred developmental paths based on transcriptional similarity and have been widely reviewed (Chen et al. 2021; Wang et al. 2021). Recent methodological work has improved the reconstruction of dynamic developmental trajectories by incorporating growth, cell division, and lineage information, and by modeling trajectories using optimal transport or related frameworks (Sha et al. 2023). For heterokairic genes, single-cell trajectories can be particularly informative. When cells from different environments are profiled jointly, environment-dependent shifts in the pseudotemporal position at which a gene is activated, or at which a cell crosses a transcriptional threshold corresponding to a developmental event, can be detected even when absolute chronological time is not identical across conditions (Chen et al. 2021; Wang et al. 2021; Sha et al. 2023). Best-practice guidelines now highlight the importance of validating pseudotime against known temporal markers and of assessing how robust inferred trajectories are to sampling biases, which is essential if heterokairy is to be quantified reliably at the cellular

TABLE 2 | Study designs and analytical approaches for identifying heterokairic genes.

Goal	Scale	Design/data	Analysis	Insight on heterokairic genes	References
Describe heterokairy in traits	Whole organism, trait	Event timing in ≥ 2 envs	Reaction norms, timing versus env. models	Which traits show timing plasticity	Spicer and Rundle (2007); Rundle and Spicer (2016); Edwards and Smallegange (2025); Smallegange (2022)
Find env.-shifted timing regulators	Bulk gene expression	Time-series transcriptomics across envs	Dynamic GRN inference, time-lag tests	Genes whose activation time shifts with env.	Ding and Bar-Joseph (2020); Sun et al. (2025); Lafuente and Beldade (2019); Huang and Agrawal (2016)
Map env. effects at the cell level	Single-cell trajectories	scRNA-seq in different envs across development	Pseudotime, trajectory comparison	Where along cell paths env. alters speed/route	Wang et al. (2021); Chen et al. (2021); Sha et al. (2023)
Quantify G × E in timing traits	Quantitative genetics	Timing phenotypes for many genotypes in envs	G × E models; QTL/GWAS	Loci whose timing effects depend on env.	Barson et al. (2015); Ong et al. (2009); Lafuente and Beldade (2019); Moczek et al. (2011)
Link variants to env.-sensitive expression	Gene regulation (eQTL)	Genotypes + expression time series in envs	eQTL with env./time interactions	Genes with expression timing shaped by genotype and env.	Lafuente and Beldade (2019); Moczek et al. (2011)
Test a specific candidate gene	Single gene/pathway	Knock-out/down/overexp. in ≥ 2 envs	Compare timing reaction norms	Direct test of the effect on timing plasticity	Denver (2009); Holzer and Laudet (2015); Poethig and Fouracre (2024)
Unbiased timing screens	Genome-wide	CRISPR screens with temporal readouts ± env.	Screen hit calling, timing metrics	Timing genes; env.-dependent hits as candidates	Cheng et al. (2023); Xu et al. (2025)
Integrate multi-layer timing data	Systems/network	Traits, expression, genetics, perturbations	Network integration, modeling	Network positions of heterokairic genes	Moss (2007); Keyte and Smith (2014); Smallegange (2022)

level (Chen et al. 2021; Wang et al. 2021). Combining single-cell trajectories with bulk time series, or with metabolic labeling that provides absolute timing information, can further refine estimates of when genes act and how that timing responds to environmental cues (Sha et al. 2023).

6.4 | Multi-Environment Transcriptomics and Expression Plasticity

Identifying heterokairic genes requires attention not only to when genes are expressed, but also to how their expression responds to environmental change. Comparative studies of developmental plasticity have used transcriptomics to map environmentally induced changes in gene expression across developmental stages and phenotypes, revealing that plastic traits are often underpinned by coordinated changes in regulatory networks (Moczek et al. 2011; Lafuente and Beldade 2019). Analyses of expression plasticity and its evolution have shown that genes with environmentally sensitive expression often exhibit characteristic network positions and regulatory features, and that genetic variation in expression plasticity can itself be mapped and selected upon (Huang and Agrawal 2016; Lafuente and Beldade 2019; Schweizer et al. 2023). Recent work has begun to align these approaches more directly with developmental timing questions. Developmental transcriptomics in plastic systems, such as nematodes with environmentally induced alternative morphs, has been used to reconstruct gene regulatory networks whose structure and activation timing differ between environments, pinpointing regulatory nodes whose timing shifts under environmental cues (Reich et al. 2025). Other studies have shown that genes exhibiting high transcriptional variability across environmental perturbations also tend to be sensitive to genetic perturbations, suggesting shared biases in regulatory architecture that determine both expression plasticity and genetic responsiveness (Tsuru and Furusawa 2025). Such patterns may help prioritize candidate heterokairic genes by highlighting genes that are both timing regulators and strongly environmentally responsive.

