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Hormonal Crosstalk, Gene Regulation, and Developmental Timing in the Molecular Control of Insect Metamorphosis

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ABSTRACT

Insect metamorphosis is often described as a sequence in which changing ecdysteroid and juvenile-hormone titres activate genetically encoded developmental programs. That description is useful but incomplete because endocrine concentration, receptor occupancy, stage identity, chromatin state, and tissue competence are not interchangeable regulatory levels. Recent work instead supports a distributed control problem: steroidogenic tissues integrate nutritional and receptor-tyrosine-kinase inputs; juvenile hormone restrains premature stage transitions through Krüppel-homolog 1-centred regulation; E93 and other transcriptional regulators reshape developmental competence; and individual tissues interpret systemic endocrine signals according to their own temporal and molecular state. This review therefore organizes metamorphic control around a proposed layered-gating model rather than a single endocrine switch. Evidence from *Drosophila*, *Bombyx*, *Tribolium*, *Blattella*, *Manduca*, mealybugs, locusts, and *Spodoptera* is used to distinguish mechanisms strongly supported in particular models from relationships that remain taxon-, stage-, or tissue-dependent. Special emphasis is placed on feedback between ecdysteroid production and neuroendocrine signals, juvenile-hormone–ecdysteroid antagonism, chromatin accessibility, post-transcriptional regulation, and tissue-specific execution. The resulting synthesis argues that developmental timing is best understood as the coincidence of endocrine availability, stage-specifying gene state, and tissue competence. This interpretation does not imply a conserved universal topology; instead, it identifies experimentally separable regulatory layers whose relative weighting varies among insect lineages and developmental contexts.

Keywords: Ecdysteroids, Juvenile hormone, E93, Krüppel-homolog 1, Developmental timing, Chromatin accessibility

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Introduction

Metamorphosis requires more than a rise or fall in a circulating hormone. Comparative evidence places endocrine transitions within an evolutionary history of changing developmental architectures, while current stage-identity models emphasize interactions among juvenile hormone (JH), Krüppel-homolog 1 (Kr-h1), Broad, and E93 [1, 2]. These perspectives suggest that the mechanistic question is not simply which hormone initiates metamorphosis, but how endocrine production, transcriptional state, and tissue responsiveness become aligned in developmental time.

A major interpretive difficulty is that the best-resolved mechanisms are concentrated in a small number of laboratory models. Shared components therefore should not be assumed to have identical regulatory weight across

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holometabolous and hemimetabolous insects, between sexes, or among tissues. This review uses a proposed layered-gating interpretation: endocrine availability defines what signals reach tissues; stage-specifying and chromatin regulators define which transcriptional programs are accessible; and tissue state determines which responses can actually be executed. These layers are analytically separable even when they are biologically coupled.

Ecdysteroid production and reception

Ecdysteroid pulses provide a central temporal signal for molts and metamorphic transitions, but their production is itself a regulated developmental output rather than an autonomous clock. The prothoracic gland and related steroidogenic tissues integrate neural, nutritional, and intracellular information before generating or releasing ecdysteroids [3]. In *Drosophila*, nutrient-dependent endocycling of prothoracic-gland cells links steroidogenic tissue growth to the timing of metamorphosis, demonstrating that endocrine capacity can be developmentally gated by cellular state [4].

Transcriptomic profiling of the *Bombyx* prothoracic gland expanded the catalogue of candidate regulators associated with ecdysteroidogenesis, while functional follow-up supported only a subset of those candidates [5]. This distinction is important: transcriptional enrichment identifies plausible components but does not by itself order them mechanistically. In *Drosophila*, the transcription factors Séance, Ouija Board, and Molting Defective cooperate in the control of ecdysone biosynthesis, illustrating that steroidogenic output can depend on a tissue-specific transcriptional ensemble rather than one master regulator [6].

Extracellular signaling adds another layer. EGFR activity in the *Drosophila* prothoracic gland promotes ecdysone biosynthesis and participates in a positive-feedback relationship with ecdysone signaling [7]. Ecdysone also feeds back on prothoracicotropic-hormone (PTTH) neurons, coupling peripheral steroid production to neuroendocrine timing [8]. Multiple receptor tyrosine kinase inputs can converge on the gland and jointly influence developmental timing and body size, arguing against a simple PTTH/Torso-only trigger [9].

