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Molecular Responses, Organismal Performance, and Fitness Interpretation Across Biological Scales of Insect Stress Physiology

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ABSTRACT

Insect stress physiology is often interpreted upward across biological scales: molecular induction is treated as protection, protection as improved performance, and improved performance as evidence of fitness benefit or adaptation. The available evidence does not justify that sequence as a default. This critical review evaluates how inference changes from molecular stress signaling through cellular, tissue and organ responses to whole-animal performance, survival and reproduction. Functional genetic studies show that stress-response genes can contribute to tolerance while producing context-dependent or opposing effects across stress regimes. Mitochondrial and redox responses likewise range from signaling and compensation to injury, and plausible energetic markers can fail to explain phenotypic differences. At tissue and organ scales, ion-transport capacity, neural function and epithelial integrity provide stronger mechanistic bridges when linked directly to systemic homeostasis, yet assay context and candidate-mechanism nulls remain consequential. Whole-animal behavior and hardening can reduce exposure or increase tolerance while simultaneously imposing costs or degrading other performance components. The review therefore argues that molecular response, physiological protection, organismal performance and fitness consequence are distinct evidential claims. A proposed cross-scale interpretation framework developed later in the review requires explicit linkage, endpoint matching, exposure realism, and validation before adaptive meaning is assigned.

Keywords: Insect stress physiology, Thermal tolerance, Molecular stress signaling, Ion homeostasis, Organismal performance, Fitness inference

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Introduction

Extreme temperature affects insects through interacting molecular, cellular, physiological, behavioral and demographic processes, but evidence collected at these levels does not automatically form a single causal chain. Broad syntheses show that thermal extremes can alter stress signaling, metabolism, water and ion balance, development, behavior, survival and reproduction, with effects depending on taxon, life stage, exposure profile and ecological setting [1–3]. The interpretive problem is therefore not whether insects respond to stress, but what a measured response permits us to conclude about the next biological scale.

Plasticity illustrates this distinction. Across insects, thermal limits are plastic, but average shifts are generally modest and heterogeneous among traits and experimental contexts [4]. Developmental thermal plasticity also

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varies across ontogeny, and persistence of early-life effects is not uniform across endpoints [5]. These findings make response magnitude an unreliable synonym for benefit: a detectable shift can represent acclimation, compensation, constraint or a cost-bearing state without demonstrating improved fitness.

Reproductive evidence further exposes the limitations of lethal tolerance as a universal proxy. Across *Drosophila* species, temperatures that impair male fertility can correspond more closely with distributional limits than lethal temperatures, showing that organisms may remain alive after a fitness-relevant function has already failed [6]. More broadly, thermal fertility loss can occur through sex-, stage- and process-specific mechanisms that are not captured by survival endpoints [7]. Even acclimation measurements require validation against the environmental regime and endpoint for which adaptive significance is claimed [8]. This review therefore treats four claims as analytically separable: a stress response occurred; a component was protected or damaged; organismal performance changed; and the change altered fitness under ecologically relevant exposure. The central task is to identify when evidence supports movement between these claims and when it does not.

Molecular stress signaling

Molecular induction has its strongest interpretive value when function is experimentally perturbed, yet even functional evidence can be conditional. In *Drosophila melanogaster*, genomic knockout of *hsp23* produced opposing effects under different thermal extremes, demonstrating that the contribution of a canonical heat-shock gene cannot be represented by a simple "more response equals more tolerance" rule [9]. Reactive oxygen species provide a second example of non-scalar interpretation. In *Mythimna separata*, stress-derived ROS participate in a regulated hemocyte pathway that enables release of an enzyme involved in growth-blocking peptide processing [10]. ROS therefore can act as pathway signals under defined conditions; this does not imply that oxidative stress generally confers protection or that greater ROS production is advantageous.

Stage and stressor further constrain molecular meaning. Transcriptomic and functional-genetic work across complex life-cycle stages shows that stress-responsive programs can differ markedly among developmental states, so a transcript that is informative in one stage need not be an equivalent biomarker in another [11]. In *Myzus persicae*, induced Hsp70 contributed functionally to UV-B tolerance, as RNAi disruption reduced stress-associated performance [12]. In *Aphis gossypii*, RNAi evidence similarly implicated Hsp70 and Cathepsin B in acute thermal tolerance [13]. These experiments strengthen claims of local functional involvement beyond expression correlation, but they do not by themselves establish lifetime fitness effects, field benefit or evolutionary adaptation.

