Chapter

Research Progress on Diapause in Flies (Diptera)

Haibin Han, Yanyan Li, Bo Zhang, Kejian Lin, Shujing Gao, Linbo Xu, Ning Wang, Wenchang Duan and Wenyuan Niu

Abstract

Diapause is a physiological process in which insects can survive in a natural environment that is not conducive to their survival, which is the result of long-term adaptation to environmental conditions. It provides a great adaptive advantage for insects, allowing insects to survive in unsuitable seasonal environments to synchronize their life cycles with those suitable for growth, development, and reproduction. The process of regulating insect diapause is a complex process interacting with multiple mechanisms. In this chapter, a review is given of the current knowledge of diapause types, environmental inducing factors, sensitive states, and the endogenous molecular mechanism associated with diapause in flies (Diptera). Research regarding both the diapause process and intrinsic mechanism is reviewed.

Keywords: diapause, stages of diapause, biotic and abiotic factors, molecular mechanisms, Diptera

1. Introduction

Diapause is a state in which insects suspend or arrest the development in response to unfavorable environmental cues. It is an adaptive mechanism with a genetic basis, regulated by the external and internal environment factors, and occurs in a specific stage during the life cycle of an insect such as embryonic, larval, pupal, and active adult stage. Once induced, diapause cannot be immediately terminated even if unfavorable conditions disappear, unless a certain break period has been experienced. Diapause is terminated with the return of appropriate environmental conditions (temperature, light, moisture, etc.), and physical and chemical conditions [1]. Diapause provides an adaptive advantage for insects, allowing them to continue surviving in unfavorable seasonal environments and ensuring that their life cycle is synchronized with conditions suitable for growth, development, and reproduction.

There are two types of diapause, obligatory diapause and facultative diapause. Obligatory diapause, also known as absolute diapause, means that insects have to enter diapause to complete their life cycle, regardless of environmental conditions. It is most found in univoltine insects (one generation per year). For example, *Anthocharis cardamines* is a univoltine butterfly species that has an obligate pupal diapause in United Kingdom [2]. Facultative diapause, also known as random diapause, means that insects start this process only when environmental conditions become adverse. It is most commonly seen in bivoltine (two generations per year) or multivoltine insects (more than two generations per year). For example, the bivoltine strain of the silkworm, *Bombyx mori*, has a facultative embryonic diapause in Japan [3]. The generation of diapause is variable, but the life stage in which diapause occurs is fixed. Thus, photoperiod, together with temperature and other environmental factors, leads to a facultative diapause in insects. For insects, the significance of diapause is not only to enable them to survive the adverse environmental conditions, but also to make the population as uniform as possible, which greatly increases the possibility of male and female mating and thus ensures the reproduction of the population. To meet energetic costs, insects use two methods that are metabolic depression and energy storage. By reducing their metabolic consumption, insects can accelerate the accumulate energy to enhance their resistance to adverse environments [4]. In recent years, the research results on diapause of the order of Diptera have increased. This chapter classifies the research progress of diapause in Diptera, including the main groups, types, stages, parental effects, diapause-inducing factors, and mechanisms of molecular regulation. It provides theoretical support for population control of Diptera pests and effective biocontrol using natural enemies [5].

2. Taxa of diapause

Diptera is the fourth largest order after Coleoptera, Lepidoptera, and Hymenoptera. In terms of diapause research, 85 species of Diptera have been studied, including Tephritidae, Culicidae, Calliphoridae, Agromyzidae, Oestridae, Muscidae, Anthomyiidae, Tachinidae, Drosophilidae, and Cecidomyidae (**Figure 1**).

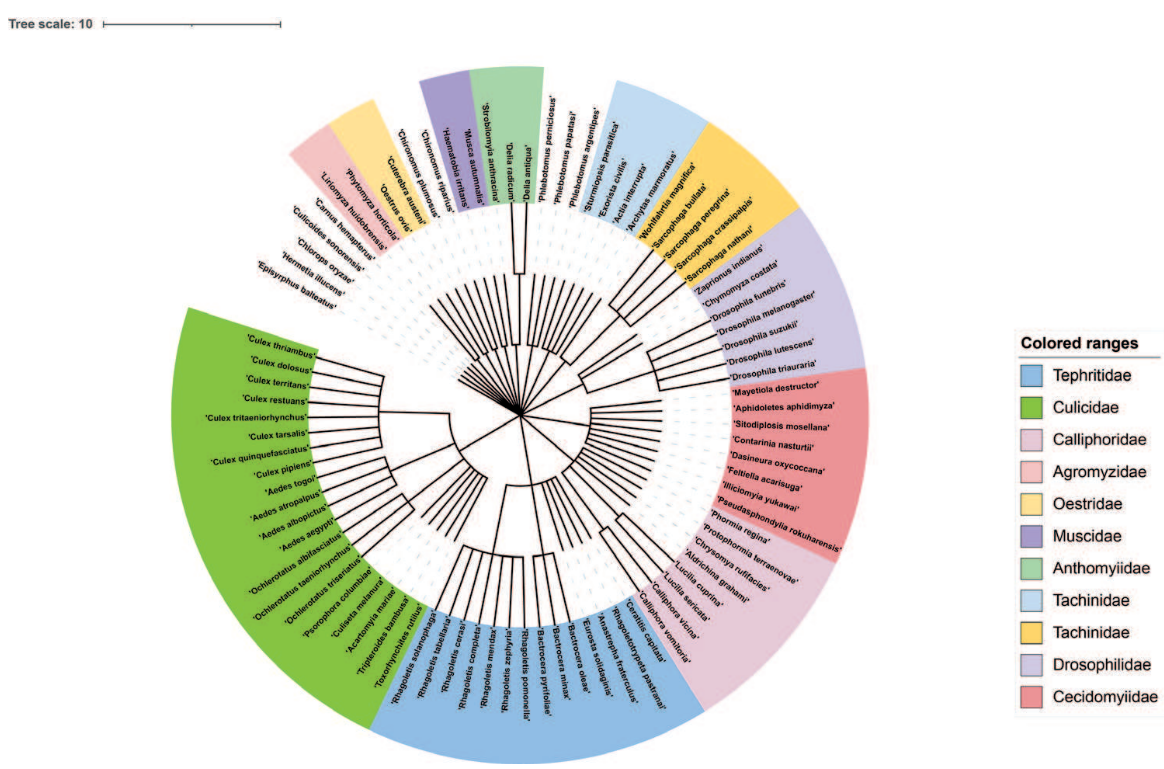

Figure 1.
Taxa of dipause in Diptera.

