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Water Balance, Stress Signaling, and Cellular Protection Underlying Molecular Mechanisms of Insect Desiccation Tolerance

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ABSTRACT

Desiccation tolerance in insects is frequently treated as a single physiological phenotype, although survival under water deficit can arise from fundamentally different mechanisms. Some insects primarily restrict water loss through the cuticle, respiratory system and excretory organs, whereas others tolerate substantial dehydration through osmotic adjustment, metabolic suppression and cellular protection. At the extreme, anhydrobiotic chironomids reorganize metabolism and stress signaling sufficiently to survive near-complete water loss. This integrative review examines how these levels interact without assuming that molecular responses observed in one taxon, life stage or dehydration regime represent universal mechanisms. Evidence is organized across cuticular and respiratory water loss, epithelial ion and water transport, compatible-solute metabolism, stress signaling, proteostasis, membrane protection and oxidative damage–repair processes. The synthesis distinguishes mechanisms that reduce the rate of dehydration from those that preserve function after water has already been lost. It further proposes that desiccation tolerance is best interpreted as a coordinated state transition in which water conservation, solute redistribution, metabolic demand, protective macromolecules and recovery capacity become limiting at different stages of dehydration and rehydration. This framework clarifies why transcriptomic induction alone cannot demonstrate protection and why extreme anhydrobiosis should not be generalized to ordinary insect drought resistance.

Keywords: Desiccation tolerance, Cuticular hydrocarbons, Malpighian tubules, Trehalose metabolism, Anhydrobiosis, Stress signaling

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Introduction

Terrestrial insects continuously exchange water with environments in which atmospheric water potential can be substantially lower than that of body fluids. Their small size and high surface-area-to-volume ratio intensify this problem, making water balance dependent on both the rate at which water is lost and the amount of loss that tissues can withstand [1]. These components are biologically separable. Reduced cuticular permeability can prolong survival without increasing intrinsic cellular tolerance, whereas osmolyte accumulation or macromolecular protection may increase tolerance to tissue dehydration without substantially slowing evaporation. Consequently, survival time under dry laboratory conditions is an integrated phenotype rather than a direct measurement of any single mechanism.

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This distinction has become increasingly important because dehydration interacts with temperature, activity and environmental exposure. Models based solely on thermal limits can misclassify mortality when prolonged heat exposure simultaneously produces substantial water loss, while laboratory desiccation assays may remove access to behavioural or dietary sources of water that operate in the field [2]. Mechanistic interpretation therefore requires explicit separation of water-loss limitation, water and ion redistribution, dehydration tolerance, and post-stress recovery.

Comparative work in *Drosophila* illustrates the value of this separation. Interspecific variation in cuticular hydrocarbon (CHC) profiles explains a substantial component of differences in water-loss resistance, but it does not imply that every desiccation-tolerant insect uses the same hydrocarbon composition or biosynthetic route [3]. Natural *D. melanogaster* populations likewise differ in basal and induced gene expression, respiration, water loss and CHC composition, demonstrating that molecular variation can accompany several physiological components of the same survival phenotype [4].

The central synthesis developed here is therefore a proposed layered constraint model of insect desiccation tolerance. It treats tolerance as the outcome of at least three distinguishable problems: limiting external water flux, maintaining internal hydromineral organization as water availability declines, and protecting or restoring cellular systems when dehydration exceeds ordinary homeostatic capacity. Extreme anhydrobiosis is used as an informative mechanistic endpoint, not as a universal model for insect drought resistance.

Cuticular and respiratory water loss

The insect cuticle is the largest interface across which uncontrolled water loss can occur, and CHCs constitute a major molecular component of its waterproofing function. Comparative manipulation in *D. melanogaster* supports a direct physical role: removal or alteration of hydrocarbons increases transpiration, whereas an intact hydrocarbon layer reduces evaporation across the body surface [5]. This moves the CHC argument beyond simple correlation between hydrocarbon abundance and survival.

