Eurasia Specialized Veterinary Publication

Entomological Research Letters

ISSN: 3108-639X

2023, Volume 3, Issue 2, Page No: 72-83
Copyright CC BY-NC-SA 4.0
Available online at: www.esvpub.com/

Oxidative Balance Constrains Insect Stress Tolerance, Aging, and Reproductive Investment Through Shared Physiological Trade-Offs

Laura Keller[1]*, Thomas Lehmann[1], Simon Brunner[2], Christoph Meier[3], David Thompson[4]

[1]Department of Oxidative Balance and Stress Tolerance, Faculty of Life Sciences, ETH Zurich, Zurich, Switzerland.
[2]Department of Aging and Reproductive Trade-Offs, Faculty of Biosciences, EPFL Lausanne, Lausanne, Switzerland.
[3]Department of Physiological Constraints and Redox Regulation, Faculty of Sciences, University of Bern, Bern, Switzerland.
[4]Department of Oxidative Stress and Life-History Integration, Faculty of Life Sciences, University of Basel, Basel, Switzerland.

***E-mail** ✉ laura.keller@usys.ethz.ch

ABSTRACT

Oxidative physiology is frequently invoked to explain insect responses to environmental stress, aging, and reproductive investment, yet the underlying evidence does not support a single continuum in which reactive oxygen species invariably represent damage, greater antioxidant activity invariably represents protection, or reproduction necessarily reduces somatic maintenance. This article develops a proposed physiological model in which oxidative balance is treated as a dynamic analytical state emerging from reactive-species generation and signaling, antioxidant and stress-protective capacity, molecular susceptibility, damage and repair, environmental demand, and life-history allocation. Evidence from insects shows that reactive oxygen species can participate in defined signaling pathways while also accumulating during damaging physiological states; antioxidant-related defenses can improve stress tolerance, but their effects depend on pathway, tissue, genotype, and exposure; and thermal, xenobiotic, nutritional, social, and activity-related challenges generate responses that vary with stage, stress history, and ecological context. Aging likewise cannot be reduced to cumulative oxidation because redox-sensitive maintenance, intervention timing, genotype, sex, activity, and laboratory–field transfer modify observed relationships. Reproductive evidence is especially heterogeneous: oxidative costs occur in some systems, are absent for particular behaviors or markers in others, and can be reversed in eusocial insects. The proposed oxidative-balance model therefore interprets stress tolerance, aging, and reproductive investment as conditionally coupled outcomes rather than obligatory consequences of a fixed oxidative budget.

Keywords: Reactive oxygen species, Antioxidant capacity, Oxidative stress, Insect aging, Reproductive Investment, Physiological trade-offs

Received: 19 September 2023
Revised: 23 November 2023
Accepted: 29 November 2023

How to Cite This Article: Keller L, Lehmann T, Brunner S, Meier C, Thompson D. Oxidative Balance Constrains Insect Stress Tolerance, Aging, and Reproductive Investment Through Shared Physiological Trade-Offs. Entomol Res Lett. 2023;3(2):72-83. https://doi.org/10.51847/PsV58cEPZY

Introduction

Oxidative physiology occupies an unusual position in insect biology because the same chemical class can participate in both information transfer and molecular injury. In *Mythimna separata* larvae, stress-derived reactive oxygen species (ROS) participate in hemocyte signaling that enables release of an enzyme involved in growth-blocking peptide processing, demonstrating a defined signaling function rather than indiscriminate toxicity [1]. Conversely, social isolation in *Camponotus fellah* workers was accompanied by ROS accumulation and shortened

© 2023 Entomological Research Letters

lifespan, with antioxidant intervention supporting an oxidative contribution to the survival effect [2]. These findings come from very different biological settings, but together they make a simple "more ROS equals more damage" interpretation inadequate.

Life-history relationships are equally resistant to a fixed oxidative-cost rule. Genetic manipulation of ROS production and scavenging in *Drosophila melanogaster* affected reproductive performance differently according to sex and pathway [3]. Selection on female reproductive timing in *Ceratitis cosyra* changed total antioxidant capacity and lipid-peroxidation phenotypes without producing a simple correspondence between less oxidative damage and greater survival [4]. In honeybee workers, reproductive activation was instead associated with improved resistance to abiotic and biotic challenges [5]. Thus, oxidative physiology can covary negatively, positively, or weakly with reproduction depending on the biological state being examined.

This article therefore proposes a conditional oxidative-balance model rather than a universal oxidative-stress scale. "Balance" is used here as an analytical description of the relation among ROS generation and signaling, protection, susceptibility, damage, repair, stress demand, and life-history investment. It is not proposed as a directly measurable universal ratio. The central question is consequently not whether an insect has "high" or "low" oxidative stress, but under which physiological and environmental conditions redox processes constrain stress tolerance, aging, or reproductive investment, and when apparently similar biomarker changes represent fundamentally different processes.

Reactive species as signals and damage sources

The distinction between signaling ROS and damaging oxidative load is fundamental. During diapause in *Helicoverpa armigera*, physiological ROS participate in signaling through insulin-pathway components associated with lifespan extension [6]. A related mechanistic study identified ROS-dependent elevation of HIF-1α phosphorylation through a CK2–MKP3–p38 route in the diapause brain [7]. These experiments provide specific examples in which ROS-dependent processes support longevity-associated physiology, but their diapause and taxonomic specificity precludes treating increased ROS as generally beneficial.

The opposite interpretive error is to infer whole-organism oxidative damage from a single responsive biomarker. Juvenile-hormone manipulation in male *Hetaerina americana* altered selected oxidative measures while catalase, glutathione, hydrogen peroxide, and damage-related endpoints did not behave as a single concordant axis [8]. Oxidative state therefore cannot be reconstructed reliably by assuming equivalence among ROS abundance, antioxidant-enzyme activity, and molecular damage. A marker can identify one component of redox physiology without diagnosing the direction of the entire system.