3. Types of diapause

Diapause can be categorized according to life stages as an egg (embryonic), larval, nymphal, or adult diapause (**Table 1**). Based on the hereditary feature, there are two types of diapause, one mandatory (obligate diapause) and one optional (facultative diapause) (**Table 1**).

Insects with obligate diapause enter diapause at a fixed developmental stage on schedule no matter how the external environmental conditions are, while those with facultative diapause can be induced into diapause at a certain stage but uncertain generation. Diapause is mainly affected by the changes in external environmental conditions. When the environmental conditions are unfavorable, insects enter the diapause, otherwise they continue to develop. Flies also experience diapause in the pupal stage (quiescent stage), during which the activity is extremely weak. *Sarcophaga similis* and *Haematobia irritans* have a pupal diapause [6], with the pupal diapause of the parasitoid fly *Exorista civilis* occurring within the insect host. Pupal diapause, common in other Diptera, is conspicuously absent in the Culicidae, whose members may pass through diapause as eggs, larvae, or adults. *Aedes albopictus* overwinters as diapausing eggs in temperate climates [7]. For *Chlorops oryzae*, diapause occurs as larvae, triggered by conditions experienced by the egg [8]. Diapause of *Procontarinia mangifera* is observed in larvae [9], Adult reproductive diapause occurs in *Drosophila suzukii* and *Protophormia terraenovae* [10]. The third-instar larvae of *Eurosta solidaginis* show obvious hibernation (winter diapause) characteristics [11]. Once *Lucilia sericata* reaches the third instar, larvae stop feeding and then enter diapause [12]. *Sitodiplosis mosellana* undergoes diapause as larvae in the soil [13].

Classification		Characteristics
Diapause induction stage	Embryonic diapause	occurs at any stage of insect embryo development; regulated by the brain-hypopharyngeal gland-diapause hormone endocrine system
	Larval diapause	occurs at any instar of larval larvae, but mostly occurs at the late larval stage
	Nymphal diapause	commonly happen in Diptera and Lepidoptera, which is regulated by brain-prothoracic-corpora allata interaction
	Adult diapause	commonly occurs in Coleoptera, Lepidoptera, Hemiptera, Diptera, Homoptera, and Orthoptera, and is regulated by the islet- corpora allata
Hereditary feature	Obligatory diapause	insects have no choice but to enter this process at some stage in their life.
	Facultative diapause	insects will start this process only when environmental conditions become adverse

Table 1.
Types of diapause.

4. Incidence of diapause

The diapause process can be divided into three phases: pre-diapause, diapause, and post-diapause. Pre-diapause occurs before the beginning of unfavorable environmental cues. Insects in this phase forecast an impending transformation in certain environmental stimuli in a special way, and change their internal neuro-endocrine system and metabolism level to enter diapause. At this stage, insects maintain normal development, which include induction and preparation stage [14]. The induction stage happens before the beginning of unfavorable environmental cues. Insects receive these specific environmental stimuli called "token stimuli" such as photoperiod, temperature, humidity, and food, to regulate their development and decide whether to enter diapause or not. Larvae of *S. mosellana* enter diapause at a long day length [15]. The preparation phase is followed by the induction phase, during which insects accumulate energy substances such as lipids, sugars, and amino acids. Lipids provide a large amount of nutrition for insects in diapause and prevent heat loss and mechanical damage [16]. Saccharides such as glycogen and trehalose, as instant sources of energy, play a crucial role in dealing with abiotic stresses [17]. Amino acids reserve help in both providing raw materials for protein synthesis of diapause insects and resistance to cold and desiccation. Amino acids are stored prior to diapause, but they help in maintaining life activities during diapause and post-diapause [16]. Previous studies of diapause in *Culex pipiens* demonstrated that during the diapause, mosquito accumulated more sugar than non-diapause mosquitoes [18].

Because of frequent observations of changing responses to various environmental conditions, diapause is divided into three eco-physiological sub-phases: initiation, maintenance, and termination [14]. The incidence of diapause is affected by a variety of factors. Many insects enter diapause at any stage of their life cycle, but for some species, the diapause stage is fixed, which can be judged by observing the color, appearance, and cocoon making of insect bodies [14, 19–21]. The maintenance phase refers to the period in which the insect remains undeveloped even under favorable developmental conditions, and the respiration and metabolic rates are at low levels. The diapause maintenance period of different insects varies greatly, ranging from a few weeks to several months or even several years. With the return of favorable environmental conditions, the intensity of diapause gradually decreases and enters the diapause termination. At this stage, insects are sensitive to temperature factors, especially low temperatures. In addition, photoperiod can induce diapause termination. Application of exogenous substances can also break insect diapause. For example, exogenous ecdysterone can terminate the diapause of *Bactrocera minax* [22].

In the termination phase, insects enter the next inactivity period if the environmental conditions are still unfavorable; however, they start resuming their physiological development [14].