Functional genetics provide stronger evidence that particular biosynthetic enzymes can alter this barrier. In *Bactrocera dorsalis*, silencing CYP4G100, a terminal hydrocarbon-biosynthesis P450 expressed strongly in oenocyte-rich tissues, decreases CHC abundance, increases water loss and reduces survival under desiccation [6]. The inference permitted by these experiments is specific: CYP4G100 contributes to cuticular waterproofing in this species. It does not establish that CYP4G copy number or expression is a universal predictor of aridity tolerance across insects.

Upstream lipid processing is also consequential. In the termite *Coptotermes formosanus*, manipulation of first-desaturase-subfamily genes alters fatty-acid-related traits and desiccation tolerance, linking hydrocarbon chemistry to the broader lipid biosynthetic network [7]. Earlier RNA-interference experiments in *Locusta migratoria* similarly identified integumentary fatty acid synthases that contribute to CHC production and cuticle permeability [8]. Together, these studies indicate that waterproofing is better represented as a biosynthetic system—fatty-acid production, elongation/desaturation and terminal hydrocarbon formation—than as a single "CHC gene."

Composition matters as much as total quantity. Hydrocarbon chain length, branching and unsaturation alter melting behaviour and molecular packing; therefore, two insects with similar total CHC abundance need not have equivalent cuticular permeability. Natural *Drosophila* populations provide a particularly useful warning against oversimplification because tolerant strains combine altered CHC composition with differences in basal transcription and respiration [4]. The waterproofing phenotype can thus be produced by several coordinated changes rather than a single increase in surface lipid.

Respiratory water loss creates a second pathway. Gas exchange exposes moist internal respiratory surfaces to the atmosphere, so activity and respiratory pattern can modify total water loss. Yet the fraction attributable to respiration varies with species, temperature, activity and measurement method. Reduced respiration during desiccation in tolerant *D. melanogaster* strains is consistent with water conservation [4], but respiration rate itself cannot be treated as a direct proxy for spiracular water loss because it also reflects metabolic demand.

Cuticular and respiratory routes should therefore remain analytically distinct. The cuticle sets a largely passive permeability boundary whose molecular composition can be modified over developmental or acclimatory time, whereas respiratory loss is coupled more directly to gas-exchange demand and behaviour. A desiccation-resistant phenotype may involve both, but evidence for one does not establish the other. This distinction becomes especially

important when interpreting transcriptomic datasets: induction of lipid-metabolism genes is mechanistically informative only when connected to lipid composition, permeability or water-flux measurements.

Ion and water transport

Once water has entered the body, survival depends on where it is retained and how ions are distributed as volume falls. Malpighian tubules and the hindgut are therefore not merely excretory organs; together they regulate fluid secretion, solute transport and reabsorption, making them central to hydromineral homeostasis [9].

Neuroendocrine control demonstrates that changing epithelial transport can alter whole-animal desiccation performance. In *Drosophila suzukii*, CAPA, leucokinin, DH44 and DH31 systems are linked to Malpighian-tubule diuretic function, while desiccation changes expression of selected neuropeptide genes [10]. The result supports a regulatory connection between environmental stress and renal physiology but does not imply that every change in peptide expression is protective.

More direct evidence comes from adult *D. melanogaster*. Tachykinin signaling through receptors in Malpighian-tubule stellate cells stimulates cation, anion and fluid transport; experimentally increasing this diuretic drive reduces survival under desiccating conditions because water is conserved less effectively [11]. This establishes an important mechanistic principle: transport systems beneficial for eliminating excess fluid under one condition can become liabilities when external water is unavailable.

The molecular machinery underlying renal transport is distributed across membrane channels, exchangers and pumps rather than one dehydration-specific pathway. Reviews of Malpighian-tubule physiology emphasize the coordinated roles of ion transport and fluid movement across secretory epithelia [12]. Aquaporins add another level because altering membrane permeability to water or small solutes changes the rate at which cells and epithelia equilibrate osmotically. Functional characterization of multiple aquaporins in *Bombus terrestris* demonstrates distinct substrate permeabilities and tissue distributions, while knockdown of a DRIP aquaporin compromises stress tolerance under cold exposure [13]. This result establishes transport function and stress relevance, but extrapolation specifically to desiccation requires direct dehydration experiments.

