

Review

Meeting the energetic demands of insect diapause: Nutrient storage and utilization

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Abstract

Insects in diapause characteristically feed very little or not at all, thus they are largely or totally dependent on energy reserves sequestered prior to the entry into diapause. Fats are the dominant reserve used during this period, but non-fat reserves are also important for some species, especially during certain phases of diapause. Metabolic depression, coupled with the low temperatures of winter, facilitates the economic utilization of reserves during the many months typical of most diapauses. Though many insects store additional lipid prior to the entry into diapause, our review of the literature indicates that this is not always the case. We provide evidence that interactions between nutrient storage and metabolism can influence the decision to enter diapause and determine how long to remain in diapause. In addition, the energy reserves expended during diapause have a profound effect on post-diapause fitness. Though the physiological and biochemical mechanisms that regulate nutrient homeostasis prior to and during diapause remain poorly known, we propose several mechanisms that have the potential to contribute to diapause-associated nutrient homeostasis. Potential players include insulin signaling, neuropeptide F, cGMP-kinase, AMP-activated protein kinase, and adipokinetic hormone.

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Contents

1. Introduction	761
2. Diapause as a distinct life history stage	761
3. Energetic challenges of diapause	761
4. Metabolic depression: a strategy for minimizing energetic demands during diapause	762
5. Nutrient reserves and diapause	762
6. Body size, reserves, and the prediapauses preparatory program	764
7. Dynamics of reserve utilization during diapause	765
8. Assessment of nutrient reserves	766
9. Key questions and future directions	768
Acknowledgments	769
References	770

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1. Introduction

Diapause poses several interesting challenges for nutrient storage and utilization. Commonly, diapause occurring in the temperate zones last for 9–10 months and, in some less-common cases, may persist for a year or more. Most diapausing insects do not feed at all during diapause or, in the case of some larvae and adults, feed very little. This implies that the insect must sequester sufficient reserves in the pre-diapause period to meet its metabolic needs during diapause and still have sufficient reserves remaining at the end of diapause to complete development and resume activity. This challenge is further exacerbated in insects that diapause during the warm summer months or in tropical regions that do not provide the low temperatures that can be exploited to suppress metabolic needs.

It is our goal in this review to identify some of the challenges of diapause as they relate to nutrient storage and utilization. A number of the prevailing perceptions about this field seem quite logical but are, in fact, not universally true. We attempt to point out some of the discrepancies that prevent us from making unwarranted generalizations. There is, for example, a perception that insects preparing for entry into diapause consistently sequester additional energy reserves, but we show that, surprisingly, this is not always the case. Though larger individuals often fare better during diapause than their smaller counterparts, we provide some examples in which the opposite is true. One of the hallmarks of diapause is the concurrent depression of metabolism, and we provide examples reflecting the wide range in extent of depression that occurs among different insects, a feature that is especially dependent upon the requirements of the life stage used for diapause. There is, however, consistency concerning the form of nutrient storage: fat reserves are clearly the most important reserve used by insects to meet their energy demand during diapause. But, we show that some insects switch from reliance on fat to use of non-fat reserves at specific times during diapause, and fascinating patterns of energy utilization, reflected in infradian cycles of oxygen consumption, suggest that energy metabolism during diapause may be more dynamic than currently appreciated. We currently know little about the physiological processes used by diapausing insects to assess their nutrient reserves and to regulate nutrient utilization, but we put forth several suggestions, based primarily on the vertebrate literature and recent progress with *Drosophila melanogaster* and *Caenorhabditis elegans*, that we feel could pave the way toward an understanding of some of the key decisions about nutrient sequestration and utilization during insect diapause.

2. Diapause as a distinct life history stage

Unlike quiescence, which is an immediate response to environmental stress that can occur at any time during the

lifecycle, diapause is a genetically programmed developmental response that occurs at a specific stage for each species. Species having an obligate diapause arrest development at the same point in the lifecycle every generation regardless of the environmental conditions, whereas in species with a facultative diapause the environment experienced by individuals, or sometimes their parents, determines whether an individual will diapause (Tauber et al., 1986; Danks, 1987; Denlinger, 2002). Examples of programmed developmental arrest are found in every stage of the insect lifecycle from embryonic blastoderm formation to adults that have already begun reproducing, illustrating the broad diversity of diapause strategies in insects. Understanding the energetic demands of diapause requires careful consideration of the diapause strategy of each species and how these energy demands may shape the insect's lifecycle.

