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Cryptobiosis in Arthropods: Mechanisms of Extreme Desiccation Tolerance and Anhydrobiosis

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Cryptobiosis represents an extraordinary biological strategy in which organisms enter a reversible state of extremely low metabolic activity and survive environmental conditions that would otherwise cause irreversible cellular damage. Among its different forms, anhydrobiosis is associated with severe or near-complete dehydration and is particularly remarkable because biological activity can resume following rehydration. Within Arthropoda, experimentally demonstrated anhydrobiosis is best characterized in the chironomid midges *Polypedilum vanderplanki* and *Polypedilum pembai*, while the encysted embryos of the crustacean *Artemia franciscana* provide an additional and extensively studied model of extreme dehydration tolerance. Research in these systems has revealed a coordinated protective network involving trehalose accumulation, glycerol and other compatible solutes, vitrification, late embryogenesis abundant (LEA) proteins, small heat-shock proteins, antioxidant responses, aquaporins, molecular chaperones, DNA repair and extensive transcriptional reprogramming. In *P. vanderplanki*, dehydration activates trehalose biosynthesis while suppressing trehalose degradation, and water movement during dehydration is regulated by aquaporins. Comparative genomics has further identified expanded gene families and lineage-specific gene clusters associated with anhydrobiosis. In *A. franciscana*, functional studies demonstrate important roles for LEA proteins, Hsp70, Hsp90, Hsf1 and trehalose metabolism in stress tolerance. Recent work has provided direct evidence that trehalose biosynthesis and transport are essential for anhydrobiotic survival in *A. franciscana*, while studies in *P. vanderplanki* cell lines have identified mechanisms governing organelle protection and trehalose removal during rehydration. These findings demonstrate that anhydrobiosis is not a passive consequence of water loss but a highly organized physiological and molecular state. Understanding these mechanisms has implications for arthropod ecology, climate resilience, biological preservation, aquaculture, biotechnology and the development of novel strategies for managing desiccation-tolerant pests.

Keywords: cryptobiosis; anhydrobiosis; arthropods; desiccation tolerance; *Polypedilum vanderplanki*; *Artemia franciscana*; trehalose; LEA proteins; vitrification; molecular chaperones

Introduction

Water is fundamental to cellular organization. It provides the medium for biochemical reactions, facilitates molecular diffusion, supports protein conformation and contributes to membrane organization. Severe water loss therefore represents one of the most damaging environmental stresses that living organisms can experience. Nevertheless, a limited number of organisms have evolved the ability to survive almost complete dehydration and later

resume normal biological activity following rehydration. This phenomenon is broadly associated with cryptobiosis, while the specific condition induced by extreme dehydration is termed anhydrobiosis.The discovery of anhydrobiosis in arthropods is historically associated with the sleeping chironomid *Polypedilum vanderplanki*. Hinton's classic 1960 study demonstrated that larvae inhabiting temporary rock pools in Africa could survive profound dehydration and subsequently resume activity following rehydration. The larvae were reported to tolerate repeated dehydration, with moisture contents falling to below 8% under experimental conditions and to below 3% under extremely dry conditions. This observation established *P. vanderplanki* as one of the most remarkable models of animal desiccation tolerance. Subsequent molecular studies demonstrated that the phenomenon is much more complex than simple dehydration resistance. Slow drying activates a coordinated physiological programme involving accumulation of trehalose, induction of LEA proteins and stress proteins, regulation of water transport and changes in antioxidant systems. Trehalose can account for approximately one-fifth of the dry body mass in desiccating *P. vanderplanki* larvae, and experimental studies demonstrated that the dehydrated larvae form a stable glassy state.

