Eurasia Specialized Veterinary Publication

Entomological Research Letters

ISSN: 3108-639X

2024, Volume 4, Issue 2, Page No: 52-62
Copyright CC BY-NC-SA 4.0
Available online at: www.esvpub.com/

Proteostasis, Membrane Stability, Metabolic Regulation, and Repair Together Define the Molecular Logic of Insect Thermal Tolerance

Maria Silva[1]*, Joao Pereira[1], Bruno Costa[2], Ricardo Alves[3]

[1]Department of Proteostasis and Thermal Tolerance, Faculty of Life Sciences, University of Coimbra, Coimbra, Portugal.
[2]Department of Membrane Stability and Metabolic Regulation, Faculty of Biosciences, University of Porto, Porto, Portugal.
[3]Department of Repair Mechanisms and Heat Tolerance, Faculty of Sciences, University of Lisbon, Lisbon, Portugal.

***E-mail** ✉ maria.silva@uc.pt

ABSTRACT

Insect thermal tolerance is commonly interpreted through individual responses such as heat-shock proteins, antioxidant enzymes, lipid remodeling, or metabolic adjustment. These processes, however, operate at different biological levels and need not vary together across taxa, life stages, or exposure regimes. This article develops a proposed molecular-physiological model in which thermal tolerance depends on coordinated preservation of proteome integrity, membrane and lipid state, energetic supply, and damage control and repair. Cellular stress signaling is treated as context-sensitive regulation rather than a universal master switch. The synthesis separates functional evidence from association, molecular response from organismal performance, and acute protection from longer-duration maintenance. Evidence across Diptera, Lepidoptera, Hemiptera, Hymenoptera, and Coleoptera indicates that component importance and direction can vary with species, developmental stage, oxygen and resource state, and exposure duration. The contribution is therefore not a conserved pathway but an evidence-bounded coordination logic that generates testable predictions about when canonical stress responses should remain compensatory or become insufficient.

Keywords: Heat shock proteins, Membrane lipids, Mitochondrial bioenergetics, Oxidative stress, Autophagy, Thermal acclimation

Received: 25 August 2024
Revised: 09 October 2024
Accepted: 10 October 2024

How to Cite This Article: Silva M, Pereira J, Costa B, Alves R. Proteostasis, Membrane Stability, Metabolic Regulation, and Repair Together Define the Molecular Logic of Insect Thermal Tolerance. Entomol Res Lett. 2024;4(2):52-62. https://doi.org/10.51847/8Dm1G0q7Y3

Introduction

Insect heat biology is often interpreted through single molecular markers, although broad synthesis shows that heat responses span protein quality control, metabolism, signaling, neuroendocrine regulation, behavior, and organismal performance [1]. This creates an inferential problem: a molecule may be heat responsive without being rate limiting, and a protective response may be activated without preserving performance.

Organismal plasticity constrains such interpretation. Across more than one hundred insect species, critical thermal limits were plastic but generally only modestly so, with upper limits less plastic than lower limits and substantial heterogeneity among life stages and study designs [2]. Large transcriptional or biochemical changes therefore should not be assumed to yield equally large shifts in thermal limits.

Temporal scale further separates different phenomena. In a natural population of *Murgantia histrionica*, high-temperature developmental performance differed markedly between experiments conducted more than a decade apart, demonstrating historical change without identifying its genetic or physiological cause [3]. Within individual

© 2024 Entomological Research Letters

lifetimes, *Drosophila suzukii* shows strong temperature-duration dependence, and reproductive impairment can occur under exposures causing limited mortality [4]. Historical change, hardening, prolonged damage, and recovery are therefore distinct analytical problems.

Developmental stage adds another boundary. In *Drosophila melanogaster*, basal heat resistance and hardening benefit differ among larvae, puparia, pupae, and adults [5]. The model developed here consequently asks not which single pathway causes heat tolerance, but how proteostasis, membrane and lipid regulation, energy metabolism, and damage control may jointly preserve function. Their integration is proposed as an organizing hypothesis, not as an empirically validated universal circuit.

Protein stability and proteostasis

Elevated temperature can destabilize proteins and increase demand for chaperoning and degradation, but "HSP induction" is too coarse to describe proteostasis. In transgenic *Drosophila* larvae, an HSP70 from a thermotolerant dipteran conferred greater thermoresistance than the corresponding endogenous construct under the tested conditions [6]. In *Monochamus alternatus*, MaltHSP70-2 was heat inducible, and RNA interference reduced thermotolerance while altering expression within the wider HSP network [7]. These experiments support gene-specific contributions, not universal sufficiency of HSP70.

Regulation is also selective. In *Chilo suppressalis*, HSF1 knockdown reduced survival and decreased expression of several, but not all, HSP genes [8]. In *Aphis gossypii*, thermal transcriptomics and targeted analysis implicated both HSP70 and Cathepsin B, linking chaperoning with proteolytic or lysosomal functions [9]. Proteostasis is therefore treated here as a domain containing protein stabilization, turnover, and damage clearance rather than a single heat-shock response.

Autophagy extends this logic while exposing cross-system entanglement. In *D. melanogaster*, heat stress altered protein aggregation and autophagic activity, and pharmacological induction of autophagy in the gut was associated with increased heat tolerance [10]. Rapamycin also affects TOR-linked translation and metabolism, so the experiment does not isolate autophagy as the only causal route.

