BMC Biology

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Multi-omics analysis reveals diapause-associated lipid remodeling in the fat body of Colorado potato beetle

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Abstract

Background The Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), is a major pest of potato that undergoes diapause (hibernation) to survive harsh winter conditions. Lipid metabolism plays a crucial role in diapause preparation and maintenance. However, the specific changes in lipid composition and their molecular regulation remain unclear. This study integrates lipidomics, transcriptomics, and proteomics to investigate diapause-associated metabolic shifts in the fat body of *L. decemlineata*.

Results We identified significant increases in monounsaturated (e.g., oleic acid) and polyunsaturated (e.g., linoleic acid) fatty acids, as well as phosphatidylethanolamines (membrane lipids) enriched in unsaturated fatty acids during diapause. Transcriptomic analysis revealed the differential expression of genes related to energy metabolism and lipid processing, while qualitative proteomics identified some calcium-associated protein isoforms (regucalcin-like and annexin B9-like) that are uniquely present in diapause. Correlation analysis suggested that specific transcripts, such as *carboxyl ester lipase*, are associated with the regulation of key lipid molecules, including oleic acid and linoleic acid, during diapause. These findings advance our understanding of lipid regulation during diapause and highlight potential molecular targets for disrupting overwintering strategies, which could inform novel pest control approaches.

Conclusions Diapause in the Colorado potato beetle is associated with changes in lipid composition in the fat body, which is mediated through changes at the transcriptome and proteome levels.

Keywords Colorado potato beetle, *Leptinotarsa decemlineata*, Diapause, Lipidome, Transcriptome, Proteome

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Background

Diapause (hibernation) is an alternative developmental pathway that enables insects to endure unfavorable seasonal conditions, including cold winters and hot summers [1, 2]. Insects typically enter diapause at a specific developmental stage, with the timing and decision to initiate diapause influenced by either external environmental factors (facultative diapause) or being an inherent part of their life cycle (obligate diapause) [1]. While diapause generally involves reduced metabolic rates, diapausing insects still require energy, which they obtain by metabolizing stored reserves due to the restricted feeding activity during this period [3, 4]. Consequently, most insects accumulate substantial energy reserves during the preparatory phase to sustain themselves through diapause and subsequent development or migration after diapause [5, 6]. Diapause is also characterized by various physiological alterations that render the insect more resistant to unfavorable conditions, including cold tolerance and dehydration resistance [7].

Lipid metabolism is essential for life, playing a crucial role in various insect activities, including long-distance flights, reproduction, starvation resistance, and diapause [8–10]. The fat body, analogous to vertebrate adipose tissue and liver, is the primary coordination center for lipid metabolism in insects. The fat body also participates in other vital processes, including hormone synthesis, production of hemolymph proteins, detoxification of harmful substances, and defense against pathogens [8, 11]. Adipocytes, the primary constituent of the fat body, store triacylglycerol (TAG) and cholesterol in intracellular lipid droplets, which are crucial for maintaining energy homeostasis and forming cellular membranes [11, 12].

TAGs, composed of three fatty acids (FAs) esterified to a glycerol backbone, are the primary long-term energy storage molecules [13]. The efficiency of TAGs in energy storage compared to sugars and amino acids stems from the anhydrous state and the high number of C-H bonds per unit weight [11, 14]. FAs, the primary energy component of TAGs, can be obtained from dietary sources or synthesized de novo from other energy sources [8, 15]. The latter is particularly important when dietary sources have insufficient lipid content, as is often the case with leaf- or sap-feeding herbivorous insects. The main processes through which lipid classes are synthesized are well established in insect models. For instance, de novo FA synthesis begins with acetyl-coenzyme A (acetyl-CoA), which, in the presence of malonyl-CoA and fatty acid synthase (FAS), undergoes sequential addition of 2-carbon units from malonyl-CoA [8, 15]. However, the regulation of the abundance of more specialized lipids, such as those involved in diapause, remains poorly understood in insects.

Biological membranes are primarily composed of a glycerophospholipid bilayer, within which numerous biologically important molecules, especially proteins, are embedded [16]. Membranes are highly dynamic structures that play central roles in initiating various molecular pathways [17]. Glycerophospholipids contain a phosphate group that is esterified to a polar headgroup. In insects, the major polar groups are choline, which forms phosphatidylcholines (PC), and ethanolamine, which forms phosphatidylethanolamines (PE). Glycerophospholipids exhibit a high degree of structural diversity, and, besides PC and PE, other hydrophilic groups, such as phosphatidylinositol (PI), are found in insects, albeit in lower quantities [18, 19]. Maintaining appropriate membrane fluidity, especially by avoiding lipid rigidification, is crucial during winter diapause. Consequently, some changes in membrane lipid composition, including PE/PC ratios and FA chain double-bond indexes, were observed during diapause in *Pieris napi* butterfly [20].

The Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), is a significant pest of potato plants in Europe and North America. This beetle is also an excellent model for studying lipid metabolism during diapause [21, 22]. During its adult stage, *L. decemlineata* undergoes diapause at depths of ~ 50 cm in the soil. The decision to enter diapause is regulated by photoperiod, with day lengths shorter than 14–15 h triggering the preparatory phase, followed by diapause [23, 24]. In cases of prolonged diapause, the beetle can remain in the soil for over two years without any feeding activity [24, 25]. *L. decemlineata* relies primarily on TAG reserves accumulated during the diapause preparatory phase to meet its energy needs during diapause maintenance and post-diapause development [26, 27]. A recent study has shown that the accumulation of TAG reserves depends on de novo lipogenesis, which is facilitated through the upregulation of enzymes such as acetyl-CoA carboxylase (ACCase) and FAS, as well as lipid transport proteins [23]. The accumulated TAG reserves require the upregulation of lipid storage droplet (LSD) proteins for proper storage [26]. A transcriptomics-based study focusing on the fat body also demonstrated the regulation of various metabolism-related genes during diapause in *L. decemlineata* [22]. Although these studies provided valuable insights into the regulation of lipid accumulation, the regulation of lipid composition during diapause remains understudied in *L. decemlineata* and other insects.

In this study, we examined the fat bodies from field-collected *L. decemlineata* larvae and adults in diapause, non-diapause, and post-diapause. The larvae served as a physiological contrast to validate our methods and to explore whether some diapause-associated changes also occur during larval development. The dissected fat

bodies were subjected to lipidomics to capture changes in lipids, such as FAs, between different life stages of *L. decemlineata* with a focus on diapause. Furthermore, fat bodies from the same life stages were analyzed using RNA-seq to reveal changes in transcript abundance levels, as well as through qualitative proteomics. The findings from lipidomics, transcriptomics, and proteomics were integrated to characterize the roles of the fat body in regulating lipid metabolism during diapause.

Results

Changes in lipid composition

In this study, we conducted a comprehensive lipidomics analysis on fat bodies dissected from *L. decemlineata* adults in diapause, non-diapause, and post-diapause, as well as larvae in their last instar stage. This analysis enabled us to capture changes in lipid composition across different life stages, with a particular focus on the diapause stage (Fig. 1a). Across all samples, 217 lipid molecules were identified, including energy-storing TAG and cellular membrane-forming phospholipids (Additional File 1).

Our analysis revealed distinct lipidomic signatures associated with different life stages of *L. decemlineata*. Principal component analysis (PCA) demonstrated the clustering of the lipid compositions depending on the life stage (PC1: 65.7% and PC2: 19.3%, Fig. 1a). As expected, the larval stage demonstrated the clearest clustering, especially along the PC1 (Fig. 1a), compared to the three adult stages. Distinct clustering was also observed between diapause and non-diapause stages. Interestingly, the post-diapause stage formed a cluster overlapping with that of the diapause stage and was tilting toward the non-diapause cluster along the PC1 (Fig. 1a). The heatmap analysis also suggested a similar pattern where lipid composition undergoes major changes between larvae and adult stages as well as between non-diapause and diapause adult stages (Additional File 2: Fig. S1a). Among adult stages, there were notable differences only when we contrasted non-diapause with diapause or post-diapause stages and the differences were less clear between diapause and post-diapause stages (Additional File 2: Fig. S1a). The correlation analysis between lipid classes showed a highly similar regulation of common lipid types including FAs, diacylglycerols, ceramides, and phosphatidylserines (Additional File 2: Fig. S1b). In contrast, TAG, the main energy storage molecule, showed a negative correlation with almost all other lipid types, including FAs and diacylglycerols, which can be combined to form TAG (Additional File 2: Fig. S1b). These negative correlations suggest a dynamic interconversion between TAG and its component lipids, as well as with other lipid classes, likely reflecting shifts in lipid metabolism related to energy homeostasis and membrane remodeling during different life stages.

