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ORIGINAL PAPER

Cross-tolerance and transcriptional shifts underlying abiotic stress in the seabird tick, *Ixodes uriae*

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Abstract
The seabird tick, *Ixodes uriae*, is a significant ectoparasite of penguins in Antarctica and of other seabirds, mainly in coastal, polar regions of the Northern and Southern hemispheres, but the tick's distribution extends into more temperate regions as well. The expansive range of this tick suggests that it is exposed to a wide range of abiotic stresses, including dehydration, heat, and cold. To better understand how *I. uriae* responds to stress exposure, we examined cross-tolerance between dehydration and thermal stress based on survival analyses and used RNA-seq to monitor transcriptional responses to cold, heat, and dehydration. Slight dehydration improved cold, but not heat tolerance, whereas severe dehydration reduced subsequent thermal tolerance. Dehydration exposure prompted transcript-level shifts underlying protein metabolism, recovery from stress, and processes allowing subsequent rehydration by water vapor uptake. Both cold and heat stress yielded expression changes involved in cuticle modification. One gene increased in expression (enzyme P450) and one decreased (transcription factor Hairy) in response to all three stresses. This study provides the groundwork for assessing stress tolerance in this bipolar ectoparasite.

Keywords Cold · Heat · Dehydration · RNA-seq · Cross-tolerance · Antarctic · Tick

Introduction

During prolonged off-host periods, ticks are vulnerable to dehydration due to their small body size and high surface area to volume ratio (Benoit and Denlinger 2010). To avert dehydration, ticks limit host seeking activity to periods of high relative humidity and retreat to sheltered microhabitats at other times (Short et al. 1989; Crooks and Randolph 2006). Cuticular modifications, decreases in respiratory water loss, and reduction of losses through excretion help minimize water loss (Benoit and Denlinger 2010; Rosendale et al. 2016b). Absorption of water vapor is used as a primary mechanism for hydration during off-host periods (Rudolph and Knülle 1974; Needham and Teel 1991; Sonenshine and Roe 2013), but it is energetically expensive and lowers off-host survival (Randolph and Storey 1999; Rosendale et al. 2017). This unique mechanism of hydration by ticks, along with other physiological aspects, makes the ecophysiology of ticks distinct from most other arthropods (Ogden and Lindsay 2016). Dehydration-related damage can be reduced by altering expression of proteins such as heat shock proteins, antioxidant enzymes, and aquaporins (Benoit 2010; Benoit and Denlinger 2010; Holmes and Benoit 2019), all of which are increased in ticks during dehydration (Rosendale et al. 2016b). In addition, molecules such as glycerol, trehalose, and other specific metabolites exhibit protective effects during dehydration by keeping cellular components from harm and act as energy sources to repair damage (Yoder et al. 2006; Michaud et al. 2008; Benoit et al. 2009a; Hagan et al. 2018). To prevent water loss, this hydrophilic species forms clusters under rocks to decrease individual water loss

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rates and resides in moist microhabitats with moisture conditions higher than the tick's critical equilibrium activity (CEA, the lowest amount of ambient moisture required for water vapor absorption to occur; Benoit et al. 2007).

The seabird tick, *Ixodes uriae*, is the prominent tick on the Antarctic continent, with a circumpolar distribution in both the northern and southern hemispheres (Wilson 1970; Barbosa et al. 2011; Muñoz-Leal and González-Acuña 2015). This tick likely originated and colonized areas in the southern hemisphere before introduction into the northern hemisphere (Dietrich et al. 2014). *I. uriae*, a three-host tick, completes its life cycle in four to five years (Hoogstraal and Mielcarek 1954; Eveleigh and Threlfall 1975); however, it can complete development in two years on the Macquarie Island, where royal penguins remain on their rookeries for six months (Murray and Vestjens 1967). At the onset of the bird breeding season, *I. uriae* in Antarctica, specifically those near Palmer Station, relies on blood of birds such as the Adelie penguin, *Pygoscelis adeliae*, for growth and reproduction. Due to the short Austral summer, where host are only present for short periods and warm periods are limited, *I. uriae* likely only feeding once (or not at all) each year as temperatures are not high enough to feed and molt while hosts are still available. This tick spends the remainder of the year, approximately eleven months (or more) outside of the short periods questing and feeding, living in aggregations underneath rocks close to the rookeries (Eveleigh and Threlfall 1975). Depending on the locality, this tick will parasitize a variety of seabird species with host-specialization yielding sympatric host races (McCoy et al. 2001). This tick has a proclivity for moisture compared to other more dry-adapted tick species (Benoit et al. 2007), and the absence of major water conservation features (e.g., reduced water loss rates) suggests Antarctica and other cool, wet regions are suitable habitats for *I. uriae* to regulate water loss and gain.