Multi-omics provides a different evidence class. In *Spodoptera frugiperda*, integrated transcriptomic and proteomic analyses identified coordinated heat-responsive changes including small HSPs and antioxidant systems, while silencing selected candidates increased mortality [11]. Functional tests strengthen inference for those nodes; the wider omics network remains largely associational. The proposed model therefore distinguishes measured co-regulation, functional dependency, and hypothesized cross-system linkage.

Membrane remodeling

"Membrane remodeling" encompasses several lipid processes that should not be conflated. In *Drosophila*, the exchangeable apolipoprotein Nplp2 supports systemic lipid flow and contributes functionally to heat acclimation [12]. This links lipid handling to thermal performance without proving an exclusive membrane-fluidity mechanism. In hymenopterans, flight-muscle fatty-acid composition covaries with thermoregulatory capacity [13]. Because body size, phylogeny, and metabolic phenotype can covary with lipid composition, this is association rather than direct membrane causation.

Stress history and diet also shape lipid state. *D. melanogaster* lines selected for heat, cold, or desiccation resistance show distinct lipid-class profiles rather than one common stress signature [14]. Separate lipidomic work found that dietary sterol signatures persist in *Drosophila* tissues and that environmental conditions can coincide with changes in membrane unsaturation [15]. Lipid state is therefore partly resource- and history-dependent.

Physical compartment is equally important. In *Plutella xylostella*, disruption of the very-long-chain-fatty-acid enzyme PxHacd2 reduced very-long-chain fatty acids, increased epidermal permeability, and altered temperature adaptability [16]. This is a lipid-dependent thermal mechanism, but it concerns epidermal barrier function rather than intracellular phospholipid bilayers. In *Bactrocera dorsalis* larvae, sublethal heat was associated with fatty-acid shifts and central metabolic reorganization, including repression of parts of aerobic tricarboxylic-acid metabolism [17]. The study links lipid and energetic responses without proving that every metabolite change is protective.

Direction is not always intuitive. In *Cyrtorhinus lividipennis* nymphs, inhibition of alkaline ceramidase increased heat survival while altering ceramide balance and antioxidant responses [18]. Thus, lipid-related effects depend on lipid class, compartment, signaling role, species, and exposure condition; a simple rule that greater pathway activity implies greater tolerance is not defensible.

energy metabolism

Energy metabolism is not a single reserve but a set of pathway capacities whose usefulness changes with temperature. Comparative flight-muscle respirometry in *Apis mellifera*, *D. melanogaster*, and *Leptinotarsa decemlineata* showed species-specific thermal sensitivity and substrate-use flexibility, with alternative fuels supporting respiration under some conditions as other routes declined [19]. The relevant capacity is therefore maintenance of ATP-producing respiration, not one universal substrate switch.

Whole-animal function can decline despite broad metabolic activation. In *Bombus eximius*, elevated temperature altered energy metabolism and coincided with poorer flight-related and pollination performance [20]. This connects metabolism to an organismal endpoint but does not identify a single metabolic cause. Conversely, *Manduca sexta* flight-muscle mitochondria did not use some alternative substrates reported in other insects for ATP production [21], underscoring taxon-specific energetic constraints.

Analytical level also matters. Key energetic enzymes show temperature-sensitive activities that differ among insect species [22], but isolated enzyme capacity is not intact-organism flux. In larval *Lymantria dispar*, metabolic rate-temperature relationships vary among instars even after accounting for body mass [23]. Enzyme activity, mitochondrial respiration, whole-animal metabolic rate, and survival must therefore remain distinct measurements.

Resource state sets another boundary. Nutrient and water restriction can alter measured thermal tolerance, indicating that energy and hydration may limit sustained protection [24]. Cold-acclimated *D. melanogaster* females better maintained mitochondrial respiration and ATP-related function under cold stress [25]. That result is not direct heat evidence; it supports only the broader principle that thermal acclimation can preserve bioenergetic function.

Because these states differ in biological level and inference, a compact comparison is more useful than a pathway list. **Table 1** separates the energetic situations that change how thermal-performance data should be interpreted.

Table 1. Energy-metabolic states that alter inference about insect thermal tolerance

Metabolic configuration	Organism or tissue and measured signal	Thermal meaning and limiting condition	Validation route and evidence state
Substrate and respiratory capacity			
Respiratory-substrate flexibility	Adult flight muscle across bee, fly, and beetle models Temperature-dependent mitochondrial respiration with alternative substrates	Multiple routes can sustain energetic throughput when one route is constrained Substrate repertoire is species specific; ex vivo capacity is not survival	Test substrate switching together with intact-animal ATP maintenance and survival Evidence-supported state
Restricted substrate repertoire	Adult hawkmoth flight muscle OXPHOS lacks some alternative substrate use reported in other insects	Compensation cannot be assumed from another taxon Endothermic physiology and ex vivo assays limit transfer	Compare homologous tissues under common substrate panels Evidence-supported boundary
Enzyme-capacity state	Multiple insect species Temperature-sensitive energetic-enzyme activity	Pathway capacity can change before system-level flux is known In vitro activity does not establish control strength in vivo	Pair enzyme assays with flux or respirometry Evidence-supported measurement state
Functional disruption and ontogenetic scaling			
Metabolic-performance disruption	Adult bumblebee Heat-associated metabolic change with reduced flight/pollination performance	Energetic disturbance can coincide with functional loss before lethality Neural or muscular impairment may contribute independently	Combine metabolic perturbation with direct performance endpoints Evidence-supported association
Ontogenetically scaled metabolism	Larval instars Stage-specific metabolic rate-temperature relationships	Development changes energetic demand and thermal sensitivity Resting metabolism is not a heat-tolerance endpoint	Match stage-specific respirometry to common performance endpoints Evidence-supported boundary
Maintenance under resource or acclimation history			
Resource-constrained maintenance	Resource-manipulated insects Tolerance changes under nutrient or water restriction	Energy and hydration can limit sustained protection Direction depends on resource and endpoint	Standardize or manipulate resource state in mechanistic heat tests Evidence-supported boundary