Environmental responses introduce an additional causal boundary. Chilling can activate insect immunity, and ROS or damage-associated signaling has been proposed as part of this response, but the mechanistic literature remains heterogeneous and incomplete [9]. Consequently, cold-associated ROS should be treated as a possible signaling channel rather than an established universal mediator of cold immunity.

The resulting distinction used throughout this article is threefold: ROS may carry information, ROS may contribute to molecular damage, and measured ROS may also accompany a response driven partly by other processes. The physiological meaning depends on compartment, duration, magnitude, life stage, and the downstream systems with which reactive species interact.

Antioxidant capacity

Antioxidant capacity is similarly misleading when reduced to one enzyme or one concentration. In *Apis cerana cerana*, manipulation of tryptophan-5-hydroxylase-1 and melatonin altered oxidative biomarkers, and melatonin treatment improved survival under cold stress, supporting a functional contribution of this antioxidant-related system to stress tolerance [10]. In *Myzus persicae*, lambda-cyhalothrin induced oxidative responses and Hsp70, while Hsp70 knockdown increased susceptibility, indicating that inducible stress proteins can contribute to protection under a specific xenobiotic challenge [11]. In *Nilaparvata lugens*, overexpressed sigma glutathione S-transferases contributed functionally to remediation of oxidative challenge associated with insecticide resistance and feeding on resistant rice [12]. These systems demonstrate deployable protective capacity, but none provides a universal measure of total antioxidant reserve.

Capacity also differs among tissues and physiological demands. In male *Teleogryllus oceanicus*, dietary antioxidants changed oxidative balance in testes and muscle, whereas increased courtship effort did not produce

the anticipated oxidative cost [13]. The result separates resource-dependent redox effects from behavioral expenditure and cautions against extrapolating a tissue assay to a whole-organism budget.

Protection may additionally depend on intrinsic molecular susceptibility. Honeybee caste lipidomics showed marked differences in membrane fatty-acid composition across queens, workers, and life-history stages, with queens retaining comparatively monounsaturated profiles while workers contained more polyunsaturated lipids [14]. Such differences can plausibly alter susceptibility to peroxidation, but the observational comparison does not establish membrane composition as the cause of queen longevity. In the proposed model, antioxidant capacity therefore includes inducible and constitutive defenses while remaining analytically distinct from the structural susceptibility of potential molecular targets.

Stress-induced oxidative demand

External challenge does not impose a uniform oxidative requirement. Acute sublethal tebuconazole exposure disrupted several components of redox homeostasis in the honeybee brain and increased a lipid-peroxidation signal, providing direct evidence that a xenobiotic can raise oxidative burden at the tissue level [15]. Yet physiological history alters the meaning of such challenge. Malathion-resistant *Tribolium castaneum* exhibited enhanced responses to oxidative stress while also showing a shorter baseline lifespan than susceptible beetles [16]. This opposition illustrates that increased defensive ability under challenge does not necessarily imply superior performance under unstressed conditions.

Thermal environments further separate oxidative demand from generalized damage. Under fluctuating thermal regimes, *Gromphadorhina coquereliana* displayed selective changes in mitochondrial metabolism and oxidative-stress physiology without wholesale energetic collapse [17]. Heat-wave responses in *Lobesia botrana* were meanwhile strongly conditioned by developmental stage and post-stress food quality [18]. The latter experiment does not establish oxidative mediation of all heat effects, but it demonstrates that recovery resources and developmental state alter the physiological consequences of a nominally similar thermal challenge.

Natural and temporally variable environments impose another interpretive boundary. Across winter conditions in honeybee colonies, temperature covaried with several physiological traits, including glycerol, vitellogenin dynamics, and virus occurrence, while antioxidant-gene expression did not display a corresponding generalized response [19]. In *Megachile rotundata*, constant and fluctuating temperature treatments produced distinct transcriptional profiles whose patterns differed substantially among developmental stages [20]. Neither transcription alone nor seasonal covariance establishes oxidative damage, but both studies show why stress demand cannot be defined only by the mean intensity of an exposure.

Stress history may even change the sign of subsequent performance. Prior anoxia exposure in *Anastrepha suspensa* reduced costs of later anoxia, demonstrating experimentally that preconditioning can improve subsequent tolerance [21]. This hormetic response should not be equated with evolutionary adaptation; it instead identifies exposure history as a physiological state variable.

These contrasts make a context–mechanism–outcome register more informative than a single oxidative-stress category because similar environmental challenges can correspond to damage, selective adjustment, conditional recovery, or preconditioning.

Table 1. Stress-induced oxidative demand changes meaning across xenobiotic, thermal, seasonal, and preconditioned physiological states

Oxidative-demand state	Dimension	Scientific content	Dimension	Scientific content
Acute neural redox disruption	**Biological setting · Evidence**	Adult honeybee brain under sublethal fungicide exposure Altered redox-homeostasis measures and lipid-peroxidation signal	**Principal boundary or uncertainty · Evidential standing**	Acute laboratory tissue response does not establish colony or population effects Evidence-supported
	Physiological interpretation · Research or test	Challenge can exceed local redox control and increase damaging load Separate acute tissue injury from longer-term organismal consequences		
	Biological setting · Evidence	Resistant versus susceptible red flour beetles	**Principal boundary or**	Strains differ genetically beyond measured redox traits