Although it may not be obvious in many insects, diapause does not entail a complete cessation of development. As evidenced by characteristic temporal patterns of gas exchange, nutrient metabolism, stress resistance, and gene expression, diapause is a dynamic process (Andre-wartha, 1952; Denlinger, 2002; Hodek, 2002; Kostal, 2006). Diapausing insects pass through a graded series of physiologically distinct developmental stages including induction, preparation, initiation, maintenance, termination, and sometimes post-diapause quiescence (recently reviewed by Kostal, 2006). Many metabolic pathways, such as the anabolic pathways leading to cell growth and proliferation, are down-regulated during diapause (Storey and Storey, 1990, 2004; Denlinger, 2002; Denlinger et al., 2005). Other pathways involved in basic cellular maintenance remain operational at reduced levels during diapause, and some metabolic pathways are up-regulated during diapause. Most obviously up-regulated are stress-resistance pathways leading to cryoprotectant and heat shock protein synthesis, however, non-stress-related metabolic pathways can also differ (Storey and Storey, 1990, 2004; Denlinger, 2002; Denlinger et al., 2005). Therefore, we believe it most useful to think of diapause as a physiologically dynamic alternative to direct development rather than a strict developmental arrest.

3. Energetic challenges of diapause

In spite of its obvious advantages, diapause is a metabolically expensive life history strategy. Costs of diapause are commonly reflected in lower post-diapause survival and reduced fecundity (Denlinger, 1981; Bradshaw et al., 1998; Han and Bause, 1998; Kroon and Veendaal, 1998; Ellers and van Alphen, 2002; Williams et al., 2003; Munyiri et al., 2004; Matsuo, 2006). The two most likely physiological mechanisms underlying these costs are damage due to diapause-associated stresses, such as desiccation or cold shock, and the depletion of metabolic reserves that could contribute to a decrease in post-diapause fitness. We should emphasize that these two

mechanisms are intertwined. For example, the production of stress resistance compounds, such as the cryoprotectant glycerol, contributes significantly to maintenance metabolism during diapause and the utilization of metabolic reserves (Storey and Storey, 1990; Denlinger, 2002). Careful biochemical dissection of diapause and post-diapause metabolism will be needed to separate what portions of the costs of diapause are due to stress damage as opposed to consumption of critical metabolic reserves.

One key challenge for many diapausing insects is that their developmental arrest encompasses significant periods at high temperature. High temperature is costly for a poikilotherm, a challenge that is especially pronounced in tropical and summer diapauses (Denlinger, 1986; Wolda, 1988). Among temperate insects, both the duration of the warm prewinter diapause period and the duration of the cold overwintering period affect survival. For example, longer warm prewinter periods and longer winter periods had a cumulative negative effect on post-diapause survival and metabolic reserves in diapausing pupae of the apple maggot, *Rhagoletis pomonella* (Feder et al., 1997a,b). Similarly, both fat reserves and fecundity decreased significantly with time spent in diapause in the parasitoid wasp, *Asobara tabida* (Ellers and van Alphen, 2002). However, not all insects show clear costs of remaining in diapause for longer periods of time. For example, the Colorado potato beetle, *Leptinotarsa decimlineata*, shows no apparent cost of diapause length on female fecundity (Peferoen et al., 1981; Jansson et al., 1989). In a few examples, wherein the diapause program includes increasing body size and metabolic reserves, diapause has even been associated with greater fecundity (Saringer and Szentkiralyi, 1980; Fantinou et al., 2004; Wang et al., 2006).

However, care must be taken when assessing costs because post-diapause fitness results from the sum of a variety of interacting life history parameters and measuring only one or a few of these can produce misleading results. For example, longer diapause periods lead to increased longevity in the bruchid beetle, *Kytorhinus sharpianus*, but also decreased fecundity (Ishihara and Shimada, 1995). Similarly, fecundity did not differ among adult female flesh flies that had experienced pupal diapauses of 50 vs. 100 days, but the fertility of females that had been in diapause for 100 days was approximately half of that for females that had only diapaused for 50 days (Denlinger, 1981). Less is known about the costs of diapause on male reproduction. Some studies have shown no effects of diapause on male performance (Denlinger, 1981). However, effects may be subtle and more difficult to detect in males than females. When the diapause period was prolonged for larvae of the seed beetle, *Exechesops leucopis*, male survival was lower, and both adult male body size and secondary sexual characters were reduced, consequences that would likely affect the ability of males to compete for females in this species (Matsuo, 2005, 2006).