Acclimation-preserved mitochondrial function	Cold-acclimated adult *Drosophila* females Better maintenance of respiration and ATP-related function under cold stress	Thermal acclimation can preserve bioenergetic function Cold-specific foundation; not direct heat evidence	Test analogous maintenance during heat acclimation Proposed transfer hypothesis

Oxidative damage and repair

Oxidative stress is more informative when linked to functional intervention than when treated as an exposure marker. In *Bombyx mori*, transgenic expression of a superoxide reductase from *Pyrococcus furiosus* reduced mortality during prolonged high-temperature exposure [26]. Because the enzyme is heterologous, the experiment shows that oxidative burden can be limiting without implying that insects naturally use this route.

Endogenous antioxidant evidence is more directly physiological. In *Bemisia tabaci* MED, catalase knockdown increased reactive oxygen species and reduced survival and fecundity under high temperature [27]. This supports a functional contribution of ROS removal but does not establish catalase as sufficient for field thermal fitness. It also distinguishes damage limitation from repair: antioxidants reduce damaging chemistry, whereas repair restores or maintains affected structures.

Life stage and biological organization broaden the repair problem. Early *D. melanogaster* embryos mount a robust heat-responsive transcriptome, with population differences involving oxidative-stress genes [28]. Transcript abundance alone, however, does not demonstrate function. In an aphid system, complementary mismatch-repair functions distributed across *Buchnera* and *Serratia* endosymbionts reduce heat-sensitive mutation accumulation in the obligate symbiont and restore host heat tolerance [29]. Repair can therefore occur at different biological levels, including across symbiotic partners, without constituting one cell-autonomous pathway.

Oxygen history can alter the damage state. After severe hypoxia and reperfusion, *D. melanogaster* exposed to higher temperatures showed greater behavioral impairment, mortality, and oxidative-stress responses than cooler groups [30]. Because hypoxia is central to that design, the evidence supports an oxygen-by-temperature boundary rather than a generic heat-only ROS mechanism.

The state-space interpretation used here is proposed: thermal failure becomes more likely when damage accumulates faster than antioxidant, proteostatic, energetic, and repair processes can maintain function. No universal threshold is implied. The figure therefore separates damage load from maintenance capacity and places organismal endpoints beyond an explicit interpretation boundary.

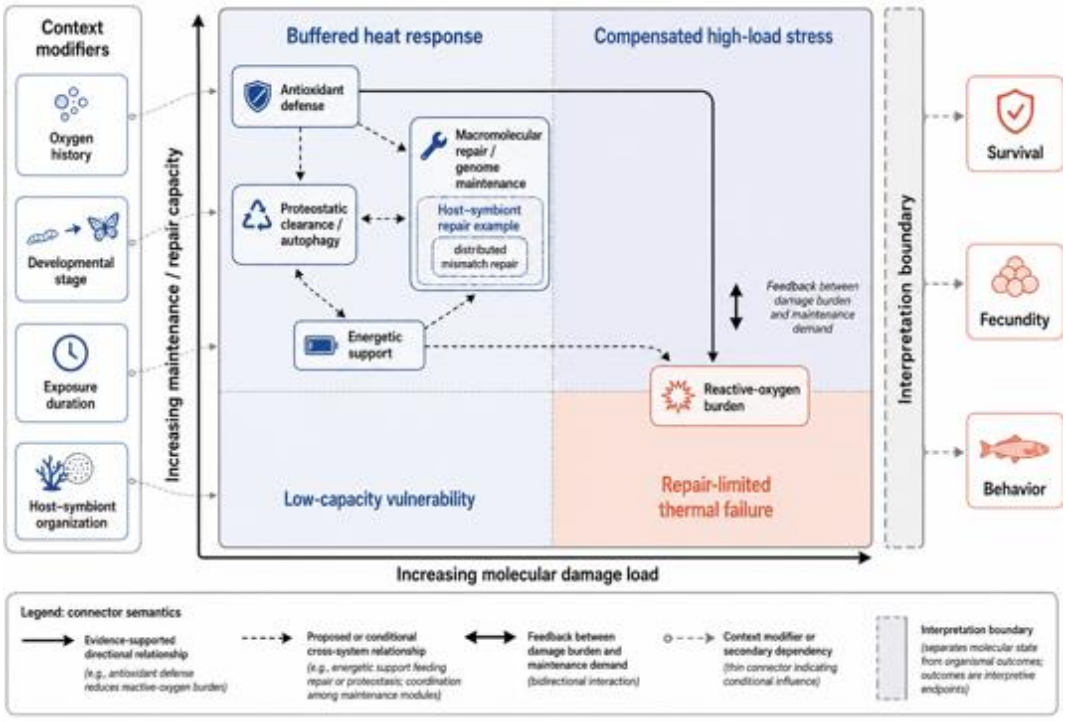


Figure 1. Damage-load and maintenance-capacity states separating buffered heat response from repair-limited thermal failure

Cellular stress signaling

Heat-responsive signaling should not be treated as a single upstream switch. In worker honeybees, heat-associated histone methylation patterns and HSP transcription differed between subspecies [31]. These observations identify chromatin state as a plausible regulatory modifier, but they do not establish that the measured marks cause thermotolerance or are heritably adaptive.