Cross-taxon comparisons expose the limits of that *Drosophila*-centered topology. In *Bombyx mori*, CRISPR disruption of *torso* reduces ecdysteroid output and delays development, yet does not abolish metamorphosis [10]. In *Manduca sexta*, nutritional and endocrine regulation of prothoracic-gland activity differs from that of *Drosophila*, indicating that similar endocrine organs can implement distinct checkpoint logic [11]. Epigenetic control also enters at the steroidogenic-tissue level: the histone demethylase KDM5 promotes prothoracic-gland endocycles and normal timing in *Drosophila* [12].

The PTTH pathway itself contains biochemical subdivisions. In *Bombyx* prothoracic glands, RSK phosphorylation participates in PTTH-stimulated ecdysone secretion [13]. In *Drosophila*, PTTH-dependent timing also contributes to environmentally responsive developmental plasticity under laboratory conditions [14]. Nutritional control changes across developmental time: a tissue- and stage-specific autophagic switch in the *Drosophila* prothoracic gland modifies premetamorphic nutritional checkpoints by altering steroidogenic capacity [15]. These results support a proposed endocrine-capacity gate, but only as an organizing synthesis; the underlying mechanisms are demonstrably stage- and taxon-dependent.

Reception adds a further boundary between hormone availability and developmental outcome. Genome-wide analysis in *Drosophila* shows that the ecdysone receptor (EcR) has direct and widespread roles in mediating transcriptional responses to ecdysone [16]. Yet receptor binding or direct transcriptional regulation should not be equated with an identical phenotypic response in every tissue. The same systemic pulse is interpreted through tissue-specific chromatin, cofactor, and developmental states considered below.

Juvenile hormone signaling

JH provides the principal antimetamorphic signal during juvenile development, with Methoprene-tolerant-centered signaling and Kr-h1 forming a core regulatory axis [17]. In *Bombyx*, Kr-h1 directly represses metamorphic gene expression and thereby prevents precocious larval-to-adult developmental programs [18]. This establishes a molecular route from JH signaling to stage identity, but it does not imply that every JH effect is mediated exclusively through Kr-h1.

JH and ecdysteroids can also interact within endocrine tissue. In the *Drosophila* ring gland, JH and 20-hydroxyecdysone exert antagonistic actions that affect the timing of developmental transitions [19]. Stage identity can nevertheless become separable from earlier size acquisition: in *Tribolium castaneum*, upregulation of E93 acts as a metamorphic trigger independently of threshold-size attainment [20]. The finding does not make

threshold size irrelevant; rather, it separates a permissive growth history from an immediate stage-specifying event.

Hemimetabolous and sexually dimorphic systems complicate a single canonical trajectory. In mealybugs, the relationship among E93, JH, and development differs between males and neotenic females, showing that sex-specific life histories can alter the expected correspondence between stage-specifier expression and adult differentiation [21]. In *Blattella germanica*, Myoglianin contributes to entry into the premetamorphic stage through endocrine-gland processes linked to JH reduction and ecdysone-related regulation [22]. These observations support conservation of interacting regulatory themes without establishing identical pathway topology across orders.

Kr-h1 itself is regulated at several molecular levels. Phosphorylation-mediated recruitment of specific cofactors modifies its antimetamorphic and vitellogenic functions in selected insect models [23]. In *Locusta migratoria*, let-7 and miR-278 target Kr-h1, linking post-transcriptional regulation to metamorphic and reproductive programs [24]. In *Drosophila*, miR-927 also targets Kr-h1 and alters developmental growth phenotypes [25]. Together, these studies argue against treating Kr-h1 transcript abundance as a complete proxy for JH pathway state.

Transcriptional and epigenetic control

Hormonal information becomes developmentally effective only when the genome is competent to respond. In *Drosophila*, hormone-dependent developmental timing is coupled to dynamic changes in chromatin accessibility, providing a mechanistic bridge between endocrine signaling and temporally restricted transcription [26]. E93 contributes to this process by providing an instructive cue for enhancer activity and accessibility, but parallel temporal inputs remain necessary at some targets [27]. E93 is therefore better interpreted as a major competence regulator than as a universally sufficient chromatin switch.