Thermal tolerance of seabird ticks is understudied (Lee and Baust 1987). The temperature tolerance for this tick ranges from −30 °C to 50 °C, one of the largest ranges for any terrestrial Antarctic arthropod (Lee and Baust 1987). Chill coma, a reversible comatose state driven by altered ion homeostasis and a failure of neuromuscular function (MacMillan et al. 2012; Findsen et al. 2013), occurs between −5 °C and −1.5 °C for most ticks including *I. uriae* (Lee and Baust 1987; Benoit et al. 2021). Extensive studies on cold tolerance conducted in other tick species identified mechanisms of cold acclimation and hardening along with gene expression changes during cold exposure (Yu et al. 2014, 2017, 2020; Rosendale et al. 2016a, 2021; Wang et al. 2017; Holmes et al. 2018). At the high end of the thermal range, ticks can tolerate short periods above 40 °C (Yoder et al. 2009; Holmes et al. 2018; Fieler et al. 2021), a response likely critical for surviving long feeding bouts on a warm-blooded host (Benoit and Denlinger 2017; Benoit et al. 2019). Prolonged exposure to temperatures near 35 °C results in increased tick mortality, but specific molecular mechanisms underlying tick heat tolerance have yet to be studied. Importantly, dehydration generates cross-tolerance to other stresses in ticks and other invertebrates (Benoit et al. 2009a,b; Sinclair et al. 2013; Rosendale et al. 2016a). This cross-tolerance between dehydration and thermal stress is due to a multitude of factors such as increases in specific metabolites, changes in cellular water levels, and altered expression of stress-related proteins (Benoit et al. 2009a; Sinclair et al. 2013). In general, cross-tolerance between cold and dehydration stress is more commonly observed than between dehydration and heat tolerance (Benoit et al. 2009a,b; Sinclair et al. 2013).

Molecular mechanisms involved in dehydration, cold, and heat tolerance have been examined in terrestrial Antarctic arthropods such as the Antarctic midge, *Belgica antarctica* (Michaud et al. 2008; Teets et al. 2012), but despite research focused on dehydration resistance and cold-hardiness in the seabird tick, little is known about the underlying mechanisms related to the tolerance and response of *I. uriae* to environmental stress (cold, heat, and dehydration). In this study, we examine the response of *I. uriae* to various stresses using a transcriptomics approach to identify genes with differential expression following cold, dehydration, and heat exposure, and identified novel and overlapping mechanisms associated with tick stress tolerance. In addition, we examine cross-tolerance between dehydration and thermal stress, both through physiological studies and by RNA-seq studies monitoring responses to cold, heat, and dehydration stress. Based on the expansive distribution of *Ixodes uriae*, the response to stress tolerance of this tick may be more substantial in comparison to those noted in other ticks, allowing survival in stressful polar regions, or other factors could underlie the survival of *I. uriae* in specific regions.

Methods

Ticks and experimental design

Tick collection and maintenance

Fed nymphs used for RNA-seq analyses were collected near two Adelie penguin colonies on Christine Island (64°47'S 64°01'W) near Palmer Station (US) on 28 Jan 2016 and held at 4 °C until sorted 6 d later. Seasonal thermal changes were monitored for this site using a HOBO thermal logger (Fig. 1). Ticks collected for comparative thermal stress experiments were obtained from Christine Island over several days in January 2017 from the same locations as in 2016 and held at 4 °C until sorted 6 d later. In both 2016 and 2017, ticks were collected from under multiple rocks to increase

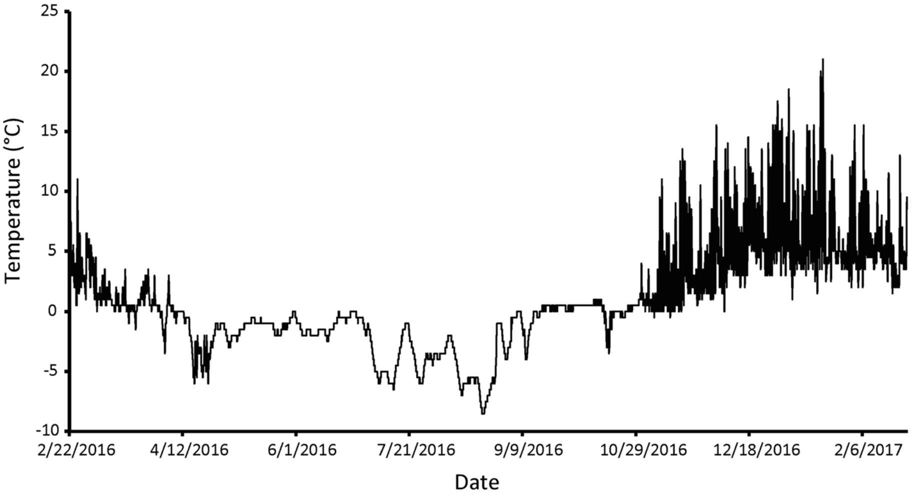

Fig. 1 Thermal conditions of a seabird tick, *Ixodes uriae*, habitat on Christine Island from Feb. 2016 to Feb. 2017 measured with a HOBO data logger. Temperatures range from −8 °C to 20 °C