Accordingly, aquaporin induction should not automatically be interpreted as “water conservation.” Increased water permeability may accelerate redistribution or controlled water loss in one physiological state but worsen dehydration in another. The direction of benefit depends on tissue, osmotic gradient, solute permeability and whether the immediate requirement is retention, redistribution or recovery. Ion and water transport are therefore state-dependent control processes rather than uniformly protective responses.

Osmolytes and metabolic adjustment

The consequences of dehydration change sharply when insects can no longer maintain normal cellular water activity. At this point, compatible solutes and metabolic reorganization can become as important as reducing external water flux. The strongest recent demonstration of this principle comes from *Aedes aegypti* eggs, which acquire pronounced desiccation tolerance late in embryogenesis. Proteomic and metabolic analyses show suppression of central carbon metabolism together with enhanced lipid utilization and polyamine production; pharmacological perturbation supports a functional contribution of this metabolic rewiring to dry survival and subsequent hatching [14]. The mechanism is therefore developmentally restricted rather than a generic property of mosquitoes.

Compatible-solute accumulation also requires cautious interpretation. In diapausing *Chymomyza costata* larvae, drought exposure induces a metabolic pattern overlapping with cold acclimation and diapause, including accumulation of several compatible solutes and energy-rich phosphagens, yet drought does not significantly increase trehalose and does not improve desiccation tolerance [15]. Trehalose elevation is consequently neither a necessary signature of every insect dehydration response nor sufficient evidence that a treatment has improved drought survival.

At the opposite extreme, trehalose is deeply integrated into anhydrobiosis in *Polypedilum vanderplanki*. Metabolome–transcriptome analysis of desiccation and rehydration shows extensive carbohydrate and intermediary-metabolism reorganization; accumulated trehalose functions not only within the dry-state protective system but also as a readily available substrate during early recovery, when pentose-phosphate-pathway activity can contribute to restoration of reducing capacity [16]. This metabolic program includes accumulation or stabilization of energy-related metabolites and conversion of potentially harmful metabolic products into less reactive forms.

Recovery also requires controlled solute removal. In the anhydrobiotic Pv11 cell line, the SLC5-family transporter STRT1 has Na^+-dependent trehalose transport activity. Disruption of Strt1 slows trehalose efflux and morphological recovery after rehydration and decreases viability of previously desiccated cells [17]. Thus, an osmolyte that is advantageous during entry into the dry state can become a source of osmotic stress if not redistributed during rehydration.

These observations motivate a distinction between osmolyte abundance, osmolyte function, and osmolyte timing. The same metabolite can operate as compatible solute, carbon reserve or recovery substrate, and its significance cannot be inferred from concentration alone. Table 1 therefore separates metabolic states according to the condition under which the same biochemical change can support, fail to support, or complicate desiccation survival.

Table 1 is required here because osmolyte accumulation becomes mechanistically misleading when metabolite identity is interpreted without developmental state, dehydration severity and recovery phase.

Table 1. When metabolic protection becomes conditional: osmolyte and energy-state interpretations across insect dehydration regimes