The discovery of *Polypedilum pembai* provided important comparative evidence. Cornette and colleagues demonstrated that larvae of this closely related Malawian species could also survive complete desiccation and recover after rehydration. Their phylogenetic analysis suggested that anhydrobiosis may have originated in a common ancestor of *P. pembai* and *P. vanderplanki*, approximately 49 million years ago. A second major arthropod model is the brine shrimp *Artemia franciscana*. Under unfavorable conditions, females can produce encysted embryos that enter diapause and possess exceptional tolerance to desiccation, freezing, anoxia and other stresses. These cysts have become a major experimental system for investigating molecular chaperones, LEA proteins, trehalose and stress-responsive regulatory mechanisms. Importantly, cryptobiosis should not be confused with ordinary dormancy or quiescence. Anhydrobiosis involves profound dehydration and a major reduction or cessation of metabolic activity. The transition is nevertheless biologically active: protective compounds accumulate before drying, stress-response genes are regulated, membranes and proteins are stabilized, and recovery mechanisms are activated during rehydration. The objective of this review is to examine the mechanisms that allow arthropods to tolerate extreme dehydration, emphasizing the experimentally demonstrated molecular processes in *P. vanderplanki*, *P. pembai* and *A. franciscana*. Particular attention is given to water loss, trehalose metabolism, vitrification, LEA proteins, molecular chaperones, antioxidant defense, DNA protection, genomic adaptation, metabolic regulation and recovery from the dry state.

Arthropod Models of Extreme Desiccation Tolerance

The sleeping chironomid *Polypedilum vanderplanki:* *Polypedilum vanderplanki* is a non-biting midge associated with temporary rock pools in semi-arid regions of Africa. Its larvae occupy shallow pools that repeatedly fill during rainfall and dry during drought. The larval stage therefore experiences recurrent dehydration and rehydration, creating strong selection for desiccation tolerance. Hinton's original observations established the remarkable ecological relationship between the larva and its temporary habitat. When pools dry, larvae become dehydrated and inactive; following rainfall, they can resume feeding within a relatively short period. Modern experimental studies have demonstrated that anhydrobiosis in this species is strongly dependent on the rate and conditions of dehydration. Slow dehydration permits the accumulation of protective molecules and the activation of cellular stress responses. Rapid dehydration, in contrast, can prevent sufficient preparation and reduce survival.

Polypedilum pembai: The discovery of *P. pembai* expanded the known arthropod diversity capable of anhydrobiosis. Cornette et al., (2017) demonstrated that larvae could reach water contents of approximately 4% and subsequently recover normal activity after rehydration. Survival remained high even after several weeks in the dry state. The close phylogenetic

relationship between *P. pembai* and *P. vanderplanki* is important because it provides a comparative system for determining which mechanisms are conserved and which are associated with ecological differences. Later population-genomic research showed that the two species experience different patterns of desiccation pressure and possess differences in genes involved in protection and repair.

Artemia franciscana: The brine shrimp *A. franciscana* represents an important crustacean model of arthropod cryptobiosis. Under favorable environmental conditions, females can produce swimming nauplii. Under unfavorable conditions, they produce encysted embryos that enter diapause. These cysts possess exceptionally high stress tolerance and can withstand prolonged periods of dehydration and freezing. Unlike *Polypedilum*, where anhydrobiosis occurs during the larval stage, *Artemia* displays its strongest cryptobiotic properties during the encysted embryonic stage. This difference demonstrates that extreme desiccation tolerance can evolve in association with different developmental stages.

Cellular Challenges Imposed by Extreme Dehydration

Extreme dehydration affects virtually every component of the cell. The removal of water reduces molecular mobility, changes macromolecular interactions and alters membrane structure. Proteins can unfold or aggregate, lipid bilayers can undergo phase transitions and nucleic acids can experience structural damage. One of the most important consequences is the loss of the aqueous environment surrounding proteins. Protein structure depends partly on interactions with water, and severe dehydration can promote aggregation. LEA proteins and other highly hydrophilic proteins are thought to reduce this problem by interacting with vulnerable macromolecules and helping maintain them in a non-aggregated state. Membranes present another major challenge. During dehydration, phospholipid bilayers can undergo phase transitions that increase leakage and compromise membrane-associated proteins. Trehalose can interact with membrane components and contribute to the preservation of membrane organization. DNA can also be damaged. Remarkably, experiments in *P. vanderplanki* showed that dehydration causes substantial nuclear DNA fragmentation and chromatin alterations even though larvae survive and later recover. This indicates that survival does not necessarily mean complete prevention of damage; rather, successful anhydrobiosis can involve tolerance of damage followed by effective repair during recovery.