4. Metabolic depression: a strategy for minimizing energetic demands during diapause

Coincident with developmental arrest, diapause is characterized by decreased intermediary and respiratory metabolism (Tauber et al., 1986; Danks, 1987; Guppy and Withers, 1999). The degree of metabolic suppression varies among insects and correlates roughly with the performance requirements of the stage of diapause. Adult monarch butterflies, *Danaus plexippus*, sustain their flight capacity even after making their pre-winter migratory flights and show little reduction in metabolic rates during overwintering dormancy (Chaplin and Wells, 1982), whereas inactive diapausing pupae of the flesh fly *Sarcophaga argyrostoma* show metabolic rates approximately 90% less than similarly inactive non-diapausing pupae (Denlinger et al., 1972, Fig. 1). Metabolic depression involves shutting down or vastly decreasing the activity of energetically expensive biochemical and physiological systems. For example, in overwintering adults of both the Colorado potato beetle *L. decimlineata* (deKort, 1990) and the tropical beetle *Stenotarsus rotundus* (Wolda and Denlinger, 1984), the diapause program includes degrading metabolically costly tissues such as the flight musculature and digestive tract which must be rebuilt after diapause.

In addition to physiological alterations that decrease metabolic rate, careful selection of a diapause site can affect diapause energetics and costs. Although depressed, metabolism remains responsive to temperature and diapausing insects experiencing lower temperatures show decreased respiration and slower consumption of reserves than insects experiencing warmer conditions; thus low temperature is a powerful force reducing the energetic costs of diapause (Thompson and Davis, 1981; Chaplin and Wells, 1982; Irwin and Lee, 2000). Evidence for reduced costs of diapause at lower overwintering temperatures have been identified in the gall fly, *Eurosta solidaginis* (Irwin and Lee, 2000, 2003), and in the rose galling wasp, *Diplolepis spinosa* (Williams et al., 2003). In both species, individuals experiencing higher temperatures during diapause in the laboratory and field had higher metabolic rates and decreased survival and fecundity (Fig. 2). Among freeze-tolerant insects, being frozen during diapause may also promote significant energetic savings. Respiratory metabolism is lower in frozen larvae of the goldenrod gall fly, *E. solidaginis*, than in supercooled larvae at the same temperature, and larvae are more likely to freeze in colder microhabitats, a feature that correlates with greater survival and post-diapause fecundity (Irwin and Lee, 2002, 2003).

5. Nutrient reserves and diapause

Many insects diapause in non-feeding life stages such as eggs, pupae, or wandering larvae, therefore all metabolic materials used during diapause originate from reserves. Among those species which diapause in life stages capable