Biological context	Observable basis and warranted reading	Failure mode and decisive check
Developmentally acquired lipid–polyamine rewiring		
Evidence-supported		
Aedes aegypti late embryos	Reduced central carbon metabolism, lipid utilization and polyamine accumulation	Restricted to a defined embryonic state; not evidence for adult mosquito tolerance
	Metabolic remodeling contributes to egg desiccation survival	Test pathway necessity across developmental stages and dehydration intensities
Compatible-solute accumulation without improved drought survival		
Evidence-supported		
Diapausing Chymomyza costata larvae	Amino-acid/solute and phosphagen shifts during drought	Shared metabolic response does not guarantee increased desiccation resistance
	Drought can engage a broader stress-metabolism program	Couple metabolomics to direct survival and water-loss phenotypes
Trehalose-centered anhydrobiotic preparation		
Evidence-supported		
Polypedilum vanderplanki larvae	Coordinated carbohydrate and intermediary-metabolite reorganization	Extreme anhydrobiosis is not equivalent to ordinary insect drought resistance
	Trehalose participates in dry-state protection and recovery metabolism	Separate entry, dry-state maintenance and rehydration functions experimentally
Rehydration-dependent trehalose clearance		
Evidence-supported		
Pv11 anhydrobiotic cells	STRT1-dependent trehalose transport and recovery	Excess intracellular solute can prolong osmotic deformation after water returns
	Solute removal is part of successful recovery, not merely reversal of accumulation	Measure transporter directionality and recovery kinetics, not dry survival alone
High osmolyte concentration interpreted as tolerance		
Proposed interpretive rule		
Cross-taxon inference	Metabolite concentration alone	Correlation may reflect stress exposure, diapause, cold acclimation or metabolic arrest
	No mechanistic conclusion without functional or physiological linkage	Require perturbation or matched water-balance measurements

Stress-signaling pathways

A molecular response to dehydration is not equivalent to a protective mechanism. Stress-responsive transcripts can represent damage, compensation, generalized stress signaling or processes required for survival. The strongest mechanistic evidence therefore comes from perturbation experiments in which a signaling component is altered and desiccation entry or recovery is measured.

The anhydrobiotic midge provides an unusually tractable example. Comparative transcriptomic work identified heat shock factor (HSF)-binding motifs enriched among desiccation-induced genes in *P. vanderplanki*. HSF knockdown in Pv11 cells suppresses induction of multiple anhydrobiosis-associated targets and substantially reduces post-desiccation survival, linking the heat-shock regulatory system to acquisition of extreme dehydration tolerance [18]. Genome-wide knockout/rescue analysis subsequently showed that HSF1 activates genes including LEA proteins and thioredoxins while repressing subsets of basal cellular functions associated with an active metabolic state [19]. HSF1 therefore coordinates both protective-gene induction and entry into a low-activity cellular program in this model.

Calcium signaling forms a second experimentally resolved pathway. Trehalose preconditioning of Pv11 cells produces a transient cytosolic Ca^{2+} increase. Perturbation experiments distinguish downstream branches: calmodulin–calcineurin–NFAT signaling contributes to tolerance of the preconditioning environment and to subsequent desiccation survival, whereas the calmodulin–CaMK–CREB branch is more specifically associated with desiccation tolerance [20]. This divergence is important because it shows that even within one signaling event, downstream branches perform non-identical functions.

Reactive oxygen species add a further complication by acting as both damage agents and signals. During *P. vanderplanki* desiccation, oxidative stress accompanies molecular damage, but experimentally induced oxidative stress can also elicit responses resembling the desiccation program. Antioxidant treatment suppresses induction of selected anhydrobiosis-associated genes, supporting a signaling role for redox change during entry into anhydrobiosis [21]. Such evidence does not imply that oxidative damage itself is beneficial; rather, controlled redox perturbation can participate in stress sensing while excessive or persistent oxidation remains damaging.

Dehydration signaling outside anhydrobiosis can involve different tissues and outputs. In adult *D. melanogaster*, water-loss dehydration stimulates ecdysone-dependent immune priming in Malpighian tubules [22]. This demonstrates that renal cells detect dehydration and alter endocrine/immune signaling, but the measured outcome is enhanced antibacterial responsiveness rather than a demonstrated increase in dehydration survival. Similarly, the desiccation relevance of renal neuropeptides must be evaluated through their effects on water flux rather than expression alone: tachykinin-driven diuresis can actively reduce survival when water conservation is required [11].

The synthesis emerging from these studies is a proposed signal-to-protection validation hierarchy. A dehydration-associated molecular change occupies the lowest evidential level when it is only correlated with stress. Evidence becomes stronger when the signal is localized to a relevant tissue, connected to a defined downstream target, experimentally perturbed, and finally linked to whole-cell or whole-animal survival. Importantly, validation remains context-specific. HSF1 and Ca^{2+} signaling are functionally supported in an anhydrobiotic cell system, whereas renal peptide signaling regulates fluid loss in ordinary *Drosophila* water balance; neither topology should be transferred wholesale to the other biological state.