Life-history state and exposure history can also alter the interpretation of molecular programs. Ant stress biology shows that heat-shock regulation can be embedded in unusual longevity-associated physiological states rather than operating as an isolated emergency pathway [14]. Social isolation in ants produces redox disturbance alongside shortened lifespan, linking social state, oxidative physiology and survival in a specific system while leaving open how broadly that pathway generalizes [15]. Cross-tolerance experiments add temporal conditionality: drought pre-exposure can enhance subsequent freezing tolerance through metabolic rearrangement without producing a reciprocal protection pattern [16]. Thus, the relevant molecular unit is often not a single marker but a state-dependent response conditioned by stage, prior stress, tissue and ecological context.

Cellular protection and damage

At the cellular level, the same measured change can indicate protection, compensation or failure depending on where it occurs relative to loss of function. Freezing-sensitive insect larvae show mitochondrial injury that differs from freeze-tolerant phenotypes, supporting mitochondria as one target of freezing damage rather than a universal cause of cold death [17]. At high temperature, *Drosophila* mitochondria undergo strong shifts in substrate use near critical thermal conditions, and the magnitude and pattern vary among species [18]. These observations are mechanistically informative, but isolated mitochondrial performance is not equivalent to intact-animal performance.

Comparative concordance can coexist with mechanistic nulls. Across *Drosophila* species, balanced mitochondrial function at low temperature is associated with cold adaptation, making mitochondrial performance a plausible component of cross-species thermal phenotype [19]. Yet an in vivo study of *D. melanogaster* lines selected for cold-related performance found that ATP homeostasis did not explain the evolved difference in chill-coma recovery [20]. The null does not make energy metabolism irrelevant; it shows that a plausible cellular marker can fail as the explanatory variable even when the broader organelle is physiologically important.

For cross-scale interpretation, cellular measurements should therefore be classified by what they directly demonstrate: signaling activity, preserved function, measurable damage, compensatory reconfiguration, or a non-diagnostic change. This classification is proposed here as an evidential checkpoint rather than a new biological pathway. Upward inference is strongest when the cellular measure is temporally linked to a defined downstream dysfunction and alternatives are tested. It is weakest when direction of expression, abundance, ATP concentration or respiratory change is itself assigned adaptive meaning without a matched functional endpoint.

Tissue and organ responses

Tissue-level physiology can bridge molecular activity and organismal failure more directly, but only when tissue identity and assay context are preserved. Thermal acclimation changes Na+/K+-ATPase activity in *D. melanogaster* in a tissue-specific manner, making whole-body or pooled measurements potentially misleading [21]. In adult flies, plasticity in Na+/K+-ATPase thermal kinetics contributes to variation in the temperature of cold-induced neural shutdown, providing a mechanistic connection between pump function and a defined neural endpoint [22]. Yet rapid cold hardening in *Locusta migratoria* also illustrates assay discordance: changes detected in axonal Na+/K+-ATPase activity and improved visual-circuit performance need not be reproduced by bulk biochemical measures [23]. A "pump response" is therefore not a single evidential object when homogenate activity, in situ axonal function and circuit output are measured differently.

Epithelial ion transport provides one of the clearest organ-to-system bridges in insect cold physiology. Loss of ion and water balance is a major component of chill injury, with gut and Malpighian-tubule function central to maintaining hemolymph homeostasis [24]. In cold-acclimated *D. melanogaster*, functional plasticity of the gut and Malpighian tubules mitigates cold-induced hyperkalemia, linking organ transport to a systemic disturbance rather than relying on enzyme abundance alone [25]. Comparative work across *Drosophila* species likewise finds cold tolerance closely associated with epithelial K+ transport capacity of Malpighian tubules and rectal pads [26]. These data support transport flux as a stronger bridge than any single transporter or enzyme assay, while still leaving room for other tissues and unmeasured regulatory processes.

Concordance becomes more persuasive when systemic state, tissue function and whole-animal outcome are measured together. In migratory locusts, cold acclimation improves chill tolerance alongside better preservation of ion balance and membrane potential, producing an internally coherent multilevel pattern [27]. Conversely, chilling can induce directional solute leak through locust gut epithelia, identifying a tissue-level failure without fully resolving the molecular lesion responsible [28]. Heat-stressed *D. melanogaster* lines provide a different boundary: cardiac performance can differ between heat-susceptible and heat-resistant phenotypes even when plausible candidate biochemical mechanisms do not differ as predicted [29]. Tissue phenotype may therefore be real while a favored molecular explanation fails.