		Greater oxidant tolerance accompanied by shorter baseline lifespan	**uncertainty · Evidential standing**	Evidence-supported
Resistance-conditioned defense	**Physiological interpretation · Research or test**	Defensive capacity and unstressed performance can move in opposite directions Compare inducible defense, constitutive costs, and baseline performance within shared backgrounds		
Selective thermal adjustment	**Biological setting · Evidence**	Cockroach under repeated fluctuating cold Specific mitochondrial/redox changes with many energetic variables maintained	**Principal boundary or uncertainty · Evidential standing**	One tropical cockroach under controlled regimes Evidence-supported
	Physiological interpretation · Research or test	Thermal challenge can induce adjustment without generalized mitochondrial dysfunction Measure multiple mitochondrial functions rather than inferring failure from one endpoint		
Recovery-resource-dependent heat state	**Biological setting · Evidence**	Grapevine moth stages exposed to heat wave and contrasting nutrition Stage- and food-dependent post-stress performance	**Principal boundary or uncertainty · Evidential standing**	Redox mediation was not established for every outcome Evidence-supported; oxidative integration proposed
	Physiological interpretation · Research or test	Consequence of thermal demand depends partly on capacity for recovery Factorially measure oxidative endpoints with developmental stage and recovery nutrition		
Seasonally distributed demand	**Biological setting · Evidence**	Winter honeybee colonies Multiple physiological responses without generalized antioxidant-transcript response	**Principal boundary or uncertainty · Evidential standing**	Seasonal covariance includes pathogens and uncontrolled environmental variation Evidence-supported
	Physiological interpretation · Research or test	Natural stress may be distributed across interacting physiological systems Link redox measurements to simultaneous ecological and physiological covariates		
Preconditioned demand state	**Biological setting · Evidence**	Soil-pupating fruit fly after prior anoxia Improved performance during subsequent anoxia	**Principal boundary or uncertainty · Evidential standing**	Controlled anoxia does not establish general adaptive hormesis Evidence-supported; state classification proposed
	Physiological interpretation · Research or test	Prior challenge can alter the physiological cost of later stress Test whether protection persists across stress intensity, timing, and stressor identity		

Aging and cumulative damage

Aging provides a particularly strong test of whether oxidative balance should be interpreted as damage accumulation alone. In *D. melanogaster*, dSirt6 overexpression increased lifespan and resistance to oxidative challenge without the anticipated major fertility penalty [22]. In a separate dietary system, early-adult methionine restriction reduced methionine sulfoxide and extended lifespan, whereas intervention later in adulthood was less

effective [23]. These findings support redox-sensitive maintenance processes while also showing that timing matters; neither manipulation demonstrates that oxidation is the sole determinant of insect aging.

Antioxidant interventions themselves can be conditional. In flies with mitonuclear incompatibilities, redox stress, respiratory complex-I suppression, and responses to N-acetylcysteine depended on genotype, sex, tissue, and treatment context [24]. Thus, an antioxidant capable of benefiting one physiological state may fail to benefit another, and treatment response cannot be interpreted independently of the underlying source of redox imbalance.

Cumulative metabolic activity provides a different route to oxidative burden. Flight experience in *Apis mellifera* workers increased ROS and oxidative-damage indicators, including 8-hydroxy-2′-deoxyguanosine, while antioxidant-enzyme responses were not uniformly elevated [25]. The evidence supports activity-associated oxidative burden but does not establish that those markers directly cause senescence.

Ecological transfer introduces a final constraint. In *Melitaea cinxia*, flight-associated oxidative injury depended on succinate-dehydrogenase genotype, and laboratory relationships were not fully concordant with patterns observed in field-derived comparisons [26]. An oxidative association produced under standardized flight challenge therefore cannot automatically be promoted to a natural fitness mechanism.

Because age-associated oxidative phenotypes may arise through redox-sensitive maintenance, timing of intervention, cumulative activity, genetic background, or context-dependent measurement, the evidence requires an explicit separation between observations and stronger aging inferences.