The distinction between stress detection and validated protection is sufficiently central that it is represented explicitly in **Figure 1** rather than collapsed into a single “desiccation signaling pathway.

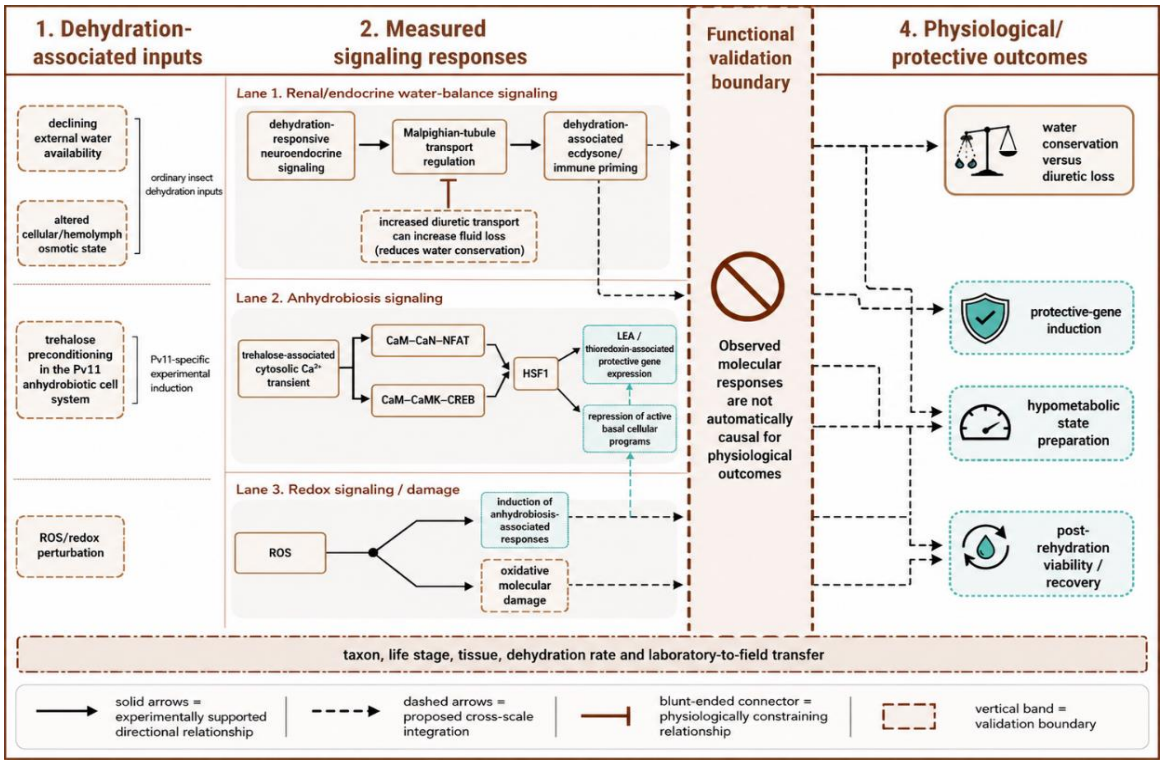


Figure 1. Dehydration-triggered signals reach protective outcomes through context-specific validation boundaries

Proteostasis and membrane protection

Extreme water loss threatens proteins through altered hydration, molecular crowding and aggregation, while membranes face phase transitions and loss of normal bilayer organization. In *Polypedilum vanderplanki*, anhydrobiosis-associated genomic regions contain LIL genes encoding unusual multi-pass transmembrane proteins, several of which localize to cellular membranes [23]. Their association with the anhydrobiotic system is strong, but localization alone does not establish the precise protective action of each protein.