5. Parental effects on diapause

Parents exhibit a greater effect on the diapause phenotype of their offspring, which defines as parental effect [23]. Parental effect is subject to natural selection, and it is the response mechanism of phenotypes to environmental heterogeneity [24, 25]. Among the parental effects, the female parent exhibits a greater influence than the male parent, so maternal inheritance is considered to be a short form of non-Mendelian parental effect (including maternal and paternal inheritance). For insects, female effects on offspring are relatively common. The parental effect of diapause in Diptera is mainly affected by environmental conditions such as diapause duration, photoperiod, temperature, and parental factors. Ordinarily, parents produce more diapausing progeny if they experience short-day length, limited resources, or low temperature [26]. In the study of *A. albopictus*, maternal photoperiod has a direct influence on egg size and embryogenesis [27].

Larval diapause in the blow fly *Calliphora vicina* is induced by their mothers to cope with short-day photoperiods. Due to the various photoperiod and temperature of the parents, the process of *C. vicina* larval diapause can vary in duration, indicating that the accumulations of diapause stimuli by parents significantly influence the intensity and incidence of progeny diapause [28]. The incidence of diapause is completely under maternal control and is not affected by the male [29]. *Lucilia sericata* has a facultative diapause in the third larval instar after cessation of feeding. Induction of the diapause is influenced by the photoperiod and temperature conditions experienced by insects in the parental generation as well as those experienced by the larvae themselves [30]. In *Sarcophaga bullata*, a maternal effect blocks the programming of diapause in progeny of females reared in short-day length that have experienced pupal diapause [31].

6. Environmental cues for diapause induction

Insect diapause is a complex process in which many mechanisms interact with each other. The major environmental signals regulating diapause onset in insects include temperature, photoperiod, food, and population density. Studies on insect diapause show that the primary cause and state of diapause can be identified and insects could be induced into diapause by artificially simulating diapause conditions in the field. Environmental cues, mostly temperature and photoperiod, control reproductive diapause in flies (Diptera), which regulate the induction, maintenance, and termination of diapause.

Seasonal change in photoperiod is the most reliable information to detect the time of year and is the major environmental signal regulating diapause onset in most insects. Photoperiod refers the period of time in a day that an organism is exposed to light or, more simply, day length. Photoperiodism is a biological response to a change in the proportions of light and dark in a daily (24 h) cycle, and the average number of daylight hours that cause insect diapause is known as "critical photoperiod." When insects respond to changes in light intensity through the brain or compound eyes, the internal "timer" automatically evaluates the length of day or night and regulates the insects to enter the diapause [32]. Usually, the diapause of Diptera is caused by short-day length. *A. albopictus* enters diapause with short-day length. The photoperiodic diapause is a crucial ecophysiological adaptation of *A. albopictus* to climate change in North America and strongly affects seasonal population dynamics, thus affecting the transmission potential of arboviruses [33]. *Chlorops oryzae* enters summer diapause under long day length, but the critical photoperiod is shortened with the increased temperature [34]. In *C. oryzae*, winter diapause in the first larval stage is induced by short-day length in the egg stage and maintained by short days in the larval stage [35]. *D. suzukii* is shown to be a typical of short-day length diapause species [9], and also, *S. similis* enters pupal diapause under short-day conditions [36].

Temperature is another major environmental signal regulating diapause, especially for Diptera. *Linothele sericata* can be stored at 7.5°C for several months and is seen as a potential replacement for honeybees, whose diapause is mainly affected by low temperatures in winter [37, 38]. The Chinese citrus fruit fly, *B. minax*, exhibits pupal diapause in the soil from November to March in the next year, and the pupal period increases with pupal weight. Temperature before entering diapause is a reasonable index to predict overwintering individuals [11]. *P. terraenovae* adults do not enter diapause at 30°C in either long-day or short-day conditions; however, between 17.5 and 27.5°C, the insect shows a long-day photoperiodic response, indicating that the diapause is mainly induced by low temperature [39].

Diapause in some species of flies is subject to both photoperiod and temperature; for example, the dominant diapause cues of *M. autumnalis* and *H. irritans* are probably both photoperiod and temperature [40]. The critical day length for *Aphidoletes aphidimyza* diapause induction is determined to be 12.7 h at 20°C. Diapause incidence is completely prevented at 30°C even though the photoperiod used is 11 L–13D. In addition, diapause induction is 100% under changing temperature conditions while maintaining the critical day length (12.7 L–11.3D), and diapause incidence is 100% in both field and greenhouse conditions under alternating temperatures of 20/16 or 25/16°C [41]. Winter diapause of *Delia antiqua* is completed under constant diapause-inducing conditions of 15°C and 12 L–12D, and the pupal period is shortened significantly [42]. The critical photoperiod for inducing diapause of *E. civilis* is between 11.8 and 11.9 h, while it can be induced into diapause at longer day lengths when the temperature is lower [43]. The incidence of *E. civilis* diapause is also influenced by the temperature and photoperiod. It is found that lower temperature prolongs the larval diapause period, and the diapause rate decreases under the high temperatures. All larvae enter diapause at 17°C, whereas the larvae become sluggish and stiff when temperature is lower than 17°C and death increases. The diapause rate is negatively correlated with light duration at 21°C. Diapause cannot be induced by short-day lengths at 25°C. Low temperature and short photoperiod are the most important prerequisites for inducing *E. civilis* diapause, among which temperature is the dominant factor, and photoperiod affects diapause induction only at certain temperatures.

7. Molecular mechanisms of diapause

7.1 Hormonal and metabolic regulation

Endogenous regulatory factors of insect diapause mainly focused on neuroendocrine systems, hormone signaling pathways, and energy metabolism pathways. Studies on *Drosophila* show that when insulin-like signals are disrupted, fruit flies stop reproducing, and their energy reserves increase [44]. Inhibiting FOXO and insulin-like polypeptide-1 (ILP-1) change many diapause phenotypes of *C. pipiens* such as increase stress resistance, fat accumulation, and delayed development [45]. Therefore, insulin signaling is thought to be an important candidate pathway to study differential regulation of diapause metabolism.