Because these cases differ in how securely tissue measurements connect to systemic function, the cross-scale evidence grid below distinguishes mechanistic bridges from scale-bound proxies, assay discordance and candidate-mechanism nulls.

Table 1. When tissue-level responses become mechanistic bridges—or remain scale-bound proxies in insect stress physiology

Tissue or organ response pattern	Dimension	Scientific content	Dimension	Scientific content
Tissue-specific pump plasticity	**Biological context · Evidence basis · Biological setting · Evidence**	Adult Drosophila under thermal acclimation Na+/K+-ATPase activity differs among tissues	**Research or test · Status · Evidential standing**	Match pump measurement to the tissue governing the downstream phenotype Evidence-derived; interpretive label proposed
	Interpretation for cross-scale inference · Boundary or uncertainty · Limits and uncertainty	Bulk biochemical change cannot be assumed to represent all organs Tissue composition and assay preparation alter the signal		

Pump-to-neural-shutdown bridge	**Biological context · Evidence basis · Biological setting · Evidence**	Adult Drosophila exposed to cold Pump thermal kinetics linked to neural shutdown temperature	**Research or test · Status · Evidential standing**	Manipulate pump function while measuring neural and organismal consequences Evidence-derived
	Interpretation for cross-scale inference · Boundary or uncertainty · Limits and uncertainty	Adjacent-level mechanism is stronger than marker correlation alone Neural shutdown is not a fitness endpoint		
Assay-context discordance	**Biological context · Evidence basis · Biological setting · Evidence**	Adult migratory locust after rapid cold hardening Axonal pump function and visual-circuit performance diverge from bulk assay expectations	**Research or test · Status · Evidential standing**	Use parallel assays on the same tissue and exposure state Evidence-derived; interpretive label proposed
	Interpretation for cross-scale inference · Boundary or uncertainty · Limits and uncertainty	Homogenate, in situ and circuit measurements are not interchangeable Discordance may reflect preparation rather than biological contradiction		
Epithelial K+ homeostasis bridge	**Biological context · Evidence basis · Biological setting · Evidence**	Drosophila cold acclimation and species comparisons Gut, Malpighian-tubule and rectal-pad transport linked to hyperkalemia and tolerance	**Research or test · Status · Evidential standing**	Test transport perturbations against hemolymph K+ and recovery endpoints Evidence-derived
	Interpretation for cross-scale inference · Boundary or uncertainty · Limits and uncertainty	Functional ion flux can connect organ physiology to systemic cold injury Other transporters and tissues can contribute		
Barrier-leak failure state	**Biological context · Evidence basis · Biological setting · Evidence**	Chilled migratory locust Directional solute leak across gut epithelia	**Research or test · Status · Evidential standing**	Pair permeability assays with targeted membrane and junctional perturbations Evidence-derived; state label proposed
	Interpretation for cross-scale inference · Boundary or uncertainty · Limits and uncertainty	Tissue injury can generate systemic ionic disturbance without identifying one molecular lesion Causal molecular origin remains unresolved		
Multilevel homeostatic concordance	**Biological context · Evidence basis · Biological setting · Evidence**	Cold-acclimated migratory locust Ion balance, membrane potential and chill tolerance shift coherently	**Research or test · Status · Evidential standing**	Perturb candidate mediators while retaining multilevel endpoints Evidence-derived; cross-scale interpretation proposed
	Interpretation for cross-scale inference · Boundary or uncertainty ·	Concordant adjacent levels strengthen, but do not complete, a causal chain Correlated intermediates may share upstream causes		

	Limits and uncertainty			
	Biological context · Evidence basis · Biological setting · Evidence	Heat-stressed Drosophila lines Cardiac performance differs while candidate biochemical differences are absent	**Research or test · Status · Evidential standing**	Retain mechanistic alternatives after candidate-marker failure Evidence-derived; interpretive label proposed
Organ phenotype with candidate null	**Interpretation for cross-scale inference · Boundary or uncertainty · Limits and uncertainty**	A genuine organ phenotype need not validate the preferred molecular mechanism Unmeasured mediators may explain the phenotype		

Note: Evidence-derived entries summarize the tissue and organ studies discussed in the preceding text. "Proposed" denotes interpretive categories introduced by this review rather than empirically validated classifications.