Chromatin state can also act upstream of hormone output. H3K27 methylation-mediated repression of Hairy influences ecdysone biosynthesis and developmental transition in *Bombyx* and *Drosophila* models [28]. The direction of regulation is consequently not confined to hormone-to-chromatin signaling; epigenetic state can modify endocrine production itself.

Post-transcriptional regulation adds another axis. Reviews of insect metamorphosis identify microRNAs and related mechanisms as important modifiers of developmental gene networks [29]. In the *Drosophila* prothoracic gland, miR-8-3p coordinates V-ATPase subunits; perturbation lowers ecdysone output and the developmental phenotype can be rescued by ecdysone feeding, strengthening pathway placement [30]. In *Spodoptera litura* midgut, multiple microRNAs change across the larval–pupal transition, while direct reporter evidence supports regulation of EcR/E75 by miR-14-3p [31]. Predicted miRNA networks should therefore be distinguished from experimentally validated regulatory edges.

Tissue-specific responses

Systemic endocrine signals do not impose a uniform cellular program. Ecdysone-responsive transcription can include repression as well as activation: in *Drosophila*, Bab2 functions as an ecdysone-responsive transcriptional repressor during development [32]. This supports a tissue-response view in which endocrine reception is filtered through local transcription-factor composition rather than translated into a single stereotyped output.

The fat body illustrates explicit hormonal coordination at the execution stage. JH and 20-hydroxyecdysone jointly control the timing of matrix-metalloproteinase-dependent fat-body cell dissociation in *Drosophila* [33]. Here the endocrine signal is upstream of a tissue-remodeling process, but the measured mechanism is specific to fat-body dissociation and should not be generalized to other organs.

Neural tissue uses steroid timing differently. In *Drosophila* brain neuroblasts, steroid-hormone signaling induces a temporal gene-expression transition that contributes to neuronal and glial diversity [34]. This is evidence for lineage-specific temporal competence, not evidence that neuroblast timing itself triggers organismal metamorphosis. Conversely, salivary-gland experiments show that steroid signaling can trigger glue-protein production outside the normal developmental program, while the magnitude and timing of the response remain constrained by tissue competence [35]. Hormone exposure is therefore neither irrelevant nor sufficient: developmental state conditions its output.

A terminal-stage example comes from the hemimetabolous cockroach *Blattella germanica*. Depletion of E93 preserves the prothoracic gland after the adult molt and permits an additional molt [36]. This striking phenotype links a stage-specifying transcription factor to termination of endocrine-organ function, but it does not by itself

explain the evolutionary origin of the final molt. Across these tissues, a common analytical lesson emerges: "response to hormone" must be decomposed into receptor engagement, local gene-regulatory competence, and execution of a tissue-specific program.

Because these cases differ simultaneously in tissue, developmental stage, and regulatory logic, a cross-scale grid resolves their interpretive differences more efficiently than a catalogue of endocrine effects.

Table 1. Tissue competence separates systemic endocrine exposure from executed metamorphic outcomes

Competence window			Execution boundary
Tissue gate	**Window and tissue context**	**Response evidence**	**Executed response versus systemic exposure**
Ecdysone-responsive repression	Drosophila developmental tissues	Bab2-dependent transcriptional repression	Hormone response can encode repression, not only activation. Cross-order conservation is unresolved. Test orthologous repressors in matched tissues and stages. Evidence-supported.
Dual-hormone remodeling gate	Drosophila fat body	JH/20E coordination of MMP-dependent cell dissociation	Tissue execution can require combined endocrine context. Specific to fat-body dissociation. Separate endocrine input from downstream remodeling machinery. Evidence-supported.
Neural temporal-competence gate	Drosophila brain neuroblasts	Steroid-induced temporal gene-expression transition	Systemic steroid timing can reshape lineage competence. Neural lineage result cannot be transferred to peripheral organs. Pair lineage-resolved timing with endocrine perturbation. Evidence-supported.
Developmentally constrained inducibility	Drosophila salivary gland	Steroid-triggered glue-protein production outside the normal program	Hormone exposure can activate an output without erasing temporal competence limits. Endpoint is salivary-gland specific. Measure responsiveness across defined developmental windows. Evidence-supported.
Stage-specifier control of endocrine-organ termination	Blattella adult prothoracic gland	E93 depletion preserves the gland and permits another molt	Stage identity can determine whether a hormone-producing organ remains functional. One hemimetabolous model; evolutionary inference is limited. Test gland termination and E93 dependency across representative orders. Evidence-supported.
Layered tissue-response gate	Cross-tissue synthesis	Convergence of receptor, local regulatory state, and tissue execution	Metamorphic response is proposed to require alignment of endocrine availability with tissue competence. Not validated as a single conserved architecture. Test with time-resolved, tissue-specific perturbation and rescue. Proposed synthesis.