6.5 | Genetic Mapping and G × E Analyses of Timing Traits

Quantitative genetic and genome-wide association approaches provide complementary routes to heterokairic genes by localizing genetic variants that affect developmental timing and assessing how their effects depend on the environment. Loci of large effect on timing traits, such as *vgll3* in salmon maturation or *LIN28B* in human puberty, demonstrate that discrete genomic regions can control developmental schedules (Barson et al. 2015; Cao et al. 2020). When phenotypes are measured across environments, genotype-by-environment interactions at such loci can be detected, indicating that allelic effects on timing change with external conditions. Similar designs applied to experimental crosses or natural populations with variation in metamorphic timing, diapause onset, or flowering time can reveal candidate heterokairic loci even before the underlying genes are identified. Integration of mapping with transcriptomics further strengthens inference. Expression quantitative trait locus (eQTL) mapping across environments and developmental stages can identify regulatory variants whose effects on gene expression timing are environment dependent, pointing to genes whose temporal regulation is under both genetic and environmental control (Moczek et al. 2011; Lafuente and Beldade 2019). When these genes are also implicated in developmental timing pathways, they become strong candidates for heterokairic status.

6.6 | Functional Testing: Perturbation Screens and Targeted Manipulation

To move from correlation to causation, candidate heterokairic genes must be functionally perturbed while developmental timing is measured under contrasting environments. Classical approaches rely on targeted loss- or gain-of-function alleles combined with environmental manipulations, allowing the effect of the gene on timing reaction norms to be assessed directly. More recently, CRISPR-based perturbation methods have expanded the scale at which timing regulators can be discovered. Massively parallel CRISPR interference or knockout screens with temporal readouts have been used to identify genes whose disruption accelerates or delays specific developmental transitions, including neuronal maturation and early embryonic lineage specification (Cheng et al. 2023; Xu et al. 2025). A recent genome-wide CRISPR screen in human pluripotent stem cells identified Menin and SUZ12, components of chromatin regulatory complexes, as regulators of human developmental timing, with perturbations altering the speed at which differentiation programs unfold (Xu et al. 2025). Although this work focused on intrinsic timing, the same screening framework could be extended by incorporating environmental manipulations (e.g., nutrient levels, growth factors, or stressors) during differentiation, and by asking which hits show environment-dependent effects on timing. Arrayed CRISPRi or CRISPRa perturbations combined with live imaging and time-lapse analysis provide a complementary route, enabling precise measurement of how individual gene perturbations shift the timing of developmental milestones across controlled environmental treatments (Cheng et al. 2023). At a smaller scale, candidate heterokairic genes highlighted by mapping or transcriptomics can be tested using CRISPR, RNAi, or conditional alleles in organisms amenable to environmental manipulation. For example, knocking down a nutrient-responsive transcription factor and observing whether developmental timing becomes less sensitive to diet would support its role as a heterokairic gene in that system. Reciprocal experiments, in which gene dosage is increased and timing plasticity is enhanced or redirected, can further clarify how the gene modulates reaction norms.

6.7 | Criteria for Upgrading Candidates to Validated Heterokairic Genes

In light of these methodological possibilities, it is useful to make the evidential standard for validated heterokairic genes explicit. A conservative set of criteria would require that: (i) the gene has a demonstrated role in controlling the timing of a defined developmental event (e.g., through mutant or perturbation phenotypes); (ii) the gene's activity or expression pattern is

shown to change across environments during the relevant developmental window; and (iii) manipulating the gene alters the reaction norm of event timing across environments, such that timing plasticity is reduced, abolished or redirected. When these conditions are met, the gene can be regarded as a validated heterokairic gene in that system. Genes that meet only the first two criteria, or where evidence for timing control or environmental modulation is indirect, remain valuable as candidates but require further experimental testing.

