Ticks and Tick-borne Diseases 13 (2022) 102033

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Dual assessment of transcriptional and metabolomic responses in the American dog tick following exposure to different pesticides and repellents

Atit Pathak [a,1], Souvik Chakraborty [a,1], Kennan Oyen [a], Andrew J. Rosendale [a,b], Joshua B. Benoit [a,*]

[a] Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45211
[b] Biology Department, Mount St. Joseph University, Cincinnati, OH, 45233

ARTICLE INFO

Keywords:
Dermacentor variabilis
Transcriptional analyses
Pesticides
Repellents
Transcriptomics
Metabolomics
Functional overlap mapping

ABSTRACT

The American dog tick, *Dermacentor variabilis*, is a major pest to humans and animals, serving as a vector to *Rickettsia rickettsii*, a bacterium responsible for Rocky Mountain spotted fever, and *Francisella tularensis*, which is responsible for tularemia. Although several tactics for management have been deployed, very little is known about the molecular response following pesticidal treatments in ticks. In this study, we used a combined approach utilizing transcriptomics and metabolomics to understand the response of the American dog tick to five common pesticides (amitraz, chlorpyrifos, fipronil, permethrin, and propoxur), and analyzed previous experimental data utilizing DEET repellent. Exposure to different chemicals led to significant differential expression of a varying number of transcripts, where 42 were downregulated and only one was upregulated across all treatments. A metabolomic analysis identified significant changes in acetate and aspartate levels following exposure to chlorpyrifos and propoxur, which was attributed to reduced cholinesterase activity. Integrating the metabolomics study with RNA-seq analysis, we found the physiological manifestations of the combined metabolic and transcriptional differences, revealing several novel biomolecular pathways. In particular, we discovered the downregulation of amino sugar metabolism and methylhistidine metabolism after permethrin exposure, as well as an upregulation of glutamate metabolism in amitraz treated samples. Understanding these altered biochemical pathways following pesticide and repellent exposure can help us formulate more effective chemical treatments to reduce the burden of ticks.

1. Introduction

Female ticks are obligate arthropod pests that rely on animals as a source of blood meals for nutrition, development, and reproduction. Consequently, their blood feeding leads to the spread of disease agents such as those causing Rocky Mountain spotted fever (vectored by *Dermacentor variabilis* and *Rhipicephalus sanguineus*) and Lyme disease (vectored by *Ixodes spp.* ticks) (Bhatia et al., 2018; Choi et al., 2016; Dantas-Torres, 2007), accounting for at least 50,000 reported cases each year in the United States (CDC, 2021). Furthermore, ticks are common agricultural pests that lead to hundreds of millions of US dollars in economic losses due to the harm to livestock from feeding and pathogen transmission (Kivaria, 2006). A comprehensive pest management strategy for reducing the spread of tick-associated diseases requires multiple tools (Perez de Leon et al., 2014; Stafford et al., 2017) including proper landscaping (Bergmeier et al., 2010; Černý et al., 2020), organic land care practices (Stafford, 2004), and management of host animals (Abbas et al., 2014; Dantas-Torres et al., 2012). Acaricides, or pesticides targeted towards ticks, can achieve a high degree of efficacy in short periods. When combined with integrated tick management strategies, these chemicals can effectively suppress tick populations (Meulbroek, 2002; Perera and Holsomback, 2005). Applying these interventions in tick hotspots such as woodland edges during the spring and summer seasons when ticks are most active can reduce the pest population.

Several factors influence the selection of different chemical products and their success as acaricides in the control of ticks (Dantas-Torres et al., 2012). Repellents are frequently used for tick bite prevention whereas liquid and granular forms of acaricides are commonly utilized for commercial tick control - although ready-to-spray (RTS) products are also available for homeowner use (Ajith Kumar et al., 2016; Butler et al.,

* Author for correspondence: Joshua B. Benoit, Department of Biological Sciences, University of Cincinnati, OH 45221, USA.
E-mail address: joshua.benoit@uc.edu (J.B. Benoit).
[1] Authors contributed equally and are considered interchangeable as first author.

https://doi.org/10.1016/j.ttbdis.2022.102033
Received 1 February 2022; Received in revised form 15 August 2022; Accepted 15 August 2022
Available online 31 August 2022

2021). Systemic and non-systemic poisons, pyrethroids, organophosphates and carbamates are major classes of acaricides used in tick control (Dryden, 2009; George, 2000), although organophosphates are no longer used due to their adverse health effects on humans and the environment (Marrs, 1993). Systemic (e.g. fipronil, imidacloprid) and non-systemic poisons (e.g. amitraz) are available for public use and prove effective for controlling ticks, mites, and lice (Elfassy et al., 2001; Pound et al., 2000). Fipronil functions by blocking GABAA-gated chloride channels in insect nerve endings, preventing chloride uptake and prompting excessive stimulation, eventually leading to death (Cole et al., 1993; Ratra and Casida, 2001). Amitraz primarily functions as an agonist in the insect's alpha-adrenergic system, causing overexcitation and ultimately paralysis or death (Baron et al., 2018; O'Neal et al., 2017; Prullage et al., 2011). Pesticides such as fipronil and amitraz can be effective in reducing tick populations, but they also cause harm to the ecosystem, humans, pets, cattle, and other beneficial arthropods (del Pino et al., 2015; Proudfoot, 2003). These chemical interventions are known to wreak havoc on the ecosystem by contaminating surface and ground water, degrading soil fertility, and harming aquatic life - all of which threaten the biodiversity of natural systems (Aktar, Sengupta, and Chowdhury, 2009; Nicolopoulou-Stamati et al., 2016).

Despite the many available control mechanisms, American dog tick (*Dermacentor variabilis*) populations have continued to grow and expand (Minigan et al., 2018). A population that once was limited to certain areas of the eastern half of the United States has experienced significant habitat growth, establishing permanent populations in several parts of southern Canada (Minigan et al., 2018; Sonenshine, 2018). This recent shift over the last few decades has been attributed to milder winters and more favorable climates for ticks, which correlates with the observed habitat expansion of four tick species (*Dermacentor variabilis, Amblyomma americanum, Amblyomma maculatum*, and *Ixodes scapularis*) into previously unsuitable geographies (Boorgula et al., 2020; Sonenshine, 2018). Current climate models predict an up to 50% expansion in suitable areas for *D. variabilis* populations within the next several decades (Sonenshine, 2018). This growing population combined with the widespread use of general insecticides may create a selective pressure, producing resistant tick populations; this phenomenon has been observed in several tick species including the brown dog tick (*Rhipicephalus sanguineus*) and the southern cattle tick (*Rhipicephalus microplus*) (Chen et al., 2007; Coles and Dryden, 2014; Feyereisen, 1995; Foil et al., 2004; George et al., 2004; Villatte et al., 2000), therefore making it increasingly difficult to manage. A critical understanding of the biomechanics of pesticides and repellant exposure in ticks is crucial for developing more effective, target-specific solutions in the future. Although the general modes of action of the five common classes of pesticides are known (Anadón et al., 1991; Gassner et al., 1997; Jackson, 2020; Jager et al., 2007; Kovacic and Somanathan, 2012; Prullage et al., 2011; Swale et al., 2014), the specific molecular and biochemical pathways altered by each of these chemical treatments in ticks are not well documented.

To better understand the responses of ticks to chemical treatments, we studied the response of *D. variabilis*, to five common pesticides (amitraz, chlorpyrifos, fipronil, permethrin, and propoxur) and DEET as a repellent. Using RNA-seq, we discovered one common transcript that was significantly upregulated and 42 transcripts that were downregulated following exposure to selected chemicals. Afterwards, these transcripts were correlated with changes in metabolite levels following exposure to specific control-based chemicals. In a subsequent experiment, we also analyzed the transcriptional changes over time for permethrin and compared it to previously published data for DEET exposure (Koloski et al., 2019), and found the majority of the transcripts to be differentially expressed during the first few hours following exposure. Overall, our study identified several novel biochemical pathways that were supported by two independent mapping pipelines and functional metabolomic analyses.

2. Materials and methods

2.1. Tick rearing and sample preparation

Colonies of female *D. variabilis* were obtained from the Tick Rearing Facility at Oklahoma State University (Stillwater, OK, USA). To prevent dehydration, groups of 20-30 ticks were collected in 50 cm^3 mesh-covered vials and reared inside 93% relative humidity (RH) (Winston and Bates, 1960), 26±1°C and 15hr:9hr light:dark chambers for storage prior to performing our experimental procedures. The ticks were randomly chosen from the stored colonies and transferred into 1.5 cm^3 mesh-covered vials. Solutions with varying concentrations of 5 different pesticide were prepared (45 μM amitraz, 0.5 μM chlorpyrifos, 0.1 μM fipronil, 0.125 μM permethrin, and 0.075 μM propoxur) by dissolving each pesticide into pure ethanol and diluting the resulting solution with DI water to ensure that the ethanol concentration was less than 5%. These concentrations were set below each pesticide's minimum lethal dose to ensure that we captured sub-lethal responses to the pesticides and not transcriptional changes due to mortality. The sub-lethal doses were determined by exposing ticks to increasing concentrations of the five pesticides and assessing survival 24 hr post-exposure. To administer the pesticide treatment, three replicates of 8-10 female *D. variabilis* were fully submerged in a randomly-chosen pesticide solution for 60 s. Control samples were submerged in <5% ethanol for equal time. Following exposure to the Adult Immersion Test (AIT), ticks were blotted dry and allowed to recover for 24 hr before being flash frozen and stored at -80°C until used for RNA-seq and metabolomic analyses. For permethrin-treated ticks, samples were also frozen at 2 hr and 6 hr following exposure to be used in a time-course experiment. These time points were chosen based on our preliminary experiments where we observed that differences in behavior appeared around 2 hr post-exposure. The 6 hr time point was selected as a "midpoint" value between 2 hr and 24 hr. Ticks were not treated with DEET; however, raw data from a similar experiment on *D. variabilis* examining RNA-seq at 0.25 hr, 4 hr, and 24 hr post-exposure to DEET was obtained from Koloski et al. (2019), where the ticks were placed on paper towels soaked with DEET for 15 min. The data from the five pesticides and DEET at 24 hr post-exposure was used for comparison with each other (denoted as pesticide-series data). The tick responses at the multiple time points post-exposure to permethrin and DEET were used in a subsequent time-course experiment (denoted as time-series data).

2.2. Transcriptome generation and expression analysis

Tick samples were prepared for analysis by cutting them into four similar-sized pieces. The fragments were homogenized in 1 ml cooled TRIzol reagent (Invitrogen, Carlsbad, CA, USA) using BeadBlaster 24 microtube homogenizers (Benchmark Scientific, Edison, NJ, USA). RNA from the mixture was separated according to the manufacturer's protocol. Afterward, DNA impurities were eliminated using DNase I (Thermo Scientific, Pittsburgh, PA, USA). The RNA was then concentrated using the GeneJET RNA Cleanup and Concentration Micro Kit (Thermo Scientific) and the concentrations were determined and quality was checked using a Nanodrop (Thermo Fisher Scientific, Waltham, MA, USA) before sequencing.

The RNA fragments were sequenced, and poly(A) library constructed by the DNA Sequencing and Genotyping Core at the Cincinnati Children's Hospital Medical Center (CCHMC). Following RNA quantification using Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) and quality assessment with a 2100 Bioanalyzer (Agilent, Santa Clara, CA), 150–500 ng of total RNA was poly(A) selected and reverse transcribed using the TruSeq Stranded mRNA Library Preparation Kit (Illumina, San Diego, CA, USA). Each sample was fitted with one of the 96 adapters containing a different 8-base molecular barcode for high-level multiplexing. We conducted 15 cycles of PCR amplification and sequenced the libraries using HiSeq 2500 (Illumina) in Rapid Mode, which resulted

in approximately 30 million high-quality, single-end reads per sample, with lengths of 75-base pairs. Raw RNA-Seq data were uploaded to the National Center for Biotechnology Information (NCBI) Sequence Read Archive: Bio-project PRJNA783667.

2.3. Quality assessment and analysis of RNA-seq datasets

The majority of analyses were conducted using the Galaxy platform (Afgan et al., 2018). Individual FASTQ files were analyzed with the FastQC tool (Andrews, 2010), to determine the quality of the raw data and to mitigate the effects of amplification bias. The Kmer test within FastQC was enabled and set at a length of 7 bp. Following this initial quality check, raw files were trimmed with Trim Galore using default settings to remove adapter sequences and biased methylation positions for the directional and non-directional sequences. Lastly, FastQC was performed on each trimmed FASTQ file to confirm quality improvement; approximately <1% raw reads were trimmed during processing (Supplement 1). *De novo* assembly was conducted according to our previous developed methods (Davies et al., 2021; Rosendale et al., 2016, 2019) using Trinity (Grabherr et al., 2011) and CLC Genomics v12 Workbench (Qiagen). Duplicated contigs were removed with the use of CD-EST-HIT (Fu et al., 2012). The final assembly for the pesticide-series data consisted of 155,220 contigs (Supplement 2), which was evaluated for completeness based upon BUSCO (Simão et al., 2015), revealing 94.2% complete and 3.4% fragmented transcripts. Similarly, for the time-series samples, the final assembly consisted of 149,441 contigs (Supplement 3), revealing 95.1% complete and 4.1% fragmented transcripts.

We quantified transcript abundance using two mapping pipelines: Salmon and Kallisto (Bray et al., 2016; Patro et al., 2017;Srivastava et al., 2019). By utilizing two mapping tools, differentially expressed contigs were identified with a higher degree of certainty by considering only those contigs that were determined to be significant through both mapping methods. The default settings were conserved for both mapping tools, using a Kmer length of 31 bp for Salmon and 200 bp for Kallisto.

TPM values obtained from DESeq2 (Love et al., 2014) were used as a proxy to establish differential transcript levels between control and pesticide-treated groups using Salmon and Kallisto output independently. The transcript-level counts were quantified using the TPM (transcripts per million). The significant expression differences were estimated using an adjusted p-value cutoff of less than 0.05 with a false discovery rate (FDR, Benjamini and Hochberg, 1995) and a $\log_2$ (fold change) $\geq |2|$. For each treatment, unique module(s) of transcripts were differentially expressed and the significant modules were matched against the reference genome protein database (available at Vectorbase or NCBI; Gulia-Nuss et al., 2016; Jia et al., 2020) of *I. scapularis, Dermacentor silvarum*, and *Drosophila melanogaster* using BLASTx (Attardo et al., 2019; Finch et al., 2020; Rosendale et al., 2019), with an expectation value (e-value) of < 0.001. The highest scoring matches for each contig were used to assign a gene ID, allowing a 70% match to our *D. variabilis* contigs. Gene ontology (GO) terms were obtained for contigs with positive BLAST hits using two GO tools, g:Profiler (Finch et al., 2020; Raudvere et al., 2019) and PANTHER (Mi et al., 2021; Mi and Thomas, 2009). GO annotations for enriched categories were analyzed with REVIGO (Supek et al., 2011) for visualization of representative functional categories associated with each treatment. A similar set of processes were conducted for the experimental groups differentiated by time-after-exposure to either permethrin or DEET.