Structural programs can also enter the stress response. In *Spodoptera frugiperda*, transcriptomic and metabolomic changes implicated chitin-related processes, and perturbation of selected chitin-related genes altered high-temperature performance [32]. Functional evidence therefore exists for particular nodes, whereas enrichment of a broader pathway remains insufficient to assign causal status to every component.

Selectivity is equally evident within canonical HSP signaling. Genome-wide analysis in *Bemisia tabaci* MED found heterogeneous heat responsiveness across the HSP superfamily, while BtaHsp90A3 silencing reduced adult survival at 42°C [33]. Heat signaling can also modify other physiological interactions: in *Helicoverpa armigera*, high-temperature exposure altered susceptibility to nucleopolyhedrovirus together with enhanced HSP expression [34]. Reduced viral susceptibility is a cross-system consequence, not evidence that heat exposure produces an overall fitness benefit.

Environmental context can reverse or amplify signaling consequences. In *D. melanogaster*, oxygen conditions interacted with mutations affecting cell-size-control pathways to alter heat tolerance [35]. The proposed model therefore treats cellular stress signaling as context-sensitive gating among proteostasis, structural maintenance, metabolism, and damage control rather than as a universal master regulator.

The distinct signaling situations require different tests of mechanism. **Table 2** converts them into a prediction-and-test ledger so that transcriptional association, functional dependency, collateral physiological effects, and context dependence are not interpreted as equivalent evidence.

Table 2. Signaling states that require different tests before they can be assigned a thermal-tolerance mechanism

Signaling condition	Biological setting with observed response	Mechanistic reading within its claim limit	Experiment that would validate the assignment	Current evidential standing
Regulatory association				
Chromatin-associated heat response	Honeybee worker subspecies Heat-linked histone methylation and HSP transcription	Regulatory state may modify heat-responsive transcription Association does not establish epigenetic causation or inheritance	Perturb chromatin regulators while measuring HSP output and thermal performance	Evidence-supported association
Structural and family-specific dependence				
Structurally coupled signaling	Fall armyworm Chitin-related omics response with selected gene perturbation	Structural/cuticular programs can participate in heat response Most pathway members remain unvalidated	Perturb individual nodes and separate developmental defects from acute thermal effects	Evidence-supported with partial functional validation
Selective HSP-family dependence	Whitefly adults Unequal HSP induction; HSP90 knockdown lowers heat survival	HSP-family members are not interchangeable One validated gene does not define family-wide necessity	Compare paralog-specific perturbations under matched heat regimes	Evidence-supported functional state
Cross-talk and contextual gating				
Stress–infection cross-talk	Lepidopteran larvae Heat-linked HSP response and changed viral susceptibility	Thermal signaling can alter a non-thermal physiological outcome Pathogen resistance is not net thermal fitness	Measure thermal survival and infection outcome jointly	Evidence-supported cross-system state
Oxygen-conditioned signaling	Adult *Drosophila* mutants Genotype-by-oxygen effects on heat tolerance	Signaling effects depend on environmental supply conditions Cell size, oxygen delivery, and metabolic signaling are intertwined	Replicate pathway perturbations across controlled oxygen states	Evidence-supported boundary
Conditional gating among systems	Cross-taxon synthesis Divergent regulatory effects across the states above	Cellular signaling may gate different downstream maintenance processes according to context	Test the same perturbation across stage, oxygen, and exposure-duration contrasts	**Proposed synthesis**

No conserved gating topology has been demonstrated

Cross-system coordination

The strongest evidence for coordination comes from studies in which one perturbation crosses mechanistic domains. Autophagy manipulation in *Drosophila* links protein-damage clearance to TOR-sensitive metabolic regulation [10]. Nplp2-dependent lipid flow links systemic lipid handling with heat acclimation [12]. In *C. lividipennis*, ceramidase inhibition altered both sphingolipid state and antioxidant response while increasing heat survival [18]. None of these experiments establishes a four-system network, but each shows that thermal physiology cannot always be partitioned into independent modules.

Multi-omics strengthens evidence for co-regulation without automatically establishing direction. In *S. frugiperda*, coordinated proteomic and transcriptomic responses include chaperone and antioxidant components, with causal support restricted to selected perturbations [11]. *B. dorsalis* larvae show stress-specific metabolic and fatty-acid remodeling [17]. Chitin-related multi-omics in *S. frugiperda* similarly combines broad association with functional evidence for only selected genes [32]. Thus, co-change should be represented differently from demonstrated dependency.

Energetic and environmental conditions further determine whether coordination preserves function. Mitochondrial substrate flexibility differs among insect species [19]. Catalase activity can directly affect ROS burden and high-temperature performance in whiteflies [27]. Oxygen history modifies oxidative outcomes in a combined-stressor *Drosophila* model [30], while oxygen also changes the effect of cell-size-control genotypes on heat tolerance [35]. The article therefore proposes a qualitative maintenance-budget logic: protection succeeds when available energetic and repair capacities are sufficient for the damage load generated under a particular context. This is not a measured optimization rule.