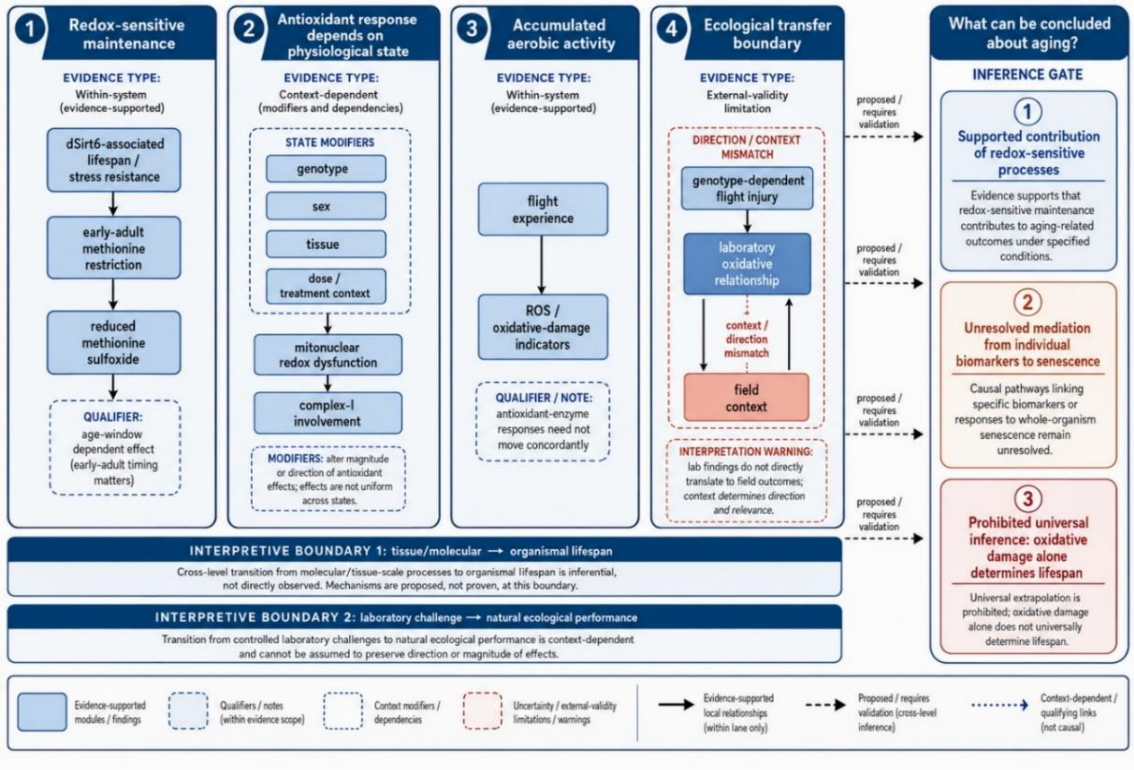


Figure 1. Aging inference depends on separating redox-sensitive maintenance, accumulated activity load, and laboratory-to-field transfer

Reproductive oxidative costs

Reproductive investment provides the clearest contradiction to a universal oxidative trade-off. In workers of several ant species, queen loss altered survival under paraquat challenge, but the direction and magnitude of the response were species dependent rather than uniform [27]. In *Temnothorax rugatulus*, fertile workers lived longer and showed altered expression of longevity- and fecundity-associated genes, with part of the phenotype changing again after restoration of the queen [28]. These social-insect systems show that reproductive or reproductive-like activation can coexist with enhanced survival, but they do not establish that reproduction generally extends insect lifespan. Social regulation, endocrine reorganization, caste physiology, and species-specific life histories remain important alternatives to a simple cost reversal.

Reproductive allocation also involves resources and molecular systems whose functions extend beyond oxidation. Metal-ion homeostasis intersects insect reproductive and immune physiology, although the mechanisms and their taxonomic distribution remain incompletely resolved [29]. In female *D. melanogaster*, limiting dietary sterols were preferentially allocated toward production of viable eggs, demonstrating resource-specific reproductive prioritization without establishing a direct oxidative allocation mechanism [30]. These observations argue against conceptualizing reproductive cost as competition for one undifferentiated energetic or antioxidant pool.

The broader evidence is therefore intentionally heterogeneous. Manipulating ROS production and scavenging can alter reproductive success differently in males and females [3]. Selection on reproductive timing can shift antioxidant and lipid-peroxidation phenotypes without a monotonic damage–survival relationship [4]. Reproductive activation in honeybee workers can coincide with greater stress resistance [5], while intensive courtship in male crickets did not generate the predicted oxidative change even though dietary antioxidants affected tissue redox state [13]. Reproductive oxidative cost is consequently best treated as a conditional outcome whose sign depends on sex, tissue, social organization, reproductive mode, limiting nutrient, marker identity, and environmental demand.

The coverage-and-gap map below is needed because the principal uncertainty is not whether reproduction can interact with oxidative physiology, but which biological contexts permit a defensible oxidative-cost inference and which require alternative explanations.

Table 2. Why reproductive oxidative cost cannot be assigned a single sign across sex, social state, behavior, and limiting-resource contexts

Reproductive-cost pattern		
Sex-dependent redox–reproduction relationship		
Biological setting · Evidence	**Interpretation permitted · Coverage gap or competing explanation**	**Decisive research need · Evidential standing**
Adult Drosophila males and females **Genetic alteration of ROS production and scavenging changes reproductive outcomes non-uniformly**	ROS handling can influence reproduction differently by sex and pathway Pleiotropy and pathway-specific effects complicate a common cost metric	Resolve tissue- and sex-specific mechanisms under matched reproductive demands Evidence-supported
Evolved reproductive timing with non-monotonic oxidative phenotype		
Biological setting · Evidence	**Interpretation permitted · Coverage gap or competing explanation**	**Decisive research need · Evidential standing**
Female marula fruit flies **Selection changes antioxidant capacity and lipid peroxidation without simple damage–survival alignment**	Reproductive evolution can alter oxidative phenotype Selection changes multiple correlated traits	Measure protection, damage, repair, and survival jointly across reproductive schedules Evidence-supported
Reproductive activation with increased stress resistance		
Biological setting · Evidence	**Interpretation permitted · Coverage gap or competing explanation**	**Decisive research need · Evidential standing**
Honeybee workers **Activated reproduction accompanies greater survival under selected challenges**	Reproduction–stress trade-off can be absent or reversed in eusocial workers Worker caste physiology and social regulation limit transfer	Compare homologous reproductive states across eusocial and solitary systems Evidence-supported
Behavioral effort without predicted oxidative cost		
Biological setting · Evidence	**Interpretation permitted · Coverage gap or competing explanation**	**Decisive research need · Evidential standing**
Male field crickets **Courtship treatment lacks expected oxidative effect while antioxidant diet changes tissue measures**	Reproductive behavior need not yield detectable oxidative cost in the assayed tissues Other energetic or physiological costs may remain	Pair behavioral effort with tissue-specific damage, repair, and performance endpoints Evidence-supported
Queen-loss response differing among ant species		
Biological setting · Evidence	**Interpretation permitted · Coverage gap or competing explanation**	**Decisive research need · Evidential standing**