Abbreviations: 20E, 20-hydroxyecdysone; JH, juvenile hormone; MMP, matrix metalloproteinase.

The same evidence can be compressed temporally by keeping systemic endocrine state separate from local competence and execution, while making explicit where stage identity and endocrine feedback alter the interpretation of a shared hormonal signal.

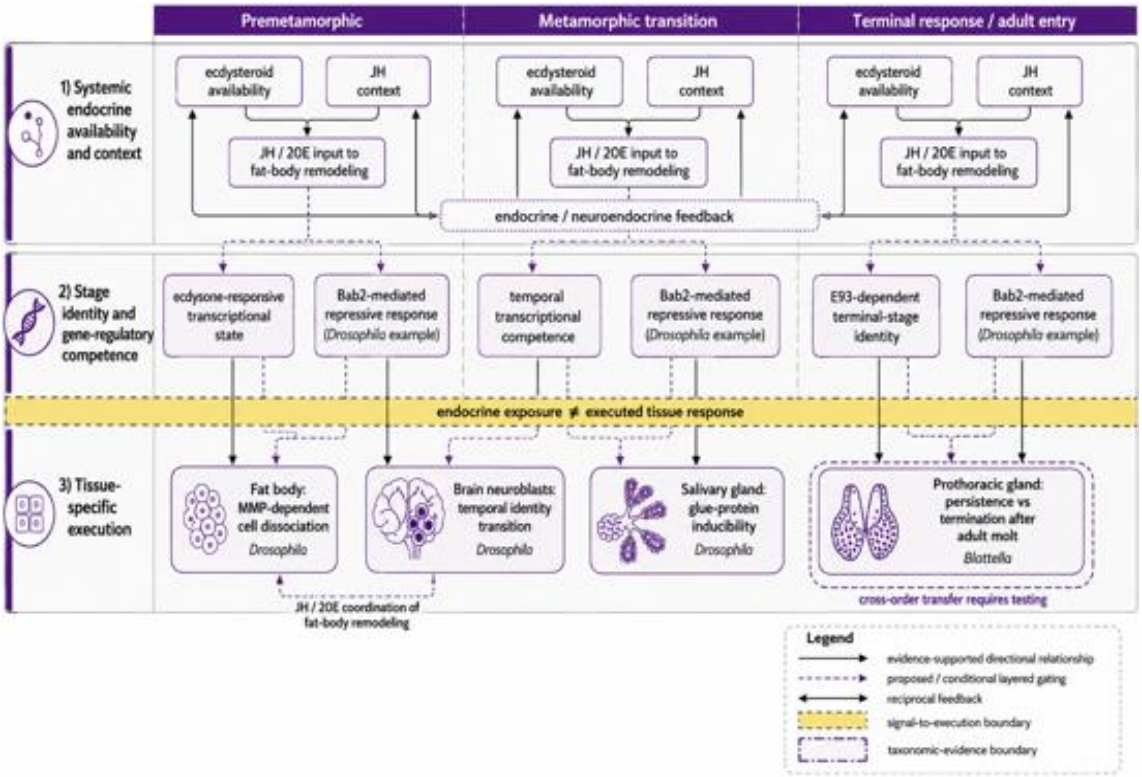


Figure 1. Temporal competence windows determine how shared endocrine signals become tissue-specific metamorphic programs

Timing, thresholds, and feedback

Developmental timing can be separated into at least two experimentally distinguishable problems: acquisition of a permissive physiological state and activation of an immediate metamorphic program. Nutrient-dependent prothoracic-gland endocycling in Drosophila links growth history to endocrine capacity [4], whereas a later autophagic switch modifies whether nutritional information continues to constrain steroidogenesis [15]. In Tribolium, E93 activation can trigger metamorphosis independently of threshold-size attainment [20]. These observations support a proposed two-gate interpretation in which permissive growth and nutritional states precede, but are not identical to, stage-specifying execution.