Broad evidence also warns against over-integration. Insect heat responses are mechanistically heterogeneous [1], and organismal thermal-limit plasticity is generally modest [2]. Coordination is consequently defined here as a proposed explanatory relation among empirically separable processes, not as a conserved molecular circuit.

To make that distinction operational, **Table 3** classifies cross-system patterns by what level of inference they justify.

Table 3. Decision matrix for distinguishing co-response, functional coupling, context conditioning, and proposed coordination

Coordination class	Pattern signature	Manuscript example	Inference ceiling and limiting boundary	Decisive test and status
Concurrent response				
Parallel co-response	Multiple molecular domains change under the same heat treatment	HSP, antioxidant, lipid, or metabolite changes in omics datasets	Systems respond concurrently Common stress severity can drive parallel change without interaction	Perturb one domain and test response in another Evidence-supported pattern
Cross-domain dependence				
Functional cross-domain coupling	One perturbation changes a second domain and thermal outcome	Autophagy–TOR context; lipid-flow–acclimation; ceramide–antioxidant coupling	A cross-domain dependency exists in the tested system Pleiotropy and taxon specificity can obscure route identity	Use orthogonal perturbations and mechanistic intermediates Evidence-supported class
Context and biological-level coupling				
Context-conditioned coupling	Effect of a pathway depends on oxygen, resource state, stage, or exposure regime	Oxygen-conditioned genotype effects; resource-limited tolerance	Mechanistic effect is conditional Moderator may act through several unmeasured processes	Factorially manipulate pathway and context Evidence-supported boundary class
Biological-level coupling	Thermal outcome depends on processes distributed across levels or partners	Host–symbiont repair contribution	Cross-level organization can alter thermal performance Specialized systems may not transfer across taxa	Disrupt the partner-specific process while preserving host state Evidence-supported specialized class
Proposed maintenance and failure states				

Coordinated maintenance state	Several domains jointly preserve function despite increasing thermal load	Integration of proteostasis, lipid state, energy supply, and repair	Generates a testable systems hypothesis Whole architecture has not been experimentally validated	Multi-domain perturbation across matched exposure durations **Proposed model class**
Coordination failure state	Damage demand exceeds available maintenance in one or more limiting domains	Endpoint loss under prolonged or contextually constrained exposure	Predicts where molecular activation may coexist with organismal failure No universal threshold or dominant domain is established	Identify the first functionally limiting domain in each taxon/stage **Proposed model class**

The nested field in **Figure 2** compresses this distinction by placing established component processes inside a proposed coordination layer while keeping contextual modifiers and organismal outcomes outside the mechanistic core.

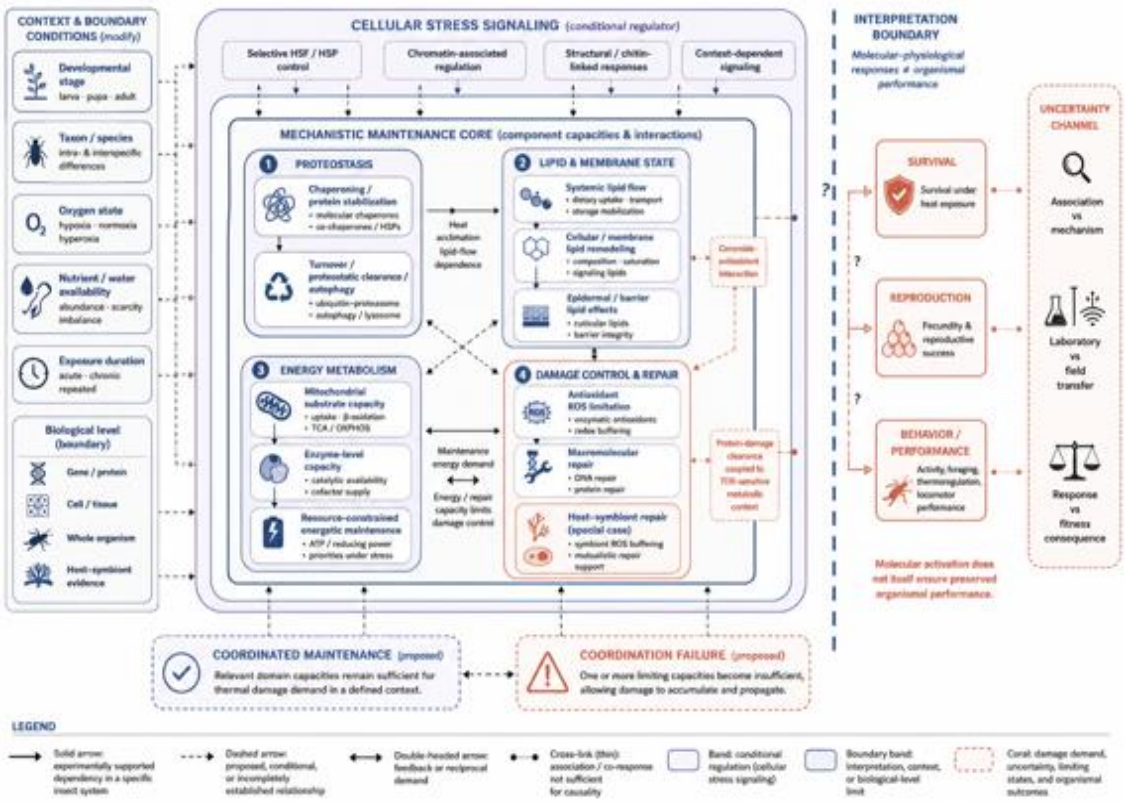


Figure 2. Nested maintenance field linking component-specific heat responses to conditional coordination and organismal failure

Integrated molecular logic of thermal tolerance

The central contribution is a proposed coordination model in which heat tolerance reflects preservation of cellular function rather than maximal activation of any single stress pathway. Direct functional evidence shows that HSP identity can alter thermoresistance [6], systemic lipid flow can contribute to heat acclimation [12], mitochondrial substrate use can constrain energetic flexibility [19], and catalase-mediated ROS removal can affect heat survival and reproduction [27]. These findings justify treating proteostasis, lipid/membrane state, energy supply, and damage control as functionally relevant domains. They do not demonstrate that the four domains form a conserved network.