Beyond this first analysis, these transcript sets underwent a weighted correlation network analysis (WGCNA) for visualizing the correlation patterns and identifying clusters (modules) of highly correlated transcripts across all treatment samples based on our previous studies (Benoit et al., 2020; Finch et al., 2020; Langfelder and Horvath, 2008). Transcripts with similar expression patterns were categorized into modules to identify groups of significant transcripts that are associated with a specific treatment sample. Prior to WGCNA, the transcript data was filtered for transcripts of zero variance. The minimum module size was set at 30 and the soft power was kept at 12. Following WGCNA, modules found to be significant ($p < 0.05$) were analyzed to understand the functions of their associated transcripts. We used CLC Genomics Workbench v12 to blast the contigs as well as the significant modules and performed GO category enrichment analyses through g:Profiler to interpret the data, as previously described (Davies et al., 2021; Rosendale et al., 2016, 2019).

2.4. Metabolic analysis of pesticide exposure

Metabolome changes in ticks were compared following exposure to different pesticides based on methods previously developed to target ticks and other blood feeding systems (Hagan et al., 2018; Rosendale et al., 2016). The polar metabolites were extracted through a modified Bligh and Dyer extraction protocol (Bligh and Dyer, 1959). Each pesticide-treated sample contained 5 ticks and was analyzed at the NMR-based Metabolomics Core at CCHMC. Dry mass for ticks was determined gravimetrically based on previous methods (Yoder et al., 2011) Dry tick samples were weighed in 2 mL standard tubes and were homogenized 3 times using 2.8 mm metal beads (Bertin Corp., Rockville, MD, USA) for 30 s each at 4000 rpm in a solution of water and cold methanol using a Minilys homogenizer (Bertin Corp.). The sample solution was diluted in a tube containing chloroform and water, resulting in a 2:2:1.8 ratio of methanol:chloroform:water. After vortexing and incubating in chilled ice for 10 min, the solution was centrifuged at 2000 g for 5 min. The polar phase was separated in a 2 ml tube and dried using a vacuum centrifuge for 2-3 hrs at room temperature. Finally, 0.6 ml of NMR buffer containing 100 mmol l−1 phosphate buffer (pH 7.3), 1 mmol l−1 TMSP (3-trimethylsilyl-2,2,3,3-d4 propionate), and 1 mg ml−1 sodium azide (prepared in deuterium oxide) was added into the tubes containing the dry samples. A 550 μL aliquot was transferred into a 5 mm NMR tube for one-dimensional ^{13}H-Nuclear Overhauser Effect Spectroscopy (NOSEY). The NMR analysis was conducted on a 600 MHz INOVA NMR spectrometer (Agilent Technologies, Santa Clara, CA, USA). In addition, we also assembled two-dimensional ^{1}H-^{13}C heteronuclear single quantum correlations (HSQC) and ^{1}H-^{1}H total correlation spectroscopy (TOCSY) using the samples. TopSpin 3.1 (Bruker, Billerica, MA, USA) was utilized to correct and align the spectral data to the internal reference standard peak, and the metabolite identities were assigned using Chenomx NMR Suite 7.8 *(Chenomx Inc., Edmonton, AB, Canada)*, two-dimensional NMR experiments, and reference spectra including those available at Human Metabolome Database (Wishart et al., 2007), the Madison Metabolomics Consortium Database (Cui et al., 2008), and the Biological Magnetic Resonance Data Bank (Ulrich et al., 2008)).

The metabolite masses were normalized by dividing each metabolite mass by the tick's dry mass. Data were analyzed using both univariate and multivariate statistical analysis approaches using Metaboanalyst (version 5.0). The average mass of each metabolite was compared using two-way ANOVA tests. The identity of each metabolite was assigned with a biomarker analysis module under Metaboanalyst. We used autoscaling to mean-center the data and divided it by the standard deviation of each variable. Differentially expressed metabolite levels were considered if the p-value was < 0.05. The differential expression of metabolites were analyzed with Metaboanalyst's Enrichment Analysis tool (MSEA) to see the fold-change of metabolites after a particular pesticide treatment. Using Enrichment Analysis's default settings, a list of metabolic pathways associated with each of the metabolites was generated. Analysis of variance (ANOVA) was performed to determine the pesticidal effect on each metabolite and to compare the absolute value of change between the treatment and control group means. Enrichment ratio was computed by calculating Hits / Expected (where hits = observed hits and expected = expected hits). Classical univariate receiver operating characteristic (ROC) curves, t-statistics, and $\log_2$ (fold change) were generated using the Biomarker Analysis tool (Xia et al.,

2013) within Metaboanalyst. For the overlap between metabolomics and transcriptomics results, the list of metabolites and metabolic pathways were manually compared with the list of differentially expressed transcripts and expressional pathways.

3. Results

3.1. Survival analysis of D. variabilis following pesticide exposure

We determined the non-lethal doses of each pesticide for *D. variabilis* after exposing them to varying concentrations. The maximum non-lethal concentrations where 100% of ticks survived following exposure to amitraz, chlorpyrifos, fipronil, propoxur, and permethrin were 45μM, 0.5μM, 0.1μM, 0.075μM, and 0.125μM, respectively (Fig. 1). These concentrations were used to prepare the samples for the RNA-seq experiments and metabolomic studies.

3.2. Synopsis of differentially expressed transcripts following pesticide exposure

The Salmon-based pipeline yielded 38,544 significantly differentially expressed transcripts ($p < 0.05$; $\log_2$ (fold change) $\geq |2|$), while the Kallisto-based pipeline yielded 22,039 transcripts (Fig. 2A). Out of the complete set of 45,577 sequences identified as differentially expressed either from the pipelines, 15,006 (32.9%) overlapped between the two mapping pipelines (Fig. 2A). Comparing the transcripts-per-million (TPM) $\log_2$ (fold-change) values from the Salmon versus the Kallisto pipelines for each transcript, a Pearson correlation coefficient of 0.807 was generated, indicating a strong correlation at the significance cut-off (two-fold difference, and a correction-based P-value < 0.05) (Fig. 2A) even though only 32.9% of the transcripts with statistically different expressions overlapped. Between 25% and 54% of the raw RNA sequence reads were mapped to transcripts (Supplement 1), which was expected based upon previous studies (Davies et al., 2021; Rosendale et al., 2016, 2019).

For the time-series data, the Salmon-based pipeline yielded 115,041 significantly differentially expressed transcripts ($p < 0.05$; $\log_2$ (fold change) $\geq |2|$) and the Kallisto-based pipeline yielded 105,061 transcripts. From the 146,691 differentially expressed unique sequences identified from either Salmon or Kallisto, 73,411 were found to overlap between the two mapping pipelines, representing 50% (Fig. 2B). A 0.817 Pearson correlation coefficient indicated a strong positive correlation between the TPM $\log_2$ (fold-change) values determined by the two mapping pipelines for the overlapping transcripts at the significance cut-off (two-fold difference and a correction-based P-value < 0.05) (Fig. 2B). Between 31% and 58% of the raw RNA-seq dataset mapped to the transcripts (Supplement 1). As our mapping methods of raw reads showed great similarity in both mapping pipelines (Fig. 2), we proceeded with the Kallisto-based data for our remaining analyses (Supplements 2-3).

3.3. Specific transcriptomic changes in D. variabilis following different chemical treatments

Principal component analysis (PCA) based on transcriptional changes from the pesticide-series data were indicative of the general modes of action of each treatment (Fig. 3A). DEET and permethrin clustered independently and appeared to be most divergent from the other pesticides and control group (Fig. 3A). For WGCNA, distinct clusters of co-expressed genes were identified (Fig. 3B). Of importance, no overlapping modules were observed between any of the six treatments, signifying that the unique sets of transcripts are representative of the changes associated with different chemical treatments (Fig. 3C). However, many unique modules for each treatment were found to be significant. Following GO term analysis, the greatest number of unique pathways were found with DEET-treated samples which included pathways such as protein methylation, ion binding, ATP binding, and intracellular transport (Supplement 5). Transcript-level changes in

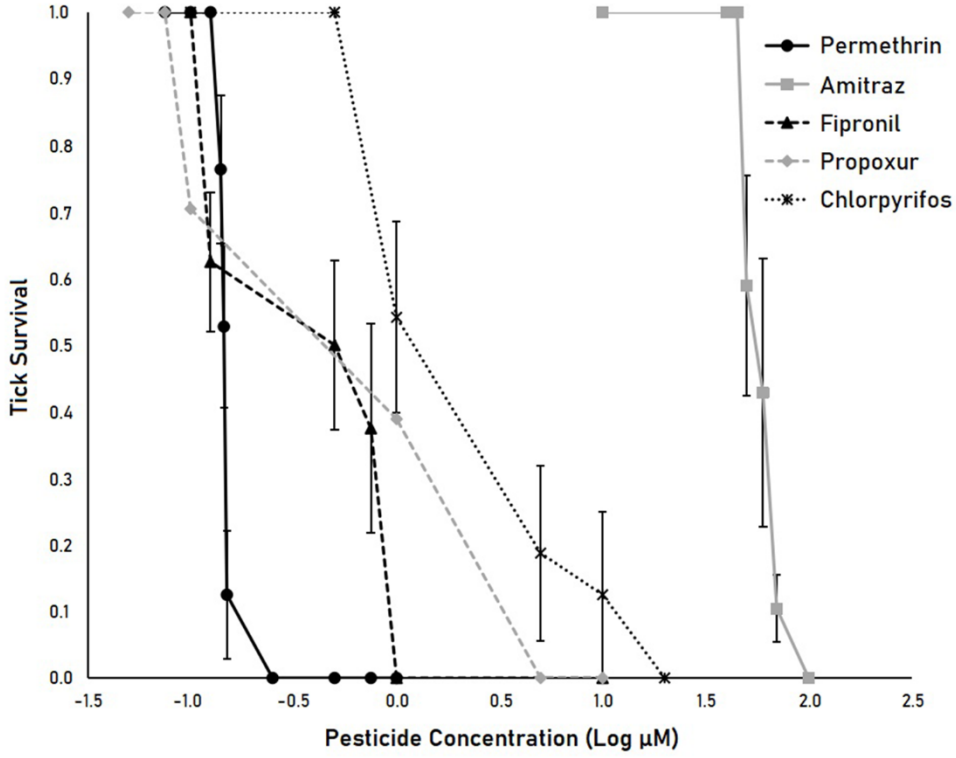

Fig. 1. *Dermacentor variabilis* survival after 24 hours post-exposure to varying concentrations of chemicals. Non-lethal treatment doses were determined using dose-dependent mortality curves for use on the experimental groups to ensure that the transcriptional changes were not due to mortality. For each treatment, 5-7 replicates were run, with 8 ticks per replicate.

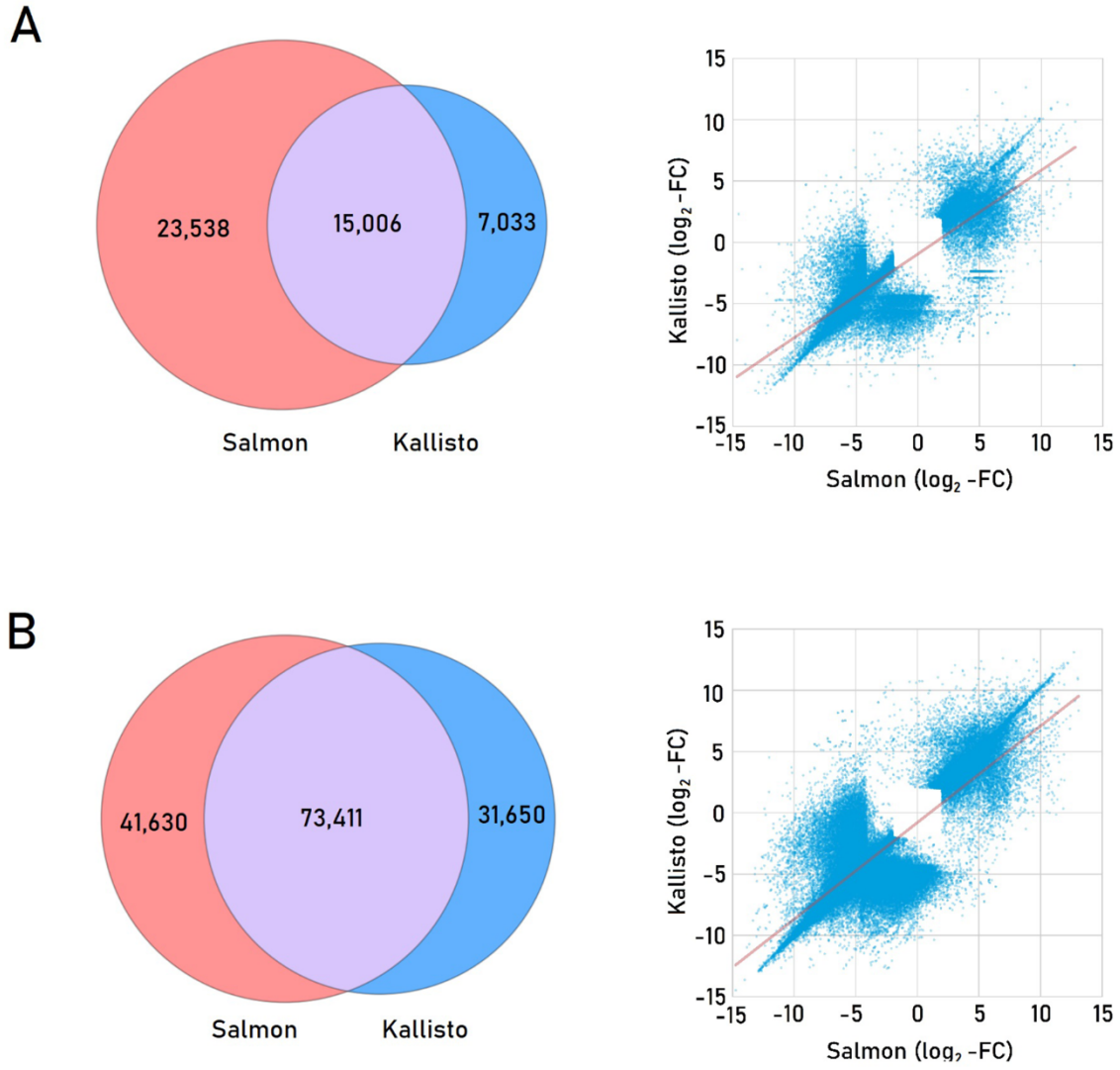

Fig. 2. Comparative analysis between two independent pipelines for RNA-seq analyses of American dog ticks (*Dermacentor variabilis*) following chemical exposure. (A) Transcripts enriched post-exposure to six chemical treatments using Salmon and Kallisto (left) and scatterplot comparing the two mapping tools, with a Pearson test ($\rho = 0.807$) (right), (B) Transcripts enriched at varied times post-exposure to Permethrin and DEET using Salmon and Kallisto (left) and scatterplot comparing the two mapping tools, with a Pearson test ($\rho = 0.817$) (right). The linear regression lines also indicate a positive correlation. The comparison revealed notable similarities between the two mapping methods. Figures were produced using Python 3.7.

propoxur included pseudouridine synthase activity, tRNA modification, RNA methylation, and ncRNA processing, while cytoplasmic translation, ribosomal large subunit biogenesis, and actin cytoskeleton were observed in permethrin-treated samples (Supplement 5). For chlorpyrifos, only hydrolase activity, acting on ester bonds, was found (Supplement 5). No unique GO terms were found for amitraz or fipronil-treated samples.