Next, we focused specifically on the lipids that were differentially abundant in diapause compared to non-diapause or post-diapause stages to identify lipids associated with diapause. Comparison between diapause and non-diapause stages revealed 9 lipids that were higher in diapause and 19 lipids that were higher in non-diapause (Fig. 1b). The lipids that were significantly higher in diapause mostly included free FAs, including monounsaturated (e.g., oleic acid) and polyunsaturated (e.g.,

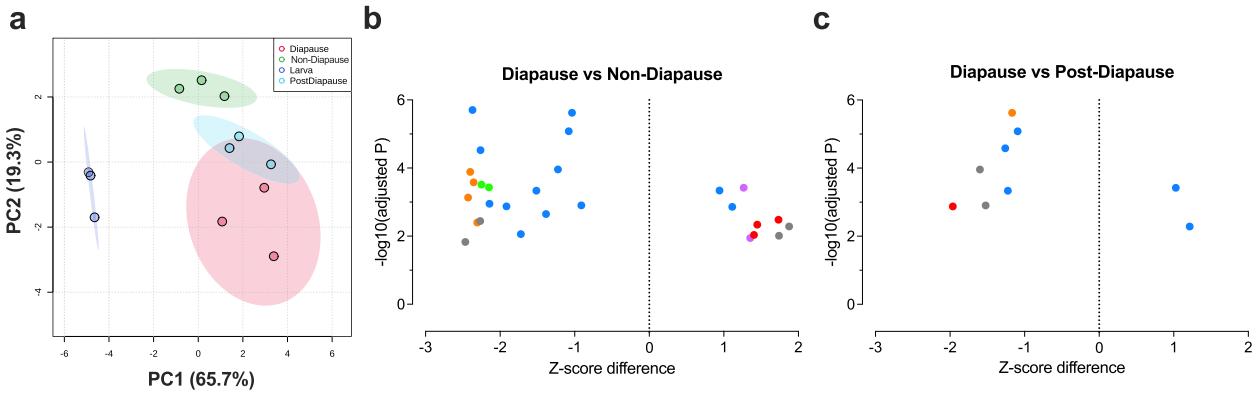


Fig. 1 Changes in lipid compositions within the fat body of different life stages of Colorado potato beetle. Lipidomics was performed through liquid chromatography with tandem mass spectrometry using three biological replicates (collected from 6 to 7 individuals). **a** Principal component analysis of lipids at different life stages. Each point represents a biological replicate ($n=3$) and the clusters are indicated by colored shading. **b**–**c** Volcano plots showing lipids that were significantly differentially abundant (adjusted $P<0.05$) in the comparison between diapause vs non-diapause (**b**) and diapause vs post-diapause (**c**). PC: phosphatidylcholine (green), PE: phosphatidylethanolamines (blue), PG: phosphatidylglycerol (purple), PI: phosphatidylinositol (red), FA: fatty acid (orange), and other lipids (gray). Further details are provided in Additional File 1 and Additional File 2: Fig. S1

linoleic acid) FAs, and membrane phospholipids such as a phosphatidylethanolamine (PE 18:1/20:2 C43 H80 N O8 P, molar mass: 769.6), a glycerophospholipid with an ethanolamine headgroup. The lipids that were higher in non-diapause compared to diapause also included phosphatidylethanolamines (membrane lipids) but with less unsaturated FA chains (e.g., PE 16:0/18:0 C39 H78 N O8 P, molar mass: 719.5), phosphatidylglycerols (membrane lipids, e.g., PG 16:0/18:1 C40 H77 O10 P, molar mass: 748.5), and phosphatidylinositols (membrane lipids, e.g., PI 18:0/18:1 C45 H85 O13 P, molar mass: 864.5). Yet, we observed other PE that contained more unsaturated carbon chains, such as PE 18:1/18:2 (C41 H76 N O8 P, molar mass: 741) that was significantly more abundant during diapause (Fig. 1b and Additional File 1). Comparison between diapause and post-diapause stages did not reveal many differentially abundant lipids (2 higher in diapause and 7 higher in post-diapause) (Fig. 1c), and most of the differentially abundant lipids overlapped with the diapause vs. non-diapause comparison, which are consistent with the PCA analysis (Fig. 1a).

Differential transcript abundance

To complement our lipidomics analysis, we performed RNA-seq on fat bodies from the same individuals to investigate changes at the mRNA level. The analysis revealed significant differences in transcript abundance between diapause, non-diapause, and post-diapause stages (Fig. 2a). The transcriptomes formed clear clusters for larvae and diapause, while the clusters for non-diapause and post-diapause adults overlapped (PC1: 31% and PC2: 21%, Fig. 2a). Next, we performed four pairwise comparisons to identify differentially abundant transcripts. The results revealed 3307 and 2477 transcripts that were more abundant in diapause when compared against non-diapause and post-diapause stages, respectively (Additional File 2: Fig. S2 and Additional File 3). In contrast, there were 2021 and 1889 transcripts that had lower abundance in diapause compared to non-diapause and post-diapause stages, respectively (Additional File 2: Fig. S2). We also compared last instar larvae and non-diapausing adults to represent developmental changes, rather than changes related to diapause. Comparison between larvae and non-diapausing adults revealed 1234 transcripts that were more abundant in larvae and 1242 transcripts that were more abundant in non-diapausing adults (Additional File 2: Fig. S2). Hence, the diapause program is associated with a higher number of differentially abundant transcripts than regular developmental changes between larvae and non-diapausing adults.

To get biological insights, we conducted gene ontology (GO) enrichment analysis on the differentially expressed transcripts (Fig. 2b–d). As expected from the analysis of the fat body, most GO terms were related to metabolic processes, including lipid metabolism. Specifically, the analysis showed that monocarboxylic acid catabolic process, organic acid catabolism, oxidative phosphorylation, fatty acid oxidation, and fatty acid beta-oxidation were among the processes that undergo alteration between diapause and non-diapause stages (Fig. 2b). Other top enriched terms were mostly related to either energy metabolism in general (e.g., ATP metabolic process) or lipid metabolism. Interestingly, amino acid metabolism (e.g., cellular amino acid catabolism) related terms were also among the most significantly enriched terms. The comparison between diapause and post-diapause yielded essentially the same enriched terms related to energy and lipid metabolism (Fig. 2d). The comparison between larvae and non-diapausing adults also revealed changes in FA metabolism-related processes but also changes in processes related to insecticide metabolism such as response to DDT and toxin metabolic process (Fig. 2c). Overall, the GO term enrichment analysis revealed changes in transcript abundance related to lipid metabolism at various life stages, which aligns with our lipidomics analysis.

Qualitative proteomics

Qualitative proteomics analysis ($n=1$ per stage) was conducted to determine the presence or absence of protein products at various life stages. Our analysis identified 1480 proteins present in at least one life stage, with a minimum number of peptide hits of 2 and a protein threshold of 95% (see Additional File 4 for details). In the larvae and adults in non-diapause, diapause, and post-diapause stages, 911, 563, 525, and 1225 proteins were detected, respectively.

Next, we focused specifically on the lipid metabolism-related protein isoforms that were identified in different life stages. PCA analysis showed that the lipid metabolism-related protein profiles of diapause and non-diapause were similar, while both were different from that of post-diapause adults and larvae (Fig. 3a). The larval and post-diapause stages were also distinctly separated in terms of lipid metabolism-related protein profiles. Subsequently, we focused on the protein isoforms that were uniquely detected, i.e., isoforms that were only present in one of the life stages based on the liquid chromatography-tandem mass spectrometry (LC–MS/MS) analysis. Interestingly, the post-diapause stage contained the highest number of lipid metabolism-related proteins uniquely identified in a stage (33 proteins) (Fig. 3b). Diapause adults had only 2 unique lipid metabolism-related proteins, while the larval stage had 16 unique proteins. The non-diapause stage did not contain any unique proteins related to