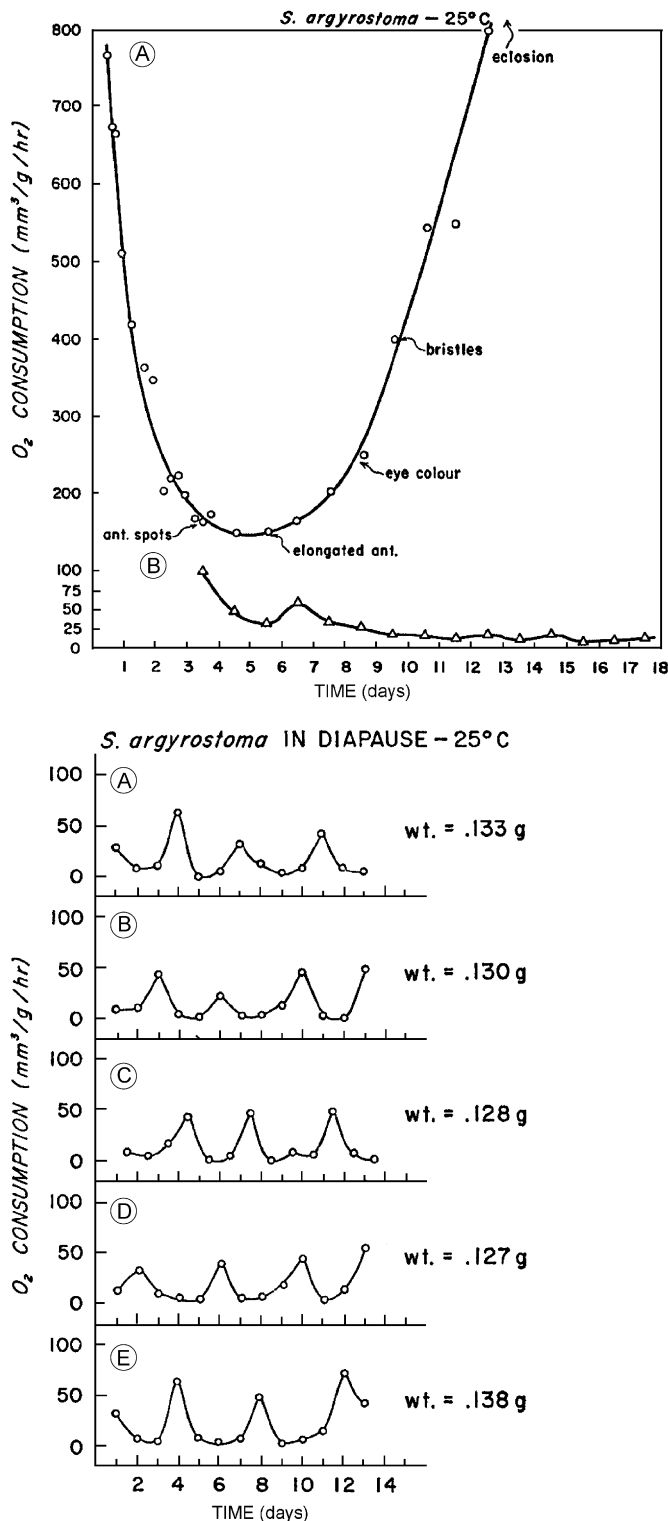


Fig. 1. Metabolic depression and cycles of oxygen consumption during pupal diapause in the flesh fly *Sarcophaga argyrostoma*. The upper panel shows oxygen consumption rate at 25°C (A) in a non-diapausing individual from the time of puparium formation until adult eclosion and (B) in a group of 10 diapausing pupae. The lower panel shows oxygen consumption patterns in individual diapausing pupa. The cyclic nature of oxygen consumption for individuals shown in the lower panel is masked in the upper panel because of the group measurements. Adapted from Denlinger et al. (1972).

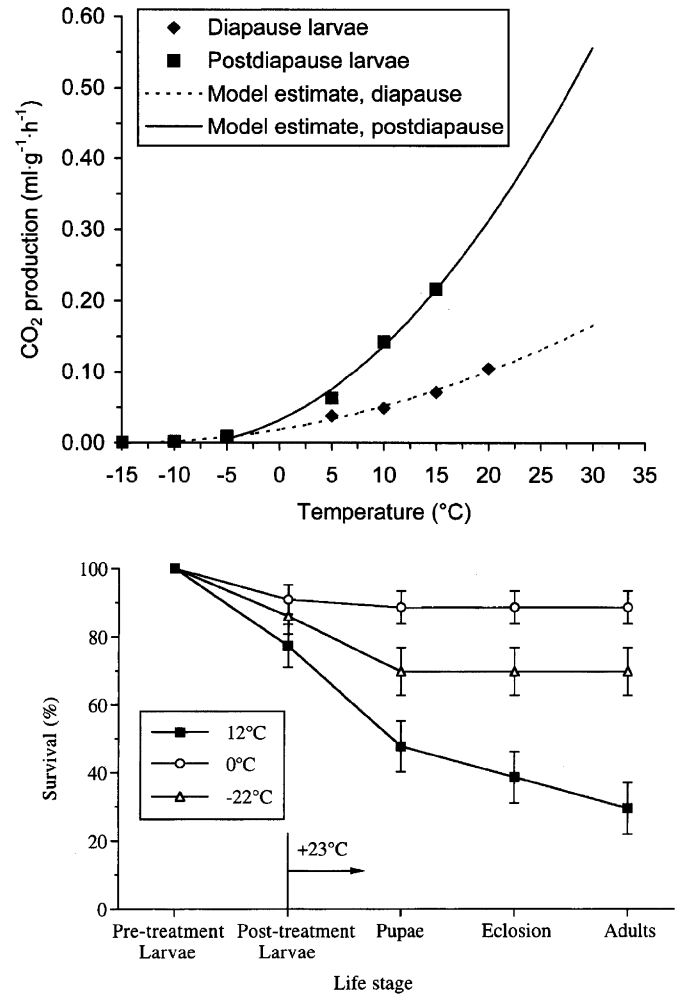


Fig. 2. Temperature during diapause affects metabolic rate and survival in the goldenrod gall fly *Eurosta solidaginis*. The upper panel shows the relationship between temperature and metabolic rate in larvae during and after diapause. The lower panel shows survival to different life stages for larvae held at different temperatures during the diapause period. Adapted from Irwin and Lee (2003) and Irwin and Lee (2000).