This distinction is important because cellular protection is broader than expression of canonical heat-shock proteins. Arthropod dehydration responses can recruit chaperones, antioxidants and membrane-remodeling processes, yet their importance depends on tissue, dehydration rate and severity [24]. Likewise, organismal water balance can be improved without requiring exceptional cellular desiccation tolerance, so resistance to water loss and tolerance of molecular dehydration must remain separate [25].

Cross-stress experiments illustrate why protein induction cannot automatically be classified as a desiccation mechanism. HSP70 contributes functionally to UV-B stress tolerance in *Myzus persicae* [26], while HSP70 and cathepsin-B perturbation modifies thermal tolerance in *Aphis gossypii* [27]. Even HSF biology varies with life-history context: a distinctive heat-shock-factor system contributes to longevity maintenance in ants [28]. These findings establish that proteostasis systems can alter stress performance, but they do not establish that the same proteins provide equivalent protection during insect dehydration.

The proposed interpretation is therefore that proteostasis and membrane protection constitute a damage-containment layer whose relevance increases as homeostatic water regulation becomes insufficient. In ordinary dehydration, this layer may complement reduced water loss; during anhydrobiosis, it becomes integral to preservation of macromolecular and membrane function and to subsequent recovery.

The distinction between preservation, stress-responsive remodeling and recovery is clearer when their different observable consequences are kept separate, as summarized in **Table 2**.

Table 2. Protective processes differ in what they preserve during dehydration and what they permit after rehydration

Biological context with observed basis	Immediate protected feature	Consequence requiring de within its limit
HSF-dependent protective transcription		
Pv11 anhydrobiotic cells **Induction of LEA-, thioredoxin- and stress-associated programs**	Protein/cellular stress management	Survival after desiccation and rehydration Strongly validated in one specialized cell system
Anhydrobiosis-associated membrane proteins		
P. vanderplanki **Membrane localization of LIL proteins**	Candidate membrane stabilization or remodeling	Specific protective function of individual proteins Localization is not equivalent to causal protection
Chaperone-mediated proteostasis		
Other insect stress systems **HSP expression plus functional perturbation**	Protein folding and damage limitation	Direct dehydration benefit Protection can be stress-, stage- and species-specific
Metabolic suppression		
Extreme dehydration preparation **Reduced biosynthetic activity with protective-gene induction**	Lower metabolic demand during water loss	Improved dry-state survival Association must be separated from energetic causation
Rehydration-linked restoration		
Anhydrobiotic cells/larvae **Solute clearance, repair and renewed biosynthesis**	Recovery of normal cellular organization	Return of viable function Dry-state preservation alone does not ensure recovery
Damage-containment layer		
Cross-scale article synthesis **Convergence of proteostasis, membrane and metabolic evidence**	Multiple cellular targets	Coordinated organismal tolerance Proposed synthesis; not yet validated as a universal insect module

Oxidative damage and repair

Redox biology creates a particularly important interpretive problem because the same dehydration episode can generate both signaling and injury. In *P. vanderplanki*, ROS increase during desiccation, and the early rehydration period can transiently exceed antioxidant capacity, producing oxidation of proteins, DNA and membrane lipids; much of this damage is subsequently repaired [21]. Metabolic evidence also indicates that recovery restores reducing capacity and redirects accumulated substrates as active metabolism resumes [16].

Consequently, antioxidant induction should not be interpreted as proof that oxidative injury has been prevented. HSF1-dependent thioredoxin expression links redox protection to the anhydrobiotic transcriptional program [19], whereas oxidative stress itself can participate upstream in induction of that program [21]. The protective state therefore requires a bounded redox perturbation: sufficient signaling to recruit defense, but not persistent damage exceeding repair capacity.

Evidence from other insects reinforces the danger of universalizing this balance. Social isolation in ants increases oxidative stress and shortens lifespan, demonstrating that sustained redox disturbance can propagate to an organismal outcome in a very different biological context [29]. Such evidence supports the general distinction between signaling-level ROS and damage-level ROS, not a shared desiccation pathway.