genetic diversity (McCoy et al. 2003) and obtain general representatives of the local population. During sorting, individuals that appeared healthy and exhibited normal leg movement were transferred to 50 ml Falcon® tubes with damp paper towels and placed in circulating cold baths at 15 °C for three weeks. The incubation temperature was increased to 20 °C after three weeks to stimulate molting. Females were separated from males after molting and maintained in a cold bath at 10 °C for 12–14 d. Females were used for all subsequent studies. As adults, only females will utilize a bloodmeal and males do not, suggesting that future studies may need to examine cold tolerance differences between males and females.

Stress exposure and cross-tolerance impacts

Cross-tolerance between dehydration and thermal stress (heat and cold) was examined for *I. uriae*. Briefly, ticks were exposed to 75% RH until they lost 0 (control), 10 (10 days), or 20% (20 days) of their water content. Ticks were then exposed to low (−20 °C and −25 °C) or high (40 °C and 45 °C) temperatures for 2 h. Following thermal stress, ticks were moved to 10 °C, 93% RH, and monitored for survival after 48 h and two weeks. Each specific treatment was replicated four times with 8 ticks per treatment. Analysis of variance followed by a Dunnett's post hoc test was used to examine significance between treatments.

Experimental treatments for RNA-seq analyses

The desiccation protocol required 58–60 h to achieve the desired 20% reduction in water content. Thus, to maintain similar post-molt ages, controls and the other treatments were held at 10 °C for 58–60 h in Falcon® tubes with damp paper towels before beginning experimentation. Controls were flash frozen in liquid nitrogen immediately after the 58–60 h incubation. Ticks in the heat and cold shock treatments were placed in dry Falcon® tubes and held 2 h at 30 °C and −5 °C, respectively, and then allowed to recover 2 h at 10 °C. For the desiccation treatment, ticks were incubated at 10 °C and ~ 4% RH on mesh screens above Drierite® in sealed Falcon® tubes. Weights were recorded every ~ 20 h until reaching 20% reduction of their body water, which was calculated assuming starting water content of 70% fresh mass (Benoit et al., 2007). Pooled initial and final masses were recorded for each treatment; initial masses were determined before stress exposure and final masses were determined after treatment and recovery. Individuals were flash frozen in liquid nitrogen following thermal exposure or final mass measurements and stored at −80 °C until performance of transcriptomic analysis.

Generation of transcriptome and analysis of differential expression

RNA extraction and sequencing

RNA extraction was performed on two samples of ~ 10–12 ticks for each RNA-Seq analysis for a total of 8 sample sets. Ticks were manually cut into quarters, placed in 1 ml of chilled TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and homogenized with a Tissue-Tearor homogenizer (BioSpec Products, Bartlesville, OK, USA). Total RNA extraction was performed using the TRIzol manufacturer's protocol. To eliminate potential genomic contamination, DNase I (Thermo Scientific, Pittsburgh, PA, USA) was used to treat RNA. RNA was then concentrated using GeneJET RNA Cleanup and Concentration Micro Kit (Thermo Scientific), and a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to determine RNA quality and concentration.

Poly(A) library construction and sequencing were carried out at the Cincinnati Children's Hospital Medical Center by the DNA Sequencing and Genotyping Core. A Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) was used for RNA quantification, and 150–500 ng total RNA was poly(A) selected and reverse transcribed with the TruSeq Stranded mRNA Library Preparation Kit (Illumina, San Diego, CA, USA). Each sample was fitted with one of 96 adapters containing a different 8-base molecular barcode for high-level multiplexing. Completed libraries were sequenced on a HiSeq 2500 System (Illumina) in Rapid Mode, following 15 cycles of PCR amplification. Approximately 30 million high-quality, single-end reads of 75 base length were generated per sample. Raw RNA-Seq data were uploaded to the National Center for Biotechnology Information (NCBI) Bioproject PRJNA659796.