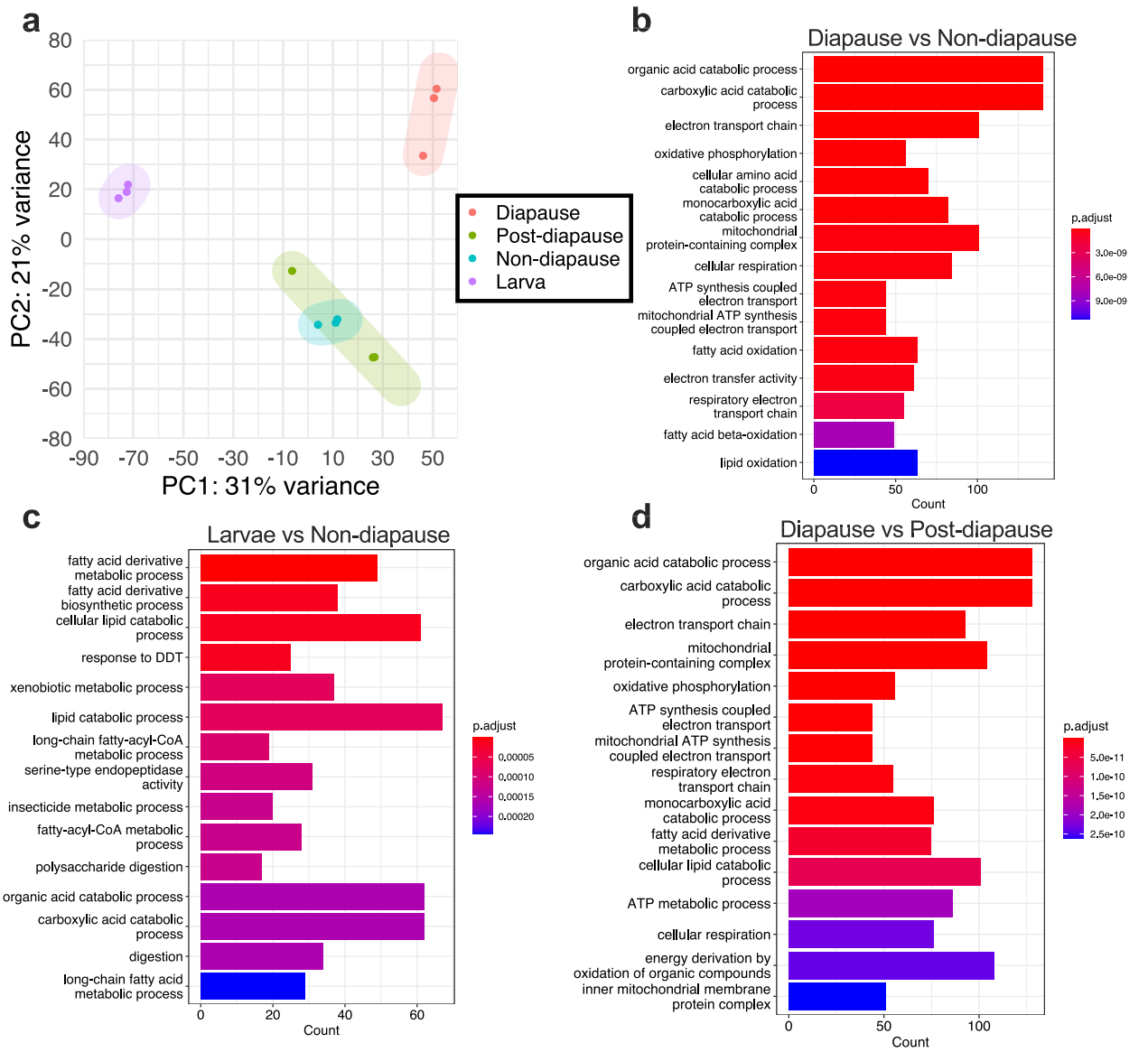


Fig. 2 Differential transcript expression in the fat body of different life stages of Colorado potato beetles. **a** Principal component analysis on the transcriptomes of larvae as well as diapause, non-diapause, and post-diapause adult Colorado potato beetle ($n=3$). **b**–**d** Gene ontology (GO) term enrichment on the differentially expressed transcripts in the comparison of different life stages. The top 15 most significant biological process hits ($P<0.05$) were selected to be shown in the plots. Due to the ambiguous nature of GO term assignments, significantly upregulated and downregulated genes were combined for the analysis. Details are provided in Additional File 3

lipid metabolism. Lipid metabolism-related proteins uniquely detected in diapause were *regucalcin-like* X1 (30.4 kDa) and annexin B9-like X1 (33.7 kDa) isoforms (Table 1). Top lipid metabolism-related proteins uniquely detected in post-diapause included fatty acid-binding protein (annotated as adipocyte isoform), other regucalcin-like X2 (24.0 kDa) and annexin B9-like X2 (35.2 kDa) isoforms, lipid storage droplets surface-binding protein 1, and a mitochondrial enoyl-CoA delta isomerase 1. Interestingly, we identified a lipase and a fatty acid synthase isoform that were uniquely found in the larval proteome.

We also investigated the most abundant proteins (highest percentage of total spectra, $n=1$) at each life stage. In diapause, top proteins included apolipophorin isoform X1 (Additional File 2: Table S1), while in non-diapause, mitochondrial 3-ketoacyl-CoA thiolase and acetyl-CoA acetyltransferase were most abundant (Additional File 2: Table S2). In post-diapause and larval stages, top proteins included calreticulin (Additional

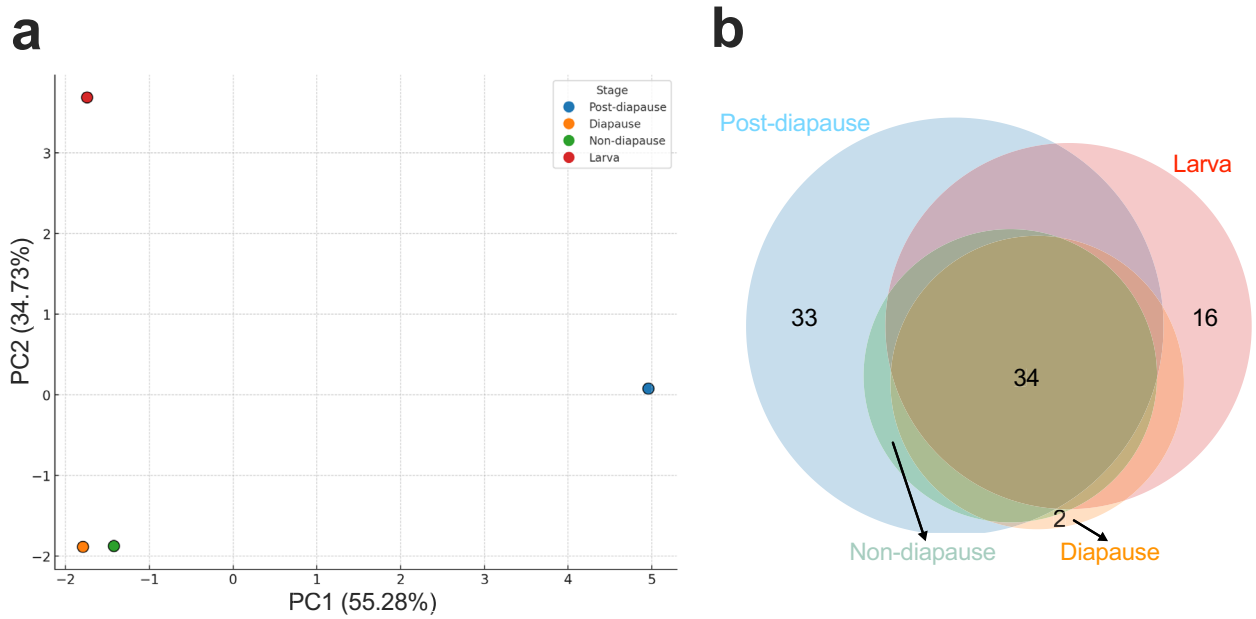


Fig. 3 Lipid metabolism related proteins isolated from fat bodies at different life stages of Colorado potato beetle. **a** Principal component analysis on lipid metabolism related protein presence or absence binary data in different life stages (peptide threshold = 1, protein threshold = 95%, $n = 1$ per life stage). **b** Venn diagram showing the number of lipid metabolism proteins present in different life stages. Post-diapause (blue), diapause (orange), non-diapause (green), larvae (red). Further details are provided in Additional File 4

Table 1 Stage-specific lipid metabolism related proteins and percentage of total spectra in different life stages of Colorado potato beetle that were identified using LC–MS/MS ($n = 1$)

Annotation*	Protein weight (kDa)	Larva	Non-diapause	Diapause	Post-diapause
Regucalcin-like isoform X1	30.4	-	-	0.08	-
Annexin B9-like isoform X1	33.7	-	-	0.01	-
Fatty acid-binding protein, adipocyte isoform X3	14.9	-	-	-	0.44
Regucalcin-like isoform X2	24.0	-	-	-	0.31
Digestive cysteine proteinase 1	62.7	-	-	-	0.12
Annexin B9-like isoform X2	35.2	-	-	-	0.11
Lipid storage droplets surface-binding protein 1 (LSD-1)	13.8	-	-	-	0.08
Enoyl-CoA delta isomerase 1, mitochondrial-like	17.1	-	-	-	0.05
Lipase 3-like	40.9	0.11	-	-	-
Fatty acid synthase	205.4	0.06	-	-	-
Estradiol 17-beta-dehydrogenase 11-like	40.3	0.05	-	-	-
Enoyl-[acyl-carrier-protein] reductase, mitochondrial	42.3	0.04	-	-	-
Long-chain fatty acid transport protein 4	70.8	0.03	-	-	-

* See Additional File 4 for details

File 2: Table S3) and fatty acid synthase (Additional File 2: Table S4), respectively. Although this analysis is limited by the lack of replication, it suggests that the fat body may produce apolipophorins, key components of lipid transport particles in insects, in abundance during diapause to help maintain energy homeostasis.

Coupling lipidomics and transcriptomics

To better understand lipid regulation during diapause, we correlated lipidomics and transcriptomics data. To that end, we selected transcript hits that contributed to the enrichment of lipid metabolism-related GO terms in our transcriptomics study and correlated the

abundance of transcripts and lipids whose abundance was significantly changed in diapause compared to non-diapause. In total, we observed 46 positive and 26 negative significant correlations ($P < 0.01$ by Pearson's correlation) between transcript and lipid pairs (6524 total pairs consisting of 233 transcripts and 24 lipids) (Fig. 4 and Additional File 5).

Oleic acid, a monounsaturated FA, is found in higher abundance in diapause compared to other adult stages (Fig. 5a). The three transcripts showing notable positive correlations with oleic acid abundance ($P < 0.01, \sim 99\%$) included *carboxyl ester lipase* (*cel*), which is capable of hydrolyzing cholesteryl esters, phospholipids, acylglycerols, and ceramide, as well as *abhydrolase domain containing 12B* (*abhd12b*), which is predicted to possess acylglycerol lipase activity. Five transcripts were negatively correlated ($P < 0.01$, 99%) with oleic acid abundance, which included a *palmitoyl-protein thioesterase* (*ppt1*) and *enoyl-CoA Delta Isomerase 1* (*eci1*). According to the qualitative proteomics results, the protein product of *ppt1* was only detectable in post-diapause (label-free quantification intensity (LFQ) = 26.1, $n = 1$). The abundance pattern of linoleic acid, a polyunsaturated FA, closely resembled that of oleic acid, with a peak in abundance during diapause compared to other adult stages. However, the direction of the correlations with the two transcripts was negative (Fig. 5b).