Water Transport and Controlled Dehydration

The first challenge in anhydrobiosis is controlled removal of water. Aquaporins are membrane proteins that facilitate water transport and therefore can influence the rate at which water leaves cells. (Kikawada et al., 2008) identified two aquaporins in *P. vanderplanki*, designated PvAQP1 and PvAQP2. Their expression patterns differed substantially. PvAQP1 was induced by dehydration and expressed broadly, whereas PvAQP2 was associated with normal water homeostasis in the fat body and was repressed during dehydration. The results suggested that PvAQP1 participates in water removal during induction of anhydrobiosis. This finding is significant because it demonstrates that anhydrobiosis begins before the organism reaches the dry state. Water loss itself is physiologically regulated. The organism actively modifies water transport while simultaneously initiating protective biochemical pathways. Thus, successful anhydrobiosis involves at least two coordinated processes: controlled water removal and simultaneous protection of cellular components against the consequences of dehydration.

Trehalose: Central Metabolic Protection

Trehalose is one of the most important small molecules associated with anhydrobiosis in arthropods. It is a non-reducing disaccharide composed of two glucose molecules and can function both as a metabolic reserve and as a protectant. In *P. vanderplanki*, slow dehydration causes a dramatic increase in trehalose. Sakurai et al., (2008) demonstrated that the accumulated sugar contributes to the formation of a glassy intracellular state and that vitrification is essential for successful anhydrobiosis. When the dry larvae were shifted from

the glassy to rubbery state, survival after rehydration declined substantially. Trehalose can stabilize biological structures through several mechanisms. It can form hydrogen-bonding interactions with proteins and membrane components, partially replacing interactions normally supplied by water. During severe drying, concentrated trehalose can also form an amorphous glass that greatly restricts molecular mobility. The importance of trehalose is supported by direct biochemical studies. Mitsumasu et al. (2010) demonstrated that dehydration-induced trehalose accumulation in *P. vanderplanki* results from increased activity of the trehalose biosynthetic pathway and reduced trehalose hydrolysis. Trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase contribute to synthesis, whereas reduced trehalase activity limits degradation. Recent research has provided even stronger functional evidence in *A. franciscana*.

Vitrification and the Glassy State

Vitrification represents a critical physical mechanism underlying anhydrobiosis. As water is removed and protective solutes become concentrated, the intracellular solution can transition into a highly viscous amorphous glass. In this state, molecular movement becomes extremely restricted. Sakurai et al. (2008) demonstrated that *P. vanderplanki* larvae form such a glassy state during anhydrobiosis. The maintenance of the glass is essential because warming or humidification that shifts the material into a rubbery state greatly reduces recovery. The importance of vitrification extends beyond trehalose concentration. Protective proteins can interact with sugars and cellular structures, creating a complex biophysical environment that minimizes molecular movement. Thus, anhydrobiosis can be understood partly as a transition from a water-dominated cellular environment to a highly stabilized glass-like state. *Artemia* cysts similarly exhibit properties consistent with biological glass formation. Experimental comparisons among cyst populations have demonstrated variation in glass transition properties and concentrations of trehalose and stress proteins, indicating that the physical properties of the dry state can contribute to differences in desiccation tolerance.

LEA Proteins and Intrinsically Disordered Protection

Late embryogenesis abundant proteins represent another major component of arthropod anhydrobiosis. LEA proteins are generally highly hydrophilic and often intrinsically disordered in their hydrated state. During dehydration, they can adopt different conformations and interact with proteins, membranes and other cellular components. In *P. vanderplanki*, transcriptomic analyses identified numerous LEA-related genes that become strongly induced during dehydration. Cornette et al., (2017) identified 44 clusters with similarity to LEA proteins, and characterized LEA proteins whose expression increased during desiccation. Subsequent genomic studies revealed that *P. vanderplanki* contains expanded clusters of genes associated with anhydrobiosis. These include multiple copies of protective genes and lineage-specific paralogues whose transcripts constitute a major portion of the mRNA pool during dehydration. The importance of LEA proteins is not restricted to insects. In *A. franciscana*, Toxopeus et al., (2014) used RNA interference to reduce group 1 LEA proteins. The resulting embryos showed significantly reduced survival after desiccation and freezing, providing direct in vivo evidence that LEA proteins contribute to protection during low-water conditions. This functional evidence is particularly important because it moves beyond correlations between LEA expression and dehydration tolerance. It demonstrates that at least some LEA proteins are necessary for optimal survival.