De novo assembly

As no genome is currently available, a *de novo* assembly of RNA-seq reads into contigs was necessary and conducted according to those previously developed in tick systems (Rosendale et al. 2016b, 2019). To generate the most complete assembly, transcripts were assembled using Trinity (v2.0, Grabherr et al. 2011), CLC Genomics Workbench (CLC Bio), and Velvet-Oases (Schulz et al. 2012). All three methods were conducted according to default settings. Coding regions for genes were identified using transdecoder v3.0 (Haas et al. 2013). All three assemblies were combined into a single contig library and redundant sequences were removed with CD-HIT-EST using a similarity cut-off of 95% (Huang et al. 2010). This combined assembly had a high number of matches to Benchmarking Universal Single-Copy Orthologs, BUSCOs, (Simão et al. 2015) and was used for further analyses (Table 1).

Contigs were identified by searching against the NCBI arthropod non-redundant (nr) protein database. In addition, contigs were compared to reference protein sequences for all members of Acari and to those from *Ixodes scapularis*. For contigs that were successfully identified, gene ontology (GO) terms (Ashburner et al., 2000) were retrieved using the GO-mapping tool (e-value of $1 \times 10-6$) and InterProScan tool in the Blast2GO program (BioBam; Conesa et al., 2005); GO annotations from these two tools were merged.

RNA-seq analyses

Illumina reads were mapped to our *I. uriae* assembly using CLC Genomics Workbench. At least 70% of each read with at least 90% identity to the reference contig was necessary for a match. Each read was allowed to match 10 different locations among the reference assembly. Expression values were based on the mapped reads to each contig per million total mapped reads (TPM). A Baggerly's test (a binomial distribution statistic, Baggerly et al. 2003) with false discovery rate (FDR, (Benjamini and Hochberg 1995) within CLC Genomics was used to assess significance. Transcripts were considered to have significant differences in expression levels compared to control when the FDR was $P < 0.05$ and fold change was at least ± 1.5. qPCR analyses were conducted according to methods previously developed for ticks (Rosendale et al. 2016a, b) with the use of an Illumina Eco qPCR system. Primers utilized were designed with Primer3 (t.44640 F-TGGGAAATGAATATTTCGAC and R-TTG AACTCCCATAGCTGTTT; o.7640 F-GATCTCAGTCTT CTCGATGC and R-AGACTTCCTGGTGAACCATT). A

Table 1 Summary statistics of *de novo* assembly for *Ixodes uriae*

	Velvet-Oases	Trinity	CLC	Combined
Total number of contigs	71,180	63,288	33,587	52,191
N50	654	687	612	687
Average contig length (bp)	611	625	589	627
Maximum contig length (bp)	8886	10,647	10,014	10,647
Total number of bases	43,479,442	39,550,856	19,784,774	32,731,020
Percentage contigs that aligned to fruit fly, BLASTX (10^-5)	51	47	49	46
Percentage contigs that aligned to *Ixodes*, BLASTX (10^-5)	69	66	68	65
Percentage Arthropod BUSCO genes that aligned to assembly, TBLASTN (10^−5)	95	96	92	97
Percentage *Ixodes* BUSCO genes that aligned to assembly, TBLASTN (10^−5)	95	97	94	97
Percentage *D. melanogaster* CORE genes that aligned to assembly, TBLASTN (10^−5)	68	68	67	69

N50, shortest sequence length at 50% of the transcriptome

[a] Fruit fly, *Drosophila melanogaster*, proteins were from the NCBI's available reference sequences (RefSeq) as of February 2015

[b] Proteins from *Ixodes* genus were from the NCBI's available RefSeq as of February 2015

[c] The arthropod set of Benchmarking sets of Universal Single-Copy Orthologs (BUSCO) were downloaded from OrthoDB (Simão et al. 2015)

[d] Core eukaryotic genes dataset for *D. melanogaster* was acquired from the CEGMA database (Parra et al. 2007)

Pearson correlation analysis was used to examine expression changes between the RNA-seq analysis and qPCR changes for these two genes.

Pathways and products with related functions that shifted during cold, heat, or dehydration stress were identified with several specific GO enrichment tools. Sets of contigs with significant differences in expression relative to control expression were compared against the *I. scapularis* genomic protein set (Gulia-Nuss et al. 2016). Matches to *I. scapularis* were submitted to PANTHER GO Analysis (Mi et al. 2019), DAVID functional annotation database (Huang et al. 2009), and g:Profiler Functional Profiling (Reimand et al. 2016). In addition, Blast2GO enrichment analysis was used to identify GO terms enriched under each stress treatment compared to the complete contig set (Conesa et al. 2005; Götz et al. 2008). For all GO analysis, enrichment was considered following a post hoc test at $P < 0.05$.