The $\log_2$ (fold change) values for each transcript were analyzed for all treatments to determine the significantly up-and-downregulated transcripts (see "Materials and Methods Section 2.3"). A comparison of differentially expressed transcripts for each pesticide found 82 common hits associated with the downregulated transcripts and one hit for upregulated transcripts (Fig. 4). The upregulated transcripts for each treatment, compared to the control, produced five enriched GO categories. Three major enriched categories include DNA metabolism, DNA biosynthetic process, and iron-binding activity (Fig. 4A). The downregulated transcripts had 8 unique GO categories associated with the enriched modules; the major categories include cell signaling, transport, and binding, negative regulation of the biological process, and DNA integration (Fig. 4B). After blasting the transcripts from each module, we found several biological, molecular, and cellular processes including molecular adapter and transducer activity, structural and cellular anatomical entity, immune system process, and interspecies interaction between organisms to be highly expressed for the DEET-treated tick groups (Supplement 4). For permethrin-treated samples, we found an increase in oxidoreductase activity, among other pathways (Supplement 5). Overall, we identified several overlapping GO categories across the different chemical treatments (Fig. 4); however, unique categories were also found following exposure to specific pesticides and repellents (Supplement 5).

3.4. Temporal transcript changes after pesticide and repellent exposure

Each treatment (DEET: 0.25hr, 4hr, 24hr; permethrin: 2hr, 6hr, 24hr; control: 24hr) clustered together and separately from the other treatment groups (Fig. 5A). There were no significant differences in the clustering patterns between different times for the same treatment (Fig. 5A). WGCNA revealed specific clusters of genes with similar expression patterns (Fig. 5B). Specifically, the early time treated samples for both permethrin and DEET shared identical significant modules

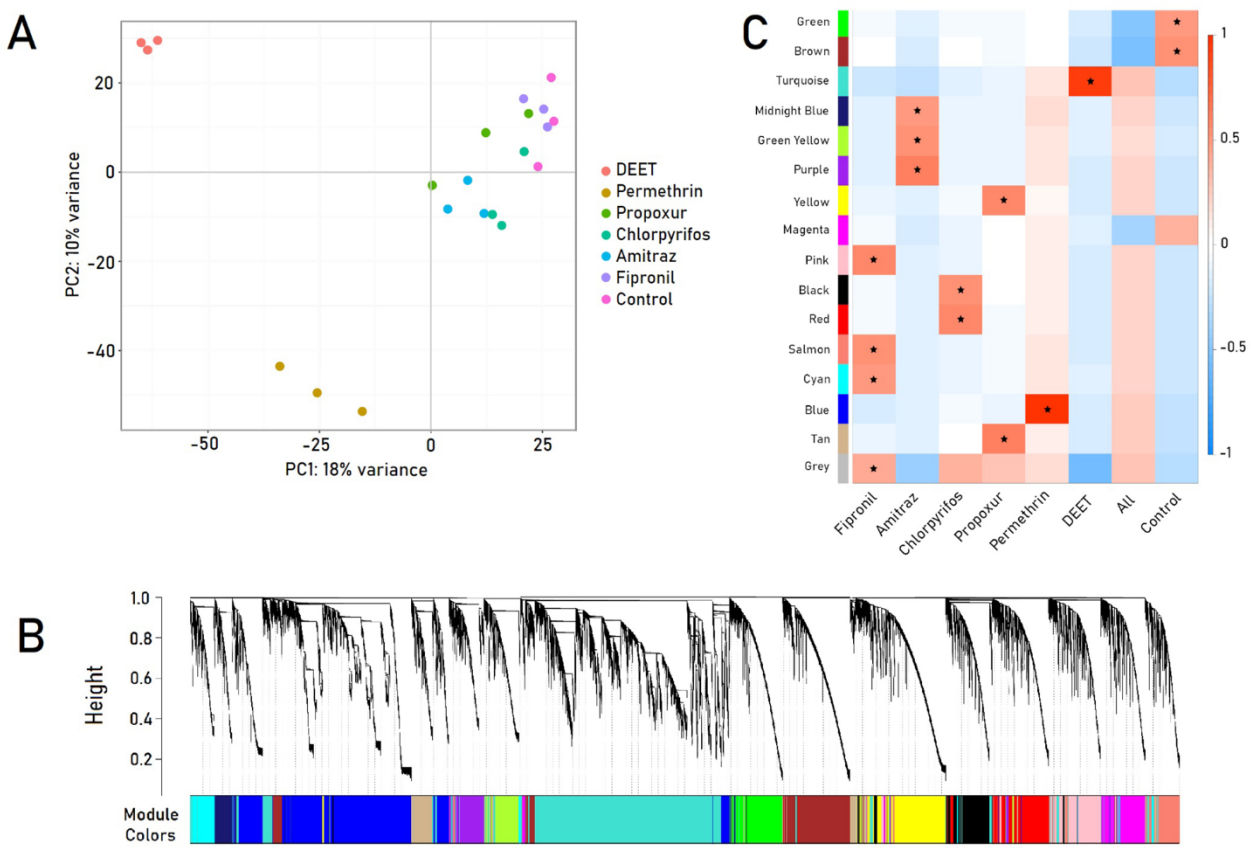

Fig. 3. Transcriptome comparison of the American dog tick, *Dermacentor variabilis, following exposure to different chemicals* (A) A principle component analysis (PCA) scores plot of the control and six treatment groups, (B) Linkage dendrogram representing average hierarchical clustering of the transcripts using WGCNA (Langfelder and Horvath, 2008). Modules, designated by color code, are branches of the clustering tree, (C) Correlation of module eigengenes to treatment groups from WGCNA, * represents values with a significant positive correlation with treatment r ($P < 0.05$).

based on WGCNA (Fig. 5C), which suggests similarity in the expression profiles of these groups (Supplement 6). We observed overlap between the early time groups within each chemical, but no overlap between the two chemical treatments (Fig. 5C). Using REVIGO, we summarized the GO terms from each module; 17 major categories were identified including oxidoreductase activity and glycerophospholipid metabolic process for early time point samples after treatment with DEET (Supplement 6). For permethrin, there were 11 major categories which included cytosol, magnesium ion binding, and rRNA binding (Supplement 6). Both permethrin and DEET were generally observed to affect a distinct cluster of transcripts (Fig. 5C). Furthermore, the largest changes in gene regulation patterns were observed during the early times (0.25hr, 2hr, 4hr, and 6hr) for both DEET and permethrin-treated samples (Supplement 6). The prominent alterations for both treatments included RNA modification, ion activity, cell cycle process, hydrolase activity and oxidoreductase activity (Supplement 6).

A $\log_2$ (fold change) enrichment analysis for each treatment-time combination identified specific up-and-downregulated transcripts, which were blasted to visualize the GO categories. We quantified the common blast hits between permethrin and DEET-treated samples for both the early and late time points after exposure (Fig. 6). Twenty enriched GO categories were associated with the upregulated transcripts at early times after exposure (permethrin: 2hr, 6hr; DEET: 0.25hr, 4hr), with the major categories being RNA processing and RNA binding (Fig. 6A). For the upregulated transcripts at the late time point (permethrin: 24hr; DEET: 24hr), the major GO categories included intracellular organelle lumen and ribonucleoprotein complex (Fig. 6B). Likewise, 33 unique GO categories were found for the downregulated transcripts at early time points, with RNA-dependent DNA biosynthetic process, ribosome, phosphotransferase activity, and alcohol group serving as acceptors, being the major categories (Fig. 6C). Meanwhile, there were 30 GO categories at the late time point, with cytosolic ribosome, RNA-dependent DNA biosynthetic process, and intracellular anatomical structure being the major categories (Fig. 6D). Several of the GO categories were observed to overlap across the different time-treatment groups (Fig. 6), but unique categories were also discovered for each chemical exposure (Supplement 7).

3.5. Transcriptome similarities between treatment groups

Out of the 15,006 significantly differentially expressed transcripts (Fig. 2A), we filtered out 3,441 false positives ($e < 0.001$); from the remaining, 260, 2,867, 64, 6,239, and 12 transcripts were unique to non-systemic pesticide (amitraz), repellent (DEET), systemic pesticide (fipronil), pyrethroid (permethrin), and nerve poisons (chlorpyrifos, propoxur), respectively (Fig. 7A). From those significantly differentially expressed transcripts, 43 were identified as common across all pesticides (Fig. 7A), with one being upregulated and 42 being downregulated. These transcripts were blasted (Fig. 4A) to reveal the specific biochemical pathways common in all of the treatment groups. In the time-series data, from the 28,356 transcripts differentially expressed during the early and late time points, 5,835 were unique to DEET exposure while 17,047 were unique to permethrin-treated samples (Fig. 7B). There were 2,466 unique common differentially expressed transcripts, whether upregulated or downregulated, present in both early and late time points of either pesticide (Fig. 7B). Moreover, core transcripts (shared or unique) were blasted against three reference protein databases (*Ixodes scapularis* RefSeq database, *Drosophila*

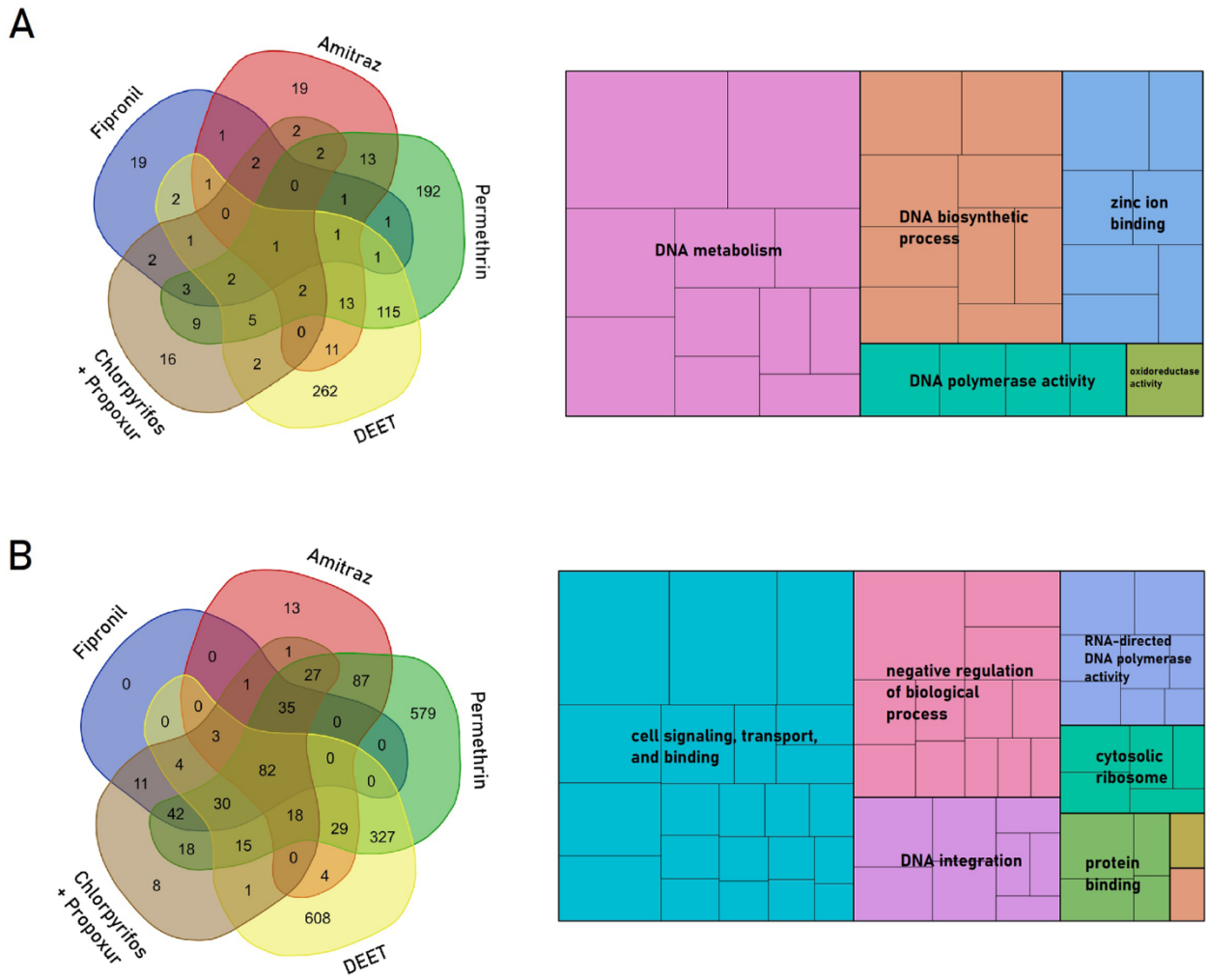

Fig. 4. Specific genes and gene ontologies (GO) of interest in pesticide and repellent-treated ticks, and their relevant molecular functions, cellular components, and biological processes visualized using treemaps. (A) Number of blast hits associated with upregulated transcripts after pesticide or repellent treatment, compared to control (left) and GO (right), (B) number of blast hits associated with downregulated transcripts after treatment, compared to control (left) and GO (right). The boxes in the treemaps represent the unique functional categories while the colors represent the major GO groups. Venn diagrams and treemaps were generated using Bioinformatics & Evolutionary Genomics (http://bioinformatics.psb.ugent.be/webtools/Venn/) and REVIGO.

melanogaster SwissProt database, and *Dermacentor silvarum* RefSeq database) and functional annotations were made, which will be helpful for developing new acaricides and repellents in future (Supplements 8-9).