Table 1. Experimentally demonstrated molecular mechanisms of arthropod anhydrobiosis

Mechanism	Arthropod model	Experimental evidence	Major function
Controlled water transport	*P. vanderplanki*	Dehydration-induced aquaporin expression	Regulates water loss
Trehalose accumulation	*P. vanderplanki*, *A. franciscana*	Biochemical analysis and gene knockdown	Stabilizes macromolecules and supports vitrification
Trehalose biosynthesis	*P. vanderplanki*, *A. franciscana*	TPS/TPP expression and RNAi studies	Builds protective sugar reserves

Mechanism	Arthropod model	Experimental evidence	Major function
Vitrification	*P. vanderplanki*	FTIR/DSC and recovery experiments	Restricts molecular mobility
LEA proteins	*P. vanderplanki*, *A. franciscana*	Expression analysis and RNAi	Protein/membrane stabilization
Small heat-shock proteins	*A. franciscana*	Functional and expression studies	Molecular chaperoning
Hsp70	*A. franciscana*	RNAi knockdown	Stress tolerance
Hsp90	*A. franciscana*	RNAi knockdown	Desiccation and low-temperature tolerance
Antioxidant systems	*P. vanderplanki*	Transcriptomic/EST analysis	Protection against oxidative damage
DNA repair	*P. vanderplanki*	DNA damage and recovery studies	Genome restoration
Specialized gene clusters	*P. vanderplanki*	Comparative genome sequencing	Molecular adaptation
Trehalose efflux during recovery	Pv11 cells	Gene knockout	Controls rehydration-associated osmotic stress

Molecular Chaperones and Heat-Shock Responses

Dehydration can destabilize proteins in ways similar to thermal stress. Molecular chaperones therefore form an important component of anhydrobiotic protection. In *A. franciscana*, p26 is a diapause-specific small heat-shock/α-crystallin protein. Liang and MacRae demonstrated that p26 is synthesized specifically during the developmental pathway leading to encystment and is associated with increased stress tolerance. Experimental work has also demonstrated that p26 can contribute to cellular protection when combined with trehalose. Mammalian cells engineered to express Artemia p26 and supplied with trehalose showed substantially improved survival following drying, demonstrating the potential synergistic relationship between protective sugars and stress proteins. Hsp70 provides another example of a stress-response protein with a functional role in crustacean anhydrobiosis. RNAi-mediated reduction of Hsp70 in *A. franciscana* cysts reduced survival after desiccation and freezing, while cyst development itself was not substantially disrupted. This distinction suggests that Hsp70 is particularly important for stress tolerance rather than for formation of the cyst itself. Hsp90 has also been shown to contribute to stress tolerance. Fatani et al., (2024) found that reducing ArHsp90 decreased tolerance of *A. franciscana* cysts to desiccation and low temperature. These findings demonstrate that arthropod anhydrobiosis depends on multiple classes of molecular chaperones rather than a single protective protein.

Heat-Shock Factor and Transcriptional Regulation

Protective proteins must be synthesized before or during entry into the dry state. Transcriptional regulation is therefore essential. In *A. franciscana*, Tan and MacRae demonstrated that heat-shock factor 1 (Hsf1) is required for stress tolerance during diapause. Manipulation of Hsf1 affected the synthesis of diapause-associated molecular chaperones and reduced stress tolerance. Studies of *P. vanderplanki* have similarly shown that dehydration activates a broad transcriptional programme involving antioxidant proteins, heat-shock proteins, LEA proteins, trehalose metabolism and transporters. The EST analysis of Cornette et al., (2017) identified 15,056 ESTs representing 4,807 UniGene clusters and demonstrated coordinated induction of multiple protective systems. Comparative transcriptomics of the Pv11 cell line subsequently demonstrated that trehalose treatment and dehydration alter expression of genes associated with stress responses, detoxification and redox regulation. These observations support a model in which anhydrobiosis is initiated through a large-scale transcriptional reprogramming rather than through the action of a few isolated genes.