Juvenile hormone (JH), a sesquiterpenoid hormone produced by the corpus allatum (CA) of insects, is one of the most important hormones in insects and plays a key role in preventing larval metamorphosis, maintaining larval state, and regulating adult developmental and physiological process. It also plays a crucial role in the expression of vitellogenin, oocyte maturation, and development. The interaction between genes associated with juvenile hormone pathway is complex, which means genes directly or indirectly participate in the regulation of JH signaling pathway. Studies on the fly *Melinda pusilla* have reported that the application of exogenous juvenile hormone can temporarily terminate diapause [46]. Diapause in *C. pipiens* adult stage is characterized by a pause in reproduction, such as stagnation of the ovaries and male accessory glands, as well as reduced mating activity. The cessation of JH production delays ovarian development, and increases stress resistance, fat, and sugar storage [47]. When *C. pipiens* females are held in diapause conditions for 22 weeks, follicles gradually grow longer. When 21-day-old diapausing mosquitoes are moved to a long daylight of 16:8(L:H) at 26°C, juvenile hormone synthesis increases rapidly and peaks 5 days later, while follicles grow to a quiescent stage. Allatectomy of young diapausing females prevents follicle growth and blood feeding when diapause is terminated prematurely, demonstrating that the physiological events are associated with juvenile hormone biosynthesis [48].

During diapause, there are a lot of significant changes that occur in energy and metabolism due to the organism need to maintain life activities under extreme environmental conditions. Expression of the trehalase gene expression and enzyme activity of *D. antiqua* in summer- and winter diapause are lower at the initial phase but increase gradually and peak in the maintenance phase [49]. The contents of glycogen and trehalose in *E. civilis* during diapause increase significantly with an increase in the diapause induction period.

7.2 Diapause-associated changes in genes

With the development of high-throughput sequencing technology, the sequencing and annotation of *Drosophila melanogaster* genome were first published in 2000 [50]. However, not all Dipteran insects have been sequenced. To simplify the genome sequencing research strategy, transcriptome sequencing can be used to study insects with or without a reference genome available. Transcriptome sequencing technology is rapid, efficient, and low-cost, and currently has been widely used in genetic research, which can carry out transcriptome analysis of a tissue or organelle in a more comprehensive way [51]. Transcriptomes would not only facilitate a better understanding of how individual genes have evolved in biological processes, but would also help to reflect what gene expression changes more precisely [52]. The transcriptome refers to the collection of all gene transcription products in a cell or tissue under certain state or physiological condition, including messenger RNA, ribosomal RNA, transport RNA, and non-coding RNA. Transcriptomics, as an important means to study cell function, can be used to investigate the amount of active gene expression at the RNA level. In recent years, with the development of second- and third-generation molecular sequencing technology, the single-cell genomics is advancing rapidly. Transcriptome based on whole-genome analysis represents the average level of all cell signals, ignoring the variation between cells [53]. Compared with traditional sequencing technology, single-cell technologies have the advantages of detecting the differences in gene expression among individual cells. At present, single-cell sequencing has been used in *Drosophila* and mosquitoes. New cell types, mechanisms of development and aging, genes controlling neural regulation, and connection have been found in *Drosophila* by single-cell RNA sequencing analysis [54]. However, single-cell sequencing has not been done to study diapause of Dipteran insects.

The use of RNA-Seq to determine genes with distinct levels of expression between diapause and non-diapause has been confined to flies, and Kyoto Encyclopedia of Genes (KEGG) analysis is performed to identify the pathways that are significantly enriched in diapause. A high-throughput RNA-Seq analysis from non-diapause and summer diapause pupae of *D. antiqua* revealed variation of cuticular and cytoskeletal components [55]. Significantly differentially regulated transcripts are identified in summer diapause (SD) and non-diapause (ND)-sensitive larvae of *D. antiqua*. Several functional terms related to lipid, carbohydrate, and energy metabolism, environmental adaption, immune response, and aging are enriched during the most sensitive SD induction period. There is much more variation of circadian clock genes in the period of ND than SD-destined larvae, which indicates that it is a key driver of integrating environmental signals to summer diapause [56]. The diapause-associated traits identified in *S. mosellana* appear to be involved in rapid spread and outbreaks. Transcriptomic sequencing performed on diapause and non-diapause larvae shows that various genes-coding metabolic enzymes are crucial for diapause [57]. The molecular mechanisms of obligatory diapause induction in *B. minax* are investigated by using high-throughput RNA-Seq data from second-instar larva, third-instar larva, and pupa stages. The cluster co-expression patterns of the differentially expressed genes reveal that significantly differentially expressed genes in the pupal stage are predicted to be related to diapause induction. All differentially expressed genes are investigated by GO functional and KEGG pathway analysis, and the results show that genes involved in processes such as 20-hydroxyecdysone (20E) biosynthesis, cell cycle, and metabolic pathways are likely related to obligatory diapause induction in *B. minax* [58]. The diapause-associated genes in *E. civilis* are related to be involved in the pathway of signal transduction, endocrine system, and carbohydrate metabolism by KEGG pathway enrichment analysis [59].

Based on transcriptome sequencing, some candidate diapause-related genes have been further studied in Diptera. For example, heat shock proteins (HSPs) have been studied in *S. mosellana*, *Sarcophaga crassipalpis*, and *Rhagoletis mendax*. Hsp90 is downregulated in *S. crassipalpis* diapause pupae and returns to pre-diapause level after diapause termination. The expression of Hsp90 is increased by heat shock or cold shock during diapause. It is showed that Hsp90 is regulated differently in diapause and diapause pupae response to heat injury [60]. DaTrypsin is a serine protease gene and is the first upregulated gene during winter and summer diapause. It may be involved in host immune defense or maintain the developmental of diapause pupae [61].