3.6. Metabolome quantification and analysis

Eight metabolites were significantly altered in ticks exposed to pesticides ($p < 0.05$) from the 57 total metabolites analyzed, with confirmation from Tukey's Honestly Significant Difference (HSD) post-hoc test (Supplement 10). The metabolites found at varying concentrations following treatment were acetate, aspartate, glucose, guanosine, inosine, lactate, mannose, and ribose (Supplement 10). Both principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed on the 57 metabolites and indicated that 45% and 16.3% of the variance was cumulatively accounted for by the first two PCs respectively (Fig. 8A) (for PCA: 48.9% and 16.4%; Supp 11). A scores plot analysis using the first two PCs indicated that two distinct clusters were discriminated at the 95% confidence level, suggesting that the metabolic profiles of these different pesticide groups differed significantly (Supplement 11). To compare the absolute value of changes between the control and the pesticide exposures, we performed a fold-change analysis individually for all the pesticides. Fold-changes were calculated as the ratios between the treatment and control group means. The significant metabolites (up-and-down regulated) were plotted using a $\log_2$ (fold change) threshold and p-values ($p < 0.05$) (Fig. 8B). The greatest number of downregulated metabolites were found associated with amitraz (20 metabolites), followed by chlorpyrifos (14 metabolites) (Fig. 8B). Amitraz also resulted in the greatest number of upregulated metabolites (5 metabolites), followed by permethrin and fipronil (4 metabolites each) (Fig. 8B). Of particular interest in chlorpyrifos-treated samples, betaine concentrations were detected at significantly higher levels.

Each of the pesticide groups were checked against control groups to classify the metabolites according to the direction of fold change. In general, we observed more down than up-regulated metabolic pathways based on MSEA (Xia and Wishart, 2010). We used over-representation analysis by providing a one-column metabolite list. Compound ID standardization was performed to map the well-annotated compounds. Overall, we discovered 17 enriched metabolic pathways across the pesticides (at p-value < 0.05) excluding DEET - for which no metabolite data was available (Fig. 9A). Amitraz was observed to yield the greatest number of enriched pathways (10 pathways) associated with upregulated metabolites, while permethrin yielded the largest number (10 pathways) associated with downregulated metabolites (Fig. 9A).

Overlap between the metabolic and transcriptomic data was

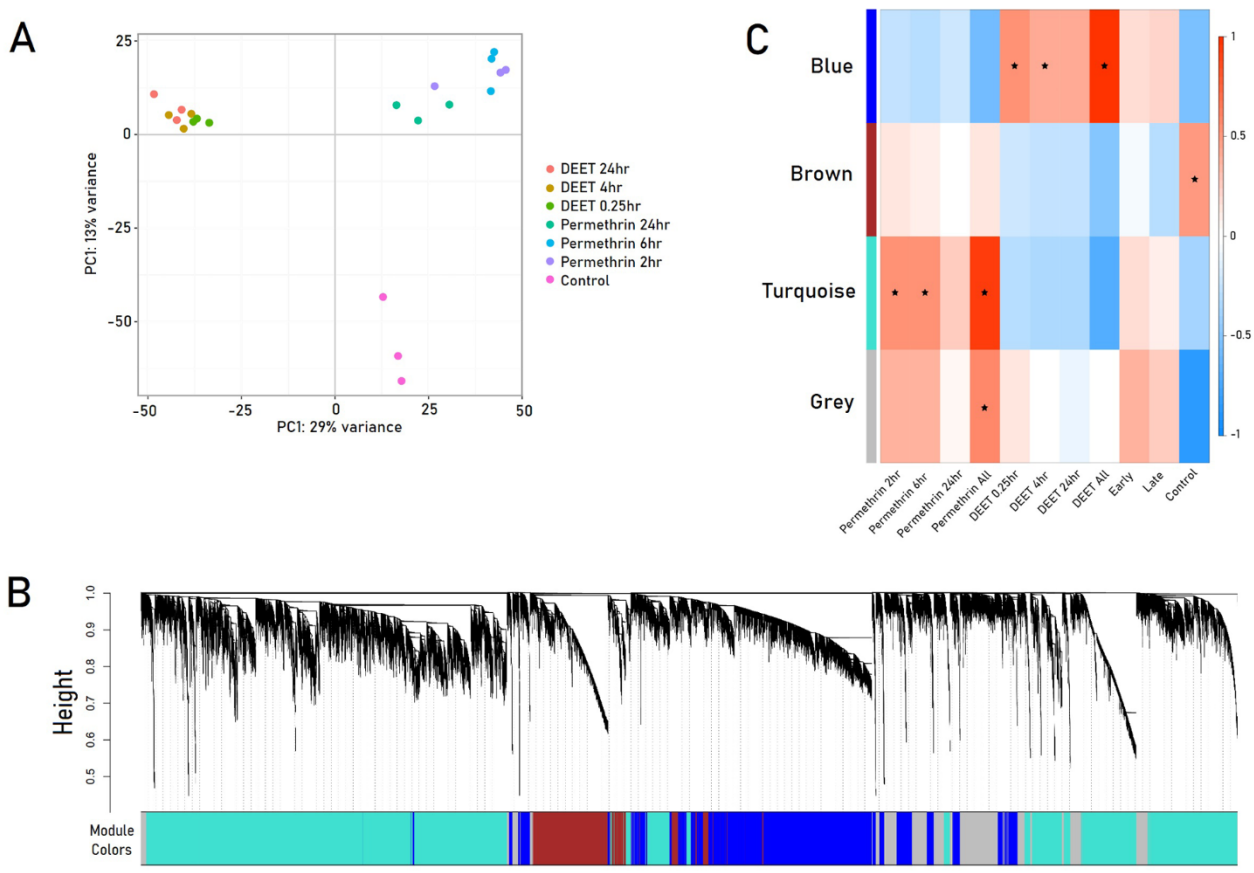

Fig. 5. Overall comparison between permethrin and DEET treated American dog tick, *Dermacentor variabilis,* at different time points of exposure. (A) A principle component analysis (PCA) scores plot of the control, permethrin, and DEET-treated ticks at various time points after exposure, (B) Linkage dendrogram representing average hierarchical clustering of the transcripts using WGCNA (Langfelder and Horvath, 2008). Modules, designated by color code, are branches of the clustering tree, (C) Correlation of module eigengenes to permethrin and DEET-treated tick samples from WGCNA, * represents values with a significant positive correlation with treatment ($P < 0.05$)

examined to determine if the changes in the metabolome could be linked to transcriptional shifts. Overall, we identified 13 enriched metabolic pathways which appeared to have functions relevant to at least one of the transcripts that were significantly changed (Fig. 9B). Some of these overlapping pathways found through our multi-"omics" approach combining transcriptomic and metabolomic data included glutamate metabolism, aspartate metabolism, ammonia recycling, and glucose-alanine metabolism in ticks treated with amitraz. For permethrin, upregulation of purine metabolism and downregulation of malate-aspartate shuttle, amino-sugar metabolism, methylhistidine metabolism and betaine metabolism were discovered. For the fipronil-treated group, only glutamate metabolism was observed to be downregulated. These results from our multi- "omics" study are corroborated by our previous analysis (Fig. 4, 6), although several novel biochemical pathways were also uncovered during this step (Fig. 9B).

4. Discussion

Although the general modes of action of pesticides and repellents commonly used to control ticks are understood based on comparison to other invertebrate systems, little is known about the effects of these chemical treatments on transcriptional and metabolic pathways in American dog ticks. In this study, we identified several molecular targets including zinc ion channel, oxidoreductase, and betaine production in American dog ticks which are affected by chemical exposure through a combined multi-omics approach. This is summarized in Fig. 10. Furthermore, our study analyzed the effect of two chemicals, DEET and permethrin, at different time points, and suggested that they have the greatest effect on American dog ticks during the first six hours following exposure. We highlight the distinct pathways targeted by permethrin and DEET and show DEET to affect twice as many biochemical pathways as permethrin. This study also used metabolic expression profiles to support our findings from the transcriptional results by comparing overlaps between the two "omics" pipelines. With the collective findings, our study provides a foundation to determine the molecular targets for pesticide treatments and may prove useful when developing tick-specific chemical control methods.

Survival analyses were conducted to identify non-lethal doses of pesticides which we used for our experiments to ensure that the molecular responses we observed are potential contributors to tick pesticide resistance. A closer look at the differentially expressed transcripts at these dosage levels revealed DEET and permethrin to increase expression of several GO categories that align with previous observations on these chemicals (Bissinger and Roe, 2010; Koloski et al., 2019). Specifically, DEET has been reported to block or alter ion channels (DeGennaro, 2015; Pellegrino et al., 2011; Swale et al., 2014); our RNA-seq analysis revealed ion binding activity to be increased after DEET exposure. This increased expression is likely a cellular response attempting to compensate for the ion channel blockage. Likewise, past studies indicate that permethrin is a potent inhibitor of mitochondrial complex I - an enzyme also known as NADH oxidoreductase (Gassner et al., 1997; Knowles, 1997; Van Leeuwen et al., 2010). Our analysis has revealed that permethrin exposure leads to increased oxidoreductase activity, which is also likely a response to the pesticide's inhibition of complex I, as increasing this complex's activity would help counter the effects of the pesticide. For the nerve poisons chlorpyrifos and propoxur, these

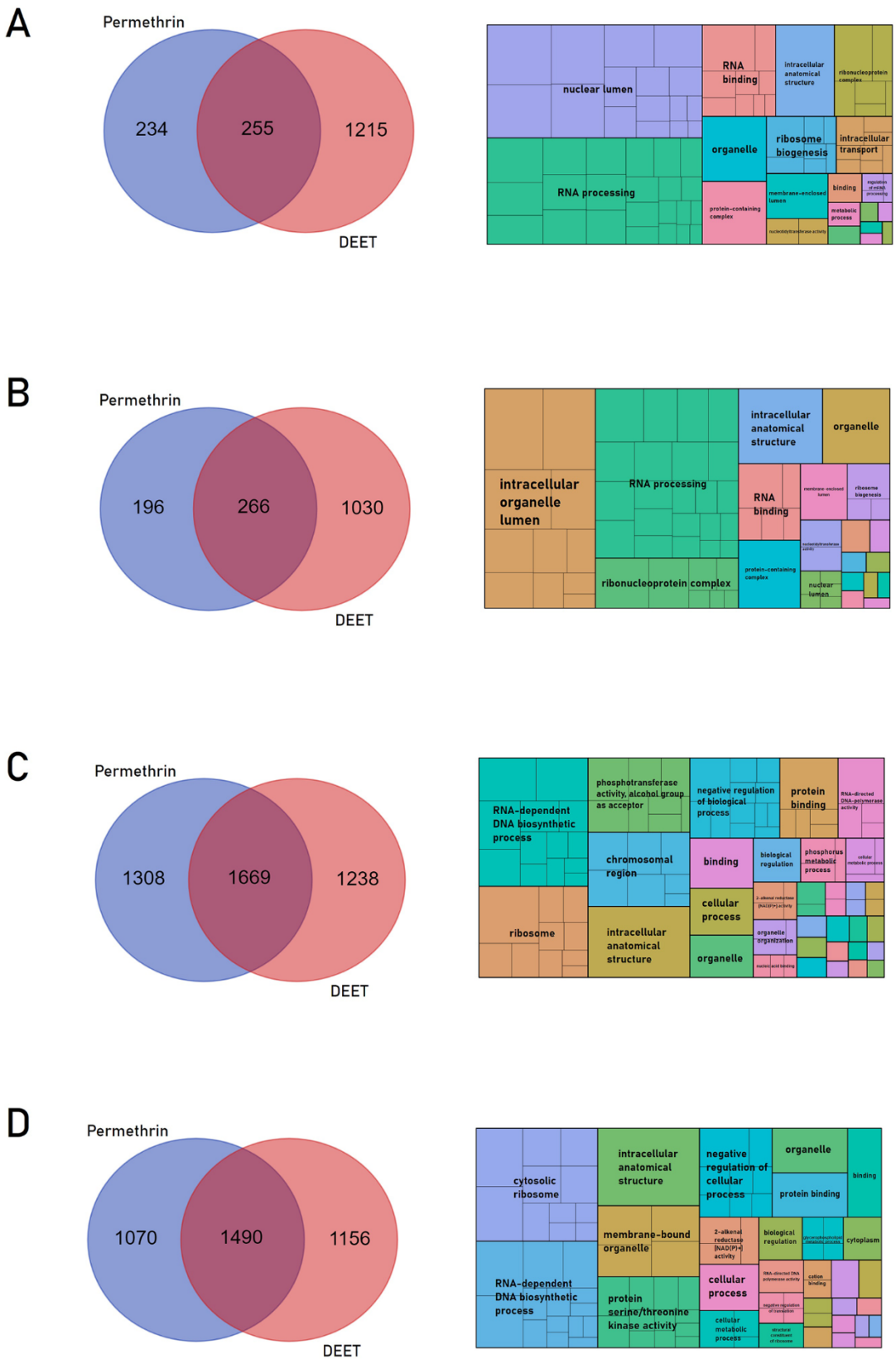

Fig. 6. Specific genes and gene ontologies (GO) of interest in permethrin and DEET exposed ticks at multiple time points, and their relevant molecular functions, cellular components, and biological processes visualized using treemaps. (A) Quantities of blast hits associated with upregulated transcripts after treatment, compared to control (left) and visualized gene ontology (GO) (right) at early times after exposure (0-6 hours), (B) upregulated blasted transcript quantities (left) and visualized gene ontology (GO) (right) at late times after exposure (24 hours or longer), (C) quantities of blasted transcripts downregulated after treatment, compared to control (left) and visualized gene ontology (GO) (right) at early times after exposure, (D) downregulated transcript quantities (left) and visualized gene ontology (GO) (right) at late times after exposure. The boxes in the treemaps represent the unique functional categories while the colors represent the major GO groups. Venn diagrams and treemaps were generated using Bioinformatics & Evolutionary Genomics (http://bioinformatics.psb.ugent.be/webtools/Venn/) and REVIGO.