of feeding, nutrient intake may be absent, minimized, or distinctly different during diapause (Tauber et al., 1986; Danks, 1987). For example, adult Colorado potato beetles, *L. decemlineata*, do not feed during diapause; they burrow into the soil and do not emerge until diapause is broken (Voss et al., 1988). In contrast, diapausing adults of the black blowfly, *Phormia regina*, are active and will feed during diapause. However, they consume 80% less than non-diapause adults (Stoffolano, 1975). Similarly, diapausing adult females of the mosquito *Culex pipiens* actively feed on sugar, but they avoid taking blood meals (Mitchell and Briegel, 1989; Bowen, 1992). Therefore, internal reserves accumulated prior to diapause are critical for providing metabolic substrates during diapause and for post-diapause processes. The importance of reserves for post-diapause processes is most apparent in species that diapause as post-feeding larvae or pupae which must contain adequate reserves at diapause termination to undergo metamorphosis, construct adult tissues, and

provide energy necessary to find food and initiate post-diapause feeding (Leather et al., 1993; Denlinger et al., 2005). Even among insects that diapause as adults, nutrient reserves are critical for restoring post-diapause functions, including the rebuilding of tissues atrophied as part of the diapause program and for providing energy for post-diapause activities, such as dispersal and reproduction (Wolda and Denlinger, 1984; deKort, 1990).

Diapausing and direct-developing insects both store metabolic reserves of the same three major groups of macronutrients: lipids, carbohydrates, and amino acids. As in other organisms, triacylglycerides are the primary form of storage lipids in insects. Triacylglycerides can be obtained directly from food or synthesized from carbohydrate or amino acid precursors via tricarboxylic acid cycle intermediates as in vertebrates (Downer and Matthews, 1976; Keeley, 1985; Steele, 1985; Canavoso et al., 2001). The fat body is the primary site of fatty acid synthesis, triacylglyceride production, and triacylglyceride storage in insects, although some triacylglycerides are stored in metabolically active tissues such as the thoracic flight musculature (Zera and Denno, 1997; Canavoso et al., 2001). The primary carbohydrate reserve in both diapausing and non-diapausing insects is the polysaccharide glycogen, while trehalose is the primary blood sugar (Steele, 1985; Danks, 1987). While some diapausing insects may contain greater trehalose concentrations than their non-diapausing counterparts, this increase in blood sugar is generally thought to function in cold and desiccation resistance rather than directly in storage. Some diapausing insects also contain increased amino acid concentrations in the blood, but, like the sugars, these additional amino acids likely function primarily in cold and desiccation resistance (Boctor, 1981; Morgan and Chippendale, 1983; Lefevre et al., 1989). However, many diapausing insects do store amino acids in specialized proteins. These proteins, which can become very abundant, were initially termed diapause proteins, but later analysis revealed that the majority belong to the storage hexamerin family of insect proteins (Telfer and Kunkel, 1991; Burmester, 1999; Denlinger et al., 2005). Storage proteins are typically accumulated prior to diapause and their constituent amino acids may be used to supply both intermediary and respiratory maintenance metabolism during diapause, and post-diapause functions such as resumption of development (Denlinger et al., 2005).

6. Body size, reserves, and the prediapause preparatory program

Diapause is programmed by environmental cues well before the onset of arrest, and diapause-destined insects often alter their physiology during the prediapause preparatory period. Changes in body size and the accumulation of energetic reserves are among the most conspicuous alterations that occur during the prediapause period (Tauber et al., 1986; Danks, 1987; Denlinger et al.,

2005). The degree of size difference between diapause and non-diapause individuals can vary dramatically among species. For example, post-feeding diapause larvae are 15% heavier than non-diapause individuals in *Pieris rapae* (Kono, 1970), and post-feeding diapause larvae are 45% heavier than non-diapause individuals in the moth *Plodia interpunctella* (Tsuji, 1958). Increased body mass is typically correlated with increased nutrient reserves, therefore larger body sizes in diapausing individuals are generally considered to be adaptive because of their greater reserves.