Results

Interactions between dehydration and thermal stress

Multiple levels of dehydration were examined to assess the impact on subsequent thermal stress (Fig. 2). A loss of 10% water increased cold tolerance of *I. uriae* at both −20 and −25 °C (Dunnett's, $P < 0.001$), but an additional loss to 20% did not further enhance cold tolerance (Fig. 2). In relation to heat stress, dehydration (10 or 20% water loss) failed to improve tick survival at either 40 or 45 °C. This indicates cross-tolerance to cold induced by dehydration stress, albeit only when water loss is not severe. Importantly, these temperatures were much greater than those within the tick microhabitat at any time of year (Fig. 1), thus ticks are likely exposed to thermal stress only when questing for, or feeding on, hosts.

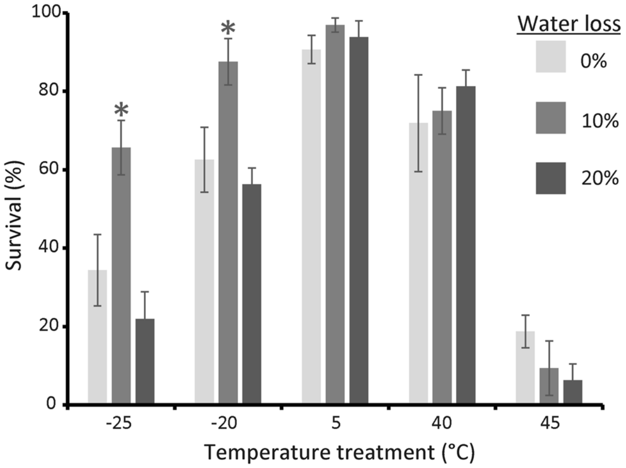

Fig. 2 Moderate dehydration improves cold, but not heat, tolerance of *Ixodes uriae*. Groups of 8 ticks (four groups) were either exposed directly to thermal stress or held at 75% relative humidity until a loss of 10% or 20% water content. Asterisks indicate results for dehydrated females differing from the corresponding control mean (ANOVA, Dunnett's, $p < 0.05$)

Transcriptome assembly and basic comparative analysis

The three *de novo* assemblies from independent programs ranged from 33,587 contigs to 71,180 contigs (Table 1), with a combined assembly of 52,191, following merging and removal of duplicate contigs. BUSCO analyses identified over 92–97% matches to the arthropod BUSCO database for each individual assembly, which was 97% for the final assembly. In addition, 97% of the BUSCO genes from the *I. scapularis* genome matched those in our assembly. Matches to benchmark sequences from *Drosophila* were much lower (Table 1), as also seen for other tick transcriptomes (Rosendale et al. 2016b, 2019; Moreira et al. 2017). Comparison of expression levels (TPM) between each replicate revealed a high correlation between each treatment (Pearson correlation coefficient: control = 0.886, dehydration = 0.999, cold = 0.910, and heat = 0.930). Pearson correlation values between each treatment (e.g., cold vs. heat) were all below 0.7. This indicates high correlation between samples of the same, but not different, stresses, suggesting that only two replicates from multiple ticks accurately represent expression differences.

Transcriptional shifts associated with thermal and dehydration stress

749 contigs with differential expression were identified when ticks were dehydrated (Fig. 3), including multiple genes with increased expression associated with oxidative stress, ion transport, and detoxification (Fig. 3, Online Resource 1–7). Those with decreased expression included multiple DNA and RNA binding factors (Fig. 3, Online Resource 2). Specific GO categories increased during dehydration were involved in the process of amide biosynthetic process and aspects underlying peptide biosynthesis (Table 2). GO categories that decreased during dehydration were associated with nucleotide and protein binding (Table 2, Online Resource 7). Following cold exposure, there were 72 contigs with differential expression (Fig. 3; Online Resource 3 and 4). The GO categories that increased were components of the structure of the cuticle; no GO categories were increased in contigs with reduced expression. Heat exposure yielded significantly different expression for 256 contigs (Fig. 3, Online Resource 5 and 6). As with cold exposure, structural constituents of the cuticle were increased during