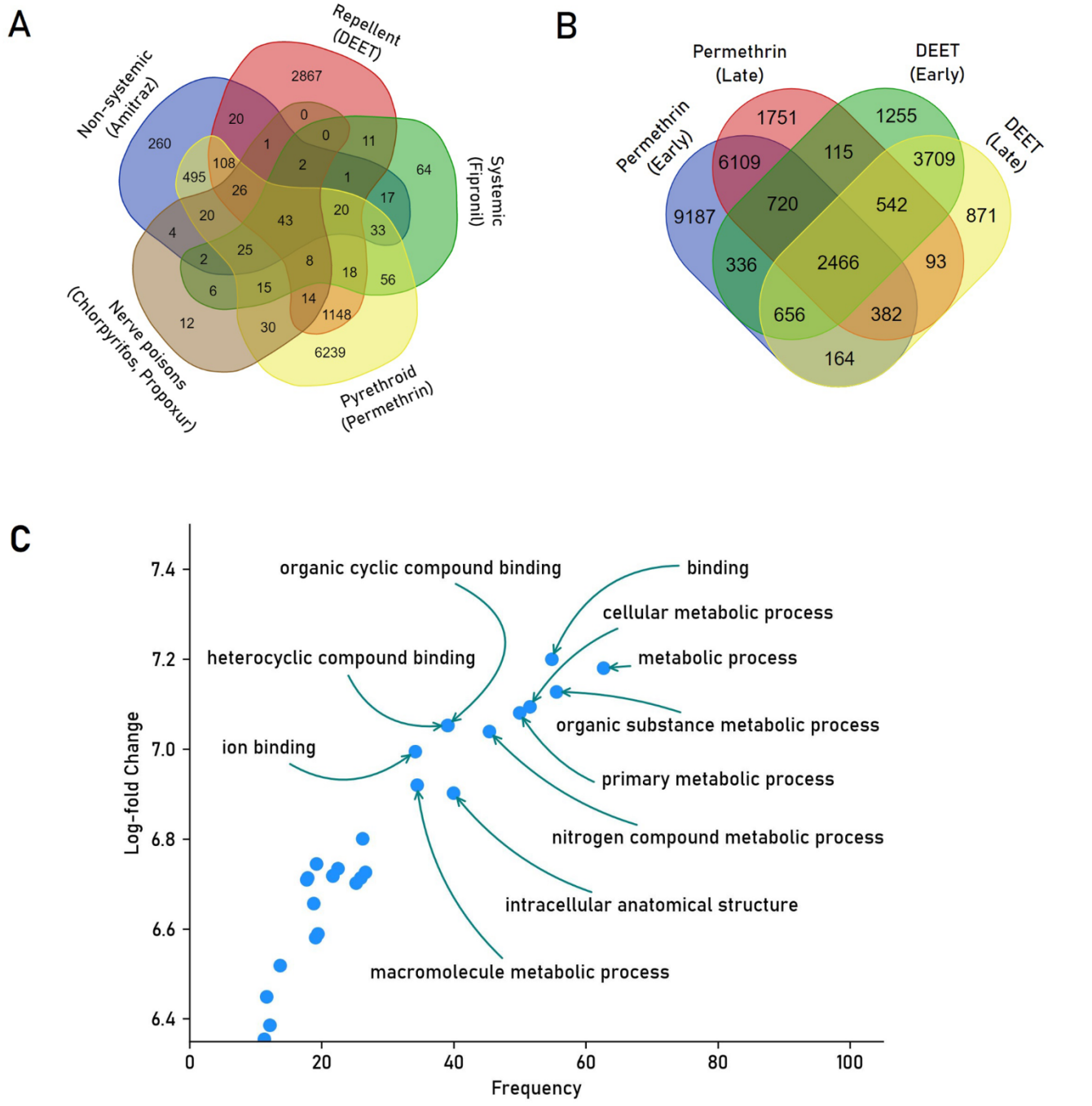

Fig. 7. Enriched transcript quantities in American dog ticks exposed to different chemical treatments. (A) Quantities of transcripts having significant differential expression in tick samples collected (A) after exposure to five treatment classes, (B) at varying time points following permethrin or DEET treatment. Venn diagrams generated using Bioinformatics & Evolutionary Genomics, (C) highly enriched sequences (> 1000 unique reads and > 6-fold difference) conserved for the common transcripts following permethrin and DEET exposure at multiple time points. The circles represent highly enriched gene ontology (GO) terms; especially notable categories are labeled.

chemicals evoked downstream effects on nucleotidyl-transferase enzyme activity and catalytic activity in ion channels, which may be due to the rapid excitatory or inhibitory synaptic transmission of neurotransmitters in the voltage-gated ion channels of the tick nervous system (Fukuto, 1990; Kovacic and Somanathan, 2012; Meijer et al., 2014). Nerve poisons inhibit acetylcholinesterase (Baxter et al., 1999; Janadaree Bandara and Parakrama Karunaratne, 2017), which might lead to an upregulation in kinase activity, as found in our propoxur treated tick samples. Serine-type peptidase activity was downregulated significantly after amitraz exposure, which interfered with cellular communication and ultimately disrupted cellular anatomical systems (Bravo et al., 2008; Fragoso-Sa et al., 2011; Neelu et al., 2016; Santos et al., 2012). Amitraz produces cytotoxic effects in ticks by producing ultrastructural changes through the inhibition of octopamine regulators (Kanapadinchareveetil et al., 2019) and resistance is associated with increased expression profiles to preserve synaptic transmission during exposure to amitraz (Baron et al., 2018). In addition to these pesticide-specific changes, we also found many other GO categories; however, these terms were found in multiple pesticide exposure profiles and are therefore unlikely to represent the unique activities of any single

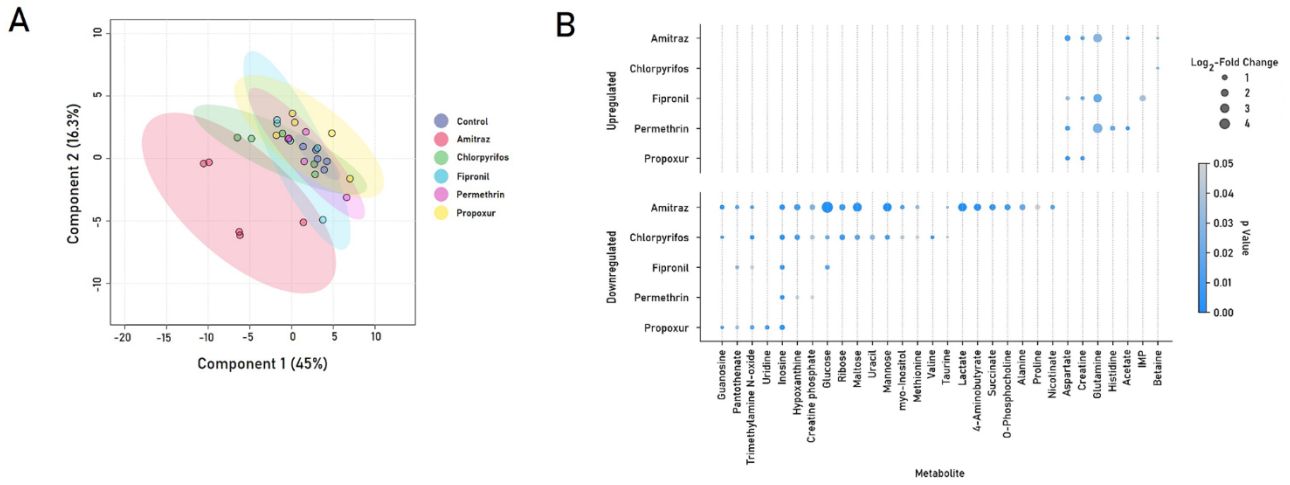

Fig. 8. Metabolome comparison between chemical treatment groups of American dog ticks, *Dermacentor variabilis*. (A) Each point of the plot represents the metabolic signature of one tick homogenate (5 ticks per homogenate). The scores plot of the partial least-squares discriminant analysis (PLS-DA) separates the metabolites in both directions of the principal components (PC1 and PC2). The separate clustering of the samples indicate that the metabolomes are significantly different. (B) dot-plot of enriched metabolites from experimental tick specimens following pesticide or repellent exposure. The upregulated metabolites are represented in the top, and the downregulated metabolites are represented in the bottom. Figure generated using Metaboanalyst (Xia et al. 2013); (Xia and Wishart 2010) and Python 3.7 (scripts uploaded to GitHub).

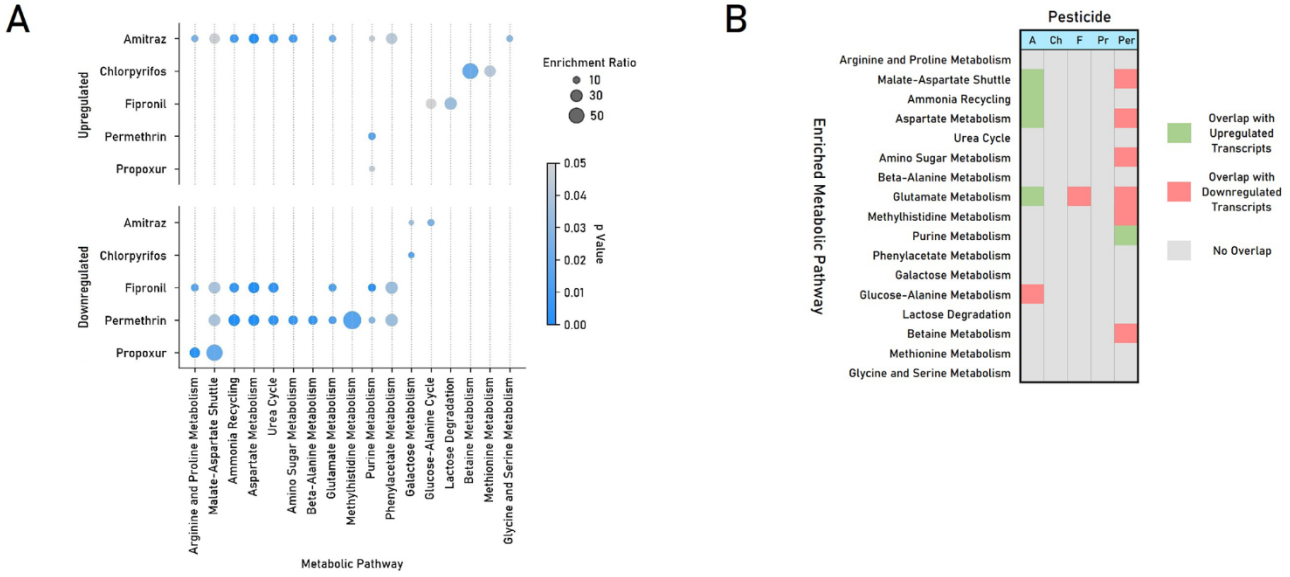

Fig. 9. Enriched metabolic pathway analysis and comparison with transcriptomes. (A) Dot-plot of seventeen enriched metabolic pathways following treatment with five pesticides. The upregulated metabolic pathways are represented in the top, and the downregulated metabolic pathways are represented in the bottom. (B) Related biomolecular pathways found in both metabolic and transcriptomic data are visualized, representing the overlap between the two analyses. Figures generated using Metaboanalyst (Xia et al. 2013); (Xia and Wishart 2010) and Python 3.7 (scripts uploaded to GitHub).

pesticide. These GO terms include broad functions such as DNA polymerase activity, ribosome activity, and mRNA processing. However, of interest is one pathway common across all treatments: neurotransmitter receptor transport. Irregular expression of this pathway likely alters the number of receptors on the cell surface, which may hinder the cell response to neurotransmitters in the extracellular matrix. Most of the pesticides used in this study shared similarities with peripheral neuropathy (Keifer and Mahurin, 1997), which is indicated by the down-regulation of this biological process (GO:BP) following treatment, as observed in our experiment.

In addition to transcript-level changes, we also observed alterations in the tick metabolome following exposure to the different pesticides. An enrichment analysis of these metabolites revealed several metabolic pathways to be affected. For ticks exposed to chlorpyrifos, betaine metabolism was found at significantly higher levels. Betaine is a metabolite which plays a role in decreasing and detoxifying homocysteine - a harmful compound (Craig, 2004). Previous studies have discovered that homocysteine levels are elevated in chlorpyrifos-exposed cells (Olsvik, et al., 2015). Thus, it is likely that chlorpyrifos exposure in ticks, which may result in increased homocysteine levels, might be triggering a biochemical response leading to increases in betaine production to counter the harmful effects of the higher levels of homocysteine. Although betaine concentrations were not detected at higher levels in our study, it may be due to a steady-state process where betaine is being used as it is being produced. Betaine, along with glycerol, underlie cross-tolerance to dehydration and cold hardening in the seabird tick, *Ixodes uriae* (Davies et al., 2021), and cold tolerance in the American dog tick (Rosendale et al., 2022). Because