Feedback further prevents timing from being reduced to a one-directional hormone cascade. EGFR signaling and ecdysone participate in positive feedback within the Drosophila prothoracic gland [7], while ecdysone-dependent regulation of PTTH neurons closes a neuroendocrine feedback loop affecting maturation [8]. Nutritional gating nevertheless differs between Manduca and Drosophila [11], and Myoglianin-associated premetamorphic control in Blattella identifies another endocrine configuration [22]. Thus, timing mechanisms should be compared as context-weighted regulatory architectures rather than assumed to represent a conserved universal checkpoint.

Finally, circulating hormone concentration does not uniquely determine response time. Direct EcR-mediated transcription [16], E93-dependent chromatin competence [27], and temporally restricted salivary-gland responsiveness [35] demonstrate separable receptor, chromatin, and tissue-response constraints. The operative developmental threshold is therefore better viewed as a coordinated state than as a single molecular concentration.

Crosstalk across regulatory layers

Metamorphic timing emerges from communication among endocrine and peripheral tissues rather than from an isolated gland-autonomous switch [37]. Crosstalk occurs at several distinguishable levels. Within endocrine tissue, JH and 20E can antagonistically affect developmental transitions in Drosophila [19]. In Blattella, Myoglianin-associated regulation connects premetamorphic timing with changes in JH- and ecdysone-related endocrine processes [22]. These examples establish specific interactions, but not an identical cross-order topology.

Regulatory information can also move between molecular layers. H3K27 methylation-dependent regulation of Hairy alters ecdysone biosynthesis, while miR-8-3p control of V-ATPase components changes steroidogenic

output [28, 30]. The shared inference is limited: epigenetic and post-transcriptional mechanisms can feed into endocrine production, but these particular pathways need not operate together in one conserved circuit.

This review therefore proposes a coupled-gating architecture in which endocrine production, stage identity, chromatin competence, and tissue execution remain separate analytical layers connected by evidence-supported or conditional cross-links. The architecture is intended to prevent three common inference failures: equating hormone presence with tissue response, equating conserved components with conserved pathway weighting, and treating molecular association as proof of causal ordering. Interorgan communication provides the systems-level basis for this interpretation, but the exact network remains taxon- and stage-dependent [37].

Because the central difficulty is deciding when an observed interaction supports a local mechanism versus a system-level inference, the following matrix separates crosstalk states from the failure modes that would otherwise be obscured in narrative prose

The distinction between evidence and system-level inference is made explicit in **Figure 2**, which places molecular observations on one side of an interpretation boundary and increasingly integrated developmental claims on the other.