A phosphatidylethanolamine and a phosphatidylinositol, two membrane lipids, showed lower abundance in diapause compared to non-diapause (Fig. 5c and d). The transcripts showing notable correlations with these lipids exhibited positive correlations ($P < 0.01, \sim 99\%$) and included *phytanoyl-CoA dioxygenase* (*phyh*) and *NAD-binding 3-hydroxyacyl-CoA dehydrogenase* (*hadh*) (Fig. 5c and d). The protein product of *phyh* was not detectable in diapause in our qualitative proteomics (LFQ = 0, $n = 1$).

Discussion

The interplay between lipid metabolism and insect physiology has been increasingly recognized, with recent insights emphasizing the role of lipid dynamics in energy regulation, stress responses, and seasonal adaptations [9, 28]. The dynamic regulation of lipid metabolism is also crucial for successful diapause in many insect species [29]. Thus, lipids serve as the primary energy source during diapause and contribute to other diapause-related changes, such as increased cold tolerance [20]. The fat body is recognized as the primary tissue responsible for lipid metabolism, and alterations in this tissue are implicated in diapause [11, 14]. Here, we present coupled lipidomics, transcriptomics, and proteomics analyses, specifically in the fat body of *L. decemlineata* across different life stages, with a focus on diapause. Our findings contribute to the understanding of lipid composition regulation during diapause at the molecular level.

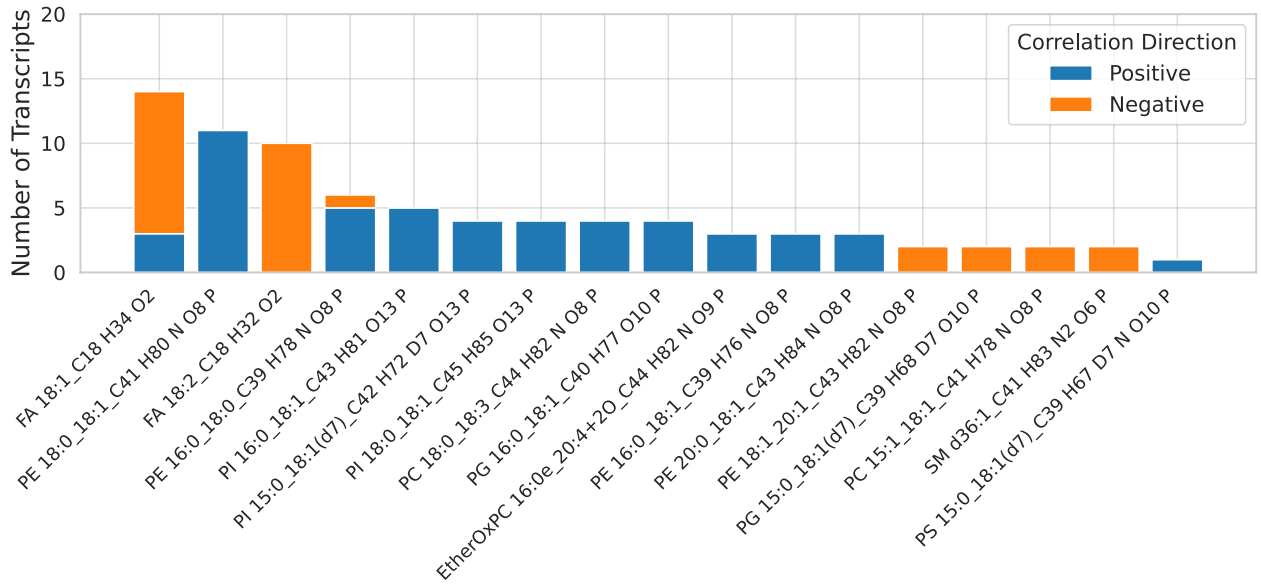


Fig. 4 Coupling transcriptomics and lipidomics in the fat body of the Colorado potato beetle. Pearson's correlations were calculated between transcripts that were differentially abundant in diapause vs. non-diapause as well as associated with lipid metabolism-related gene ontology terms and lipids that were differentially abundant in diapause vs. non-diapause adult stages (Fig. 2). The plot shows the number of significant positive and negative correlations with transcripts per lipid (adjusted $P < 0.01$ by Pearson's correlation, $n = 3$). Further details are provided in Additional File 5

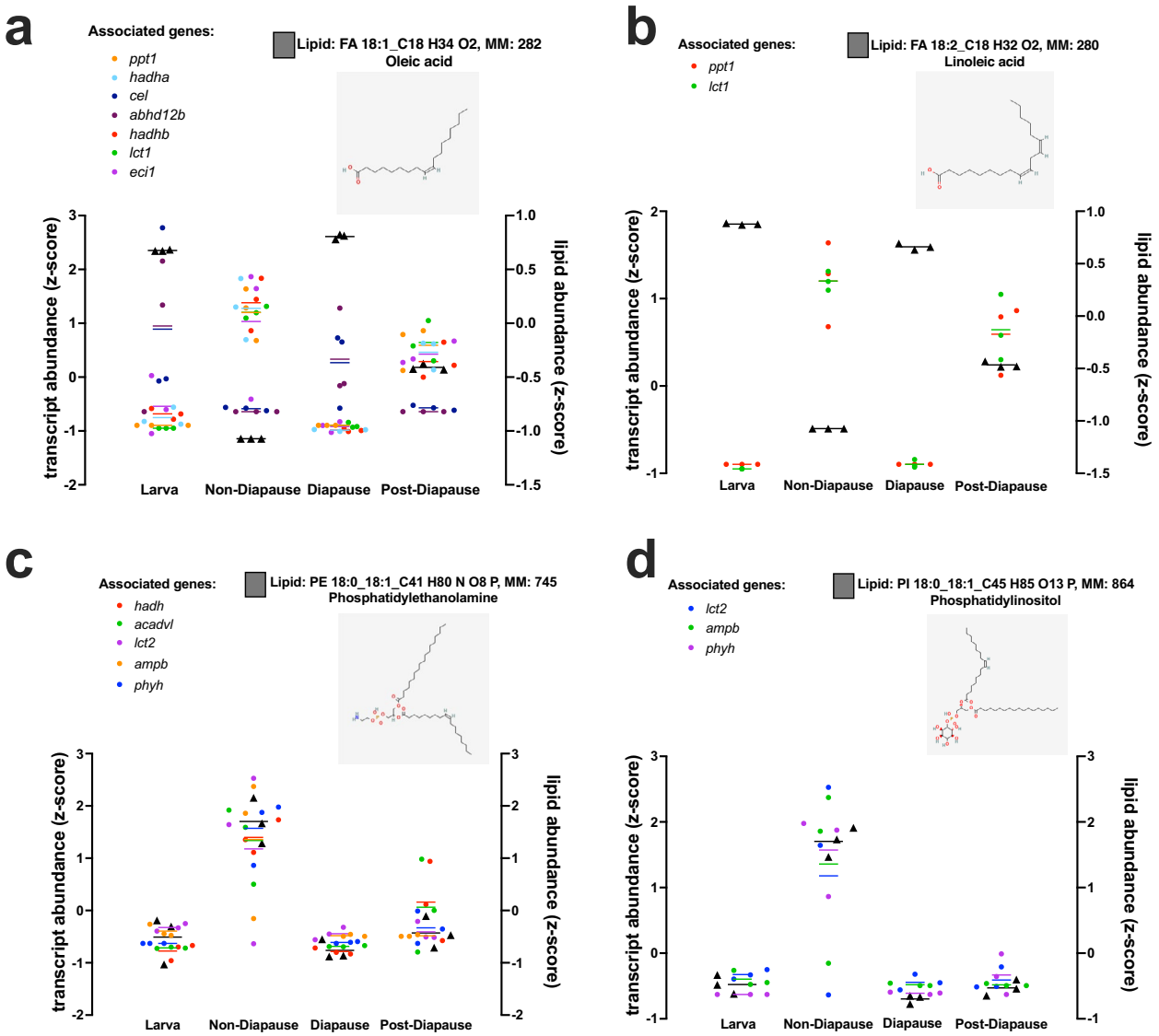


Fig. 5 Selected lipids and associated transcripts in the fat bodies of Colorado potato beetles. The most significant positive and negative Pearson's correlations between lipids and transcripts that were differentially abundant in diapause compared to non-diapause or post-diapause Colorado potato beetles are shown. Plots show the z-score normalized (triangles, $n=3$) abundance of **a** oleic acid, **b** linoleic acid, **c** phosphatidylethanolamine (PE) 18:0/18:1, and **d** phosphatidylinositol (PI) 18:0/18:1 along with the *z*-score normalized (dots, $n=3$) abundance of the significantly correlating transcripts (positive or negative Pearson's correlation above 99% and $P<0.01$, $n=3$). The lines denote the mean *Z*-score per stage. The lipid structures were retrieved from PubChem. Further details are provided in Additional File 5

In this study, we observed that the lipidome of diapausing *L. decemlineata* is characterized by an increased abundance of unsaturated FAs, such as oleic and linoleic acid. These changes were consistent with the transcriptomic data, where many genes involved in FA metabolism were differentially expressed in diapause compared to non-diapause stages. The changes in the lipid composition, supported by differential gene expression, are presumably one of the many adaptations of the diapause program to the cold weather and restricted access to host plants during winter [3, 4, 8]. Our proteomics data did not capture many of the robust changes observed at the transcriptomic level, likely due to its qualitative nature ($n=1$) and the need for further optimization of the protein isolation protocol from fat body samples.