Eusocial ant workers **Paraquat survival changes after queen loss but is not taxonomically uniform**	Social reproductive state can modify oxidant tolerance Species-specific colony regulation	Use comparative manipulations that separate fertility from queen-associated social effects Evidence-supported
Fertility positively associated with worker longevity		
Biological setting · Evidence	**Interpretation permitted · Coverage gap or competing explanation**	**Decisive research need · Evidential standing**
Temnothorax workers **Fertile state accompanies longer survival and partly reversible molecular changes**	Fertility and longevity can covary positively in a social insect Endocrine and social-state reorganization may drive both traits	Experimentally separate reproductive activity from regulatory state Evidence-supported
Cross-system resource coupling		
Biological setting · Evidence	**Interpretation permitted · Coverage gap or competing explanation**	**Decisive research need · Evidential standing**
Multiple insects and female Drosophila **Reproduction shares metal-handling physiology with immunity; sterols are preferentially routed to viable eggs**	Resource identity matters to reproductive allocation Resource competition is not automatically oxidative competition	Measure whether specific resource limitation alters redox protection or repair directly Evidence-supported; oxidative coupling proposed

Allocation among protection, repair, and reproduction

Life-history trade-offs can involve oxidative mechanisms, but the evidence does not justify treating every trade-off as competition for a fixed antioxidant budget. In *Lestes viridis*, experimentally accelerated larval development increased oxidative damage and shortened subsequent adult lifespan; mitochondrial uncoupling attenuated the relationship, providing a defined case in which oxidative damage contributed mechanistically to a development–lifespan trade-off [31]. This is important proof of principle, but it remains a single developmental system rather than evidence that oxidative damage universally mediates rapid-development costs.

Allocation is also difficult to infer from biomarkers alone. In male *Hetaerina americana*, relationships between sexual signaling and oxidative physiology differed among total antioxidant capacity, hydrogen peroxide, catalase, and superoxide dismutase, with some associations absent or directionally different [32]. An increase in one defensive marker therefore cannot by itself establish that resources were reallocated toward somatic protection or away from reproduction.

The synthesis proposed here consequently treats protection, repair, and reproduction as conditionally coordinated processes rather than fixed compartments of one physiological budget. Protection may reduce formation of damage; repair may remove or replace damaged molecules after exposure; reproduction may compete for some limiting resources while remaining independently regulated for others. These processes can also be co-regulated by endocrine, nutritional, social, or developmental state. The model therefore predicts trade-offs only when environmental demand, resource identity, physiological reserve, and reproductive state create a genuine constraint; it does not prescribe fixed allocation fractions.

Oxidative-balance model

The proposed oxidative-balance model integrates four separable domains: reactive-species generation and signaling; effective protection and molecular susceptibility; damage accumulation and repair; and life-history demand. Their interaction is treated as state dependent. Oxidative balance is therefore not a biochemical ratio and cannot be diagnosed by one ROS measure, antioxidant enzyme, or damage product. A signaling-dominant state may contain elevated ROS without major injury, whereas a damage-dominant state requires evidence that molecular injury has exceeded prevention or repair. Likewise, high antioxidant activity may represent effective protection, compensatory induction under high demand, or a pathway-specific response with little information about whole-organism reserve.

State dependence is supported by the contrasting systems reviewed above. Fluctuating thermal exposure can produce selective physiological adjustment rather than generalized dysfunction [17]. Antioxidant intervention can change direction according to genotype, sex, tissue, and redox background [24]. Social reproductive state can coincide with increased longevity rather than obligatory somatic cost [28], and nutrient identity can determine

which resources are preferentially routed into viable reproduction [30]. The new synthesis is the proposition that these modifiers can be represented within one conditional state architecture; the exact integrated architecture has not itself been empirically validated.

Testing that architecture requires measurements at levels that should not be collapsed. A validated density-gradient approach has demonstrated that highly purified, active mitochondria can be isolated from multiple insect species and orders, providing a methodological route for direct organelle-level assessment [33]. Such measurements can strengthen mechanistic inference, but mitochondrial function must still be connected experimentally to tissue damage, organismal tolerance, reproduction, and lifespan before cross-level causation is established.

A comparative adjudication matrix is useful because the same observed biomarker pattern can support different explanations depending on physiological context.

Table 3. Adjudicating competing explanations for physiological states predicted by the oxidative-balance model

Focal state or distinction	Foundational dimensions	Interpretive and testing dimensions	
	Interpretation favored by the model · Competing explanation or boundary	**Discriminating test · Biological setting**	**Evidential standing · Evidence**
Signal-dominant reactive state	Reactive species participate primarily in signaling Unmeasured damage may coexist	Combine pathway perturbation with molecular-damage and performance assays Acute regulated response or diapause physiology	Proposed state; components evidence-supported Elevated ROS with pathway activation but limited evidence of injury
Compensated high-demand state	Protection is meeting increased oxidative demand Marker induction may occur without major oxidative involvement	Measure damage, reserve, repair, and performance simultaneously Thermal, xenobiotic, or metabolic challenge	Proposed state Increased defensive activity with maintained function
Damage-dominant state	Demand exceeds effective prevention and repair Damage may be correlated rather than mediating	Manipulate the candidate oxidative pathway and test rescue Sustained challenge or accelerated physiological demand	Proposed state; mediation requires experiment Molecular oxidation accompanied by impaired function or survival
Reproduction-compatible maintenance state	Reproduction does not impose an obligatory oxidative trade-off Social endocrine regulation or resource specialization may explain covariance	Compare reproductive and non-reproductive states while controlling social and nutritional context Reproductively active social insects or resource-specific allocation	Proposed state Reproductive investment without expected stress or longevity penalty
Context-reversal state	Oxidative meaning is conditional on background and environment Assay artifacts or unmeasured ecological variables	Replicate standardized markers across laboratory and natural settings Laboratory versus field or contrasting genotypes	Evidence-bounded proposed classification Direction of oxidative association changes across contexts
Multilevel validation state	Cross-level agreement strengthens mechanistic interpretation Concordance alone does not prove causality	Combine organelle-quality validation with pathway intervention and organismal outcomes Organelle, tissue, and whole-organism measurements collected together	Proposed validation criterion Concordant or discordant responses across biological levels

These states are not ordered stages, and an insect need not progress through them sequentially. The state-space representation below instead treats transitions as conditional on demand, protection, repair, reproductive state, and environmental history.