7.3 Diapause-associated changes in proteins

Proteins are complex molecules that play a central role in biological processes. Proteomics is used to elucidate the expression and function of protein on the basis of genome research. Changes in protein expression during diapause can be explored by two-dimensional gel electrophoresis and mass spectrometry. Isobaric tag for relative and absolute quantitation (iTRAQ) can quantitatively analyze proteins from different sources in a single assay, and is used to study quantitative changes in the proteome by tandem mass spectrometry. Due to its high efficiency and sensitivity, iTRAQ has the potential to further advance the study of molecular mechanisms involved in diapause. Proteomic analysis of Diptera has also been reported. A proteomic approach was used to investigate the proteins extracted from larvae of *S. mosellana* at different developmental stages, which include pre-diapause, over-summering diapause, over-wintering diapause, and post-diapause. The results showed that two small Hsps play key roles in stress tolerance during diapause [62]. Proteins synthesized by pupal brains of the flesh fly *S. crassipalpis* were examined during diapause and non-diapause using pulse labeling and two-dimensional electrophoresis, and it was found that a cluster of about 15 brain proteins appears to be specific to diapausing pupae [63]. Ninety-five differently expressed proteins were identified in the diapause of *E. civilis* by using iTRAQ proteomics, and Hsps were found to be the key diapause-associated proteins. These proteins are mainly involved in material and energy metabolism [64]. Proteomic changes are studied in diapausing versus non-diapausing *D. antiqua* using two-dimensional differential gel electrophoresis, and it is found that some identified differential proteins may play an important role in physiological processes such as heat resistance, chromosome separation, and folic acid metabolism [65].

7.4 Diapause-associated changes in metabolite profiles

Metabolome refers to a collection of small molecular compounds that participate in the metabolism of an organism or cell with a relative molecular weight of less than 1000 DA in a specific physiological period. Metabolomics is a new discipline that simultaneously conducts qualitative and quantitative analysis of small molecule metabolite. It can be used to investigate how metabolites change with time when the organism is stimulated [66]. The cellular activities of living organisms are jointly undertaken by genes, proteins, and small molecule. The metabolic level can reflect the functional changes of macromolecules and amplify the small changes in gene expression. Various techniques are widely used to determine metabolic phenotypes, including liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC/MS), and nuclear magnetic resonance (NMR). LC-MS analysis does not require sample volatileness and thermal stability, and is suitable for compounds with high boiling points, strong polarity, and poor thermal stability. Most metabolites involved in life science have these qualities, so LC-MS has broad application. In recent years, metabolomics has been applied in the area of medicine and microbiology, but there are still relatively few studies on insect diapause. The metabolic profiles of diapause and non-diapause *B. minax* pupae show that proline, trehalose, N-acetylglutamate, and alanine significantly contribute to cold tolerance during diapause [67]. In the metabolomics analysis of diapause and non-diapause pupae of *E. civilis*, L-proline, L-phenylalanine, L-histidine, and L-tyrosine are significantly different, which provides a foundation for mechanistic follow-up studies in insect diapause [68].

8. Conclusion

Knowledge of diapause in Diptera is essential for the development of effective pest management strategies and to increase the shelf-life of parasitoids used in the biological control industry. This chapter summarizes the recent progress on diapause of Diptera. We do believe that further studies should be investigated in the diapause of Diptera. Current research studies suggest that histone modification, DNA methylation, RNA methylation, and small noncoding RNAs all may be involved in the regulation of diapause in Diptera. However, it remains unclear whether they regulate the hormonal and physiological changes associated with diapause of Diptera; research has primarily focused on physiological changes associated with pre-diapause with limited attention given to post-diapause. Studies showed that the indices of insects, such as oviposition quantity, oviposition duration, and life span, increased positively during post-diapause phase. The accumulation and consumption of energy storage substances in pre-diapause and diapause will affect the biological characteristics in post-diapause phase. Combined with biological characteristics in post-diapause, studies on development rate, feeding, individual size, diapause maintain environmental conditions, and nutritional supplements after the diapause are necessary. Existing studies on diapause in Diptera are mainly based on single omics, and studies using multi-omics are still vacant. Therefore, for a deeper understanding of the complex molecular landscape of diapause in Diptera, all the available omics data should be utilized in combination rather than treating them individually.

Acknowledgements

This study was supported by the Science and technology planning project of Inner Mongolia Autonomous Region (2021GG0057, 2020ZY0001, 2019BS03011).

Author details

Haibin Han[1]*, Yanyan Li[2], Bo Zhang[3], Kejian Lin[1], Shujing Gao[1], Linbo Xu[1], Ning Wang[1], Wenchang Duan[2] and Wenyuan Niu[4]

1 Institute of Grassland Research, Chinese Academy of Agricultural Sciences, Hohhot, China

2 Inner Mongolia Agricultural University, Hohhot, China

3 Zhejiang University, Hangzhou, China

4 Garden Bureau of Chahar Right Front Banner, Ulanqab, China

*Address all correspondence to: hhb.25@163.com

References

[1] Denlinger DL. Regulation of diapause. Bulletin of the Entomological Society of America. 1979;**47**:93-122. DOI: 10.1146/annurev.ento.47.091201.145137

[2] Stålhandske S, Lehmann P, Pruisscher P, Leimar O. Effect of winter cold duration on spring phenology of the orange tip butterfly *Anthocharis cardamines*. Ecology and Evolution. 2015;**5**:5509-5520. DOI: 10.1002/ece3.1773

[3] Yokoyama T, Saito S, Shimoda M, Kobayashi M, Takasu Y, Sezutsu H, et al. Comparisons in temperature and photoperiodic-dependent diapause induction between domestic and wild mulberry silkworms. Scientific Reports. 2021;**11**(1):8052. DOI: 10.1038/s41598-021-87590-4

[4] Sim C, Denlinger DL. Insulin signaling and FOXO regulate the overwintering diapause of the mosquito *Culex pipiens*. Proceedings of the National Academy of Sciences. 2008;**105**(18):6777-6781. DOI: 10.1073/pnas.0802067105