Even though size differences may not be apparent, diapausing individuals of many species accumulate greater fat, glycogen, or protein reserves than non-diapause individuals as part of the diapause preparatory program (See Table 4, pp. 21–22 in Danks, 1987). For example, last instar diapause larvae of the pink bollworm, *Pectinophora gossypiella*, accumulate 50% greater lipid reserves than non-diapausing last instar larvae (Adkisson et al., 1963), and diapause-destined adult *C. pipiens* mosquitoes accumulate approximately twice the lipid and carbohydrate stores as equivalently aged non-diapause adults (Mitchell and Briegel, 1989, Fig. 3). Similarly, both post-feeding diapause larvae of the southwestern corn borer *Diatraea grandiosella* (Brown and Chippendale, 1978) and diapausing adult females of the Colorado potato beetle *L. decemlineata* (de Kort and Koopmanschap, 1994) accumulate markedly greater quantities of storage hexamerins than their non-diapausing counterparts.

From an adaptive standpoint, it makes good functional sense for diapause-destined individuals to increase their reserves as a strategy to deal with the energetic demands of the diapause period. However, not all species show diapause-associated increases in reserves or body size. For example, neither diapausing post-feeding larvae of the fly *Calliphora vicina* (Saunders, 1997) nor diapausing pupae

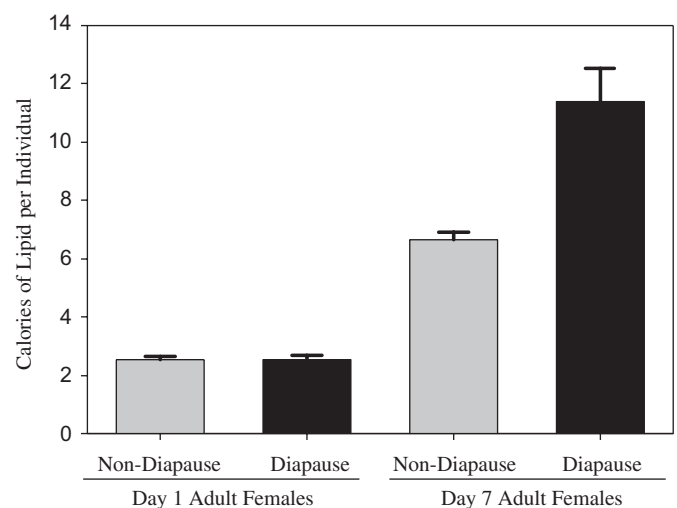


Fig. 3. Diapausing adult females of the mosquito *Culex pipiens* accumulate approximately twice the lipid stores as non-diapausing individuals during the first 7 days after eclosion. Bars represent means \pm s.e. Adapted from Mitchell and Briegel (1989).

of the tobacco hornworm *Manduca sexta* (Siebert, 1986) are heavier or store significantly greater quantities of lipid than their non-diapausing counterparts. In *C. vicina*, this results in adults from diapausing larvae eclosing with less teneral fat reserves than adults from non-diapausing larvae (Saunders, 2000). In some cases, diapausing individuals are even smaller than non-diapausing individuals. Diapause pupae of the butterfly *Papilio polyxenes* (Blau, 1981) and larvae of the budworm *Choristoneura fumiferana* (Harvey, 1961) are both smaller than their non-diapausing counterparts, presumably due to declining plant quality and increased costs of prolonged feeding.

It is unclear what proportion of diapausing species increase storage of metabolic reserves or body size as part of their diapause strategy because few species have been studied in detail. From a life history perspective, it is important to understand the relative costs and benefits of accumulating additional reserves or size prior to diapause. Some species that accumulate greater reserves are known to feed more or longer in preparation for diapause, perhaps increasing the risk of attracting natural enemies or not completing development before the onset of inclement conditions (Masaki, 1977; Tauber et al., 1986). In addition, whether a species increases lipid reserves prior to diapause or not may be related to the environment experienced during the diapause preparatory period. For example, bolls of cotton plants have greater lipid content than squares. While diapause-destined larvae of the pink bollworm, *P. gossypiella*, will feed on both structures, diapause-destined larvae only accumulate greater lipid reserves than non-diapause larvae when fed high-fat bolls, and not when fed lower-fat squares (Clark and Chadbourne, 1962). This observation highlights the importance of ecological conditions for diapause physiology and careful consideration of experimental conditions when performing research on diapause physiology.