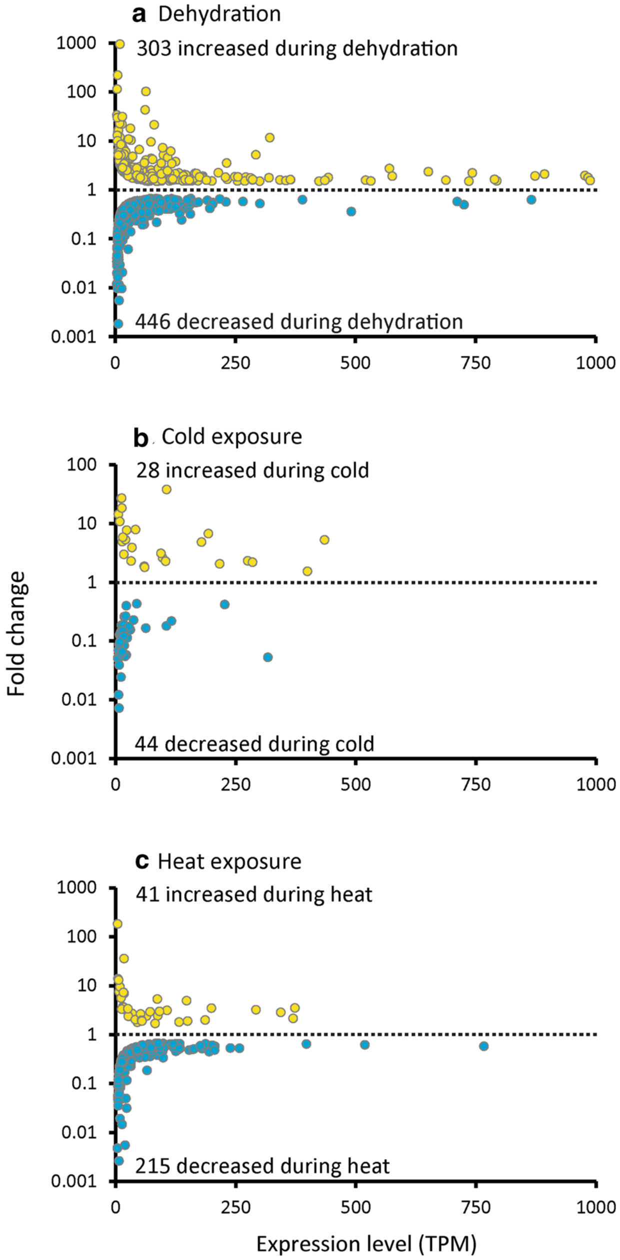

Fig. 3 Fold changes in transcript expression based on RNA-seq analysis. Yellow indicates higher expression following stress exposure and blue is higher expression in controls. a. Dehydration exposure (20% loss of water content), b. Cold exposure (−5 °C for 2 h followed by 2 h recovery at 10 °C), and c. Heat exposure (30 °C for 2 h followed by 2 h recovery at 10 °C). A Baggerly's test (a binomial distribution statistic, (Baggerly et al. 2003)) with false discovery rate was used to denote significance ($p < 0.05$)

heat stress, but no GO categories were enriched in contigs that were reduced during heat exposure.

Overlap in gene expression profiles between the three stress exposures revealed much more similarity between heat and dehydration stress (Fig. 4). Only a single contig had increased transcript levels during exposure to all three stresses, a contig predicted to be a cytochrome P450 (Fig. 4a, c). Similarly, only a single contig was consistently decreased during cold, heat, and dehydration stress, a contig predicted to be hairy-like transcription factor (Fig. 4b, c). qPCR validation of these two genes confirmed that all three stresses increased the transcript levels for the P450 and decreased levels for hairy-like transcription factor (Pearson correlation = 0.894).

Discussion

Cross-tolerances between stresses are observed in many invertebrate systems, even in other tick species (Benoit et al. 2009a; Sinclair et al. 2013; Rosendale et al. 2016a). Here, we showed that dehydration yielded a significant improvement in low temperature tolerance but did not improve tolerance to higher temperatures. A similar response has been observed in other invertebrate systems, most likely since cold and dehydration stress are more similar than dehydration and heat stress in respect to the biochemical and molecular response (Benoit et al. 2009a,b; Sinclair et al. 2013). The lack of increased heat tolerance following dehydration exposure is consistent with results from many other invertebrates. Only invertebrates capable of extreme dehydration, such as midges and tardigrades (tolerate losing over 75% of their water content), have elevated high temperature tolerance in response to dehydration (Benoit et al. 2009a; Hengherr et al. 2009; Sogame and Kikawada 2017). Cross-tolerance occurs in response to a combination of factors that respond similarly to each type of stress, predominantly shifts in gene and protein expression profiles, specific metabolites, and changes in cell components such as membranes (Overgaard et al. 2005, 2007, 2014; Michaud et al. 2008; Marteaux et al. 2017). Specific mechanisms for cross-tolerance between dehydration and cold were not directly assessed, but our RNA-seq studies suggest mechanisms underlying this cross-tolerance. Most likely a combination of changes in expression of stress associated genes and specific biochemical factors (amino acids, betaine, glycerol; Rosendale et al. 2021) underlie the cross-tolerance between cold and dehydration in *I. uriae*.