We recently showed that the accumulation of adequate fat stores is crucial during the diapause preparation phase in *L. decemlineata* [23]. Notably, if a sufficient amount of lipid intermediates is not accumulated, *L. decemlineata* delays its entrance into diapause

by several days, likely to feed more for adequate fat accumulation. The previous study also showed that the increase in the activity of de novo lipogenesis enzymes ACCase and FAS was involved in the rapid accumulation of fat stores during the diapause preparatory phase. The results of the current study suggest that the regulation of lipids at the gene expression level is not limited to the accumulation of fat stores but also involves shifts in lipid profiles, including an increased abundance of unsaturated free FAs for diapause. A limitation of the current study was that the diapause preparatory phase was not analyzed; hence, it remained unclear whether these shifts in lipid profiles already occurred during the preparatory phase or during diapause.

Interestingly, the transcriptomics study of the fat body revealed that more transcripts increased in abundance during diapause compared to other life stages. This is in contrast to the notion that diapause entails an overall downregulation of genes for suppressed activity [2, 30, 31]. Furthermore, many enriched GO terms among transcripts differentially abundant with respect to diapause were related to lipid metabolism. Hence, the current study suggests that the fat body remains metabolically active during diapause, presumably to maintain energy homeostasis and membrane integrity throughout overwintering. Previous studies in *L. decemlineata* also support the conclusion that regulation of lipid metabolism and other metabolic changes are key features of diapause [20, 32]. For instance, *lipid storage droplet-2* is upregulated to support the accumulation of substantial TAG stores before diapause in *L. decemlineata* [26] and the Sunn pest *Eurygaster maura*, a hemipteran with a mandatory hibernation [12].

Our findings demonstrate that *L. decemlineata* accumulates unsaturated FAs, particularly oleic and linoleic acids, during diapause, likely to maintain membrane fluidity and cold tolerance [33–35]. The unsaturation of the carbon chains in lipids increases fluidity at low temperatures and is expected to enhance survival rates during winter [36]. The fluidity may also benefit the cell membrane and lipid droplets, which are crucial for energy homeostasis during diapause [35, 37]. Interestingly, a membrane lipid phosphatidylethanolamine (PE 18:0/18:1) had lower abundance in diapause compared to other adult stages, while we observed PE containing more unsaturated carbon chains (e.g., PE 18:1/18:2 and PE 18:1/20:2) increasing in abundance in diapause (Additional File 1). Both are incorporated into the glycerophospholipid bilayer of membranes that exhibit highly diverse composition and structure, which influences biophysical properties and resistance to cold [38]. We hypothesize that the increase in fluidity provided by the increase in unsaturated carbon chains is important for the survival of *L. decemlineata* under cold winter conditions.

Our efforts to couple transcriptomics and lipidomics resulted in candidate genes that may directly or indirectly regulate the dynamic changes in lipid profiles throughout the life cycle, including diapause. For instance, *carboxyl ester lipase*, which hydrolyzes dietary fat, cholesteryl esters, and fat-soluble vitamins [39], showed increased transcript abundance and was positively correlated with rising oleic acid levels during diapause. This suggests that the *carboxyl ester lipase* may contribute to the mobilization of stored lipids, releasing oleic acid from larger lipid reserves to support energy homeostasis or membrane remodeling necessary for maintaining cellular function during diapause. Another interesting gene candidate that decreased in transcript abundance during diapause was annotated as *phytanoyl-CoA dioxygenase*, which catalyzes the alpha-oxidation of phytanic acid, a branched FA resistant to β-oxidation [40, 41]. It is suggested to be required for eggshell formation in the silkworm (*Bombyx mori*) [42]. However, we did not observe a significant change in the levels of phytanic acid in this study (Additional File 1). Hence, this putative *phytanoyl-CoA dioxygenase* might be downregulated during diapause to establish diapause lipid composition (e.g., decrease in saturated carbon chains in membranes) through complex and indirect regulation of the lipid network. A limitation of the current study was the limited functional annotation available for this non-model insect, which prevented us from confidently assigning transcripts to lipid synthesis or breakdown pathways. Consequently, the mechanisms underlying these putative regulatory relationships remain unknown. These hypotheses at the individual gene and lipid levels therefore require functional validation.

Calcium signaling has been implicated in lipid metabolism through the regulation of intracellular calcium levels and interaction with lipid-related enzymes [43]. Interestingly, we identified two calcium-associated proteins, namely regucalcin- and annexin-like isoforms, that were uniquely present in the diapausing *L. decemlineata* proteome. Both proteins could be involved in establishing appropriate lipid metabolism during diapause. Calcium-binding proteins, including annexins, regucalcin, and calmodulin, have been implicated in lipid metabolism and stress responses in various insect species, *L. decemlineata* and *Drosophila montana* [8, 44, 45]. We recently demonstrated that calmodulin, calcineurin, and regucalcin influence fat storage and mobilization, particularly in the context of diapause and starvation in *L. decemlineata* [44]. More specifically, knocking down *regucalcin* transcripts significantly reduced lipid stores, indicating its role in lipid

homeostasis [44]. Overexpression of *regucalcin* leads to TAG catabolism in rat liver, which is analogous to that in the insect fat body, and similarly, it may be necessary for energy homeostasis during diapause [46]. In another study, we found that *calreticulin* and *calnexin*, two ER-resident calcium-binding chaperones, are expressed in the fat body of *L. decemlineata* during diapause, also suggesting a conserved role for calcium-associated proteins in diapause-related metabolic adjustments [47]. Annexin isoforms, on the other hand, are known to bind to membrane regions, exposing negatively charged phospholipids through calcium activation and stabilizing membrane defects [48]. Hence, annexin could be required for the maintenance of cold-resistant membranes during diapause. Moreover, the protein product of an *enoyl-CoA delta-isomerase*, which plays a role in the metabolism of unsaturated FAs [49], was uniquely identified in post-diapause and had higher transcript abundance in post-diapause compared to diapause. Together with the observation that unsaturated FAs are higher in diapause but then decrease in abundance during post-diapause, we suggest that this enoyl-CoA delta isomerase may be important for the catabolism or conversion of unsaturated FAs during post-diapause, as they are no longer required for cold tolerance. However, due to the qualitative nature of our proteomics analysis ($n=1$), these findings require further confirmation.

Interestingly, we observed that the lipids and transcripts of interest were differentially abundant in diapause compared to both other non-diapausing adult stages and showed similar abundance in the larval stage (Fig. 5). This suggests that the part of the lipid regulation observed in adult diapause might be derived from the lipid regulation happening during larval development. This hypothesis is a potential solution to the observation that diverse diapause behaviors are observed among closely related insects [50]. The repurposing of an existing lipid regulatory mechanism in the life cycle (larval development) for adult diapause may have occurred in *L. decemlineata* and other insects. A similar case of repurposing ancient genetic mechanisms for achieving changes in lipid metabolism during the diapause program was shown in the African turquoise killifish [51].

Overall, this study provides comprehensive evidence supporting the hypothesis that diapause requires specific changes in lipid profiles, which are in turn regulated by differential gene expression in the fat body. A key contribution of this work is the generation of multiple testable hypotheses regarding the biological functions of changes in lipid composition and associated gene regulation during diapause. For instance, we hypothesize that the *carboxyl ester lipase* identified in our dataset contributes to the increase in oleic acid levels during diapause to support membrane remodeling or energy mobilization. Looking ahead, these hypotheses are amenable to experimental validation using RNA interference-based functional studies [23, 52]. Genes identified in this study could be selectively silenced using RNA interference, and the downstream effects on lipid profiles could be assessed to test the correlative links identified here. In addition, core diapause traits such as survival under cold stress and post-diapause fitness could be evaluated to determine the functional roles of candidate genes in lipid regulation and diapause success.

Conclusions

This study provides a comprehensive multi-omics analysis of diapause-associated metabolic shifts in the Colorado potato beetle. We demonstrated that diapause is characterized by increased unsaturated lipid species, likely contributing to membrane fluidity and cold resistance. Transcript-lipid correlation analysis identified key candidate genes, such as *carboxyl ester lipase* and *palmitoyl-protein thioesterase*, that may regulate lipid dynamics during diapause. Proteomics revealed calcium-associated proteins that could influence lipid storage and mobilization. Future research should focus on functional validation of these candidates, particularly through gene knockdowns or metabolic assays, to establish their roles in diapause regulation. Additionally, further replicates in proteomics experiments will strengthen these findings. The insights gained here enhance our understanding of insect diapause and open avenues for targeting lipid metabolism in pest management strategies.

Methods

Colorado potato beetles

Four life stages of the Colorado potato beetle (*L. decemlineata*) were sampled from a pesticide-free potato field at the Molecular Entomology Lab, Ankara University (Ankara, Turkey), where one to two generations occur per year (Fig. 6). The sample sets included the following: (1) actively feeding 4th instar larvae (collected in June 2018), (2) non-diapausing actively feeding adults that are not destined for diapause (July 2019), (3) diapausing adults (January 2019), and (4) post-diapause adults (May 2019). To confirm the non-diapause status of the July adult group, thirty individuals were maintained in field cages containing potted potato plants. Their continued feeding and mating behavior over a one-month observation period, with no signs of burrowing behavior, verified that they were not preparing for diapause.