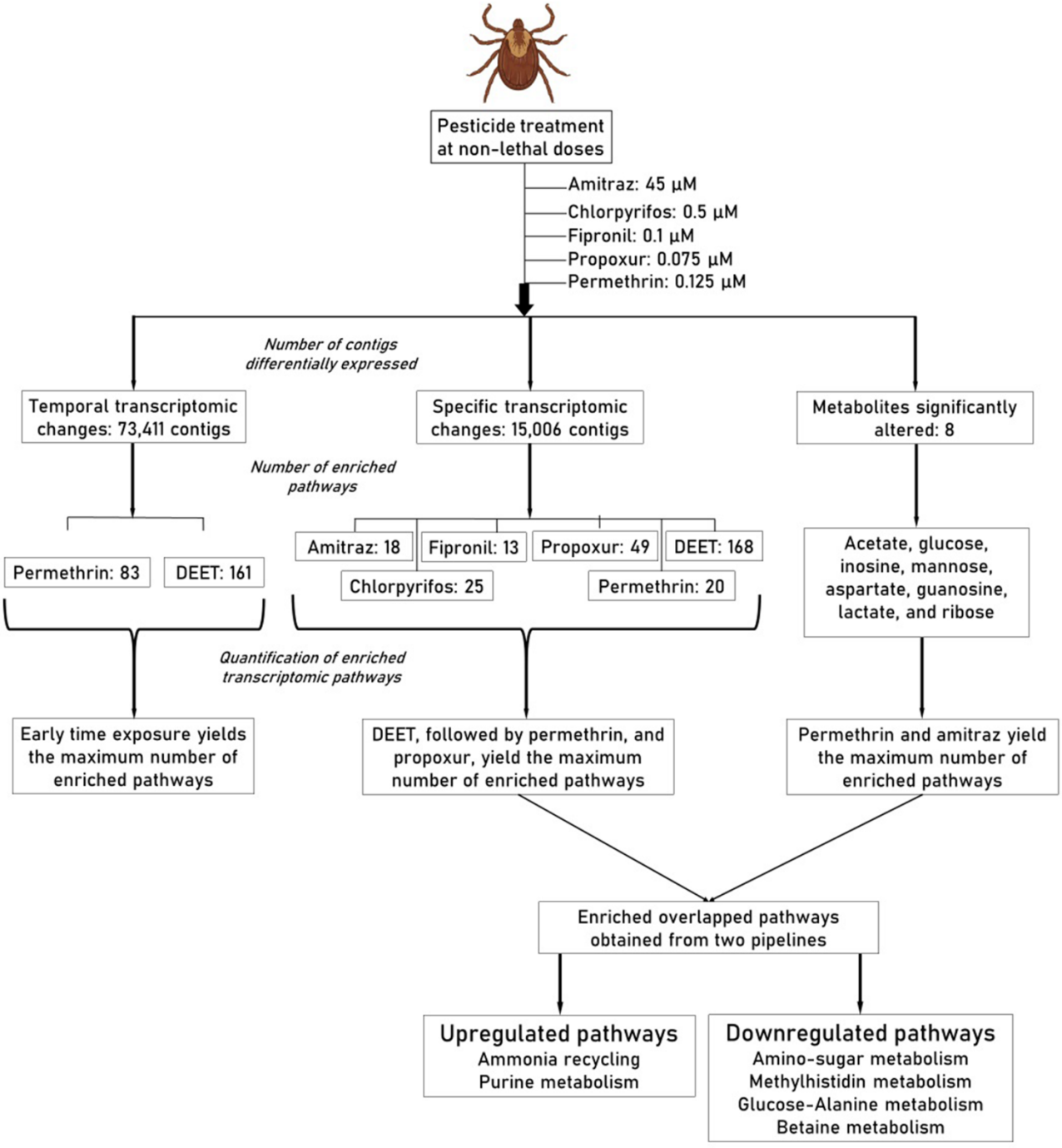

Fig. 10. Summary of experimental pipeline of *Dermacentor variabilis* RNA-seq analysis following pesticide or repellent treatment. The general workflow used for chemical treatment, sample preparation, quantification of transcriptomic and metabolic changes, biomolecule quantification, and experimental data analysis. The greatest number of transcripts were enriched following DEET and permethrin exposure, as well as during early time points. Eight different metabolites from a collection of 53, were significantly altered after the treatments. The impact of two independent pipelines to identify novel biochemical pathways are highlighted based on experimental evidence from this study.

these molecules are critical to hardening genes, they may also be relevant in genes related to pesticide resistance in *D. variabilis*. The enrichment analysis on permethrin-treated ticks revealed purine metabolism to be upregulated. Previous studies on *Drosophila melanogaster* shared similar results, where exposure to permethrin reportedly leads to increases in purine metabolism (Brinzer et al., 2015). In addition to these metabolic changes, we also found decreased levels of maltose, mannose, lactate, and glucose in ticks that were treated with amitraz (Fig. 8B). Decreases in glucose can likely be attributed to the suppression of glucose-alanine cycle and galactose metabolism (Fig. 9A), as found in study. We confirmed a significant number of overlaps using our multi-omics approach in our amitraz and permethrin-treated samples, although overlap was also found in fipronil-treated ticks (Fig. 9B). In addition to these findings, we uncovered novel altered pathways such as amino sugar metabolism, which was downregulated following permethrin treatment. These overlapping pathways are likely prime targets for future pesticide development.

When analyzing the effect of permethrin and DEET at multiple times,

we report transcript expression to be markedly different in the early (<6 hr) versus later (24 hr) hours. In particular, we noticed significantly decreased transcriptomic responses during later time points in both permethrin and DEET-treated samples (Fig. 7B). Earlier studies with rats discovered that permethrin is metabolized and excreted relatively early and the greatest toxicity symptoms are observed between 3 to 6 hours after treatment (Anadón et al., 1991). As a result, during the later time points, the effects of permethrin become less significant, with the intracellular calcium concentrations and Na^{+}/K^{+} ATPase activity reverting back to their normal state (Anadón et al., 1991). A different study on American dog ticks exposed to DEET yielded similar results; ticks undergo a gradual homeostatic recovery over a 24 hr time course following exposure (Koloski et al., 2019). These studies correspond with our findings that the greatest transcriptional differences were observed during the early time points (Fig. 7B). In addition to time-course differences, our experiment highlights the differences in the molecular targets of permethrin and DEET in American dog ticks. One of the major pathways affected by permethrin during early time points is magnesium ion binding. In DEET-treated samples, we found glycophospholipid metabolic processes, intracellular transport, and regulation of mitotic cell cycle to be significantly altered (Supplement 6). These findings are similar to previous research where it has been shown that permethrin inhibits calcium magnesium-ATPase, ultimately leading to excess neurotransmitter release (Linnett, 2008). The distinct expression profiles of permethrin and DEET-exposed ticks underscore previously reported differences in the modes of action between the two chemicals (Gassner et al., 1997; Swale et al., 2014). In particular, permethrin is known to interfere with sodium channels in arthropods, altering neural transmission and causing paralysis or death (USEP, 2008; Thomas et al., 2020; Tomlin, 1997), while DEET reduces transcripts encoding cytochrome P450 and acetylcholinesterase, causing muscle spasms (Koloski et al., 2019). Altogether, our results highlight the fundamental differences between the modes of action of the two distinct classes of chemical treatments and quantify the differences in their actions over time.

The transcriptional profiles determined in this study may represent aspects that are key in pesticide-resistant tick populations (Adelman et al., 2011; Zhu et al., 2013). For example, cytochrome P450 plays a role in metabolic detoxification of acaricides, leading to pesticide resistance among cattle ticks (Le Gall et al., 2018). Extensive research has previously been conducted on bedbugs, wherein it was discovered that strains resistant to pyrethroids contain increased expression of certain genes (Zhu et al., 2013). Another study conducted by Mamidala et al. (2012) compared the transcriptomes of pesticide-resistant (PR) and pesticide-susceptible (PS) strains of the bed bug, *Cimex lectularius,* and found a significant number of the upregulated transcripts in PR bed bugs to be related to defense genes that help confer resistance (Mamidala et al., 2012). In our experiment, those transcripts we identified as highly upregulated following exposure to pesticides may likewise be associated with defense genes that play a role in resistance. These molecular defense mechanisms can be further studied to devise future treatments that limit the defensive response ticks exhibited when exposed to DEET repellent and other pesticides. For example, designing new chemicals or nanoparticles that block nerve transmission in ticks for longer periods of time could prove effective for managing the pest by controlling the sensory pathways. This approach may be even more effective if targeted towards the Haller's organ - the tick's main sensory organ. A recent study reports that Haller's organ is capable of detecting volatile compounds including DEET; if the organ is dysfunctional, it can lead to the suppression of detoxification, lipid metabolism, and immunity responses in the tick, thus increasing its vulnerability to the repellent (Koloski and Cassone, 2021). A deeper understanding of the neurobiology behind the Haller's organ will be helpful for formulating new chemicals that can specifically target this organ, rather than relying on general repellents or poisons.

Applying chemicals is a proven method for preventing tick population growth outdoors. However, a recent expansion of tick habitats due to climate change and reports of pesticide-resistant tick populations call for the development of more-effective control options and strategies for applying treatments in a more efficient, timely manner. This study provides an in-depth overview of American dog ticks' system biology including its expressional and metabolomic alterations following exposure to six chemical treatments. Our cross-disciplinary approach revealed that different treatments induce unique molecular changes in the tick system, underscoring the different modes of action of each chemical intervention. These studies lay the groundwork for future projects examining tick-pesticide dynamics at the molecular and biochemical level.

Supplementary Material 1: Percent of raw reads of *Dermacentor variabilis* mapped to *Ixodes scapularis* genome and number of reads annotated per sample. Results of each RNA-seq treatment following chemical exposure of *Dermacentor variabilis* mapped to predicted genes from reference gene set. RNA-seq datasets are available under the following NCBI Bioprojects: PRJNA783667

Supplementary Material 2: RNA-seq results for *Dermacentor variabilis* gene set following treatments. Expression values are in transcripts per million (TPM). RNA-seq datasets are available under the following NCBI Bioprojects: PRJNA783667

Supplementary Material 3: RNA-seq results for *Dermacentor variabilis* gene set following different times after identical treatments. Expression values are in transcripts per million (TPM). RNA-seq datasets are available under the following NCBI Bioprojects: PRJNA783667

Supplementary Material 4: Representative categories for the gene ontologies of significantly enriched WGCNA modules for six chemical-treated *Dermacentor variabilis* samples. Frequency denotes the percent of the GO term in the protein annotation database, where higher GO term frequencies imply more general terms while lower frequencies imply more specific ones.

Supplementary Material 5: Pathways enriched in *Dermacentor variabilis* following chemical treatment. The GO categories are grouped based on biological process, cellular component, or molecular function.

Supplementary Material 6: Representative categories for the gene ontologies of significantly enriched WGCNA modules for permethrin and DEET treated *Dermacentor variabilis* samples at multiple time points. Frequency denotes the percent of the GO term in the protein annotation database, where higher GO term frequencies imply more general terms while lower frequencies imply more specific ones.

Supplementary Material 7: Pathways enriched in *Dermacentor variabilis* at varied time points following permethrin and DEET treatment. The GO categories are grouped based on biological process, cellular component, or molecular function.

Supplementary Material 8: Functional annotation of core transcripts after different chemical treatments. The core transcripts following exposure to several treatments were blasted against three databases: *Ixodes scapularis* RefSeq database, *Drosophila melanogaster* SwissProt database, and *Dermacentor variabilis* RefSeq database.

Supplementary Material 9: Functional annotation of core transcripts following permethrin and DEET treatment at varied time points. The core transcripts after permethrin and DEET exposure at multiple time points were blasted against three databases: *Ixodes scapularis* RefSeq database, *Drosophila melanogaster* SwissProt database, and *Dermacentor variabilis* RefSeq database.

Supplementary Material 10: Quantification of significantly altered metabolites after pesticidal exposure. One-way analysis of variance (ANOVA) reveals eight metabolites to be significantly changed (left), and concentrations of those eight metabolites in the *Dermacentor variabilis* following pesticide exposure.

Supplementary Material 11: Principal component analysis (PCA) scores plot of control and pesticide-treated tick samples. Both the principal components PC1 and PC2 clustered separately. These PCs explain 65.3% of the observed variance.

CRediT authorship contribution statement

Atit Pathak: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Souvik Chakraborty:** Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Kennan Oyen:** Data curation, Investigation, Funding acquisition, Methodology, Writing – review & editing. **Andrew J. Rosendale:** Data curation, Investigation, Funding acquisition, Methodology, Writing – review & editing. **Joshua B. Benoit:** Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request (metabolomic results) or has been submittd to the required data repository.

Acknowledgements

We thank the anonymous reviewers for their constructive comments on the paper. This work was supported by the United States Department of Agriculture's National Institute of Food and Agriculture Grant 2016-67012-24652 to A.J.R. Funding was also provided by the University of Cincinnati as a Faculty Development Research Grant and, in part, through the National Science Foundation DEB-1654417 and National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number R01AI148551 to J.B.B. K.J.O was funded by the David H. Smith Conservation Fellowship.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2022.102033.

References

Abbas, R.Z., Zaman, M.A., Colwell, D.D., Gilleard, J., Iqbal, Z., 2014. Acaricide resistance in cattle ticks and approaches to its management: the state of play. Vet. Parasitol. 203, 6–20. https://doi.org/10.1016/j.vetpar.2014.03.006.

Adelman, Z.N., Kilcullen, K.A., Koganemaru, R., Anderson, M.A.E., Anderson, T.D., Miller, D.M., 2011. Deep sequencing of pyrethroid-resistant bed bugs reveals multiple mechanisms of resistance within a single population. PLoS One 6, e26228. https://doi.org/10.1371/journal.pone.0026228.

Afgan, E., Baker, D., Batut, B., van den Beek, M., Bouvier, D., Cech, M., Chilton, J., Clements, D., Coraor, N., Grüning, B.A., Guerler, A., Hillman-Jackson, J., Hiltemann, S., Jalili, V., Rasche, H., Soranzo, N., Goecks, J., Taylor, J., Nekrutenko, A., Blankenberg, D., 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic Acids Res. 46, W537–W544. https://doi.org/10.1093/nar/gky379.

Ajith Kumar, K.G., Sharma, A.K., Kumar, S., Ray, D.D., Rawat, A.K.S., Srivastava, S., Ghosh, S., 2016. Comparative in vitro anti-tick efficacy of commercially available products and newly developed phyto-formulations against field collected and resistant tick lines of *Rhipicephalus* (*Boophilus*) *microplus*. J. Parasit. Dis. 40, 1590–1596. https://doi.org/10.1007/s12639-015-0736-3.

Aktar, M.W., Sengupta, D., Chowdhury, A., 2009. Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip. Toxicol. 2, 1–12. https://doi.org/10.2478/v10102-009-0001-7.

Anadón, A., Martinez-Larrañaga, M.R., Diaz, M.J., Bringas, P., 1991. Toxicokinetics of permethrin in the rat. Toxicol. Appl. Pharmacol. 110, 1–8. https://doi.org/10.1016/0041-008x(91)90284-l.

Andrews, S, 2010. FastQC: A quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/. https://doi.org/10.1093/nar/gky379.

Attardo, G.M., Abd-Alla, A.M.M., Acosta-Serrano, A., Allen, J.E., Bateta, R., Benoit, J.B., Bourtzis, K., Caers, J., Caljon, G., Christensen, M.B., Farrow, D.W., Friedrich, M., Hua-Van, A., Jennings, E.C., Larkin, D.M., Lawson, D., Lehane, M.J., Lenis, V.P., Lowy-Gallego, E., Macharia, R.W., Malacrida, A.R., Marco, H.G., Masiga, D., Maslen, G.L., Matetovici, I., Meisel, R.P., Meki, I., Michalkova, V., Miller, W.J., Minx, P., Mireji, P.O., Ometto, L., Parker, A.G., Rio, R., Rose, C., Rosendale, A.J., Rota-Stabelli, O., Savini, G., Schoofs, L., Scolari, F., Swain, M.T., Takáč, P., Tomlinson, C., Tsiamis, G., Van Den Abbeele, J., Vigneron, A., Wang, J., Warren, W. C., Waterhouse, R.M., Weirauch, M.T., Weiss, B.L., Wilson, R.K., Zhao, X., Aksoy, S., 2019. Comparative genomic analysis of six *Glossina* genomes, vectors of African trypanosomes. Genome Biol. 20, 187. https://doi.org/10.1186/s13059-019-1768-2.