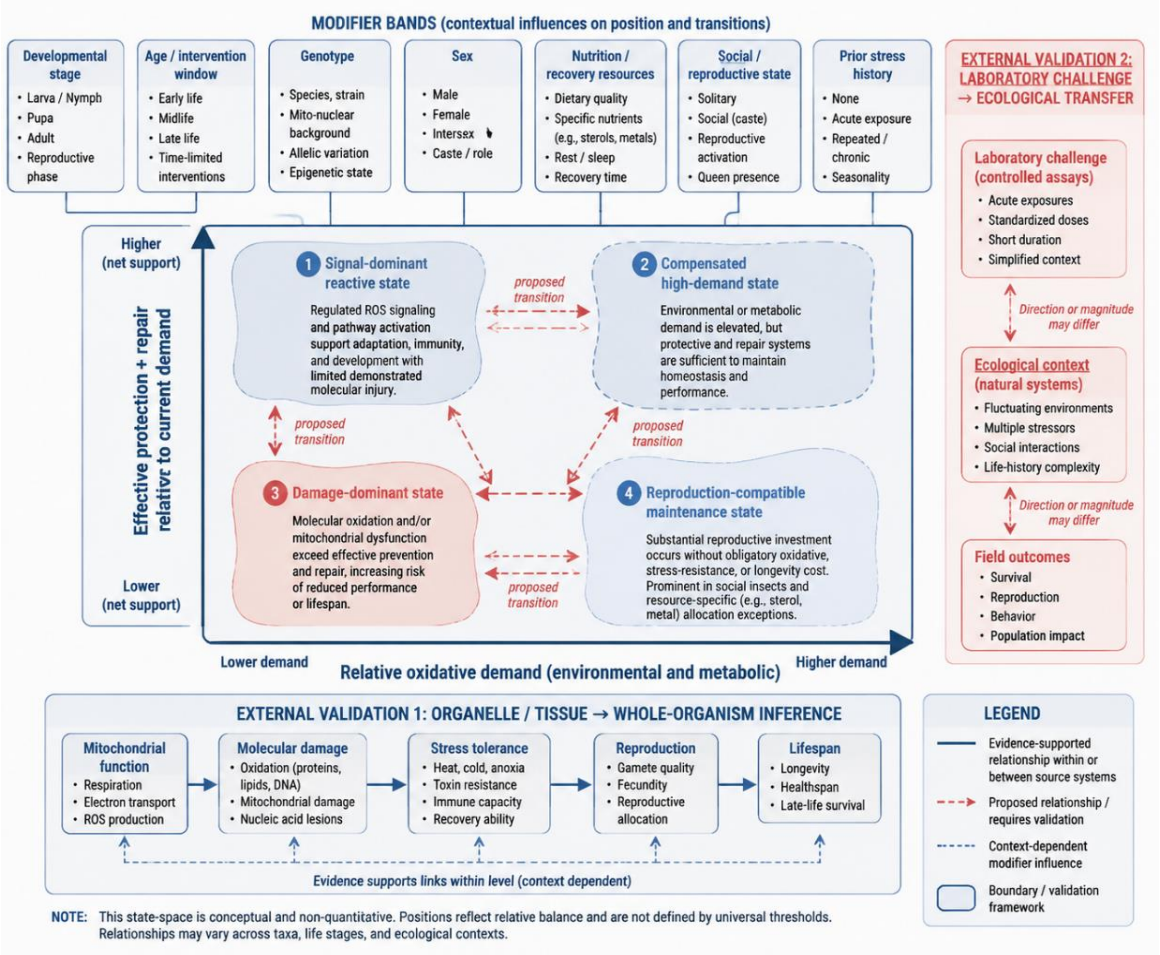


Figure 2. Physiological state determines whether oxidative demand is absorbed, repaired, or expressed as life-history cost

Predictions

The proposed model generates conditional rather than universal biomarker predictions. When two insects experience similar external stress, the one with greater effective protective or repair capacity should not necessarily show lower antioxidant activity; it may instead show greater inducible activity while accumulating less molecular damage. Because antioxidant and damage markers can diverge within the same experiment [8], discrimination among model states requires simultaneous measurement of generation, protection, damage, and performance rather than reliance on one endpoint.

Stress history, developmental stage, and recovery resources should alter the probability that a challenge is expressed as damage rather than compensation. Heat responses already differ with stage and post-stress nutrition [18], while prior anoxia can improve subsequent tolerance [21]. The proposed prediction is that factorial manipulation of these modifiers will separate compensated-demand states from damage-dominant states more effectively than stress intensity alone.

Timing and intrinsic physiological background should similarly modify intervention effects. Early-adult methionine restriction produced stronger lifespan effects than later intervention [23], and antioxidant treatment in mitonuclear-stressed flies depended on genotype and sex [24]. The model therefore predicts interaction effects rather than a common directional benefit from antioxidant manipulation.

Reproduction–longevity predictions should differ across solitary and eusocial contexts. Species-specific responses to queen loss [27] indicate that even among social insects the sign is not fixed. Validation should therefore compare reproductive state, oxidative damage, repair, stress tolerance, and survival prospectively across taxa instead of assuming that reproductive effort has one oxidative consequence.

Results and Discussion

The assembled evidence rejects both a purely damaging interpretation of reactive species and an equally broad interpretation in which ROS elevations are intrinsically beneficial signals. Stress-derived ROS can participate in defined signaling [1], whereas oxidative processes can also mediate lifespan loss under particular social conditions [2]. The proposed model reconciles these observations by requiring evidence about pathway, compartment, exposure history, damage, and performance before assigning physiological meaning.