Dehydration stress yielded substantial transcriptional changes, with 749 contigs showing differential expression. Importantly, dehydration levels used in this experiment were likely stressful and ecologically relevant for *I. uriae* but are far below the amount of water loss that causes mortality (Benoit et al. 2007). A previous study on the American dog tick, *Dermacentor variabilis*, examined gene expression and metabolite changes associated with tick dehydration (Rosendale et al. 2016b). For *D. variabilis*, the response to dehydration seems to have two major physiological roles, 1. prevent and repair damage associated with dehydration and

Table 2 Enrichment of specific gene ontology (GO) categories following exposure to dehydration, cold, and heat. Categories included were enriched in 3 of the 4 programs used to establish GO enrichments (Panther, G:Profiler, DAVID, and Blast2GO)

Dehydration increased	Dehydration decreased	Cold increased	Heat increased
Amide biosynthetic process	Binding	Structural constituent of cuticle	Structural constituent of cuticle
Cellular amide metabolic process	Nucleic acid binding		
Nucleobase-containing smallmolecule metabolic process	Protein binding		
Nucleoside monophosphate metabolic process			
Organonitrogen compound biosynthetic process			
Organonitrogen compound metabolic process			
Peptide biosynthetic process			
Peptide metabolic process			
Purine-containing compound metabolic process			
Purine nucleotide metabolic process			
Ribosephosphate metabolic process			
Small molecule metabolic process			
Structural constituent of ribosome			
Translation			

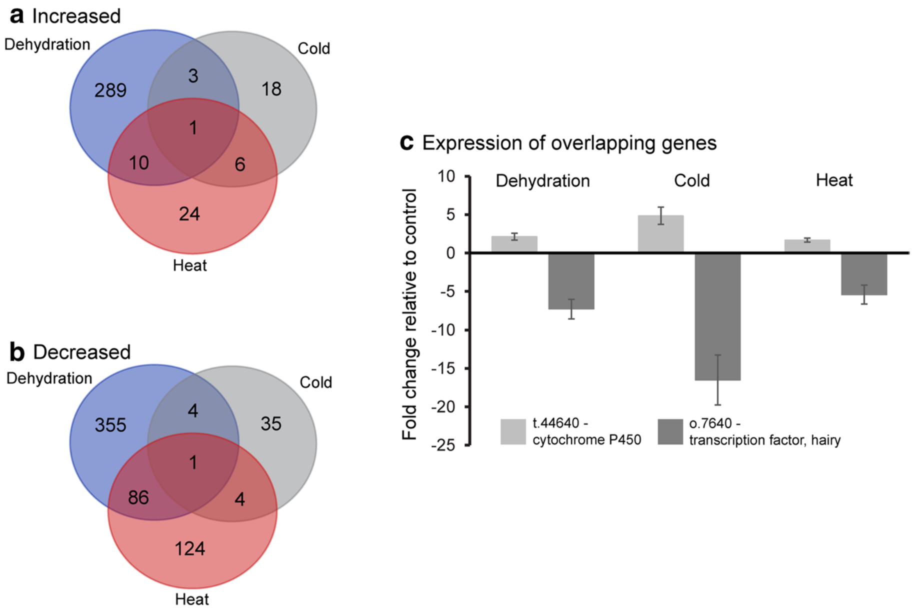

Fig. 4 Comparative analysis between transcript expression differences in relation to stress. Overlap in transcripts with significant increases (**a**) and decreases (**b**) in expression levels between cold, dehydration, and heat stress. Genes that overlap between all three treatments were identified with BLAST. The intersections between species were generated with Venn tools (http://bioinformatics.psb.ugent.be/webtools/Venn/). **c** Fold expressional differences between the individual genes with significant increased or decreased expression following all stress types

2. increase processes that promote hydration when conditions become more favorable (Rosendale et al. 2016b). The transcriptional response we noted in *I. uriae* is similar to that in *D. variabilis*, in that it included expression of multiple cytochrome P450 genes and other detoxification genes, cell structure components, and membrane structure components. As in *D. variabilis*, there was increased expression of genes associated with GABA-associated processes and glycerol production, which likely contribute to increased glycerol production in the salivary glands and induce subsequent salivation when relative humidity levels increase above the point that allows water vapor uptake (Lindsay and Kaufman 1986; Lucien et al. 1995; Benoit et al. 2007). Lastly, stress proteins and factors that alter membranes increased during dehydration, a response also noted in the American dog tick (Rosendale et al. 2016b). These results suggest there are general transcriptional changes associated with dehydration in both prostriate and metastriate ticks.