Whole-animal performance

Whole-animal performance is not a scalar endpoint. A response may reduce immediate exposure, preserve one task or impose a delayed cost, and these effects can coexist. In *Drosophila*, threat responses are accompanied by cardiac and metabolic changes that reduce subsequent starvation survival, demonstrating that an apparently defensive state can carry measurable downstream costs [30]. Heat-avoidance behavior in *Drosophila* is both robust and plastic and can alter the temperatures an animal actually experiences [31]. Behavior therefore modifies realized dose before internal physiology is interpreted, but laboratory avoidance capacity does not guarantee that appropriate microhabitats, time or mobility are available in the field.

Endpoint discordance is equally important after physiological conditioning. Automated phenotyping shows that heat hardening can improve a thermotolerance-related endpoint while reducing motility in *D. melanogaster* [32]. Calling the hardened phenotype simply "better" would erase a within-organism trade-off whose ecological consequence is unresolved; lower activity could represent impairment, energy conservation or a context-dependent cost. Performance claims should consequently name the task measured rather than treat thermotolerance, locomotion and recovery as interchangeable indicators of quality.

Finally, field inference requires an exposure model commensurate with the biology. In natural *Drosophila* populations, time-integrated thermal exposure provided stronger prediction of temperature-associated mortality and selection than static critical limits in the evaluated system [33]. The important lesson is not that one dynamic model transfers universally, but that duration, sequence and cumulative dose can determine whether a laboratory performance metric retains meaning outside the assay. Whole-animal performance is therefore the first level at which behavior, physiology and exposure history converge, yet it remains an intermediate evidential layer until survival and reproduction are measured under conditions relevant to actual ecological opportunity.

Survival and reproduction

Survival and reproduction expose the largest inferential gap between stress tolerance and biological consequence because failure can occur in one axis while another remains apparently intact. Experimental heatwaves in *Tribolium castaneum* impaired male sperm function and produced effects extending into offspring traits; developmental heat stress in *Drosophila melanogaster* imposed adult male reproductive costs; and short temperature exposures reduced sperm viability in *Bombus impatiens* [34–36]. The convergence is limited: differing taxa, schedules and endpoints support recurrent male reproductive vulnerability, not a universal threshold or mechanism.

Exposure history further changes the meaning of a stress response. In adult female *D. melanogaster*, daily and weekly mild heat schedules produced different transcriptomic, metabolic, fertility and longevity-related outcomes [37]. Peak temperature alone therefore does not define repeated stress. Recovery interval, cumulative dose, age and state may contribute to schedule dependence. Reproductive interpretation should report intensity, duration, frequency and recovery rather than treating nominal temperature as the complete exposure.

The word fitness also requires discipline. Male sterilization temperatures can reveal vulnerability that lethal limits miss [6], but fertility thresholds are still not lifetime reproductive success. Heatwave-induced sperm dysfunction in *T. castaneum* is directly reproductive and can extend across generations [34], whereas developmental heat

effects on mating and offspring production in *D. melanogaster* reveal genotype- and trait-dependent costs [35]. Bumble-bee sperm viability is biologically closer to male fertility than worker thermal limits but remains short of colony growth or population persistence [36]. Repeated heat effects on female fertility and longevity likewise represent fitness-related endpoints rather than complete realized fitness [37]. The appropriate inference is therefore endpoint-specific: survival, mating success, gamete function, fecundity, offspring production and transgenerational effects should not be collapsed into a single scalar measure.

The conditionality matrix below makes explicit where survival and reproductive endpoints diverge and what additional evidence is needed before a demographic interpretation is justified.

Table 2. Survival can remain an incomplete descriptor when reproductive failure depends on sex, stage, exposure history, and endpoint