The same conditionality applies to life-history trade-offs. Reproduction can show sex-dependent oxidative effects [3], no detectable oxidative response to a specific behavioral effort [13], or positive associations with survival in eusocial workers [28]. These are not interchangeable experiments, and their heterogeneity should not be averaged into a universal direction. Rather, they identify sociality, sex, tissue, resource identity, and reproductive state as candidate modifiers of when oxidative constraints become limiting.

Measurement remains a central limitation. Antioxidant transcription can remain comparatively unresponsive while other seasonal physiological variables change [19], and laboratory oxidative relationships can differ from field patterns [26]. Consequently, neither marker concordance nor laboratory challenge alone establishes ecological relevance. Future tests should combine pathway perturbation, multiple molecular-damage endpoints, validated organelle measurements, organismal performance, and field-relevant exposure regimes.

The model is therefore best regarded as a falsifiable organizing hypothesis. It would be weakened if stress, aging, and reproduction consistently showed the same relationships regardless of stage, genotype, social state, nutrition, or environmental history. It would gain support if factorial studies showed that these modifiers predict when signaling remains regulated, when protection compensates for demand, and when damage becomes functionally consequential. Such validation must be prospective; the existing literature establishes the component phenomena, not the integrated model itself.

Conclusion

Oxidative physiology constrains insect performance through conditional relationships rather than a universal ROS–damage–trade-off sequence. Reactive species can signal or damage; antioxidant activity can protect or reflect elevated demand; cumulative damage can contribute to aging without constituting a complete theory of aging; and reproduction can impose costs, show no measurable oxidative cost, or coexist with enhanced survival. The proposed oxidative-balance model organizes these contrasts around state-dependent generation, protection, repair, damage, and life-history demand while preserving taxonomic, developmental, environmental, and measurement boundaries. Its value lies in generating discriminating tests, not in claiming that a single oxidative metric already predicts insect stress tolerance, lifespan, or reproductive investment.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Matsumoto H, Ochiai M, Imai E, Matsumura T, Hayakawa Y. Stress-derived reactive oxygen species enable hemocytes to release activator of growth blocking peptide (GBP) processing enzyme. J Insect Physiol. 2021;131:104225. doi:10.1016/j.jinsphys.2021.104225
2. Koto A, Tamura M, Wong PS, Aburatani S, Privman E, Stoffel C, et al. Social isolation shortens lifespan through oxidative stress in ants. Nat Commun. 2023;14(1):5493. doi:10.1038/s41467-023-41140-w
3. Turnell BR, Kumpitsch L, Reinhardt K. Production and scavenging of reactive oxygen species both affect reproductive success in male and female Drosophila melanogaster. Biogerontology. 2021;22(4):379-96. doi:10.1007/s10522-021-09922-1
4. Malod K, Roets PD, Oosthuizen C, Blount JD, Archer CR, Weldon CW. Selection on age of female reproduction in the marula fruit fly, Ceratitis cosyra (Walker) (Diptera: Tephritidae), decreases total