Dissection

The fat bodies (including white and yellow tissues) from *L. decemlineata* adults and larvae were dissected on ice in

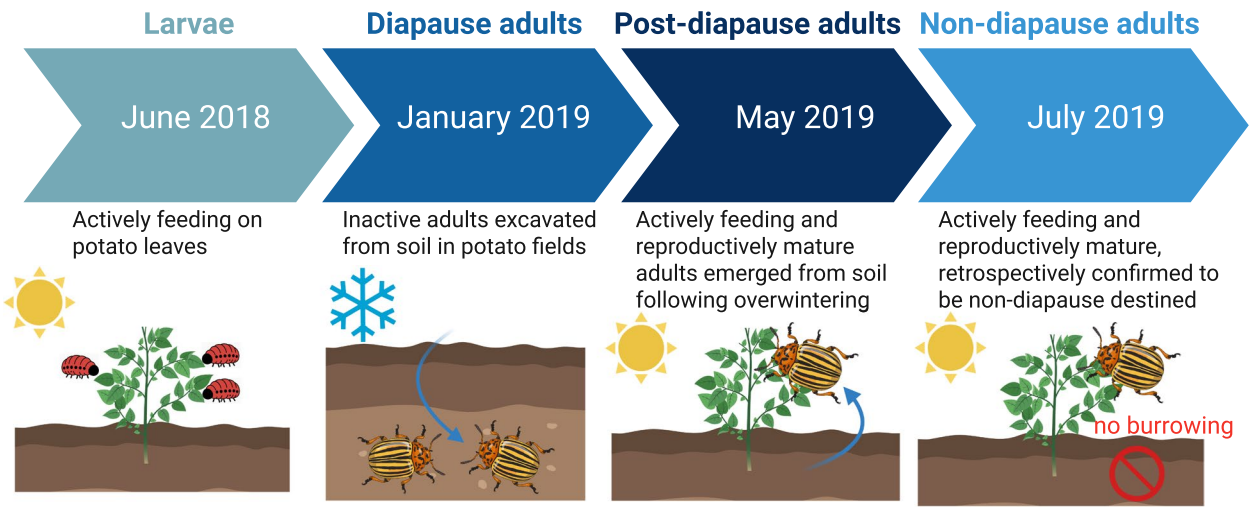


Fig. 6 Timeline of Colorado potato beetle *L. decemlineata* samples collected from a potato field in Ankara, Turkey

phosphate-buffered saline (pH ~ 7.4) using forceps under a stereomicroscope within 24 h following collection from the experimental field. For RNA-seq, 10 mg of fat body tissue was collected from 6 to 7 females or larvae in 1.5 mL Eppendorf tubes and immediately flash-frozen using liquid nitrogen ($n=3$ biological replicates). For lipidomics, 10 mg of fat body tissue was collected from 6 to 7 females or larvae in 2 mL autosampler glass vials and immediately filled with CO_2 to prevent oxidation ($n=3$ biological replicates). For qualitative proteomics, 10 mg of fat bodies were dissected from 5 to 10 larvae or adults in a droplet of ice-cold protease inhibitor cocktail (Roche Diagnostics GmbH, Indianapolis, USA) and transferred into ice-cold Ringer solution (153 mM NaCl, 2.68 mM KCl, 1.36 mM CaCl2·2H2O) containing protease inhibitor cocktail ($n=1$ biological replicate). Samples were shipped on dry ice for downstream RNA-seq, lipidomics, and qualitative proteomics analyses.

Lipidomics

The lipidomics service was provided by the Mass Spectrometry Core Facility (University of Wisconsin–Madison). Lipidomic analysis was performed using an integrated LC–MS system comprising an Agilent InfinityLab Poroshell 120 EC-C18 1.9 µm (2.1×50 mm) column maintained at 50 °C. This system was connected to an Agilent HiP 1290 Multisampler, Agilent 1290 Infinity II binary pump, and column compartment, which in turn were Linked to an Agilent 6546 Accurate Mass Q-TOF dual ESI mass spectrometer. Chromatographic separation was achieved using a mobile phase of ACN (60:40 v/v) containing 10 mM ammonium formate and 0.1% formic acid (Phase A) and IPA:ACN (90:9:1 v/v) with the same additives (Phase B). The gradient commenced at 15% Phase B, increasing to 99% over 13.8 min before returning to initial conditions, with a consistent flow rate of 0.5 mL/min. Injection volumes were 1 µL for the positive ion mode and 5 µL for the negative ion mode. Mass spectrometric detection employed both positive and negative ionization settings, adjusting parameters such as source gas temperature, flow, and various voltages to optimize the detection of lipid species. Reference masses were introduced for calibration and quality control.

Data from the LC–MS runs were captured using the Agilent Mass Hunter Workstation for data acquisition. Quality control samples and process blanks were injected systematically to validate the consistency of the data. Post-acquisition data analysis was conducted using Profinder software for retention time alignment and peak area extraction against a lipid library created with Lipid Annotator. This library included detailed lipid identities with mass-to-charge ratios and retention times. The results, including integration discrepancies and annotations for multiple occurrences of identical m/z values representing structural isomers, were compiled into. csv files for both ionization modes. Negative ionization results were used for downstream data analysis. MetaboAnalyst v6.0 was used to perform one-way ANOVA followed by Fisher's LSD tests after default filtering of the lipidomics data. False Discovery Rate (FDR) correction was applied to the results to control for multiple comparisons. R v4.3 was used to *Z*-normalize the normalized peak intensity values [53].

RNA-seq

Total RNA was isolated from each sample using the innuPREP RNA Mini Kit 2.0 (Analytik Jena, Germany), following the manufacturer's protocol. RNA quality and

quantity were assessed using Bioanalyzer 2100 (Agilent Technologies). For each life stage, three biological replicates were used. mRNA was enriched using the NEBNext® Poly(A) mRNA Magnetic Isolation Module (New England Biolabs), followed by library preparation using the NEBNext® Ultra™ II RNA Library Prep Kit for Illumina (New England Biolabs), according to the manufacturer's instructions. Libraries were sequenced on an Illumina HiSeq 2500 platform at the National Research Council in Saskatoon, Canada.

De novo assembly and functional annotation

Erroneous k-mers from paired-end reads were discarded from the libraries using Rcorrector (v1.0.5) with default settings; and unfixable reads were discarded using the "FilterUncorrectabledPEfastq.py" function in "Transcriptome Assembly Tools" [54]. Adaptor sequences were removed using TrimGalore! (v0.6.7), and reads with a quality score greater than 30 were retained. The cleaned reads from all RNA-seq libraries were assembled de novo using Trinity v2.1 [55] with default settings. In total, 74,529 transcripts, including putative isoforms, were assembled. The de novo assembly had an N50 value of 2041 and a BUSCO (v5.4.2) completeness score of 92.2% (*S*: 49.3%, *D*: 42.9%, *F*: 2.9%, *M*: 4.9%, *n*: 2124) when compared against the Endopterygota lineage (BUSCO. v4 datasets). The transcripts were annotated using eggNOG-mapper v2 [56].

Differentially abundant transcripts

Read count per transcript was calculated using Salmon (v1.8) by mapping the cleaned reads onto the de novo Colorado potato beetle transcriptome. Transcripts with less than 10 total read counts across all 12 libraries were excluded from the analysis. The R package "DESeq2" (v1.4) was used to identify differentially expressed transcripts between Colorado potato beetles at life stages. Transcripts with adjusted P values (Benjamini–Hochberg method) below 0.05 and Log_2 fold change (LFC) values below −1.0 or above 1.0 were considered to have significantly decreased or increased in abundance, respectively.

Proteomics

Proteins were extracted using 2.5% sodium dodecyl sulfate, 5% β-mercaptoethanol, and subjected to in-solution digestion LC–MS/MS analysis at the University of Victoria Genome British Columbia Proteomics Centre, Canada. Samples were denatured, reduced, alkylated, and digested using a Preomics iST Sample Preparation kit (Preomics GmbH, Martinsried, Germany) according to the manufacturer's protocol. Samples were resuspended in the loading buffer at a concentration of 1 µg/µL.

Peptide mixtures (~1 µg) were separated by on-line reverse phase chromatography using a Thermo Scientific EASY-nLC 1000 system with a reversed-phase pre-column and an in-house prepared reverse phase nano-analytical column, coupled on-line with an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with a Nanospray Flex NG source. Solvents used were 2% acetonitrile, 0.1% formic acid (solvent A), and 90% acetonitrile, 0.1% formic acid (solvent B). Samples were separated by a 140-min gradient and analyzed using the Orbitrap Fusion instrument with specified parameters for ion trap with HCD fragmentation. Raw data files were generated using XCalibur 4.2.28.14 (Thermo Scientific) software and analyzed with Proteome Discoverer 2.2.0.388 software suite (Thermo Scientific).

Enrichment and pathway analysis

The "enricher" function in the R package "ProfileClusterer" was utilized to analyze the enrichment status of GO terms associated with the differentially abundant transcripts in the pairwise comparisons larvae vs non-diapause, diapause vs non-diapause, and diapause vs post-diapause. Transcripts with at least one GO annotation served as the background. Transcripts that decreased or increased in abundance were not distinguished during the enrichment analyses due to the ambiguous nature of GO term annotations with regard to the directionality of the involvement in the associated process. We selected the 15 most significantly (i.e., lowest *P* values) enriched biological process GO terms for the enrichment plots.