Thermal stress elicited fewer differentially expressed transcripts compared to dehydration. Direct cold exposure failed to induce robust transcriptional changes in *D. variabilis*; significant changes were noted only in response to rapid cold hardening (RCH) applied before a more extensive cold exposure (Rosendale et al. 2021). Gene expression changes in relation to cold were also examined in *Dermacentor silvarum* and revealed responses following prolonged exposure to low temperatures (4 °C) (Yu et al. 2017, 2020). The large number of differentially expressed genes in *D. silvarum* is likely because the cold treatment was more akin to RCH in *D. variabilis* (Rosendale et al. 2021), rather than the stress of subzero temperature exposure examined in this study. Cuticle change was the only significant GO category enriched in relation to cold exposure, a response also noted for other invertebrates, including both *D. variabilis* and *D. silvarum* (Dunning et al. 2013, 2014; MacMillan et al. 2016). As with cold, cuticle factors were enriched during heat exposure, as noted in other insect and mite systems (Nguyen et al. 2009; Tian et al. 2020; Quan et al. 2020). The specific cuticle proteins increased during cold and heat represent five of the six overlapping genes, suggesting that heat and cold stress both induce changes in the cuticle of *I. uriae*. The identification of cuticle changes in relation to thermal stress for *I. uriae*, *D. variabilis*, and *D. silvarum* suggests that this a critical feature of tick thermotolerance. The specific mechanism why cuticle changes occur under both heat and cold need to be established by future functional studies, but are likely to be associated with maintaining cuticle integrity as severe thermal stress can damage the cuticle of invertebrates. Other expression changes associated with cold and heat stress are quite diverse, ranging from stress-associated factors to salivary gland proteins.

Only two genes showed overlapping expression in response to the three types of stress: one (P450) was upregulated and the other (a transcription factor) downregulated. P450s are critical in detoxification, metabolic aspects, and responses to most environmental factors (Feyereisen 1999, 2012, 2020). Based on comparison to other systems, this specific P450 is likely involved in lipid metabolism (Li et al. 2004), suggesting that specific fat metabolic processes may play critical roles in relation to tick stress. The transcript that was suppressed is a specific transcription factor that is most closely related to *hairy*, a transcript responsive to stress in other systems and is linked to many functional processes that range from development, tissue morphogenesis, metabolism, and membrane change (Li et al. 2004; Liu et al. 2006; Zhou et al. 2008; Azad et al. 2009). The observed decrease in Hairy could alter specific biological processes during stress exposure, but functional studies are needed to determine its role as this transcription factor can have a multitude of role.

In summary, this study provides transcript expression differences in association with abiotic stress for the seabird tick and identifies cross-tolerance between dehydration stress and cold tolerance. Importantly, many of the stress tolerance transcript changes related to cold and dehydration stress are similar between *I. uriae* (prostriate tick) and *D. variabilis/D. silvarum* (metastriate ticks), suggesting some commonality in stress responsive genes among ticks. In our experiments, we found no unique responses related to cold, heat, and dehydration exposure that would allow this tick to inhabit the extreme environments of the polar regions. Cold and dehydration resistance is similar to that observed in other *Ixodes* species (Lee and Baust 1987; Vandyk et al. 1996; Benoit et al. 2007; Herrmann and Gern 2013), indicating these two stress factors are not likely major determinants of the distribution of *I. uria*e. Heat tolerance has not yet been thoroughly examined as a limiting factor in ticks. Heat could limit survival by direct exposure (Yoder et al. 2009; Holmes et al. 2018) by increasing metabolism that, in turn, reduces finite nutrient reserves during starvation (Rosendale et al. 2019), or by interactions between dehydration and heat exposure that impact survival (Rosendale et al. 2017). Future studies that examine the dynamics between stress exposure, gene expression changes, and longevity are needed to link specific transcript changes with tick survival. In addition, as *I. uria*e has wide distributional and host ranges (Wilson 1970; McCoy et al. 2001; Barbosa et al. 2011; Muñoz-Leal and González-Acuña 2015), examining stress tolerance and the molecular response among these different populations may be of interest as considerable variation has been noted among populations of other invertebrate species, which could shed light on why this tick has northern and southern polar distribution (Hallas et al. 2002; Hoffmann et al. 2002).

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Author contributions This study was designed and conceived by J.B.B and A.J.R. The manuscript was written by B.D. and J.B.B. RNA-seq studies were conducted by A.J.R., B. D., and J.B.B. Physiological studies were conducted by J.D.G and J.B.B. Samples were collected by J.D.G., J.B.B., R.E.L., and D.L.D. Figures were prepared by B.D. and J.B.B. All authors were responsible for editing the manuscript and have approved publication.

Data availability All datasets used for RNA-seq analyses are available under the NCBI Bioproject PRJNA659796.

Declarations

Conflict of interest We do not declare any conflict of interests.

Informed consent Not applicable to current study.

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