Oxidative Stress and Antioxidant Protection

Although metabolism becomes strongly suppressed during anhydrobiosis, oxidative damage remains a significant concern. Dehydration can alter cellular redox balance, while rehydration can rapidly restore oxygen consumption and mitochondrial activity. The early EST study of *P. vanderplanki* identified multiple antioxidant-related genes among transcripts induced during dehydration. These findings suggested that oxidative stress is an important component of dehydration injury and that antioxidant responses are integral to survival. DNA damage studies provide additional evidence. Gusev et al., (2014) found that dehydration produced severe nuclear DNA fragmentation in *P. vanderplanki* larvae, yet the larvae remained viable and subsequently recovered. This suggests that successful anhydrobiosis involves both damage limitation and repair. The combination of antioxidant defense, protective proteins and DNA repair therefore allows arthropods to tolerate molecular damage that would otherwise compromise survival.

Genome-Level Adaptation to Anhydrobiosis

The genome of *P. vanderplanki* provides evidence that anhydrobiosis has a distinctive genetic basis. Gusev et al., (2014) compared the genome of *P. vanderplanki* with that of the closely related desiccation-sensitive *P. nubifer*. They identified clusters of duplicated genes encoding proteins with potential protective functions. Many of these paralogues were strongly expressed during dehydration. The significance of these expanded gene clusters is that anhydrobiosis may depend not simply on modification of universal stress-response pathways but also on lineage-specific molecular innovations. A particularly interesting example is the discovery of LIL proteins in *P. vanderplanki*. These proteins possess multiple transmembrane domains and are associated with gene clusters linked to anhydrobiosis. Their strong induction during dehydration suggests that membrane-associated mechanisms may represent an additional layer of protection beyond classical LEA proteins and trehalose. Population-genomic research comparing *P. vanderplanki* and *P. pembai* has further demonstrated differences in adaptation to extreme desiccation. In *P. pembai*, additional copies of protein L-isoaspartyl methyltransferase (PIMT) genes showed evidence of positive selection, consistent with ecological differences in the frequency of dehydration–rehydration cycles.

Cellular Reorganization and Metabolic Depression

Anhydrobiosis requires profound metabolic restructuring. In the dry state, conventional metabolism cannot continue because water is insufficient to support normal biochemical reactions. Yet the cell must retain the ability to recover. Research using the Pv11 cell line has provided direct insight into this process. Belott et al., (2024) demonstrated that preconditioning before dehydration induced autophagy and reduced mitochondrial respiration by more than 70%. Mitochondrial membrane potential was lost during dehydration but rapidly restored following rehydration, indicating that mitochondrial structures can remain sufficiently protected for recovery. This finding supports the concept of metabolic preparation. Before dehydration, the cell does not simply shut down; instead, it reorganizes energy metabolism and cellular structures in anticipation of a period of extremely low activity. The ability to preserve organelles is equally important. Membrane-bound and membraneless cellular compartments must survive dehydration-induced changes in molecular crowding and physical state. Recent observations in Pv11 cells indicate that some organelles and condensate-like structures remain protected or can rapidly reform following rehydration.

Rehydration: The Other Half of Anhydrobiosis

Successful anhydrobiosis cannot be evaluated solely by survival in the dry state. The ultimate test is whether normal biological function can be restored after water returns. Rehydration presents its own risks. Rapid water entry can create osmotic stress, alter membrane properties and restore metabolic activity faster than protective systems can respond. Therefore, controlled removal of protective molecules may be essential. Recent research in Pv11 cells

identified STRT1, a sodium-dependent trehalose transporter, as an important component of recovery. Knockout of STRT1 reduced survival after rehydration, and the transporter appears to facilitate trehalose export during recovery. This suggests that trehalose is protective during dehydration but must subsequently be removed efficiently to prevent osmotic disturbances. This discovery changes the conceptual model of anhydrobiosis. Protection is not simply about accumulating a large amount of a protective compound. The organism must also control its distribution, intracellular concentration and removal during recovery.