Despite the observation that many species change their feeding patterns and accumulate greater reserves in association with diapause, little is known about the underlying physiological regulation of these shifts in feeding and intermediary metabolism. Recent work on the mosquito *C. pipiens* shows that the increased sugar feeding and lack of blood feeding associated with the accumulation of fat reserves and inhibition of reproduction during the prediapause period is correlated with changes in intermediary metabolism, specifically increased transcript abundance for the liposynthetic enzyme fatty acid synthase and decreased expression of trypsins used in blood meal digestion (Robich and Denlinger, 2005). Studies detailing diapause-associated changes in intermediary metabolism and feeding physiology are needed across taxa with different diapause strategies to expand our understanding of the metabolic processes underlying prediapause reserve accumulation. The ultimate goal in this area is to understand the underlying neurological and endocrine signaling mechanisms that regulate diapause-associated shifts in feeding patterns and intermediary metabolism.

7. Dynamics of reserve utilization during diapause

Numerous authors have shown that stored reserves decline during diapause and that individuals experiencing longer diapause periods contain fewer reserves after diapause (Tauber et al., 1986; Danks, 1987). For example, both the fly *C. vicina* (Saunders, 2000) and the parasitoid wasp *A. tabida* (Ellers and van Alphen, 2002) diapause as post-feeding larvae, and the fat content of resulting post-diapause adults decreases linearly with time spent in diapause. Similarly, storage protein content declines during diapause in larvae of the southwestern corn borer, *D. grandiosella* (Chippendale, 1973), and in adults of the Colorado potato beetle, *L. decemlineata* (Lefevre et al., 1989). Species may differ in their relative utilization of each reserve class during diapause. For example, the mobilization of fat reserves to fuel oxidative metabolism during diapause is evident in the low respiratory quotients, near 0.7, exhibited by some insects (Boell, 1935; Lees, 1955). However, there are also examples of insects displaying higher RQ values indicative of metabolizing a combination of fuels potentially including fats, carbohydrates, and amino acids (Schneiderman and Williams, 1953; Lees, 1955).

Patterns of reserve utilization can also change noticeably as individuals pass through different stages of diapause development. For example, total body fat decreases rapidly during the first half of diapause in pupae of the flesh fly *Sarcophaga crassipalpis*, while fat content remains relatively constant during the second half of diapause (Adedokun and Denlinger, 1985). Conversely, fat-free dry mass decreases little during the first half of diapause, and then drops rapidly during the second half of diapause in this species. This pattern suggests that diapausing *S. crassipalpis* pupae utilize fat stores as the primary metabolic fuel during the first half of diapause and other substrates, perhaps carbohydrates or proteins, in the second half of diapause. Similarly, RQ values in the solitary bee *Megachile rotundata* are near 0.7 during the first 3 months of overwintering, but are higher (0.8–1.0) during the last 3 months, suggesting that patterns of substrate catabolism change from early to late diapause in this species as well (Yocum et al., 2005). Unfortunately, data on lipid content and other stored nutrients, metabolites, and metabolic enzymes are rarely combined with measures of respiratory metabolism in the same animal over the course of diapause.

Intermediary and respiratory metabolism can also exhibit regular cycles of activity over time scales of hours or days during diapause (Hayes et al., 1972; Denlinger et al., 1972; Crozier, 1979a, b). This phenomenon has been best studied in the flesh fly *S. crassipalpis*, wherein diapausing pupae exhibit regularly timed pulses of oxygen consumption lasting for 12–36 h followed by several days of undetectable oxygen uptake (Denlinger et al., 1972; Slama and Denlinger, 1992, Fig. 1). Akin to the periodic arousals observed in mammalian hibernation, the