Failure mode or boundary	Evidence	Interpretive consequence · Biological setting	Research or test · Evidential standing
Survival retained while male fertility fails			
Fertility threshold is not population growth	Sterilizing and lethal temperatures diverge	Reproductive vulnerability can precede mortality Cross-species Drosophila thermal comparisons	Measure lethal and reproductive limits under matched exposures Evidence-derived
Heatwave sperm-function damage			
Protocol- and sex-specific; transgenerational outcome is not universal	Sperm function and reproductive performance decline after experimental heatwaves	Sublethal heat can damage reproduction despite organismal survival Adult male red flour beetles	Couple male reproductive assays with offspring performance and recovery Evidence-derived
Developmental heat with adult reproductive cost			
Genotype and trait effects differ; genetic variance is not realized adaptation	Adult male mating, fertility and offspring traits vary after stressful regimes	Early thermal history can reappear as adult reproductive impairment Drosophila developmental exposure	Follow developmental treatments into adult reproductive endpoints Evidence-derived
Gamete sensitivity below worker-lethal framing			
Sperm viability alone does not establish colony-level consequence	Short heat exposure lowers sperm viability	Gametes can be more vulnerable than commonly used whole-animal thresholds Adult male bumble bees	Link gamete damage to mating, queen insemination and colony outcomes Evidence-derived
Frequency-dependent repeated stress			
Laboratory schedule may not reproduce field thermal histories	Daily versus weekly stress changes molecular and fertility/longevity phenotypes	Exposure schedule is part of the phenotype's causal context Adult female Drosophila	Manipulate frequency and recovery independently while retaining fitness-related endpoints Evidence-derived
Endpoint substitution			
Proxy distance from demographic consequence varies	Survival, locomotion, fertility or gamete measures used interchangeably	Different endpoints occupy different positions on the path to realized fitness Any stress study using one proxy for "fitness"	Name the endpoint and validate each inferential transition Proposed synthesis

Note: "Proposed synthesis" identifies an interpretive category introduced by this review; evidence-derived rows summarize the patterns described in the preceding section without implying a shared quantitative threshold.

These relationships are represented in **Figure 1** as alternative trajectories rather than a single upward pathway, because the same nominal stress can terminate in survival, reproductive impairment, delayed cost, or unresolved demographic consequence.

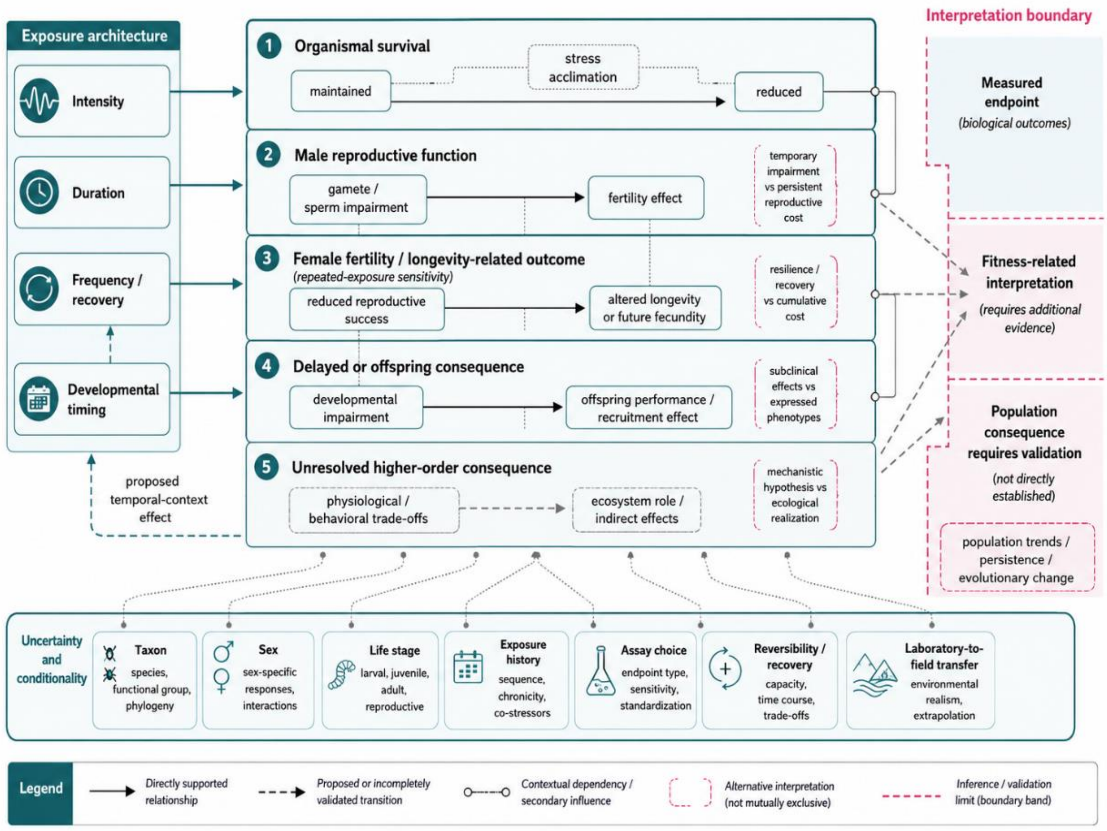


Figure 1. Divergent survival and reproductive trajectories under sublethal and repeated thermal stress