Baron, S., Barrero, R.A., Black, M., Bellgard, M.I., van Dalen, E.M.S., Fourie, J., Maritz-Olivier, C., 2018. Differentially expressed genes in response to amitraz treatment suggests a proposed model of resistance to amitraz in *R. decoloratus* ticks. Int. J. Parasitol. Drugs Drug Resist. 8, 361–371. https://doi.org/10.1016/j.ijpddr.2018.06.005.

Baxter, G.D., Green, P., Stuttgen, M., Barker, S.C., 1999. Detecting resistance to organophosphates and carbamates in the cattle tick *Boophilus microplus*, with a propoxur-based biochemical test. Exp. Appl. Acarol. 23, 907–914. https://doi.org/10.1023/a:1006364816302.

Benoit, J.B., Bose, J., Bailey, S.T., Polak, M., 2020. Interactions with ectoparasitic mites induce host metabolic and immune responses in flies at the expense of reproduction-associated factors. Parasitology 147, 1196–1205. https://doi.org/10.1017/S0031182020000918.

Bergmeier, E., Petermann, J., Schröder, E., 2010. Geobotanical survey of wood-pasture habitats in Europe: diversity, threats and conservation. Biodivers. Conserv. 19, 2995–3014. https://doi.org/10.1007/s10531-010-9872-3.

Bhatia, B., Hillman, C., Carracoi, V., Cheff, B.N., Tilly, K., Rosa, P.A., 2018. Infection history of the blood-meal host dictates pathogenic potential of the Lyme disease spirochete within the feeding tick vector. PLoS Pathog. 14, e1006959 https://doi.org/10.1371/journal.ppat.1006959.

Bissinger, B.W., Roe, R.M., 2010. Tick repellents: Past, present, and future. Pestic. Biochem. Physiol. 96, 63–79. https://doi.org/10.1016/j.pestbp.2009.09.010.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917. https://doi.org/10.1139/o59-099.

Boorgula, G.D.Y., Peterson, A.T., Foley, D.H., Ganta, R.R., Raghavan, R.K., 2020. Assessing the current and future potential geographic distribution of the American dog tick, *Dermacentor variabilis* (Say) (Acari: Ixodidae) in North America. PLoS One 15, e0237191. https://doi.org/10.1371/journal.pone.0237191.

Bravo, M., Henríquez, H., Coronado, A., 2008. In vitro efficacy of amitraz and cypermethrin on *Boophilus microplus* from dairy farms in Lara State. Venezuela. Ann. N. Y. Acad. Sci. 1149, 246–248. https://doi.org/10.1196/annals.1428.032.

Bray, N.L., Pimentel, H., Melsted, P., Pachter, L., 2016. Erratum: Near-optimal probabilistic RNA-seq quantification. Nat. Biotechnol. 34, 888. https://doi.org/10.1038/nbt0816-888d.

Brinzer, R.A., Henderson, L., Marchiondo, A.A., Woods, D.J., Davies, S.A., Dow, J.A.T., 2015. Metabolomic profiling of permethrin-treated *Drosophila melanogaster* identifies a role for tryptophan catabolism in insecticide survival. Insect Biochem. Mol. Biol. 67, 74–86. https://doi.org/10.1016/j.ibmb.2015.09.009.

Butler, R.A., Chandler, J.G., Vail, K.M., Holderman, C.J., Trout Fryxell, R.T., 2021. Spray and pour-on acaricides killed Tennessee (United States) field-collected *Haemaphysalis longicornis* nymphs (Acari: Ixodidae) in laboratory bioassays. J. Med. Entomol. https://doi.org/10.1093/jme/tjab115.

CDC, 2021. Tickborne Disease Surveillance Data Summary [WWW Document]. URL. https://www.cdc.gov/ticks/data-summary/index.html (accessed 9.28.21).

Černý, J., Lynn, G., Hrnková, J., Golovchenko, M., Rudenko, N., Grubhoffer, L., 2020. Management options for *Ixodes ricinus*-associated pathogens: a review of prevention strategies. Int. J. Environ. Res. Public Health 17. https://doi.org/10.3390/ijerph17061830.

Chen, A.C., He, H., Davey, R.B., 2007. Mutations in a putative octopamine receptor gene in amitraz-resistant cattle ticks. Vet. Parasitol. 148, 379–383. https://doi.org/10.1016/j.vetpar.2007.06.026.

Choi, E., Pyzocha, N.J., Maurer, D.M., 2016. Tick-borne illnesses. Curr. Sports Med. Rep. 15, 98–104. https://doi.org/10.1249/JSR.0000000000000238.

Cole, L.M., Nicholson, R.A., Casida, J.E., 1993. Action of phenylpyrazole insecticides at the GABA-gated chloride channel. Pestic. Biochem. Physiol. 46, 47–54. https://doi.org/10.1006/pest.1993.1035.

Coles, T.B., Dryden, M.W., 2014. Insecticide/acaricide resistance in fleas and ticks infesting dogs and cats. Parasit. Vectors 7, 8. https://doi.org/10.1186/1756-3305-7-8.

Craig, S.A.S., 2004. Betaine in human nutrition. Am. J. Clin. Nutr. 80, 539–549. https://doi.org/10.1093/ajcn/80.3.539.

Cui, Q., Lewis, I.A., Hegeman, A.D., Anderson, M.E., Li, J., Schulte, C.F., Westler, W.M., Eghbalnia, H.R., Sussman, M.R., Markley, J.L., 2008. Metabolite identification via the Madison Metabolomics Consortium Database. Nat. Biotechnol. 26, 162–164. https://doi.org/10.1038/nbt0208-162.

Dantas-Torres, F., 2007. Rocky Mountain spotted fever. Lancet Infect. Dis. 7, 724–732. https://doi.org/10.1016/S1473-3099(07)70261-X.

Dantas-Torres, F., Chomel, B.B., Otranto, D., 2012. Ticks and tick-borne diseases: a One Health perspective. Trends Parasitol 28, 437–446. https://doi.org/10.1016/j.pt.2012.07.003.

Davies, B., Rosendale, A.J., Gantz, J.D., Lee, R.E., Denlinger, D.L., Benoit, J.B., 2021. Cross-tolerance and transcriptional shifts underlying abiotic stress in the seabird tick, *Ixodes uriae*. Polar Biol. 44, 1379–1389. https://doi.org/10.1007/s00300-021-02887-4.

DeGennaro, M., 2015. The mysterious multi-modal repellency of DEET. Fly 9, 45–51. https://doi.org/10.1080/19336934.2015.1079360.

del Pino, J., Moyano-Cires, P.V., Anadon, M.J., Díaz, M.J., Lobo, M., Capo, M.A., Frejo, M.T., 2015. Molecular mechanisms of amitraz mammalian toxicity: a

comprehensive review of existing data. Chem. Res. Toxicol. 28, 1073–1094. https://doi.org/10.1021/tx500534x.
Dryden, M.W., 2009. Flea and tick control in the 21st century: challenges and opportunities. Vet. Dermatol. 20, 435–440. https://doi.org/10.1111/j.1365-3164.2009.00838.x.
Elfassy, O.J., Goodman, F.W., Levy, S.A., Carter, L.L., 2001. Efficacy of an amitraz-impregnated collar in preventing transmission of *Borrelia burgdorferi* by adult Ixodes scapularis to dogs. J. Am. Vet. Med. Assoc. 219, 185–189. https://doi.org/10.2460/javma.2001.219.185.
Feyereisen, R., 1995. Molecular biology of insecticide resistance. Toxicol. Lett. 82-83, 83–90. https://doi.org/10.1016/0378-4274(95)03470-6.
Finch, G., Nandyal, S., Perretta, C., Davies, B., Rosendale, A.J., Holmes, C.J., Gantz, J.D., Spacht, D.E., Bailey, S.T., Chen, X., Oyen, K., Didion, E.M., Chakraborty, S., Lee, R.E., Denlinger, D.L., Matter, S.F., Attardo, G.M., Weirauch, M.T., Benoit, J.B., 2020. Multi-level analysis of reproduction in an Antarctic midge identifies female and male accessory gland products that are altered by larval stress and impact progeny viability. Sci. Rep. 10, 19791. https://doi.org/10.1038/s41598-020-76139-6.
Foil, L.D., Coleman, P., Eisler, M., Fragoso-Sanchez, H., Garcia-Vazquez, Z., Guerrero, F. D., Jonsson, N.N., Langstaff, I.G., Li, A.Y., Machila, N., Miller, R.J., Morton, J., Pruett, J.H., Torr, S., 2004. Factors that influence the prevalence of acaricide resistance and tick-borne diseases. Vet. Parasitol. 125, 163–181. https://doi.org/10.1016/j.vetpar.2004.05.012.
Fragoso-Sa, H., Garcia-Vaz, Z., Tapia-Pere, G., Ortiz-Naje, A., Rosario-Cr, R., Rodriguez-, I., 2011. Response of Mexican Rhipicephalus (Boophilus) microplus ticks to selection by amitraz and genetic analysis of attained resistance. J. Entomol. 8, 218–228. https://doi.org/10.3923/je.2011.218.228.
Fukuto, T.R., 1990. Mechanism of action of organophosphorus and carbamate insecticides. Environ. Health Perspect. 87, 245–254. https://doi.org/10.1289/ehp.9087245.
Fu, L., Niu, B., Zhu, Z., Wu, S., Li, W., 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 28, 3150–3152. https://doi.org/10.1093/bioinformatics/bts565.
Gassner, B., Wüthrich, A., Scholtysik, G., Solioz, M., 1997. The pyrethroids permethrin and cyhalothrin are potent inhibitors of the mitochondrial complex I. J. Pharmacol. Exp. Ther. 281, 855–860.
George, J.E., 2000. Present and future technologies for tick control. Ann. N. Y. Acad. Sci. 916, 583–588. https://doi.org/10.1111/j.1749-6632.2000.tb05340.x.
George, J.E., Pound, J.M., Davey, R.B., 2004. Chemical control of ticks on cattle and the resistance of these parasites to acaricides. Parasitology 129 Suppl, S353–S366. https://doi.org/10.1017/s0031182003004682.
Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B.W., Nusbaum, C., Lindblad-Toh, K., Friedman, N., Regev, A., 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29, 644–652. https://doi.org/10.1038/nbt.1883.
Gulia-Nuss, M., Nuss, A.B., Meyer, J.M., Sonenshine, D.E., Roe, R.M., Waterhouse, R.M., Sattelle, D.B., de la Fuente, J., Ribeiro, J.M., Megy, K., Thimmapuram, J., Miller, J. R., Walenz, B.P., Koren, S., Hostetler, J.B., Thiagarajan, M., Joardar, V.S., Hannick, L.I., Bidwell, S., Hammond, M.P., Young, S., Zeng, Q., Abrudan, J.L., Almeida, F.C., Ayllón, N., Bhide, K., Bissinger, B.W., Bonzon-Kulichenko, E., Buckingham, S.D., Caffrey, D.R., Caimano, M.J., Croset, V., Driscoll, T., Gilbert, D., Gillespie, J.J., Giraldo-Calderón, G.I., Grabowski, J.M., Jiang, D., Khalil, S.M.S., Kim, D., Kocan, K.M., Koči, J., Kuhn, R.J., Kurtti, T.J., Lees, K., Lang, E.G., Kennedy, R.C., Kwon, H., Perera, R., Qi, Y., Radolf, J.D., Sakamoto, J.M., Sánchez-Gracia, A., Severo, M.S., Silverman, N., Šimo, L., Tojo, M., Tornador, C., Van Zee, J. P., Vázquez, J., Vieira, F.G., Villar, M., Wespiser, A.R., Yang, Y., Zhu, J., Arensburger, P., Pietrantonio, P.V., Barker, S.C., Shao, R., Zdobnov, E.M., Hauser, F., Grimmelikhuijzen, C.J.P., Park, Y., Rozas, J., Benton, R., Pedra, J.H.F., Nelson, D.R., Unger, M.F., Tubio, J.M.C., Tu, Z., Robertson, H.M., Shumway, M., Sutton, G., Wortman, J.R., Lawson, D., Wikel, S.K., Nene, V.M., Fraser, C.M., Collins, F.H., Birren, B., Nelson, K.E., Caler, E., Hill, C.A., 2016. Genomic insights into the *Ixodes scapularis* tick vector of Lyme disease. Nat. Commun. 7, 10507. https://doi.org/10.1038/ncomms10507.
Hagan, R.W., Didion, E.M., Rosselot, A.E., Holmes, C.J., Siler, S.C., Rosendale, A.J., Hendershot, J.M., Elliot, K.S.B., Jennings, E.C., Nine, G.A., Perez, P.L., Rizlallah, A. E., Watanabe, M., Romick-Rosendale, L.E., Xiao, Y., Rasgon, J.L., Benoit, J.B., 2018. Dehydration prompts increased activity and blood feeding by mosquitoes. Sci. Rep. 8, 6804. https://doi.org/10.1038/s41598-018-24893-z.
Jackson, E.R., 2020. The Pesticide Manual: A World Compendium. J. AOAC Int. 64, 1270–1271. https://doi.org/10.1093/jaoac/64.5.1270a.
Jager, T., Crommentuijn, T., van Gestel, C.A.M., Kooijman, S.A.L.M., 2007. Chronic exposure to chlorpyrifos reveals two modes of action in the springtail *Folsomia candida*. Environ. Pollut. 145, 452–458. https://doi.org/10.1016/j.envpol.2006.04.028.
Janadaree Bandara, K.M.U., Parakrama Karunaratne, S.H.P., 2017. Mechanisms of acaricide resistance in the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* in Sri Lanka. Pestic. Biochem. Physiol. 139, 68–72. https://doi.org/10.1016/j.pestbp.2017.05.002.
Jia, N., Wang, J., Shi, W., Du, L., Sun, Y., Zhan, W., Jiang, J.-F., Wang, Q., Zhang, B., Ji, P., Bell-Sakyi, L., Cui, X.-M., Yuan, T.-T., Jiang, B.-G., Yang, W.-F., Lam, T.T.-Y., Chang, Q.-C., Ding, S.-J., Wang, X.-J., Zhu, J.-G., Ruan, X.-D., Zhao, L., Wei, J.-T., Ye, R.-Z., Que, T.C., Du, C.-H., Zhou, Y.-H., Cheng, J.X., Dai, P.-F., Guo, W.-B., Han, X.-H., Huang, E.-J., Li, L.-F., Wei, W., Gao, Y.-C., Liu, J.-Z., Shao, H.-Z., Wang, X., Wang, C.-C., Yang, T.-C., Huo, Q.-B., Li, W., Chen, H.-Y., Chen, S.-E., Zhou, L.-G., Ni, X.-B., Tian, J.-H., Sheng, Y., Liu, T., Pan, Y.-S., Xia, L.-Y., Li, J., Genome, Tick, Zhao, F., Cao, W.-C., Microbiome Consortium (TIGMIC), 2020. Large-scale comparative analyses of tick genomes elucidate their genetic diversity and vector capacities. Cell 182, 1328–1340.e13. https://doi.org/10.1016/j.cell.2020.07.023.
Kanapadinchareveetil, S., Chandrasekhar, L., Pious, A., Kartha, H.S., Ravindran, R., Juliet, S., Nair, S.N., Ajithkumar, K.G., Ghosh, S., 2019. Molecular, histological and ultrastructural characterization of cytotoxic effects of amitraz on the ovaries of engorged females of *Rhipicephalus* (*Boophilus*) *annulatus*. Exp. Parasitol. 204, 107732 https://doi.org/10.1016/j.exppara.2019.107732.
Keifer, M.C., Mahurin, R.K., 1997. Chronic neurologic effects of pesticide overexposure. Occup. Med. 12, 291–304.
Kivaria, F.M., 2006. Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania. Trop. Anim. Health Prod. 38, 291–299. https://doi.org/10.1007/s11250-006-4181-2.
Knowles, C.O., 1997. Mechanisms of resistance to acaricides. In: Sjut, V. (Ed.), Molecular Mechanisms of Resistance to Agrochemicals. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 57–77.
Koloski, C.W., Cassone, B.J., 2021. Transcriptional profiling of Dermacentor variabilis (Acari: Ixodidae) provides insights into the role of the Haller's organ in spatial DEET recognition. Ticks Tick Borne Dis. 13, 101827 https://doi.org/10.1016/j.ttbdis.2021.101827.
Koloski, C.W., LeMoine, C.M.R., Klonowski, A.R., Smith, C.M., Cassone, B.J., 2019. Molecular evidence for the inhibition of cytochrome p450s and cholinesterases in ticks by the repellent DEET. Ticks Tick Borne Dis. 10, 515–522. https://doi.org/10.1016/j.ttbdis.2018.12.006.
Kovacic, P., Somanathan, R., 2012. Propoxur: a novel mechanism for insecticidal action and toxicity. Rev. Environ. Contam. Toxicol. 218, 141–150. https://doi.org/10.1007/978-1-4614-3137-4_4.
Langfelder, P., Horvath, S., 2008. WGCNA: an R package for weighted correlation network analysis. BMC Bioinform. 9, 559. https://doi.org/10.1186/1471-2105-9-559.
Le Gall, V.L., Klafke, G.M., Torres, T.T., 2018. Detoxification mechanisms involved in ivermectin resistance in the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*. Sci. Rep. 8, 12401. https://doi.org/10.1038/s41598-018-30907-7.
Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550. https://doi.org/10.1186/s13059-014-0550-8.
Mamidala, P., Wijeratne, A.J., Wijeratne, S., Kornacker, K., Sudhamalla, B., Rivera-Vega, L.J., Hoelmer, A., Meulia, T., Jones, S.C., Mittapalli, O., 2012. RNA-Seq and molecular docking reveal multi-level pesticide resistance in the bed bug. BMC Genomics 13, 6. https://doi.org/10.1186/1471-2164-13-6.
Marrs, T.C., 1993. Organophosphate poisoning. Pharmacol. Ther. 58, 51–66. https://doi.org/10.1016/0163-7258(93)90066-m.
Meijer, M., Dingemans, M.M.L., van den Berg, M., Westerink, R.H.S., 2014. Inhibition of voltage-gated calcium channels as common mode of action for (mixtures of) distinct classes of insecticides. Toxicol. Sci. 141, 103–111. https://doi.org/10.1093/toxsci/kfu110.
Meulbroek, L., 2002. The promise and challenge of integrated risk management. Risk Manag. Insur. Rev. 5, 55–66. https://doi.org/10.1111/1098-1616.00006.
Mi, H., Ebert, D., Muruganujan, A., Mills, C., Albou, L.-P., Mushayamaha, T., Thomas, P. D., 2021. PANTHER version 16: a revised family classification, tree-based classification tool, enhancer regions and extensive API. Nucleic Acids Res. 49, D394–D403. https://doi.org/10.1093/nar/gkaa1106.
Mi, H., Thomas, P., 2009. PANTHER pathway: an ontology-based pathway database coupled with data analysis tools. Methods Mol Biol. 563, 123–140.
Minigan, J.N., Hager, H.A., Peregrine, A.S., Newman, J.A., 2018. Current and potential future distribution of the American dog tick (*Dermacentor variabilis*, Say) in North America. Ticks Tick Borne Dis 9, 354–362. https://doi.org/10.1016/j.ttbdis.2017.11.012.
Neelu, S., Veer, S., Shyma, K.P., 2016. In vitro efficacy of commercial preparation of amitraz in *Rhipicephalus (Boophilus) microplus* ticks in north Gujarat. India. Ruminant Science 5, 33–37.
Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., Hens, L., 2016. Chemical pesticides and human health: the urgent need for a new concept in agriculture. Front.. Public Health 4, 148. https://doi.org/10.3389/fpubh.2016.00148.
Olsvik, P.A., Berntssen, M.H.G., Søfteland, L., 2015. Modifying effects of vitamin E on Chlorpyrifos toxicity in Atlantic salmon. PLoS One 10, e0119250. https://doi.org/10.1371/journal.pone.0119250.
O'Neal, S.T., Brewster, C.C., Bloomquist, J.R., Anderson, T.D., 2017. Amitraz and its metabolite modulate honey bee cardiac function and tolerance to viral infection. J. Invertebr. Pathol. 149, 119–126. https://doi.org/10.1016/j.jip.2017.08.005.
Patro, R., Duggal, G., Love, M.I., Irizarry, R.A., Kingsford, C., 2017. Salmon provides fast and bias-aware quantification of transcript expression. Nat. Methods 14, 417–419. https://doi.org/10.1038/nmeth.4197.
Pellegrino, M., Steinbach, N., Stensmyr, M.C., Hansson, B.S., Vosshall, L.B., 2011. A natural polymorphism alters odour and DEET sensitivity in an insect odorant receptor. Nature 478, 511–514. https://doi.org/10.1038/nature10438.
Perera, J., Holsomback, J., 2005. An integrated risk management tool and process. In: 2005 IEEE Aerospace Conference, pp. 129–136. https://doi.org/10.1109/AERO.2005.1559306.
Perez de Leon, A.A., Teel, P.D., Li, A., Ponnusamy, L., Roe, R.M., 2014. Advancing integrated tick management to mitigate burden of tick-borne diseases. Outlooks on Pest Management 25, 382–389. https://doi.org/10.1564/v25_dec_10.
Pound, J.M., Miller, J.A., George, J.E., 2000. Efficacy of amitraz applied to white-tailed deer by the "4-poster" topical treatment device in controlling free-living lone star