[5] Hand SC, Denlinger DL, Podrabsky JE. Mechanisms of animal diapause: Recent developments from nematodes, crustaceans, insects, and fish. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 2016;**310**(11):R1193-R1211. DOI: 10.1152/ajpregu.00250.2015

[6] Lysyk TJ, Moon RD. Diapause recruitment and survival of overwintering *Haematobia irritans* (Diptera: Muscidae). Environmental Entomology, Annapolis: Entomological Society of America. 2001;**30**(6):1090-1097. DOI: 10.1603/0046-225X-30.6.1090

[7] Suman DS, Wang Y, Gaugler R. The insect growth regulator pyriproxyfen terminates egg diapause in the Asian tiger mosquito, *Aedes albopictus*. PLoS One. 2015;**10**(6):e0130499. DOI: 10.1371/journal.pone.0130499

[8] Takeda M. Photoperiodic induction, maintenance and termination of winter diapause in two geographic ecotypes of the rice stem maggot, *Chlorops oryzae* Matsumura (Diptera: Chloropidae). Applied Entomology and Zoology. 1996;**31**(3):379-388. DOI: 10.1007/BF01904716

[9] Amouroux P, Normand F, Delatte H. Diapause incidence and duration in the pest mango blossom gall midge, *Procontarinia mangiferae* (Felt), on Reunion Island. Bulletin of Entomological Research. 2014;**104**(5):661-670. DOI: 10.1017/S0007485314000480

[10] Zhai Y, Lin Q, Zhang J. Adult reproductive diapause in *Drosophila suzukii* females. Journal of Pest Science. 2016;**89**(3):679-688. DOI: 10.1007/s10340-016-0760-9

[11] Irwin JT, Bennett VA, Lee RE. Diapause development in frozen larvae of the goldenrod gall fly, *Eurosta solidaginis* Fitch (Diptera: Tephritidae). Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology. 2001;**171**(3):181-188. DOI: 10.1007/s003600000154

[12] Tachibana SI, Numata H. Effects of temperature and photoperiod on the termination of larval diapause in *Lucilia sericata* (Diptera : Calliphoridae). Zoological Science. 2004;**21**(2):197-202. DOI: 10.2108/zsj.21.197

[13] Wise IL, Lamb RJ. Diapause and emergence of *Sitodiplosis mosellana* (Diptera : Cecidomyiidae) and its parasitoid *Macroglenes penetrans* (Hymenoptera: Pteromalidae). Canadian Entomologist. 2004;**136**(1):77-90. DOI: 10.4039/N03-032

[14] Košťál V. Eco-physiological phases of insect diapause. Journal of Insect Physiology. 2006;**52**(2):113-127. DOI: 10.1016/j.jinsphys.2005.09.008

[15] Wu JX, Li CQ, Li YP. Advances in research on diapause of the wheat blossom midges. Chinese Journal of Applied Entomology. 2004;**41**(6):499-503. DOI: 10.3969/j.issn.0452-8255.2004.06.001

[16] Hahn DA, Denlinger DL. Energetics of insect diapause. Annual Review of Entomology. 2011;**56**(1):103-121. DOI: 10.1146/annurev-ento-112408-085436

[17] Ren XY, Qi XY, An T. Research on the accumulation, transformation and regulation of nutrients in diapause insects. Journal of Applied Entomology. 2016;**53**(4):685-695. DOI: 10.7679/j.issn.2095-1353.2016.086

[18] Mitchell CJ, Briegel H. Inability of diapausing *Culex pipiens* (Diptera: Culicidae) to use blood for producing lipid reserves for overwinter survival. Journal of Medical Entomology. 1989;**26**(4):318-326. DOI: 10.1111/j.1365-2761.1989.tb00328.x

[19] Veerman A, Slagt ME, Alderlieste MFJ. Photoperiodic induction of diapause in an insect is vitamin A dependent. Experientia. 1985;**41**(9):1194-1195. DOI: 10.1007/BF01951725

[20] Paolucci S, Zande LV, Beukeboom LW. Adaptive latitudinal cline of photoperiodic diapause induction in the parasitoid *Nasonia vitripennis* in Europe. Journal of Evolutionary Biology. 2013;**26**(4):705-718. DOI: 10.1111/jeb.12113

[21] Powell CP, Bass MH. Some effects of photoperiod, temperature, and food on the induction of diapause in the Boll Weevil. Journal of Economic Entomology. 1968;**61**(3):624-625. DOI: 10.1093/jee/61.3.624

[22] Chen Z, Dong Y, Wang Y. Pupal diapause termination in *Bactrocera minax*: an insight on 20-hydroxyecdysone induced phenotypic and genotypic expressions. Scientific Reports. 2016;**6**:27440. DOI: 10.1038/srep27440

[23] Holbrook GL, Schal C. Maternal investment affects offspring phenotypic plasticity in *Aviviparous cockroach*. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**(15):5595-5597. DOI: 10.1073/pnas.0400209101

[24] Reznik SY, Voinovich ND. Maternal regulation of *Trichogramma embryophagum* diapause photoperiodic sensitivity of adult females. Biological Control. 2011;**57**(2):158-162. DOI: 10.1016/j.biocontrol.2011.02.005

[25] Melanie G, Hans VD, Bengt K. Reproductive plasticity, ovar-ian dynamics and maternal effects in response to temperature and flight in *Pararge aegeria*. Journal of Insect Physiology. 2010;**56**(9):1275-1283. DOI: 10.1016/j.jinsphys.2010.04.009

[26] Savalli UM, Czesak ME, Fox CW. Paternal investment in the seed beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae): Variation among populations. Annals of the Entomological Society of America. 2000;**93**(6):1173-1178. DOI: 10.1023/A:1005834807873