Coupling of transcriptomics and lipidomics

Transcripts that were differentially expressed in diapause vs non-diapause or diapause vs post-diapause comparisons and that had GO terms associated with lipid metabolism (e.g., fatty acid oxidation, fatty acid beta-oxidation, lipid oxidation, fatty acid catabolic process, fatty acid derivative metabolic process, and/or cellular lipid catabolic process) were filtered and the normalized expressions at different life stages were obtained using DESeq2 (median of ratios method). Next, we tested Pearson's correlations (R v4.3) between the selected transcripts and lipids whose abundance changed significantly in diapause vs non-diapause or diapause vs post-diapause comparisons using the *Z*-score normalized values at larvae, non-diapause, diapause, and post-diapause.

Abbreviations

ABHD12B	Abhydrolase domain containing 12B
ACCase	Acetyl-coenzyme A carboxylase
Acetyl-CoA	Acetyl-coenzyme A
cel	Carboxyl ester lipase
eci1	Enoyl-CoA delta isomerase 1
FA	Fatty acid

FAS	Fatty acid synthase
FDR	False discovery rate
GO	Gene ontology
hadh	NAD-binding 3-hydroxyacyl-CoA dehydrogenase
LC–MS/MS	Liquid chromatography-tandem mass spectrometry
LFC	Log_2 fold change
LFQ	Label-free quantification intensity normalized protein abundance
LSD	Lipid storage droplet
PC	Phosphatidylcholine
PCA	Principal component analysis
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
phyh	Phytanoyl-CoA dioxygenase
PI	Phosphatidylinositol
ppt1	Palmitoyl-protein thioesterase
TAG	Triacylglycerol

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12915-025-02408-3.

Additional File 1. Title of data: Differentially abundant lipids. Description of data: Provides a list of all lipids that show differential abundance across pairwise comparisons of the four life stages.

Additional File 2. Title of data: Supporting figures and tables. Description of data: Contains figures S1-S2, and tables S1-S4. Figure S1 – Overview of changes in lipid compositions within the fat body of different life stages of Colorado potato beetle. Figure S2 – Overview of differential transcript expression in the fat body of different life stages of Colorado potato beetles. Table S1 – Top 10 proteins with highest percentage of total spectra in diapausing *L. decemlineata*, identified using LC-MS/MS (n = 1). Table S2 – Top 10 proteins with highest percentage of total spectra in non-diapause *L. decemlineata*, identified using LC-MS/MS (n = 1). Table S3 – Top 10 proteins with highest percentage of total spectra in post-diapause *L. decemlineata*, identified using LC-MS/MS (n = 1). Table S4 – Top 10 proteins with highest percentage of total spectra in *L. decemlineata* larvae, identified using LC-MS/MS (n = 1).

Additional File 3. Title of data: Differentially expressed transcripts. Description of data: Provides a list of all transcripts that show differential expression across pairwise comparisons of the four life stages.

Additional File 4. Title of data: Protein abundances. Description of data: Provides a list of identified proteins and their abundances across four developmental life stages.

Additional File 5. Title of data: Correlation analysis between lipids and transcripts. Description of data: Provides a list of correlations between all lipid and transcript pairs.

Acknowledgements
We would like to thank Prof. Dr. Mevlüt Emekci and Prof. Ahmet Güray Ferizli. Figure 6 was created with BioRender.

Authors' contributions
UT and DC conceptualized the study and wrote the manuscript. DC and GG conducted the experiments, with support from SB, DB, CD, and MNL. UT, DH, and RLG supervised the study. DC and CC performed the bioinformatics analyses. DC, GG, and UT curated and analysed the data. DC, UT, GG, DH, and RLG edited and revised the manuscript. All authors read and approved the final manuscript.

Funding
This research was funded through a grant by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) (grant #: 118o976).

Data availability
All data generated or analysed during this study are included in this published article, its supplementary information files and publicly available repositories. The lipidomics data are publicly available via figshare (https://doi.org/10.6084/m9.figshare.29881754.v1) [57] and processed data are provided in Additional File 1. The proteomics data are publicly available via figshare (https://doi.org/10.6084/m9.figshare.29881817.v2) [58] and processed data are provided in Additional File 4. The RNA sequencing and assembly data are available via the National Center for Biotechnology Information Sequence Read Archive with accession number PRJNA1240628 [59] and processed data are provided in Additional File 3.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 25 March 2025 Accepted: 3 September 2025
Published online: 02 October 2025

References

1. Denlinger DL, editor. Insect Diapause. In: Insect Diapause. Cambridge: Cambridge University Press; 2022. p. i–ii.
2. Denlinger DL. Regulation of diapause. Annu Rev Entomol. 2002;47:93–122. https://doi.org/10.1146/annurev.ento.47.091201.145137.
3. Hahn DA, Denlinger DL. Energetics of insect diapause. Annu Rev Entomol. 2011;56:103–21. https://doi.org/10.1146/annurev-ento-112408-085436.
4. Hahn DA, Denlinger DL. Meeting the energetic demands of insect diapause: nutrient storage and utilization. J Insect Physiol. 2007;53:760–73. https://doi.org/10.1016/j.jinsphys.2007.03.018.
5. Sinclair BJ. Linking energetics and overwintering in temperate insects. J Therm Biol. 2015;54:5–11. https://doi.org/10.1016/j.jtherbio.2014.07.007.
6. Sinclair BJ, Marshall KE. The many roles of fats in overwintering insects. J Exp Biol. 2018;221(Suppl_1):jeb161836. https://doi.org/10.1242/jeb.161836.
7. MacRae TH. Gene expression, metabolic regulation and stress tolerance during diapause. Cell Mol Life Sci. 2010;67:2405–24. https://doi.org/10.1007/s00018-010-0311-0.
8. Toprak U, Hegedus D, Doğan C, Güney G. A journey into the world of insect lipid metabolism. Arch Insect Biochem Physiol. 2020;104:e21682. https://doi.org/10.1002/arch.21682.
9. Toprak U. Recent insights into insect physiology and biochemistry. Arch Insect Biochem Physiol. 2025;118:e70034. https://doi.org/10.1002/arch.70034.
10. Doğan C, Güney G, Güzel KK, Can A, Hegedus DD, Toprak U. What you eat matters: nutrient inputs alter the metabolism and neuropeptide expression in Egyptian cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae). Front Physiol. 2021. https://doi.org/10.3389/fphys.2021.773688.
11. Arrese EL, Soulages JL. Insect fat body: energy, metabolism, and regulation. Annu Rev Entomol. 2010;55:207–25. https://doi.org/10.1146/annurev-ento-112408-085356.
12. Toprak U, Guz N, Gurkan MO, Hegedus DD. Identification and coordinated expression of perilipin genes in the biological cycle of sunn pest, *Eurygaster maura* (Hemiptera: Scutelleridae): implications for lipolysis and lipogenesis. Comp Biochem Physiol B Biochem Mol Biol. 2014;171:1–11. https://doi.org/10.1016/j.cbpb.2014.02.001.
13. Aguilar JG dos S. An overview of lipids from insects. Biocatal Agric Biotechnol. 2021;33:101967. https://doi.org/10.1016/j.bcab.2021.101967.
14. Downer RGH, Matthews JR. Patterns of lipid distribution and utilisation in insects. Am Zool. 1976;16:733–45. https://doi.org/10.1093/icb/16.4.733.
15. Gondim KC, Atella GC, Pontes EG, Majerowicz D. Lipid metabolism in insect disease vectors. Insect Biochem Mol Biol. 2018;101:108–23. https://doi.org/10.1016/j.ibmb.2018.08.005.