The model further distinguishes compensation from successful maintenance. A strong molecular response can indicate effective protection, but it can also indicate that a system is approaching a limiting state. Chaperone induction, antioxidant activation, or metabolic reorganization therefore cannot be interpreted by magnitude alone. Their meaning depends on whether damaged structures are stabilized or removed, ATP-producing capacity remains adequate, and organismal performance is preserved. This distinction is proposed because most studies

measure only subsets of these levels simultaneously. It predicts multiple failure routes: protein damage may become limiting despite adequate energy, energetic limitation may prevent repair despite strong stress signaling, or membrane/barrier disruption may increase demand on otherwise competent protective systems.

Cellular stress signaling is positioned as conditional regulation. HSF1 controls only a subset of HSP responses in one lepidopteran [8], chromatin marks are associated with subspecies-specific heat transcription in honeybees [31], HSP90 function is selective within the whitefly HSP family [33], and genotype effects on heat tolerance vary with oxygen conditions in *Drosophila* [35]. Accordingly, the proposed model contains no master switch.

The proposed coordination logic is asymmetric: evidence that one component contributes to tolerance establishes that component as potentially limiting, but absence of a response in another taxon does not establish irrelevance. Different insects may reach comparable tolerance through different combinations of pathway capacity, constitutive protection, inducible response, tissue specialization, or behavioral avoidance. Comparative validation should therefore test functional equivalence rather than require identical molecular signatures.

Repair is similarly plural. Autophagic clearance can affect heat tolerance [10], while an aphid's heat tolerance can depend on mismatch-repair functions distributed across bacterial symbionts [29]. These mechanisms can be grouped as maintenance of damaged biological structures only at the conceptual level; they should not be represented as one biochemical pathway.

The model therefore makes a limited claim: thermal tolerance is expected to depend on whether the relevant systems can collectively maintain function under a specified thermal load. Because average plasticity of organismal limits is modest [2] and tolerance depends strongly on stress duration and endpoint [4], predictive accuracy cannot be assumed. Prospective validation of the proposed architecture remains necessary.

Predictions across acute and chronic exposure

The first prediction concerns temporal reweighting. Duration-dependent loss of survival and reproduction demonstrates that thermal intensity cannot be interpreted without exposure time [4]. Functional HSP70 evidence [7] and catalase-dependent ROS control [27] support rapid protective components, but the proposed model predicts that prolonged exposure will increasingly reveal energetic depletion, accumulated macromolecular damage, and repair limitations. No universal acute/chronic cutoff is proposed.

The second prediction is developmental. Heat hardening varies by life stage in *Drosophila* [5], larval metabolic thermal sensitivity changes across instars in *Lymantria* [23], and early embryos have a distinct heat-responsive transcriptional state [28]. The model therefore predicts that the first limiting domain—and hence the most informative biomarker or perturbation—will differ among developmental stages.

Third, context should alter the relationship between molecular activation and organismal outcome. Nutrient and water status can modify thermal tolerance [24]. Oxygen history modifies temperature-associated oxidative outcomes after hypoxia [30], and oxygen conditions alter genotype effects on *Drosophila* heat tolerance [35]. Resource and oxygen state are therefore prioritized, but not exhaustive, boundary conditions.

Finally, validation should combine evidence levels rather than rely on expression alone. Meta-analysis shows that organismal thermal plasticity remains modest despite widespread acclimation responses [2]. Multi-omics can identify candidate coordination patterns while functional perturbation is required for causal edges [11]. Directionally unexpected ceramidase effects illustrate why pathway activity cannot be assigned a protective sign *a priori* [18]. Mitochondrial substrate-use differences show why a mechanism validated in one taxon may fail in another [19]. A stringent test of the proposed model should therefore measure molecular state, perturb selected nodes, and quantify organismal endpoints across more than one exposure duration, with field transfer evaluated separately.

Prospective tests should keep endpoints separate. Survival, reproduction, locomotor performance, and critical thermal limits can diverge because they fail at different rates and may depend on different tissues. A molecular predictor should therefore be evaluated against a prespecified endpoint rather than an undifferentiated label of "thermal tolerance." Repeated or chronic exposure should also include recovery measurements, because a response that preserves acute survival may impose energetic or reproductive costs visible only after stress cessation.

Field transfer is a separate validation step. Laboratory perturbations identify mechanistic dependencies, but natural thermal regimes combine fluctuating temperature with resource variation, hydration constraints, pathogens, and behavioral thermoregulation. The proposed model predicts that laboratory-supported edges will generalize most

reliably when the field context preserves the same limiting process; this prediction remains untested at the architecture level.