Why molecular response does not necessarily equal adaptive benefit

Functional molecular evidence is necessary for many mechanistic claims, but it is not sufficient for adaptive interpretation. Knockout or RNAi studies show that Hsp23, Hsp70 and Cathepsin B can contribute causally to defined stress phenotypes [9, 12, 13]. That evidential gain is local: perturbation establishes a functional contribution under the tested treatment. It does not establish that the response increases lifetime reproductive success, that the benefit persists under field exposure, or that the underlying variant or inducible program was shaped by selection for the inferred function. The distinction is especially clear when the sign of a gene's contribution changes with the stress regime.

Null and discordant findings are therefore central evidence, not inconveniences. ATP homeostasis failed to explain evolved differences in chill-coma recovery among selected *D. melanogaster* lines [20]. In locusts, assay scale altered the apparent relationship between Na+/K+-ATPase activity and neural performance [23]. Heat-resistant and heat-susceptible *D. melanogaster* differed in cardiac performance without the expected candidate biochemical explanation [29]. Heat hardening improved one thermotolerance-related phenotype while reducing motility [32]. Together, these examples show four different failure modes: plausible marker without phenotypic explanation, assay non-equivalence, organ phenotype with candidate-mechanism null, and benefit in one endpoint accompanied by cost in another.

Plasticity itself also cannot be labeled adaptive by default. Across insects, thermal-limit plasticity is widespread but generally modest and heterogeneous [4]. Acclimation measurements require explicit validation against the environmental conditions and outcomes for which adaptive significance is claimed [8]. Developmental heat exposure can reveal reproductive costs even while quantitative-genetic variation suggests potential for response [35]. Repeated mild heat can produce transcriptomic change alongside schedule-dependent fertility and longevity effects [37]. This review therefore proposes a four-term evidence vocabulary: response for a measured change, functional contribution for experimentally supported involvement, benefit for improvement in a relevant performance or fitness-related endpoint under the tested context, and adaptation only when appropriate heritable evolutionary evidence supports that claim.

The trait–process–consequence map below prevents these terms from being treated as interchangeable stages of a single confirmed mechanism.

Table 3. Why stress-responsive traits cannot be promoted directly to adaptive benefit

Inferential state	Molecular response	Functional contribution	Cellular or organ concordance	Organismal benefit	Fitness-related benefit	Evolutionary adaptation
Biological or evidential context	Transcript, protein, metabolite or redox change	Gene or pathway perturbation alters tolerance	Molecular change accompanies preserved tissue function	A defined task, tolerance or recovery endpoint improves	Fertility, survival or offspring production improves	Heritable change increases fitness in the relevant environment
Evidence basis · Permitted interpretation · Evidence	Stress-associated molecular shift The system responded under the measured condition	Knockout/RNAi or comparable manipulation Candidate contributes to the defined phenotype	Matched cellular, transport, neural or cardiac measure Mechanistic bridge becomes more plausible	Whole-animal performance measurement Benefit is supported for that endpoint and context	Explicit reproductive or survival endpoint Consequence is closer to realized fitness	Selection and evolutionary evidence Adaptive interpretation may be justified
Boundary or failure mode	Direction of change does not specify protection	Context, pleiotropy and endpoint dependence can change effect	Concordance can share an upstream cause	Other performance components may worsen	Proxy may omit mating ecology, density and population dynamics	Plasticity or genetic variance alone is insufficient
Required next test · Status · Evidential standing	Perturb the candidate and measure downstream function Proposed synthesis	Test across relevant stresses, stages and performance outcomes Evidence-derived category; synthesis proposed	Manipulate the mediator while retaining adjacent-level endpoints Proposed synthesis	Measure trade-offs and ecologically relevant exposure Proposed synthesis	Measure lifetime and demographic consequences where required Proposed synthesis	Demonstrate heritable fitness advantage and environmental relevance Proposed evidential boundary

Note: The table does not constitute a validated scoring system. Its categories are proposed interpretive boundaries grounded in the empirical contrasts reviewed above.