Table 3 reconciles these apparently conflicting interpretations by separating the biological phase in which a redox observation is made from the conclusion that observation can support.

Table 3. Reconciling apparently conflicting redox evidence across dehydration and recovery

Redox evidence pattern — evidential organization		Interpretive reach, restraint, and verification	
Redox evidence pattern	**Dehydration/recovery state**	**What is directly observed, competing interpretation, and required discriminating test**	**Interpretation supported**

Transient ROS rise with protective-gene induction	Entry into anhydrobiosis	Redox change accompanies signaling response ROS may simultaneously initiate molecular injury Manipulate ROS while measuring both signaling and damage	ROS can participate in stress induction
Protein, lipid or DNA oxidation	Severe dehydration/early rehydration	Molecular damage markers increase Damage magnitude need not predict eventual death Couple damage kinetics to repair and viability	Protective capacity has been exceeded locally
Antioxidant/thioredoxin induction	Preparation or recovery	Redox-defense machinery increases Induction may reflect prior damage rather than successful protection Functional loss/rescue of defined antioxidant components	Cells recruit redox-control capacity
Rapid post-rehydration repair	Recovery	Oxidative lesions decline after water returns Declining markers may also reflect turnover or selection of surviving cells Track lesions and repair pathways longitudinally	Repair capacity contributes to successful recovery
Elevated ROS associated with reduced performance	Non-desiccation insect stress	Redox disturbance and organismal impairment co-occur Mechanism and thresholds remain context-dependent Perturb redox state independently of the initiating stress	Persistent oxidative stress can be harmful
Signal–damage–repair balance	Cross-scale article synthesis	Integration of phase-specific findings No universal optimum or threshold is established Prospective multilevel validation required	Redox meaning depends on position in the dehydration cycle

Because oxidative protection cannot be represented faithfully as a simple antioxidant-versus-damage dichotomy, **Figure 2** places dehydration severity and recovery capacity in a qualitative state space.

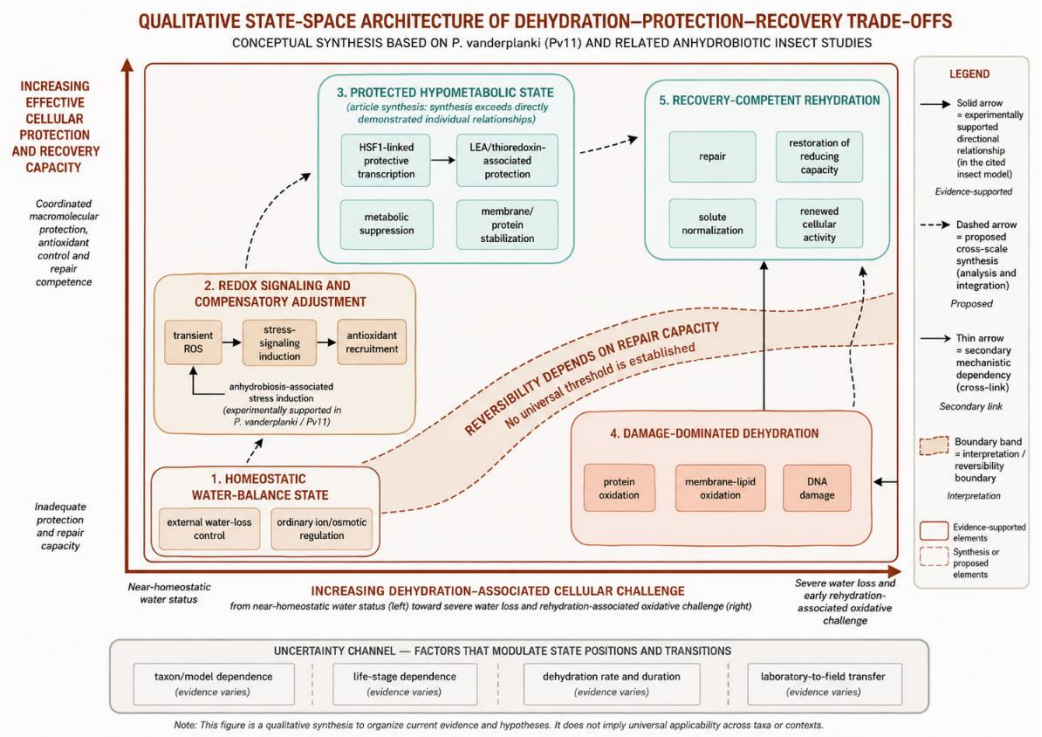


Figure 2. Redox protection changes meaning across dehydration and rehydration states