Conclusion

Insect thermal tolerance is better represented as a problem of maintaining function across interacting cellular constraints than as the output of one molecular switch. Proteostasis, lipid and membrane state, energy metabolism, and damage control each have independent empirical support, yet their relative importance varies with taxon, developmental stage, exposure duration, oxygen and resource state, and measurement level. The model advanced here is explicitly proposed: it organizes those components into a conditional coordination logic and identifies failure as insufficient maintenance relative to thermal damage demand. Its value lies in generating tests that distinguish co-response from dependency, molecular protection from organismal performance, and laboratory mechanism from field generalization.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. González-Tokman D, Córdoba-Aguilar A, Dáttilo W, Lira-Noriega A, Sánchez-Guillén RA, Villalobos F. Insect responses to heat: Physiological mechanisms, evolution and ecological implications in a warming world. Biol Rev Camb Philos Soc. 2020;95(3):802-21. doi:10.1111/brv.12588
2. Weaving H, Terblanche JS, Pottier P, English S. Meta-analysis reveals weak but pervasive plasticity in insect thermal limits. Nat Commun. 2022;13(1):5292. doi:10.1038/s41467-022-32953-2
3. Ardelan A, Tsai A, Will S, McGuire R, Amarasekare P. Increase in heat tolerance following a period of heat stress in a naturally occurring insect species. J Anim Ecol. 2023;92(10):2039-51. doi:10.1111/1365-2656.13995
4. Ørsted M, Willot Q, Olsen AK, Kongsgaard V, Overgaard J. Thermal limits of survival and reproduction depend on stress duration: A case study of Drosophila suzukii. Ecol Lett. 2024;27(3):e14421. doi:10.1111/ele.14421
5. Moghadam NN, Ketola T, Pertoldi C, Bahrndorff S, Kristensen TN. Heat hardening capacity in Drosophila melanogaster is life stage-specific and juveniles show the highest plasticity. Biol Lett. 2019;15(2):20180628. doi:10.1098/rsbl.2018.0628
6. Shilova VY, Zatsepina OG, Garbuz DG, Funikov SY, Zelentsova ES, Schostak NG, et al. Heat shock protein 70 from a thermotolerant Diptera species provides higher thermoresistance to Drosophila larvae than correspondent endogenous gene. Insect Mol Biol. 2018;27(1):61-72. doi:10.1111/imb.12339
7. Li H, Li S, Chen J, Dai L, Chen R, Ye J, et al. A heat shock 70kDa protein MaltHSP70-2 contributes to thermal resistance in Monochamus alternatus (Coleoptera: Cerambycidae): Quantification, localization, and functional analysis. BMC Genomics. 2022;23(1):646. doi:10.1186/s12864-022-08858-1
8. Dong CL, Feng Z, Lu MX, Du YZ. Chilo suppressalis heat shock proteins are regulated by heat shock factor 1 during heat stress. Insect Mol Biol. 2023;32(1):69-78. doi:10.1111/imb.12814
9. Liu J, Liu Y, Li Q, Lu Y. Heat shock protein 70 and Cathepsin B genes are involved in the thermal tolerance of Aphis gossypii. Pest Manag Sci. 2023;79(6):2075-86. doi:10.1002/ps.7384
10. Willot Q, du Toit A, de Wet S, Huisamen EJ, Loos B, Terblanche JS. Exploring the connection between autophagy and heat-stress tolerance in Drosophila melanogaster. Proc Biol Sci. 2023;290(2006):20231305. doi:10.1098/rspb.2023.1305
11. Yang CL, Meng JY, Zhou JY, Zhang JS, Zhang CY. Integrated transcriptomic and proteomic analyses reveal the molecular mechanism underlying the thermotolerant response of Spodoptera frugiperda. Int J Biol Macromol. 2024;264(Pt 1):130578. doi:10.1016/j.ijbiomac.2024.130578