7 | Synthesis and Future Directions

The distinction between heterochrony and heterokairy has clarified how evolutionary and plastic changes in developmental timing should be framed. Heterochrony has been treated as an evolutionary shift in developmental schedules among lineages, whereas heterokairy has been defined as environmentally induced variation in the timing of developmental events within genotypes or populations (Spicer and Rundle 2007; Keyte and Smith 2014; Rundle and Spicer 2016). On this basis, heterokairic genes have been proposed as heterochronic regulators whose activity is modulated by environmental context in ways that generate heterokairy. This conceptual pairing places genes explicitly at the interface of environment, development, and evolution, without conflating plastic and evolved differences in timing (Moss 2007; Spicer and Rundle 2007; Keyte and Smith 2014; Rundle and Spicer 2016).

Across systems, a recurring pattern has been revealed (Figure 2). In nematodes, nuclear hormone receptors and FOXO factors (*daf-12, daf-16*) and their associated *let-7* family microRNAs form an environmentally responsive module that coordinates dauer decisions with larval timing (Antebi et al. 2000; Moss 2007; Hammell et al. 2009). In insects, ecdysone pulses act through the let-7-C cluster to time metamorphic remodeling, with hormonal dynamics shaped by nutrition and season (Sempere et al. 2002). In plants, the miR156–SPL module integrates sugar and hormone signals to regulate the schedule of vegetative phase change (Poethig and Fouracre 2024). In vertebrates, the *Lin28/let-7* axis and the Hippo cofactor vgll3 influence puberty and maturation timing in ways that depend on growth and energy balance (Moss 2007; Barson et al. 2015). These examples suggest that heterokairic genes often recur in similar integrator classes across taxa (such as endocrine receptors, nutrient-sensing/FOXO nodes, and conserved timing microRNAs), indicating convergence on comparable network positions where endocrine and metabolic signals intersect developmental timing modules.

At the same time, it has become apparent that only a minority of environmentally regulated timing genes currently meet a strict definition of "validated" heterokairic genes. For many systems, evidence is strongest for heterochronic function or for environmental responsiveness, but not yet for the full causal chain linking external cues, gene activity, and quantified heterokairy. This gap has partly reflected methodological limitations: developmental time series were often collected in a single environment, expression studies were not aligned with precise timing phenotypes, and functional tests rarely combined gene perturbation with controlled environmental variation (Moss 2007; Keyte and Smith 2014).

Recent advances now offer an opportunity to close this gap. Environment × genotype × time designs, together with time-series transcriptomics and regulatory network inference, allow regulators whose activation time shifts between environments to be identified systematically (Lafuente and Beldade 2019; Ding and Bar-Joseph 2020). Single-cell trajectory methods provide a complementary view at the cellular level, making it possible to detect environment-dependent changes in the pseudotemporal position of fate decisions and in the onset of regulatory modules (Hammell et al. 2009; Sha et al. 2023). Genome-wide CRISPR-based screens with temporal readouts have demonstrated that intrinsic timing regulators can be recovered in an unbiased way and could, in principle, be extended to multi-environment settings to pinpoint genes whose effects on developmental speed are context dependent (Xu et al. 2025).

Several priorities emerge from this synthesis. First, validated heterokairic genes need to be established in a broader range of taxa and developmental contexts by applying explicit criteria: demonstration of a timing role, environmental modulation of activity during the relevant window, and an effect on timing reaction norms when the gene is perturbed. Second, the organization of timing networks should be compared across species with contrasting ecologies to test whether particular network architectures or classes of heterokairic genes are associated with specific life histories or environmental regimes. Third, the evolvability and robustness of heterokairy should be examined by asking how changes in the sensitivity, feedback structure, or redundancy of heterokairic genes alter reaction norms for developmental time and the potential for genetic accommodation.

Finally, there is a need for closer integration between trait-based heterokairy studies and molecular analyses. Many systems in which heterokairy has been documented at the phenotypic level have not yet been examined with the tools now available for transcriptomic and genetic dissection, while several model systems with well-characterized timing networks have only recently begun to be studied across environments. By bringing these approaches together, the distribution, mechanisms, and evolutionary consequences of heterokairic genes can be characterized more fully, and developmental timing plasticity can be placed on the same mechanistic footing as other, more familiar forms of phenotypic plasticity.

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Data Availability Statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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