[27] Lacour G, Vernichon F, Cadilhac N. When mothers anticipate: Effects of the prediapause stage on embryo development time and of maternal photoperiod on eggs of a temperate and a tropical strains of *Aedes albopictus* (Diptera: Culicidae). Journal of Insect Physiology. 2014;**71**:87-96. DOI: 10.1016/j.jinsphys.2014.10.008

[28] McWatters HG, Saunders DS. Maternal temperature has different effects on the photoperiodic response and duration of larval diapause in blow fly (*Calliphora vicina*) strains collected at two latitudes. Physiological Entomology. 1998;**23**(4):369-375. DOI: 10.1046/j.1365-3032.1998.234101.x

[29] McWatters HG, Saunders DS. The influence of each parent and geographic origin on larval diapause in the blow fly, *Calliphora vicina*. Journal of Insect Physiology. 1996;**42**(8):721-726. DOI: 10.1016/0022-1910(96)00030-3

[30] Shin-Ichiro TACHIBANA, Hideharu. Maternal induction of larval diapause and its sensitive stage in the blow fly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae). Entomological. Science. 2004;**7**(3):231-235. DOI: 10.1111/j.1479-8298.2004.00068.x

[31] Reynolds JA, Peyton JT, Denlinger DL. Changes in microRNA abundance may regulate diapause in the flesh fly, *Sarcophaga bullata*. Insect Biochemistry and Molecular Biology. 2017;**84**:1-14. DOI: 10.1016/j.ibmb.2017.03.002

[32] Emerson KJ, Bradshaw WE, Holzapfel CM. Complications of complexity: Integrating environmental, genetic and hormonal control of insect diapause. Trends in Genetics: TIG. 2009;**25**(5):217-225. DOI: 10.1016/j.tig.2009.03.009

[33] Armbruster PA. Photoperiodic diapause and the establishment of *Aedes albopictus* (Diptera: Culicidae) in North America. Journal of Medical Entomology. 2016;**53**(5):1013-1023. DOI: 10.1093/jme/tjw037

[34] Takeda M. Effects of photoperiod and temperature on larval development and summer diapause in two geographic ecotypes of the rice stem maggot, *Chlorops oryzae* Matsumura (Diptera: Chloropidae). Applied Entomology and Zoology. 1997;**32**(1):63-74. DOI: 10.1007/BF01991956

[35] Takeda M, Nagata T. Effects of temperature and winter diapause on survival and development in bivoltine and trivoltine ecotypes of the rice stem maggot, *Chlorops oryzae* Matsumura (Diptera: Chloropidae), reared on a winter host. Applied Entomology and Zoology. 1998;**33**(1):85-96. DOI: 10.1086/320066

[36] Goto SG, Numata H. Alteration of the pupal diapause program and regulation of larval duration by photoperiod in the flesh fly *Sarcophaga similis* Meade (Diptera: Sarcophagidae). Applied Entomology and Zoology. 2009;**44**(4):603-609. DOI: 10.1303/aez.2009.603

[37] Ichikawa A, Ikeda M, Goto SG. Cold storage of diapausing larvae and post-storage performance of adults in the blowfly *Lucilia sericata* (Diptera: Calliphoridae). Applied Entomology and Zoology. 2020;**55**(3):321-327. DOI: 0.1007/s13355-020-00685-8

[38] Zhou Z, Dong X, Su Q. Effects of pre-diapause temperature and body weight on the diapause intensity of the overwintering generation of *Bactrocera minax* (Diptera: Tephritidae). Journal of Insect Science. 2020;**20**(1):12. DOI: 10.1093/jisesa/ieaa005

[39] Numata H, Shiga S. Induction of adult diapause by photoperiod and temperature in *Protophormia terraenovae* (Diptera: Calliphoridae) in Japan. Environmental Entomology. 1995;**24**(6):1633-1636. DOI: 10.1093/ee/24.6.1633

[40] Fowler FE, Chirico J, Sandelin BA. Seasonality and diapause of *Musca autumnalis* (Diptera: Muscidae) at its southern limits in North America, with observations on *Haematobia irritans* (Diptera: Muscidae). Journal of Medical Entomology. 2015;**52**(6):1213-1224. DOI: 10.1093/jme/tjv123

[41] Masafumi Y, Eizi. Effect of photoperiod and temperature on the induction of diapause in a Japanese strain of *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). Applied Entomology and Zoology. 2012;**47**(1):17-26. DOI: 10.1007/s13355-011-0084-4

[42] Nomura M, Ishikawa Y. Biphasic effect of low temperature on completion of winter diapause in the onion maggot, *Delia antiqua*. Journal of Insect Physiology. 2000;**46**(4):373-377. DOI: 10.1016/S0022-1910(99)00120-1

[43] Hondelmann P, Poehling HM. Diapause and overwintering of the hoverfly *Episyrphus balteatus*. Entomologia Experimentalis Et Applicata. 2007;**124**(2):189-200. DOI: 10.1111/j.1570-7458.2007.00568.x

[44] Tatar M, Yin C. Slow aging during insect reproductive diapause: why butterflies, grasshoppers and flies are like worms. Experimental Gerontology. 2001;**36**(4):732-738. DOI: 10.1016/S0531-5565(00)00238-2

[45] Sim C, Denlinger DL. Insulin signaling and the regulation of insect diapause. Frontiers in Physiology. 2013;**4**:189. DOI: 10.3389/fphys.2013.00189

[46] Agui N, Mihara M, Kurahashi H. Effect of juvenile hormone analogue on ovarian development of the reproductive-diapausing parasitic fly *Melinda pusilla*. Medical Entomology and Zoology. 2016;**42**(4):311-317. DOI: 10.7601/mez.42.311

[47] Kang DS, Denlinger DL, Sim C. Suppression of allatotropin simulates reproductive diapause in the mosquito *Culex pipiens*. Journal of Insect Physiology. 2014;**64**:48-53. DOI: 10.1016/j.jinsphys.2014.03.005