antioxidant capacity and lipid peroxidation. J Insect Physiol. 2020;125:104084. doi:10.1016/j.jinsphys.2020.104084
5. Kennedy A, Herman J, Rueppell O. Reproductive activation in honeybee (Apis mellifera) workers protects against abiotic and biotic stress. Philos Trans R Soc Lond B Biol Sci. 2021;376(1823):20190737. doi:10.1098/rstb.2019.0737
6. Zhang XS, Wang T, Lin XW, Denlinger DL, Xu WH. Reactive oxygen species extend insect life span using components of the insulin-signaling pathway. Proc Natl Acad Sci U S A. 2017;114(37). doi:10.1073/pnas.1711042114
7. Su X, Su Z, Xu W. ROS elevate HIF-1α phosphorylation for insect lifespan through the CK2-MKP3-p38 pathway. Biochim Biophys Acta Mol Cell Res. 2023;1870(2):119389. doi:10.1016/j.bbamcr.2022.119389
8. Martínez-Lendech N, Osorio-Beristain M, Franco B, Pedraza-Reyes M, Obregón A, Contreras-Garduño J. Does juvenile hormone prompt oxidative stress in male damselflies? J Exp Biol. 2019;222(Pt 5). doi:10.1242/jeb.194530
9. El-Saadi MI, MacMillan HA, Ferguson LV. Cold-induced immune activation in chill-susceptible insects. Curr Opin Insect Sci. 2023;58:101054. doi:10.1016/j.cois.2023.101054
10. Fan W, Li G, Zhang X, Wang Y, Wang C, Xu B, et al. The role of melatonin and Tryptophan-5-hydroxylase-1 in different abiotic stressors in Apis cerana cerana. J Insect Physiol. 2021;128:104180. doi:10.1016/j.jinsphys.2020.104180
11. Dong B, Liu XY, Li B, Li MY, Li SG, Liu S. A heat shock protein protects against oxidative stress induced by lambda-cyhalothrin in the green peach aphid Myzus persicae. Pestic Biochem Physiol. 2022;181:104995. doi:10.1016/j.pestbp.2021.104995
12. Zhang Y, Yang B, Yu N, Luo G, Gao H, Lin X, et al. Insecticide resistance associated overexpression of two sigma GST genes assists Nilaparvata lugens to remedy oxidative stress from feeding on resistant rice variety. Pestic Biochem Physiol. 2022;188:105230. doi:10.1016/j.pestbp.2022.105230
13. Simmons LW, Lovegrove M, Lymbery SJ. Dietary antioxidants, but not courtship effort, affect oxidative balance in the testes and muscles of crickets. J Exp Biol. 2018;221(Pt 20). doi:10.1242/jeb.184770
14. Martin N, Hulbert AJ, Brenner GC, Brown SHJ, Mitchell TW, Else PL. Honey bee caste lipidomics in relation to life-history stage and the long life of the queen. J Exp Biol. 2019;222(Pt 24). doi:10.1242/jeb.207043
15. Mackei M, Sebők C, Vöröházi J, Tráj P, Mackei F, Oláh B, et al. Detrimental consequences of tebuconazole on redox homeostasis and fatty acid profile of honeybee brain. Insect Biochem Mol Biol. 2023;159:103990. doi:10.1016/j.ibmb.2023.103990
16. Rauf A, Wilkins RM. Malathion-resistant Tribolium castaneum has enhanced response to oxidative stress, immunity, and fitness. Pestic Biochem Physiol. 2022;184:105128. doi:10.1016/j.pestbp.2022.105128
17. Lubawy J, Chowański SP, Colinet H, Słocińska M. Mitochondrial metabolism and oxidative stress in the tropical cockroach under fluctuating thermal regimes. J Exp Biol. 2023;226(17). doi:10.1242/jeb.246287
18. Iltis C, Louâpre P, Vogelweith F, Thiéry D, Moreau J. How to stand the heat? Post-stress nutrition and developmental stage determine insect response to a heat wave. J Insect Physiol. 2021;131:104214. doi:10.1016/j.jinsphys.2021.104214
19. Prado A, Brunet JL, Peruzzi M, Bonnet M, Bordier C, Crauser D, et al. Warmer winters are associated with lower levels of the cryoprotectant glycerol, a slower decrease in vitellogenin expression and reduced virus infections in winter honeybees. J Insect Physiol. 2022;136:104348. doi:10.1016/j.jinsphys.2021.104348
20. Torson AS, Yocum GD, Rinehart JP, Nash SA, Kvidera KM, Bowsher JH. Physiological responses to fluctuating temperatures are characterized by distinct transcriptional profiles in a solitary bee. J Exp Biol. 2017;220(Pt 18):3372-80. doi:10.1242/jeb.156695
21. Visser B, Williams CM, Hahn DA, Short CA, López-Martínez G. Hormetic benefits of prior anoxia exposure in buffering anoxia stress in a soil-pupating insect. J Exp Biol. 2018;221(Pt 6). doi:10.1242/jeb.167825
22. Taylor JR, Wood JG, Mizerak E, Hinthorn S, Liu J, Finn M, et al. Sirt6 regulates lifespan in Drosophila melanogaster. Proc Natl Acad Sci U S A. 2022;119(5). doi:10.1073/pnas.2111176119
23. Kosakamoto H, Obata F, Kuraishi J, Aikawa H, Okada R, Johnstone JN, et al. Early-adult methionine restriction reduces methionine sulfoxide and extends lifespan in Drosophila. Nat Commun. 2023;14(1):7832. doi:10.1038/s41467-023-43550-2

24. Camus MF, Rodriguez E, Kotiadis V, Carter H, Lane N. Redox stress shortens lifespan through suppression of respiratory complex I in flies with mitonuclear incompatibilities. Exp Gerontol. 2023;175:112158. doi:10.1016/j.exger.2023.112158
25. Margotta JW, Roberts SP, Elekonich MM. Effects of flight activity and age on oxidative damage in the honey bee, Apis mellifera. J Exp Biol. 2018;221(Pt 14). doi:10.1242/jeb.183228
26. Pekny JE, Smith PB, Marden JH. Enzyme polymorphism, oxygen and injury: A lipidomic analysis of flight-induced oxidative damage in a succinate dehydrogenase d (Sdhd)-polymorphic insect. J Exp Biol. 2018;221(Pt 6). doi:10.1242/jeb.171009
27. Majoe M, Libbrecht R, Foitzik S, Nehring V. Queen loss increases worker survival in leaf-cutting ants under paraquat-induced oxidative stress. Philos Trans R Soc Lond B Biol Sci. 2021;376(1823):20190735. doi:10.1098/rstb.2019.0735
28. Negroni MA, Macit MN, Stoldt M, Feldmeyer B, Foitzik S. Molecular regulation of lifespan extension in fertile ant workers. Philos Trans R Soc Lond B Biol Sci. 2021;376(1823):20190736. doi:10.1098/rstb.2019.0736
29. Cardoso-Jaime V, Broderick NA, Maya-Maldonado K. Metal ions in insect reproduction: A crosstalk between reproductive physiology and immunity. Curr Opin Insect Sci. 2022;52:100924. doi:10.1016/j.cois.2022.100924
30. Zanco B, Rapley L, Johnstone JN, Dedman A, Mirth CK, Sgrò CM, et al. Drosophila melanogaster females prioritise dietary sterols for producing viable eggs. J Insect Physiol. 2023;144:104472. doi:10.1016/j.jinsphys.2022.104472
31. Janssens L, Stoks R. Rapid larval development under time stress reduces adult life span through increasing oxidative damage. Funct Ecol. 2018;32(4):1036-45. doi:10.1111/1365-2435.13068
32. Martínez-Lendech N, Golab MJ, Osorio-Beristain M, Contreras-Garduño J. Sexual signals reveal males' oxidative stress defences: Testing this hypothesis in an invertebrate. Funct Ecol. 2018;32(4):937-47. doi:10.1111/1365-2435.13051
33. Yang XJ, Zhao ZS, Zhang YM, Ying JP, Wang SH, Yuan ML, et al. A method for isolating highly purified and active mitochondria from insects. J Insect Physiol. 2022;140:104402. doi:10.1016/j.jinsphys.2022.104402