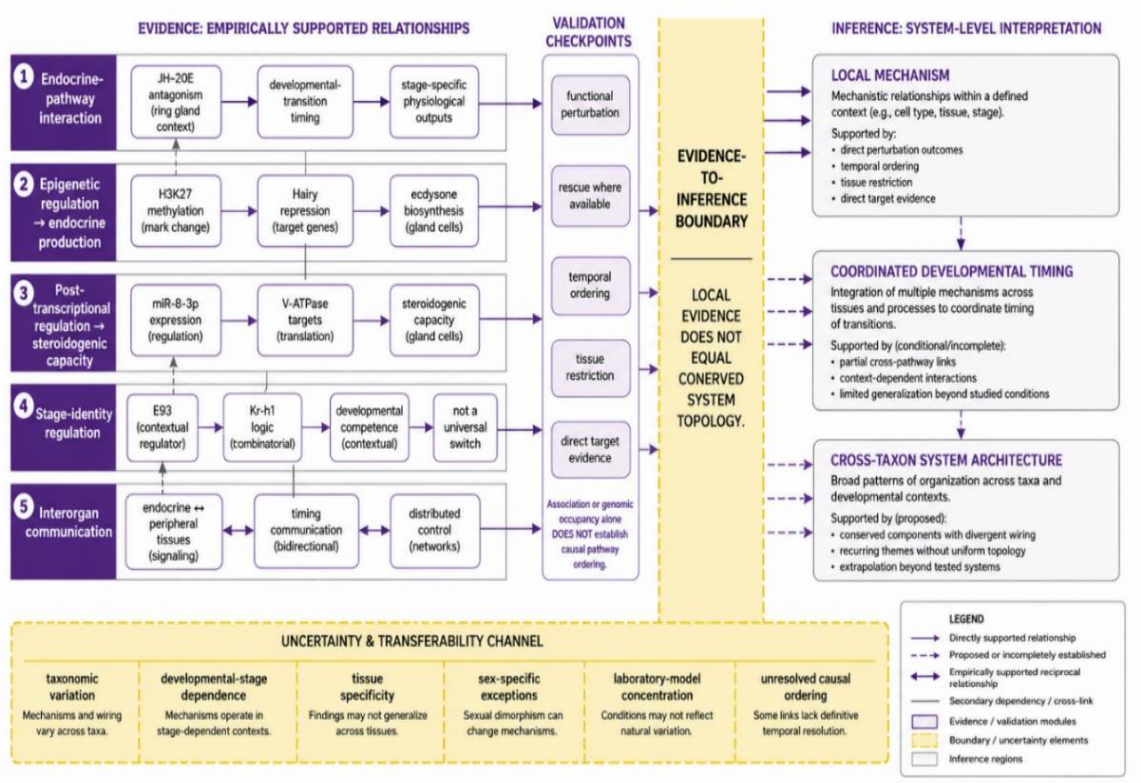


Figure 2. Where crosstalk evidence ends and system-level inference begins in metamorphic timing

Emerging molecular technologies

The principal methodological challenge is no longer simply identifying more metamorphosis-associated genes. Prothoracic-gland transcriptomics can expand candidate discovery [5], but functional perturbation is required before a candidate is assigned a causal position. CRISPR analysis of Bombyx torso illustrates how direct genetic intervention can reveal that a strongly implicated pathway component is important without being absolutely required for metamorphosis [10].

Genome-wide EcR mapping provides evidence for direct receptor targets [16], whereas chromatin-accessibility profiling can identify changing regulatory competence [26]. E93 perturbation combined with enhancer analysis further separates an instructive temporal regulator from parallel inputs that remain necessary at particular loci [27]. Histone-state analysis similarly becomes most informative when paired with functional manipulation [28].

The emerging priority is therefore methodological integration rather than data volume alone. Tissue-restricted perturbation, rescue, endocrine measurement, temporal sampling, and genomic readouts should be combined wherever feasible. Such combinations can distinguish molecular association from pathway placement and local mechanism from organism-level timing.

Unresolved mechanistic problems

The largest unresolved problem is taxonomic transfer. Comparative analysis shows that nutritional regulation of prothoracic-gland activity differs between Manduca and Drosophila [11], while mealybug E93/JH relationships expose sex-specific departures from canonical expectations [21]. Torso function in Bombyx likewise demonstrates that a conserved signaling component need not carry identical developmental weight [10].

A second problem is causal ordering. Ecdysone-dependent feedback on PTTH neurons is established in Drosophila [8], E93 can act as an immediate metamorphic trigger in Tribolium [20], and E93-dependent chromatin remodeling contributes to temporal competence [27]. What remains unresolved across orders is whether corresponding nutritional, endocrine, chromatin, and stage-identity events form a conserved sequence, parallel gates, or partially reciprocal processes.

Progress will require experiments that measure several regulatory levels within the same developmental series rather than reconstructing a pathway from different species and tissues. Transcriptomic discovery [5], direct receptor mapping [16], functional miRNA analysis [30], and interorgan approaches [37] provide complementary tools, but none alone resolves complete system causality. Cross-order validation should therefore prioritize matched developmental stages, tissue-specific perturbation, endocrine measurements, rescue, and time-resolved molecular readouts.

Conclusion

Insect metamorphosis is most coherently interpreted as a problem of temporal alignment among endocrine production, stage-identity regulation, chromatin competence, and tissue-specific execution. The evidence does not support reducing this control system to one hormone pulse, one transcription factor, or one conserved pathway topology. Instead, experimentally separable regulatory layers interact with different weights across tissues, stages, sexes, and insect lineages.

The principal synthesis advanced here is a proposed coupled-gating view: metamorphic progression occurs when endocrine availability and developmental competence become sufficiently aligned for specific tissues to execute their stage-appropriate programs. This formulation accommodates feedback, molecular crosstalk, and taxonomic variation without treating them as exceptions to an otherwise universal linear cascade. Its value ultimately depends on future tests that resolve causal ordering within individual species while expanding beyond the small set of experimentally dominant models.

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