16. Gennis RB. Biomembranes: molecular structure and function. New York, NY: Springer; 1989. https://doi.org/10.1007/978-1-4757-2065-5.
17. Casares D, Escribá PV, Rosselló CA. Membrane lipid composition: effect on membrane and organelle structure, function and compartmentalization and therapeutic avenues. Int J Mol Sci. 2019;20:2167. https://doi.org/10.3390/ijms20092167.
18. Jones HE, Harwood JL, Bowen ID, Griffiths G. Lipid composition of subcellular membranes from larvae and prepupae of *Drosophila melanogaster*. Lipids. 1992;27:984–7. https://doi.org/10.1007/BF02535576.
19. Hammad LA, Cooper BS, Fisher NP, Montooth KL, Karty JA. Profiling and quantification of *Drosophila melanogaster* lipids using liquid chromatography/mass spectrometry. Rapid Commun Mass Spectrom. 2011;25:2959–68. https://doi.org/10.1002/rcm.5187.
20. Lehmann P, Pruisscher P, Posledovich D, Carlsson M, Käkelä R, Tang P, et al. Energy and lipid metabolism during direct and diapause development in a pierid butterfly. J Exp Biol. 2016;219:3049–60. https://doi.org/10.1242/jeb.142687.
21. Adamski Z, Bufo SA, Chowański S, Falabella P, Lubawy J, Marciniak P, et al. Beetles as model organisms in physiological, biomedical and environmental studies - a review. Front Physiol. 2019;10:319. https://doi.org/10.3389/fphys.2019.00319.
22. Lebenzon JE, Torson AS, Sinclair BJ. Diapause differentially modulates the transcriptomes of fat body and flight muscle in the Colorado potato beetle. Comp Biochem Physiol Part D Genomics Proteomics. 2021;40:100906. https://doi.org/10.1016/j.cbd.2021.100906.
23. Cedden D, Güney G, Toprak U. The integral role of de novo lipogenesis in the preparation for seasonal dormancy. Proc Natl Acad Sci. 2024;121:e2406194121. https://doi.org/10.1073/pnas.2406194121.
24. de Kort C a. d. Thirty-five years of diapause research with the Colorado potato beetle. Entomol Exp Appl. 1990;56:1–13. https://doi.org/10.1111/j.1570-7458.1990.tb01376.x.
25. Margus A, Lindström L. Prolonged diapause has sex-specific fertility and fitness costs. Evol Ecol. 2020;34:41–57. https://doi.org/10.1007/s10682-019-10024-1.
26. Güney G, Toprak U, Hegedus DD, Bayram Ş, Coutu C, Bekkaoui D, et al. A look into Colorado potato beetle lipid metabolism through the lens of lipid storage droplet proteins. Insect Biochem Mol Biol. 2021;133:103473. https://doi.org/10.1016/j.ibmb.2020.103473.
27. Yocum GD, Buckner JS, Fatland CL. A comparison of internal and external lipids of nondiapausing and diapause initiation phase adult Colorado potato beetles, *Leptinotarsa decemlineata*. Comp Biochem Physiol B Biochem Mol Biol. 2011;159:163–70. https://doi.org/10.1016/j.cbpb.2011.03.007.
28. Toprak U, Musselman LP. From cellular biochemistry to systems physiology: new insights into insect lipid metabolism. Insect Biochem Mol Biol. 2021;133:103585. https://doi.org/10.1016/j.ibmb.2021.103585.
29. Toprak U. The role of peptide hormones in insect lipid metabolism. Front Physiol. 2020. https://doi.org/10.3389/fphys.2020.00434.
30. Kankare M, Parker DJ, Merisalo M, Salminen TS, Hoikkala A. Transcriptional differences between diapausing and non-diapausing *D. montana* females reared under the same photoperiod and temperature. PLoS ONE. 2016;11:e0161852. https://doi.org/10.1371/journal.pone.0161852.
31. Güney G, Cedden D, Körnig J, Ulber B, Beran F, Scholten S, et al. Physiological and transcriptional changes associated with obligate aestivation in the cabbage stem flea beetle (*Psylliodes chrysocephala*). Insect Biochem Mol Biol. 2024;173:104165. https://doi.org/10.1016/j.ibmb.2024.104165.
32. Lebenzon JE, Denezis PW, Mohammad L, Mathers KE, Turnbull KF, Staples JF, et al. Reversible mitophagy drives metabolic suppression in diapausing beetles. Proc Natl Acad Sci. 2022;119:e2201089119. https://doi.org/10.1073/pnas.2201089119.
33. Hazel JR. Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? Annu Rev Physiol. 1995;57:19–42. https://doi.org/10.1146/annurev.ph.57.030195.000315.
34. Wu G, Baumeister R, Heimbucher T. Molecular mechanisms of lipid-based metabolic adaptation strategies in response to cold. Cells. 2023. https://doi.org/10.3390/cells12101353.
35. Batz ZA, Armbruster PA. Diapause-associated changes in the lipid and metabolite profiles of the Asian tiger mosquito, Aedes albopictus. J Exp Biol. 2018;221:jeb189480. https://doi.org/10.1242/jeb.189480.
36. Teets N, Denlinger D. Physiological mechanisms of seasonal and rapid cold-hardening in insects. Physiol Entomol. 2013;38:105–16. https://doi.org/10.1111/phen.12019.
37. Lehmann P, Westberg M, Tang P, Lindström L, Käkelä R. The diapause lipidomes of three closely related beetle species reveal mechanisms for tolerating energetic and cold stress in high-latitude seasonal environments. Front Physiol. 2020. https://doi.org/10.3389/fphys.2020.576617.
38. Tomcala A, Tollarová M, Overgaard J, Simek P, Kostál V. Seasonal acquisition of chill tolerance and restructuring of membrane glycerophospholipids in an overwintering insect: triggering by low temperature, desiccation and diapause progression. J Exp Biol. 2006;209:4102–14. https://doi.org/10.1242/jeb.02484.
39. Johansson BB, Fjeld K, El Jellas K, Gravdal A, Dalva M, Tjora E, et al. The role of the carboxyl ester lipase (*CEL*) gene in pancreatic disease. Pancreatology. 2018;18:12–9. https://doi.org/10.1016/j.pan.2017.12.001.
40. Ala-Nisula T, Sah-Teli SK, Ronkainen V-P, Dimova EY, Koivunen P. Human phytanoyl-CoA dioxygenase domain-containing 1 (PHYHD1) is a putative oxygen sensor associated with RNA and carbohydrate metabolism. FEBS Lett. 2023;597:1651–66. https://doi.org/10.1002/1873-3468.14666.
41. Jansen GA, Waterham HR, Wanders RJA. Molecular basis of Refsum disease: sequence variations in Phytanoyl-CoA hydroxylase (PHYH) and the PTS2 receptor (PEX7). Hum Mutat. 2004;23:209–18. https://doi.org/10.1002/humu.10315.
42. Chen A, Liao P, Li Q, Zhao Q, Gao M, Wang P, et al. Phytanoyl-CoA dioxygenase domain-containing protein 1 plays an important role in egg shell formation of silkworm (*Bombyx mori*). PLoS ONE. 2021;16:e0261918. https://doi.org/10.1371/journal.pone.0261918.
43. Toprak U, Doğan C, Hegedus D. A comparative perspective on functionally-related, intracellular calcium channels: the insect ryanodine and inositol 1,4,5-trisphosphate receptors. Biomolecules. 2021;11:1031. https://doi.org/10.3390/biom11071031.
44. Doğan C, Hänniger S, Heckel DG, Coutu C, Hegedus DD, Crubaugh L, et al. Characterization of calcium signaling proteins from the fat body of the Colorado Potato Beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae): implications for diapause and lipid metabolism. Insect Biochem Mol Biol. 2021;133:103549. https://doi.org/10.1016/j.ibmb.2021.103549.
45. Vesala L, Salminen TS, Kankare M, Hoikkala A. Photoperiodic regulation of cold tolerance and expression levels of *regucalcin* gene in *Drosophila montana*. J Insect Physiol. 2012;58:704–9. https://doi.org/10.1016/j.jinsphys.2012.02.004.
46. Yamaguchi M, Murata T. Involvement of regucalcin in lipid metabolism and diabetes. Metabolism. 2013;62:1045–51. https://doi.org/10.1016/j.metabol.2013.01.023.
47. Doğan C, Hänniger S, Heckel DG, Coutu C, Hegedus DD, Crubaugh L, et al. Two calcium-binding chaperones from the fat body of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) involved in diapause. Arch Insect Biochem Physiol. 2021;106:e21755. https://doi.org/10.1002/arch.21755.
48. Lin Y-C, Chipot C, Scheuring S. Annexin-V stabilizes membrane defects by inducing lipid phase transition. Nat Commun. 2020;11:230. https://doi.org/10.1038/s41467-019-14045-w.
49. van Weeghel M, te Brinke H, van Lenthe H, Kulik W, Minkler PE, Stoll MSK, et al. Functional redundancy of mitochondrial enoyl-CoA isomerases in the oxidation of unsaturated fatty acids. FASEB J. 2012;26:4316–26. https://doi.org/10.1096/fj.12-206326.
50. Batz ZA, Clemento AJ, Fritzenwanker J, Ring TJ, Garza JC, Armbruster PA. Rapid adaptive evolution of the diapause program during range expansion of an invasive mosquito. Evolution. 2020;74:1451–65. https://doi.org/10.1111/evo.14029.
51. Singh PP, Reeves GA, Contrepois K, Papsdorf K, Miklas JW, Ellenberger M, et al. Evolution of diapause in the African turquoise killifish by remodeling the ancient gene regulatory landscape. Cell. 2024. https://doi.org/10.1016/j.cell.2024.04.048.
52. Cedden D, Güney G, Rostás M, Bucher G. Optimizing dsRNA sequences for RNAi in pest control and research with the dsRIP web platform. BMC Biol. 2025;23:114. https://doi.org/10.1186/s12915-025-02219-6.
53. Pang Z, Lu Y, Zhou G, Hui F, Xu L, Viau C, et al. Metaboanalyst 6.0: towards a unified platform for metabolomics data processing, analysis and

interpretation. Nucleic Acids Res. 2024. https://doi.org/10.1093/nar/gkae253.
54. Song L, Florea L. Rcorrector: efficient and accurate error correction for Illumina RNA-seq reads. Gigascience. 2015;4:48. https://doi.org/10.1186/s13742-015-0089-y.
55. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, et al. Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. Nat Biotechnol. 2011;29:644–52. https://doi.org/10.1038/nbt.1883.
56. Cantalapiedra CP, Hernández-Plaza A, Letunic I, Bork P, Huerta-Cepas J. EggNOG-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale. Mol Biol Evol. 2021;38:5825–9. https://doi.org/10.1093/molbev/msab293.
57. Cedden D. Raw lipidomics data files for Multi-Omics Analysis Reveals Diapause-Associated Lipid Remodeling in the Fat Body of Colorado Potato Beetle. figshare. 2025. https://doi.org/10.6084/m9.figshare.29881754.v1.
58. Cedden D. Raw proteomics data for Multi-Omics Analysis Reveals Diapause-Associated Lipid Remodeling in the Fat Body of Colorado Potato Beetle. figshare. 2025. https://doi.org/10.6084/m9.figshare.29881817.v2.
59. Cedden D. RNA-seq in the fat body of Colorado potato beetle and wheat stink bug. NCBI Sequence Read Archive. 2025. https://identifiers.org/ncbi/bioproject:PRJNA1240628.

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