Table 2. Integrated sequence of arthropod anhydrobiosis and recovery

Stage	Major physiological event	Molecular mechanisms	Functional outcome
Environmental drying	Declining water availability	Stress sensing and transcriptional activation	Initiation of preparation
Early dehydration	Controlled water loss	Aquaporins, osmotic regulation	Progressive dehydration
Protective preparation	Accumulation of solutes and proteins	Trehalose synthesis, LEA proteins, Hsps	Cellular stabilization
Advanced dehydration	Metabolic depression	Reduced respiration, energy conservation	Lower metabolic demand
Dry state	Near-complete water loss	Vitrification, molecular shielding	Long-term survival
Damage management	DNA/protein protection	Antioxidants, chaperones, repair systems	Maintenance of cellular integrity
Rehydration	Rapid restoration of water	Water uptake and osmotic regulation	Restoration of cellular hydration
Protective-molecule removal	Declining trehalose requirement	Trehalose transporters	Prevention of osmotic injury
Metabolic recovery	Mitochondrial reactivation	Restoration of respiration	Return of energy production
Developmental recovery	Resumption of growth/activity	Gene-expression reprogramming	Return to active life

Ecological Significance of Extreme Desiccation Tolerance

Anhydrobiosis has profound ecological value in environments where water availability is unpredictable. In *P. vanderplanki* and *P. pembai*, temporary aquatic habitats repeatedly disappear during dry periods. Anhydrobiosis allows larvae to remain viable until rainfall restores the habitat. The repeated nature of dehydration–rehydration cycles may have shaped the molecular architecture of these species. The comparative population-genomic study of *P. vanderplanki* and *P. pembai* is particularly important because it indicates that differences in ecological exposure can be associated with differences in the evolution of protective genes. For *A. franciscana*, cryptobiotic cysts allow embryos to persist under hypersaline and dry conditions. When suitable conditions return, cysts can resume development and hatch. This strategy contributes to the persistence of populations in temporary saline habitats. Extreme desiccation tolerance may also influence geographical distribution. Species able to withstand prolonged dry periods can persist in environments that impose severe water stress on other organisms. However, tolerance of dehydration does not necessarily imply unlimited ecological advantage. Survival must ultimately be balanced against developmental timing, reproduction, food availability and interactions with other organisms.

Implications for Agriculture and Pest Biology

Desiccation tolerance is particularly relevant to agricultural entomology because insects frequently encounter drought, low humidity, high temperature and periods without host plants. Although complete anhydrobiosis is rare among crop pests, partial mechanisms associated with dehydration tolerance may influence seasonal survival. The molecular pathways identified in anhydrobiotic arthropods provide useful conceptual models for

understanding how pests survive dry periods. Trehalose accumulation, aquaporin regulation, antioxidant defense and stress proteins are all relevant to insect water balance and environmental stress responses. From a pest-management perspective, identifying developmental stages with high desiccation tolerance could improve predictions of pest carryover between seasons. Similarly, understanding stress-protective pathways may help identify physiological weaknesses that could be exploited in integrated pest management. However, it would be inappropriate to assume that mechanisms identified in *P. vanderplanki* or *A. franciscana* automatically apply to agricultural pests. Complete anhydrobiosis is unusual within Arthropoda, and most insects possess only partial desiccation tolerance. Therefore, translation into pest management requires species-specific experimental validation.