ticks (Acari: Ixodidae). J. Med. Entomol. 37, 878–884. https://doi.org/10.1603/0022-2585-37.6.878.
Proudfoot, A.T., 2003. Poisoning with amitraz. Toxicol. Rev. 22, 71–74. https://doi.org/10.2165/00139709-200322020-00001.
Prullage, J.B., Tran, H.V., Timmons, P., Harriman, J., Chester, S.T., Powell, K., 2011. The combined mode of action of fipronil and amitraz on the motility of *Rhipicephalus sanguineus*. Vet. Parasitol. 179, 302–310. https://doi.org/10.1016/j.vetpar.2011.03.041.
Ratra, G.S., Casida, J.E., 2001. GABA receptor subunit composition relative to insecticide potency and selectivity. Toxicol. Lett. 122, 215–222. https://doi.org/10.1016/s0378-4274(01)00366-6.
Raudvere, U., Kolberg, L., Kuzmin, I., Arak, T., Adler, P., Peterson, H., Vilo, J., 2019. g: Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). Nucleic Acids Res. 47, W191–W198. https://doi.org/10.1093/nar/gkz369.
Rosendale, A.J., Dunlevy, M.E., McCue, M.D., Benoit, J.B., 2019. Progressive behavioural, physiological and transcriptomic shifts over the course of prolonged starvation in ticks. Mol. Ecol. 28, 49–65. https://doi.org/10.1111/mec.14949.
Rosendale, A.J., Leonard, R.K., Patterson, I.W., Arya, T., Uhran, M.R., Benoit, J.B., 2022. Metabolomic and transcriptomic responses of ticks during recovery from cold shock reveal mechanisms of survival. J. Exp. Biol. 225, jeb236497. https://doi.org/10.1242/jeb.236497.
Rosendale, A.J., Romick-Rosendale, L.E., Watanabe, M., Dunlevy, M.E., Benoit, J.B., 2016. Mechanistic underpinnings of dehydration stress in the American dog tick revealed through RNA-Seq and metabolomics. J. Exp. Biol. 219, 1808–1819. https://doi.org/10.1242/jeb.137315.
Santos, F.C.C., Vogel, F.S.F., 2012. Amitraz and cypermethrin resistance ticks *Rhipicephalus (Boophilus) microplus* in cattle herds located in Rio Grande do Sul from 2005 to 2011. Revist. Portug. Ciên. Vet. 107, 121–124.
Simão, F.A., Waterhouse, R.M., Ioannidis, P., Kriventseva, E.V., Zdobnov, E.M., 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31, 3210–3212. https://doi.org/10.1093/bioinformatics/btv351.
Sonenshine, D.E., 2018. Range expansion of tick disease vectors in North America: implications for spread of tick-borne disease. Int. J. Environ. Res. Public Health 15, 478. https://doi.org/10.3390/ijerph15030478.
Srivastava, A., Malik, L., Smith, T., Sudbery, I., Patro, R., 2019. Alevin efficiently estimates accurate gene abundances from dscRNA-seq data. Genome Biol 20, 65. https://doi.org/10.1186/s13059-019-1670-y.
Stafford, K.C., 2004. Tick management handbook; an integrated guide for homeowners, pest control operators, and public health officials for the prevention of tick-associated disease. Connecticut Agricultural Experiment Station.
Stafford, K.C., Williams, S.C., Molaei, G., 2017. Integrated pest management in controlling ticks and tick-associated diseases. J. Integr. Pest. Manag. 8, 28. https://doi.org/10.1093/jipm/pmx018.
Supek, F., Bošnjak, M., Škunca, N., Šmuc, T., 2011. REVIGO summarizes and visualizes long lists of gene ontology terms. PLoS One 6, e21800. https://doi.org/10.1371/journal.pone.0021800.
Swale, D.R., Sun, B., Tong, F., Bloomquist, J.R., 2014. Neurotoxicity and mode of action of N, N-diethyl-meta-toluamide (DEET). PLoS One 9, e103713. https://doi.org/10.1371/journal.pone.0103713.
Thomas, D.B., Klafke, G., Busch, J.D., Olafson, P.U., Miller, R.A., Mosqueda, J., Stone, N. E., Scoles, G., Wagner, D.M., Perez-De-Leon, A., 2020. Tracking the increase of acaricide resistance in an invasive population of cattle fever ticks (Acari: Ixodidae) and implementation of real-time PCR assays to rapidly genotype resistance mutations. Ann. Entomol. Soc. Am. 113, 298–309. https://doi.org/10.1093/aesa/saz053.
Tomlin, C., 1997. The Pesticide Manual: A World Compendium. British Crop Protection Council, Alton, UK.
Ulrich, E.L., Akutsu, H., Doreleijers, J.F., Harano, Y., Ioannidis, Y.E., Lin, J., Livny, M., Mading, S., Maziuk, D., Miller, Z., Nakatani, E., Schulte, C.F., Tolmie, D.E., Wenger, R.K., Yao, H., Markley, J.L., 2008. BioMagResBank. Nucleic Acids Res. 36, D402–408. https://doi.org/10.1093/nar/gkm957.
U.S.E.P.A, 2008. Reregistration eligibility decision (RED) for permethrin.
Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W., Tirry, L., 2010. Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: a review. Insect Biochem. Mol. Biol. 40, 563–572. https://doi.org/10.1016/j.ibmb.2010.05.008.
Villatte, F., Ziliani, P., Marcel, V., Menozzi, P., Fournier, D., 2000. A high number of mutations in insect acetylcholinesterase may provide insecticide resistance. Pestic. Biochem. Physiol. 67, 95–102. https://doi.org/10.1006/pest.2000.2478.
Winston, P.W., Bates, D.H., 1960. Saturated solutions for the control of humidity in biological research. Ecology 41, 232–237. https://doi.org/10.2307/1931961.
Wishart, D.S., Tzur, D., Knox, C., Eisner, R., Guo, A.C., Young, N., Cheng, D., Jewell, K., Arndt, D., Sawhney, S., Fung, C., Nikolai, L., Lewis, M., Coutouly, M.A., Forsythe, I., Tang, P., Shrivastava, S., Jeroncic, K., Stothard, P., Amegbey, G., Block, D., Hau, D. D., Wagner, J., Miniaci, J., Clements, M., Gebremedhin, M., Guo, N., Zhang, Y., Duggan, G.E., MacInnis, G.d., Weljie, A.M., Dowlatabadi, R., Bamforth, F., Clive, D., Greiner, R., Li, L., Marrie, T., Sykes, B.D., Vogel, H.J., Querengesser, L., 2007. HMDB: the Human Metabolome Database. Nucleic Acids Res. 35, D521–D526. https://doi.org/10.1093/nar/gkl923.
Xia, J., Broadhurst, D.I., Wilson, M., Wishart, D.S., 2013. Translational biomarker discovery in clinical metabolomics: an introductory tutorial. Metabolomics 9, 280–299. https://doi.org/10.1007/s11306-012-0482-9.
Xia, J., Wishart, D.S., 2010. MSEA: a web-based tool to identify biologically meaningful patterns in quantitative metabolomic data. Nucleic Acids Res. 38, W71–W77. https://doi.org/10.1093/nar/gkq329.
Yoder, J.A., Hedges, B.Z., Benoit, J.B., 2011. Water balance of the American dog tick, *Dermacentor variabilis*, throughout its development with comparative observations between field-collected and laboratory-reared ticks. Int. J. of Acarol. 38, 334–343.
Zhu, F., Gujar, H., Gordon, J.R., Haynes, K.F., Potter, M.F., Palli, S.R., 2013. Bed bugs evolved unique adaptive strategy to resist pyrethroid insecticides. Sci. Rep. 3, 1456. https://doi.org/10.1038/srep01456.