[48] Janice R, Ming-Hsuan C, Roger M. Juvenile hormone biosynthesis in diapausing and nondiapausing *Culex pipiens* (Diptera: Culicidae). Journal of Medical Entomology. 1999;**36**(3):355-360. DOI: 10.1093/jmedent/36.3.355

[49] Guo Q, Hao YJ, Li Y. Gene cloning, characterization and expression and enzymatic activities related to trehalose metabolism during diapause of the onion maggot *Delia antiqua* (Diptera: Anthomyiidae). Gene. 2015;**565**(1):106-115. DOI: 10.1016/j.gene.2015.03.070

[50] Peng W, Feng MJ, Chen H. Progress on genome sequencing of Dipetran insects. Hereditas. 2020;**42**(11):1093-1109. DOI: 10.16288/j.yczz.20-130

[51] Zhang QL, Yuan ML. Progress in insect transcriptomics based on the next generation sequencing technique. Acta Entomologica Sinica. 2013;**56**(12):1489-1508. DOI: 10.16380/j.kcxb.2013.12.005

[52] Stark R, Grzelak M, Hadfield J. RNA sequencing: The teenage years. Nature Reviews Genetics. 2019;**20**(11):631-656. DOI: 10.1038/s41576-019-0150-2

[53] Zeb Q, Wang C, Shafiq S. An Overview of Single-Cell Isolation Techniques. USA: Elsevier, Academic

Press; 2019. pp. 101-135. DOI: 10.1016/B978-0-12-814919-5.00006-3

[54] McLaughlin CN, Brbic M, Xie Q. Single-cell transcroptomes of developing and adult olfactory receptor neurons in *Drosophila*. eLife. 2021;**10**:e63856. DOI: 10.1101/2020.10.08.332130

[55] Hao YJ, Zhang YJ, Si FL. Insight into the possible mechanism of the summer diapause of *Delia antiqua* (Diptera: Anthomyiidae) through digital gene expression analysis. Insect Sci. 2016;**23**(3):438-451. DOI: 10.1111/1744-7917.12323

[56] Ren S, Hao YJ, Chen B. Global Transcriptome sequencing reveals molecular profiles of summer diapause induction stage of onion maggot, *Delia antiqua* (Diptera: Anthomyiidae). G3-Genes Genomes. Genetics. 2018;**8**(1):207-217. DOI: 10.1534/g3.117.300393

[57] Gong ZJ, Wu YQ, Miao J. Global Transcriptome analysis of orange wheat blossom midge, *Sitodiplosis mosellana* (Gehin) (Diptera: Cecidomyiidae) to identify candidate transcripts regulating diapause. PLoS One. 2013;**8**(8):e71564. DOI: 10.1371/journal.pone.0071564

[58] Zhou Z, Dong X, Li C. Comparative transcriptome analysis of different developmental stage of *Bactrocera minax* (Diptera: Tephritidae): Implication of the molecular basis of its obligatory diapause induction. Comparative Biochemistry and Physiology D-Genomics and Proteomics. 2021;**38**:100818. DOI: 10.1016/j.cbd.2021.100818

[59] Zhang B, Han HB, Xu LB. Transcriptomic analysis of diapause-associated genes in *Exorista civilis* Rondani (Diptera: Tachinidae). Archives of Insect Biochemistry and Physiology. 2021;**107**(2):e21789. DOI: 10.1002/arch.21789

[60] Rinehart JP, Denlinger DL. Heat-shock protein 90 is down-regulated during pupal diapause in the flesh fly, *Sarcophaga crassipalpis*, but remains responsive to thermal stress. Insect Molecular Biology. 2000;**9**(6):641-645. DOI: 10.1046/j.1365-2583.2000.00230.x

[61] Chen B, Kayukawa T, Jiang HB. DaTrypsin, a novel clip-domain serine proteinase gene up-regulated during winter and summer diapauses of the onion maggot, *Delia antiqua*. Gene;**347**(1):115-123. DOI: 10.1016/j.gene.2004.12.026

[62] Zhao J, Huang Q, Zhang G. Characterization of two small heat shock protein genes (Hsp17.4 and Hs20.3) from *Sitodiplosis mosellana*, and their expression regulation during diapause. Insects. 2021;**12**(2):119. DOI: 10.3390/insects12020119

[63] Joplin K, Yocum G, Denlinger D. Diapause specific proteins expressed by the brain during the pupal diapause of the flesh fly, *Sarcophaga crassipalpis*. Journal of Insect Physiology. 1990;**36**(10):775, 781-779, 783. DOI: 10.1016/0022-1910(90)90051-G

[64] Han HB, Jiang S, Lin KJ. Proteomic analysis of diapause proteins in the dominant parasitoid tachinid fly *Exorista civilis*. Journal of Plant Protection. 2021;**48**(6):1359-1369. DOI: 10.13802/j.cnki.zwbhxb.2021.2021892

[65] Jiang T, Zhu YL, Peng YC. Universal and differential transcriptional regulatory pathways involved in the preparation of summer and winter diapauses in *Pieris melete*. Bulletin of Entomological Research. 2021;**111**(3):371-378. DOI: 10.1017/S0007485321000018

[66] Dunn WB, Broadhurst D, Begley P. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Nature Protocols. 2011;**6**(7):1060-1083. DOI: 10.1038/nprot.2011.335

[67] Wang J, Fan H, Xiong KC. Transcriptomic and metabolomic profiles of Chinese citrus fly, *Bactrocera minax* (Diptera: Tephritidae), along with pupal development provide insight into diapause program. PLoS One. 2017;**12**(7):e0181033. DOI: 10.1371/journal.pone.0181033

[68] Zhang B, Han HB, Xu LB. Metabolomic analysis of diapause-associated metabolites in tachinid parasite *Exorista civilis* based on liguid chromatograph mass spectrometry. Journal of Plant Protection. 2022;**49**(2):705-706. DOI: 10.13802/j.cnki.zwbhxb.2021.2020228