Biotechnology and Biological Preservation

The molecular mechanisms of anhydrobiosis have important applications in biotechnology. Trehalose-mediated stabilization, protein-based molecular shielding and controlled vitrification provide models for preserving biological materials in a dry state. One of the most striking demonstrations comes from the Pv11 cell line derived from *P. vanderplanki*. Watanabe et al., (2016) demonstrated that Pv11 cells could be air-dried to approximately 6% residual moisture, stored at room temperature for up to 251 days and subsequently resume proliferation after rehydration. This finding is important because it demonstrates that principles of arthropod anhydrobiosis can be transferred from whole organisms to cultured animal cells. The system may eventually contribute to dry preservation of biological materials, although the current approach remains highly specialized. The success of Pv11 cells depends on species-specific preconditioning and cannot simply be transferred to conventional insect or mammalian cell lines. Recent genetic studies have further strengthened the potential of Pv11 as a biotechnology platform. Genome editing has been used to investigate calcium signaling and other regulatory pathways involved in anhydrobiosis, providing experimental access to mechanisms that were previously difficult to manipulate.

Future Research Directions

Future research should focus on identifying the minimal molecular requirements for anhydrobiosis. Although trehalose, LEA proteins and molecular chaperones are clearly important, experimental evidence indicates that no single component is sufficient. The ability of *P. vanderplanki* cells to survive prolonged drying requires coordinated regulation of metabolism, membrane organization, protein stabilization and recovery. Comparative genomics offers a particularly promising approach. Comparing closely related anhydrobiotic and non-anhydrobiotic species can reveal genes specifically associated with the evolution of extreme desiccation tolerance. The *P. vanderplanki–P. nubifer* and *P. vanderplanki–P. pembai* comparisons provide useful examples. Functional genomics should increasingly replace purely correlative studies. RNA interference, CRISPR/Cas9 and gene-expression analysis can determine whether candidate genes are actually required for survival. The recent discovery of the role of trehalose transport in Pv11 recovery illustrates the value of this approach. Another important area is the physics of the dry state. Understanding how sugars, proteins, membranes and organelles interact during vitrification could identify general principles applicable across biological systems. Repeated dehydration–rehydration cycles also deserve greater attention. Most mechanistic studies examine controlled laboratory dehydration, whereas natural populations may experience numerous cycles. Long-term studies should therefore investigate cumulative DNA damage, protein repair, mitochondrial stability and reproductive consequences. Finally, research should connect molecular tolerance with ecological performance. The ability to survive a dry state is only one component of fitness. Future studies should determine how desiccation tolerance influences development, fecundity, dispersal and population persistence under realistic environmental conditions.

Conclusion

Cryptobiosis and anhydrobiosis represent some of the most remarkable survival strategies known in arthropods. Experimental studies of *Polypedilum vanderplanki*, *P. pembai* and *Artemia franciscana* demonstrate that extreme dehydration tolerance depends on coordinated regulation of water transport, metabolism, protective solutes, proteins, membranes, DNA and recovery processes. Trehalose is a central component of this system, but it does not act alone. In *P. vanderplanki*, trehalose accumulation contributes to vitrification, while LEA proteins and other stress proteins stabilize cellular structures. Aquaporins regulate water movement, antioxidants mitigate oxidative damage and DNA repair mechanisms restore genomic integrity. In *A. franciscana*, LEA proteins, p26, Hsp70, Hsp90 and Hsf1 have experimentally demonstrated roles in stress tolerance. Most importantly, recent RNAi experiments provide direct evidence that trehalose biosynthesis and transport are essential for the anhydrobiotic survival of *A. franciscana*. The latest studies also show that recovery is an active process. Trehalose must be removed after rehydration, mitochondria must regain function and cellular structures must be reorganized. The discovery of STRT1 in Pv11 cells demonstrates that the molecular machinery required for recovery is as important as the machinery responsible for entering the dry state. Thus, anhydrobiosis should be viewed as a complete physiological programme consisting of preparation, controlled dehydration, molecular stabilization, metabolic depression, long-term maintenance and regulated recovery. Continued integration of comparative genomics, molecular genetics, cell biology, biophysics and ecological studies will provide a deeper understanding of how arthropods survive at the limits of cellular hydration and may reveal new principles for biological preservation, environmental adaptation and sustainable management of stress-tolerant arthropod populations.

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