12. Rommelaere S, Boquete JP, Piton J, Kondo S, Lemaitre B. The exchangeable apolipoprotein Nplp2 sustains lipid flow and heat acclimation in Drosophila. Cell Rep. 2019;27(3):886-99.e6. doi:10.1016/j.celrep.2019.03.074
13. Rodríguez E, Weber JM, Darveau CA. Diversity in membrane composition is associated with variation in thermoregulatory capacity in hymenopterans. Comp Biochem Physiol B Biochem Mol Biol. 2018;224:115-20. doi:10.1016/j.cbpb.2017.11.017
14. Ko L, Harshman L, Hangartner S, Hoffmann A, Kachman S, Black P. Changes in lipid classes of Drosophila melanogaster in response to selection for three stress traits. J Insect Physiol. 2019;117:103890. doi:10.1016/j.jinsphys.2019.103890
15. Knittelfelder O, Prince E, Sales S, Fritzsche E, Wöhner T, Brankatschk M, et al. Sterols as dietary markers for Drosophila melanogaster. Biochim Biophys Acta Mol Cell Biol Lipids. 2020;1865(7):158683. doi:10.1016/j.bbalip.2020.158683
16. Lei G, Zhou H, Chen Y, Vasseur L, Gurr GM, You M, et al. A very long-chain fatty acid enzyme gene, PxHacd2 affects the temperature adaptability of a cosmopolitan insect by altering epidermal permeability. Sci Total Environ. 2023;891:164372. doi:10.1016/j.scitotenv.2023.164372
17. Ren L, Zhang H, Zhou J, Wu Y, Liu B, Wang S, et al. Unique and generic crossed metabolism in response to four sub-lethal environmental stresses in the oriental fruit fly, Bactrocera dorsalis Hendel. Ecotoxicol Environ Saf. 2023;264:115434. doi:10.1016/j.ecoenv.2023.115434
18. Chen M, Shi XX, Wang N, Zhang C, Shi ZY, Zhou WW, et al. Alkaline ceramidase (ClAC) inhibition enhances heat stress response in Cyrtorhinus lividipennis (Reuter). Front Physiol. 2023;14:1160846. doi:10.3389/fphys.2023.1160846
19. Menail HA, Cormier SB, Ben Youssef M, Jørgensen LB, Vickruck JL, Morin P Jr, et al. Flexible thermal sensitivity of mitochondrial oxygen consumption and substrate oxidation in flying insect species. Front Physiol. 2022;13:897174. doi:10.3389/fphys.2022.897174
20. Kuo Y, Lu YH, Lin YH, Lin YC, Wu YL. Elevated temperature affects energy metabolism and behavior of bumblebees. Insect Biochem Mol Biol. 2023;155:103932. doi:10.1016/j.ibmb.2023.103932
21. Wilmsen SM, Dzialowski E. Substrate use and temperature effects in flight muscle mitochondria from an endothermic insect, the hawkmoth Manduca sexta. Comp Biochem Physiol A Mol Integr Physiol. 2023;281:111439. doi:10.1016/j.cbpa.2023.111439
22. Léger A, Cormier SB, Blanchard A, Menail HA, Pichaud N. Investigating the thermal sensitivity of key enzymes involved in the energetic metabolism of three insect species. J Exp Biol. 2024;227(10):jeb247221. doi:10.1242/jeb.247221
23. Powers SD, Grayson KL, Martinez E, Agosta SJ. Ontogenetic variation in metabolic rate-temperature relationships during larval development. J Exp Biol. 2024;227(14):jeb247912. doi:10.1242/jeb.247912
24. Mitchell KA, Boardman L, Clusella-Trullas S, Terblanche JS. Effects of nutrient and water restriction on thermal tolerance: A test of mechanisms and hypotheses. Comp Biochem Physiol A Mol Integr Physiol. 2017;212:15-23. doi:10.1016/j.cbpa.2017.06.019
25. Colinet H, Renault D, Roussel D. Cold acclimation allows Drosophila flies to maintain mitochondrial functioning under cold stress. Insect Biochem Mol Biol. 2017;80:52-60. doi:10.1016/j.ibmb.2016.11.007
26. Jiang L, Huang C, Wang B, Guo H, Sun Q, Xia F, et al. Enhanced heat tolerance in transgenic silkworm via overexpression of Pyrococcus furiosus superoxide reductase. Insect Biochem Mol Biol. 2018;92:40-4. doi:10.1016/j.ibmb.2017.11.010
27. Liang P, Ning J, Wang W, Zhu P, Gui L, Xie W, et al. Catalase promotes whitefly adaptation to high temperature by eliminating reactive oxygen species. Insect Sci. 2023;30(5):1293-308. doi:10.1111/1744-7917.13157
28. Mikucki EE, O'Leary TS, Lockwood BL. Heat tolerance, oxidative stress response tuning and robust gene activation in early-stage Drosophila melanogaster embryos. Proc Biol Sci. 2024;291(2029):20240973. doi:10.1098/rspb.2024.0973
29. Ling X, Guo H, Di J, Xie L, Zhu-Salzman K, Ge F, et al. A complete DNA repair system assembled by two endosymbionts restores heat tolerance of the insect host. Proc Natl Acad Sci U S A. 2024;121(51):e2415651121. doi:10.1073/pnas.2415651121

30. Habib P, Jung J, Wilms GM, Kokott-Vuong A, Habib S, Schulz JB, et al. Posthypoxic behavioral impairment and mortality of Drosophila melanogaster are associated with high temperatures, enhanced predeath activity and oxidative stress. Exp Mol Med. 2021;53(2):264-80. doi:10.1038/s12276-021-00565-3
31. Alattal YZ, Alghamdi AA. Linking histone methylation states and hsp transcriptional regulation in thermo-tolerant and thermo-susceptible A. mellifera L. subspecies in response to heat stress. Insects. 2023;14(3):225. doi:10.3390/insects14030225
32. Yan X, Zhao Z, Feng S, Zhang Y, Wang Z, Li Z. Multi-omics analysis reveal the fall armyworm Spodoptera frugiperda tolerate high temperature by mediating chitin-related genes. Insect Biochem Mol Biol. 2024;174:104192. doi:10.1016/j.ibmb.2024.104192
33. Zheng HY, Qin PH, Yang K, Liu TX, Zhang YJ, Chu D. Genome-wide identification and analysis of the heat-shock protein gene superfamily in Bemisia tabaci and expression pattern analysis under heat shock. Insects. 2022;13(7):570. doi:10.3390/insects13070570
34. Shen ZJ, Liu YJ, Cheng J, Li Z, Michaud JP, Liu XX. High temperature exposure reduces the susceptibility of Helicoverpa armigera to its nucleopolyhedrovirus (HearNPV) by enhancing expression of heat shock proteins. Pest Manag Sci. 2022;78(6):2378-89. doi:10.1002/ps.6868
35. Privalova V, Sobczyk Ł, Szlachcic E, Labecka AM, Czarnoleski M. Heat tolerance in Drosophila melanogaster is influenced by oxygen conditions and mutations in cell size control pathways. Philos Trans R Soc Lond B Biol Sci. 2024;379(1896):20220490. doi:10.1098/rstb.2022.0490