Cross-scale inference errors

Cross-scale error begins when differences among biological levels are treated as measurement noise rather than changes in the object being measured. Stress-responsive transcription can differ among life-cycle stages [11]; Na+/K+-ATPase acclimation is tissue-specific [21]; in situ axonal and bulk biochemical assays can yield different pictures of cold hardening [23]; gut and Malpighian-tubule transport cannot be replaced by a generic enzyme proxy when hyperkalemia is the systemic endpoint [25]; and cardiac phenotypes need not validate the candidate biochemical mechanism [29]. Transcript abundance, enzyme activity, epithelial flux and organ performance are therefore distinct evidence classes.

A second error is endpoint–dose mismatch. Male fertility limits can reveal thermal vulnerability not captured by lethal limits [6]. Time-integrated exposure can outperform static critical temperatures for explaining mortality patterns in a natural *Drosophila* system [33]. Short-term heat damage to bumble-bee sperm shows that a gamete-level endpoint can be sensitive under conditions that a worker-centered lethal framing would miss [36]. None of these observations establishes a universally superior metric; each shows that the relevant endpoint and temporal representation must match the inference being attempted.

The third error is externalizing laboratory responses without validating the exposure ecology. Plastic changes in thermal limits are heterogeneous [4], and developmental responses vary in persistence across ontogeny [5]. Acclimation therefore requires tests of whether laboratory treatments represent the environmental state against which benefit is claimed [8]. Behavioral heat avoidance can alter realized thermal dose [31], while fluctuating developmental heat can expose costs and genotype dependence [35]. Repeated stress frequency also changes molecular and fitness-related outcomes [37]. A proposed minimum validation chain is consequently: identify the biological level measured; preserve the actual temporal exposure; specify the endpoint; test the adjacent-level link where mechanism is claimed; and validate field or evolutionary transfer separately.

Integrative interpretation framework

The evidence supports an interpretive architecture, not a universal stress pathway. Broad insect stress physiology spans molecular, cellular, organ, organismal and reproductive responses whose coupling varies among taxa and environments [1]. Acclimation research shows why environmental validation must accompany claims of adaptive relevance [8], while stage-specific functional evidence shows that biological level and developmental state cannot be treated as interchangeable [11]. The framework proposed here therefore records, for every claim, the biological level, evidence class, exposure context, endpoint and inference status.

Confidence in a cross-scale explanation should increase qualitatively when adjacent levels are connected within the same system and by an appropriate manipulation. Gene knockout can establish a local contribution to tolerance [9]; mitochondrial injury can identify a cellular target without proving organismal causation [17]; pump kinetics can connect to neural shutdown [22]; ion balance, membrane potential and chill tolerance can shift coherently during acclimation [27]; defensive physiology can be linked to a downstream survival cost [30]; and reproductive heat damage can be followed into offspring consequences [34]. These examples differ in causal depth, but they share a useful principle: unmeasured levels should remain unmeasured rather than being silently filled by inference.

Validation must also be transition-specific. Heterogeneous plasticity constrains generalization from response to benefit [4]. Fertility–lethality mismatches constrain survival-based fitness inference [6]. Time-integrated field mortality constrains static-threshold transfer [33]. Gamete sensitivity constrains organism-level thermal generalization across sex and trait [36]. Exposure-frequency effects constrain attempts to treat molecular signatures as exposure-independent predictors [37]. The proposed framework therefore uses no numerical certainty grade. Instead, each transition is classified qualitatively as observed, experimentally supported, inferred, proposed or unvalidated, with explicit uncertainty attached to taxon, stage, sex, assay, exposure history and environment. Its purpose is to prevent biological distance from being mistaken for evidential continuity.

Conclusion

Insect stress physiology is most informative when biological scales are connected without being collapsed. Molecular induction can identify response, perturbation can establish local function, cellular and organ measurements can reveal protection or injury, and whole-animal assays can quantify performance, yet none of these automatically establishes fitness benefit or adaptation. Reproductive endpoints, temporal exposure, trade-offs and laboratory-to-field transfer repeatedly create points at which an apparently coherent upward narrative can fail. The principal contribution of this review is therefore an evidence-bounded interpretation strategy: name the biological level, match the exposure and endpoint, retain null and discordant evidence, distinguish functional contribution from benefit, and validate each cross-scale transition that carries a stronger claim. This approach preserves mechanistic ambition while preventing stress markers, performance proxies and evolutionary language from becoming interchangeable.

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