Cross-scale integration

The preceding mechanisms become biologically meaningful only when their levels are connected without being collapsed. Comparative work on Argentine ants shows that body size and CHC composition can influence desiccation resistance at the organismal level [30], whereas RNAi of a diuretic-hormone receptor in *Lymantria*

dispar links neuroendocrine transport control to water content and stress performance [31]. These are different causal routes to a superficially similar survival phenotype.

Life stage adds another boundary. Functional-genetic evidence across complex insect life cycles demonstrates that stress-responsive mechanisms can differ markedly among developmental stages [32]. Broader quantitative syntheses similarly show that physiological plasticity is pervasive but frequently modest and heterogeneous [33], while developmental history can alter both the magnitude and persistence of tolerance phenotypes [34].

Direct CHC manipulation in *D. melanogaster* further separates reduced transpiration from dehydration tolerance itself [35]. At an ecological level, *Sitophilus oryzae* symbionts can modify cuticle-associated water retention and dry-condition performance [36], illustrating that apparent "host desiccation physiology" can also depend on biotic partners.

The article therefore proposes a cross-scale limiting-layer principle: desiccation survival is constrained by whichever layer—external water flux, hydromineral regulation, metabolic adjustment, cellular protection or recovery—is least able to accommodate the current dehydration state. This is an organizing synthesis rather than a validated universal model.

Major knowledge gaps

The first priority is experimental linkage across levels. Candidate genes should be connected to water flux, ion balance, molecular damage and recovery rather than validated only by expression. Methodological work on acclimation emphasizes that treatment-induced phenotypes must be separated from evolutionary adaptation and tested with measurements appropriate to the claimed mechanism [37]. The same discipline is required for desiccation biology.

Second, exposure architecture remains poorly standardized. Dehydration rate, temperature, humidity, access to food or free water, activity and recovery interval can change which physiological constraint becomes limiting. Research on insect thermal extremes demonstrates how exposure duration and interacting stresses alter mechanistic interpretation [38]; analogous factorial designs are needed for dehydration.

Third, taxonomic and ecological coverage remains uneven. *Drosophila*, mosquitoes and *P. vanderplanki* provide powerful experimental systems, but their mechanisms span ordinary resistance to exceptional anhydrobiosis. Laboratory assays also rarely reproduce fluctuating humidity, microclimatic buffering, behavioural water acquisition or simultaneous thermal stress. Ecological studies of climate extremes show that organismal responses cannot automatically be translated into population persistence or community consequence [39].

Future progress therefore requires matched measurements across dehydration and rehydration, explicit life-stage and sex comparisons, tissue-resolved functional perturbation, direct membrane and proteostasis assays, and field-relevant exposure sequences. The strongest tests of the proposed limiting-layer principle would manipulate one mechanistic layer while measuring whether another becomes the new constraint.

Conclusion

Insect desiccation tolerance is not a single molecular program. Water-loss barriers, renal transport, metabolic adjustment, stress signaling, proteostasis, membrane preservation, redox control and recovery solve different problems at different stages of dehydration. Their relative importance shifts with taxon, life stage, exposure rate and severity.The central synthesis is that resistance should be interpreted as a sequence of interacting constraints rather than as the presence of a particular metabolite, transcript or stress pathway. Extreme anhydrobiosis demonstrates what cellular protection can achieve, but it should not define ordinary insect drought resistance. Progress will depend on experiments that connect molecular perturbation to water balance, damage and recovery across biological scales.

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