frequency of oxygen consumption peaks is temperature dependent, and periods of high oxygen consumption are associated with both changes in gene expression and increased synthesis of multiple classes of proteins (Denlinger et al., 1972; Joplin and Denlinger, 1989; Craig and Denlinger, 2000). Interestingly, these multi-day cycles of oxygen consumption in flesh flies are associated with increased titers of juvenile hormone (JH) and followed by increased JH esterase activity, suggesting that JH plays an important regulatory role (Denlinger et al., 1984; Denlinger and Tanaka, 1989). Periodic cycles of CO₂ release have been observed in numerous diapausing insects, but a firm link between the oxygen consumption cycles and CO₂ release has not been established (Schneiderman and Williams, 1955; Buck, 1962; Slama, 1988; Lighton, 1996; Chown et al., 2006). Clearly more work is needed to integrate respiratory patterns with intermediary metabolism in diapausing insects. Because JH is known to affect many aspects of intermediary metabolism in insects, it is a likely candidate for coordinating dynamics of metabolism during diapause in flesh flies and other insects (Stepien et al., 1988; Riddiford, 1994; Wyatt and Davey, 1996; Farkas et al., 2002). Future work focusing on the endocrine and biochemical regulation of metabolic cycles during diapause may provide an excellent forum for exploring the regulation of intermediary and respiratory metabolism in insects in general.

Rapid environmentally induced shifts in intermediary metabolism and substrate utilization are also well documented in diapausing insects. Many species convert glycogen stores to cryoprotectants in direct response to low temperature (Chino, 1958; Baust and Miller, 1970; Morrissey and Baust, 1976; Yaginuma and Yamashita, 1991). For example, during early fall, larvae of the goldenrod gall fly, *E. solidaginis*, accumulate significant glycogen reserves which are converted into glycerol late in the fall in response to low temperatures (Storey and Storey, 1990). Cryoprotectant production in several insect species is responsive to cold, such that low temperatures experienced in the fall, and even temperature drops during winter, promote the conversion of glycogen stores into glycerol (Storey and Storey, 1990). Carbohydrate-based cryoprotectants can also be metabolically recycled into storage compounds or catabolized to meet current energetic demands. For example, glycogen stores increase as cryoprotectants are cleared and reconverted back into glycogen at the end of the cold season in the goldenrod gall fly, *E. solidaginis* (Storey and Storey, 1986). Moving diapausing larvae of *E. solidaginis* from 3 to 13 °C during diapause leads to a decrease in sorbitol and an increase in glycogen content compared to individuals left at 3 °C (Storey and Storey, 1983). Therefore, conversion of glycogen into cryoprotectants and reconversion of cryoprotectants back into glycogen or other metabolic substrates is a dynamic process that can occur multiple times during diapause.

Although numerous factors may ultimately interact to control the dynamics of conversion of glycogen into

glycerol in insects, a temperature-dependent phosphorylation mechanism plays an important role. Glycogen phosphorylase is activated to break down glycogen through phosphorylation by the enzyme glycogen phosphorylase kinase and deactivated through dephosphorylation by glycogen phosphorylase phosphatase. Temperature-dependent activation of glycogen phosphorylase occurs under cold conditions because low-temperature exposure in the 0–5 °C range inactivates glycogen phosphorylase phosphatase while the reduction in glycogen phosphorylase kinase activity at 0–5 °C is less pronounced and in line with *Q*₁₀ predictions for the enzyme (Ziegler et al., 1979; Hayakawa and Chino, 1982; Churchill and Storey, 1989). Therefore, glycogen phosphorylase is more likely to be activated and provide glucose substrates for glycerol synthesis at low temperatures. This provides an elegant system for direct environmental control of metabolic flux via protein-phosphorylation reactions. Biochemical mechanisms similar to those underlying carbohydrate/cryoprotectant metabolism during diapause could possibly also contribute to the regulation of lipid and protein stores during diapause, and careful biochemical dissection of substrate–product stoichiometry and enzyme activity in cryoprotectant metabolic pathways should serve as a model for exploring biochemical regulation of lipid and protein metabolism during diapause.

8. Assessment of nutrient reserves

Insects are capable of assessing their internal nutritional status, a feature that has important consequences for behavior, such as foraging and mating, and life histories, such as the timing of metamorphosis and reproduction (Nijhout, 1994; Simpson et al., 1995; Hoshizaki, 2005; Swevers et al., 2005; Wu and Brown, 2006). We are beginning to understand that insects, like vertebrates, integrate information about the quality and quantity of current intake using both nervous stimuli, such as stretch receptors and compound-specific sensory receptors, and endocrine signals, primarily from the digestive tract acting on the brain. Much less is known about how insects perceive nutrient reserves, but it is clear that the fat body plays a dynamic role in signaling nutrient status in insects and is critical in modulating important life history traits such as longevity, metamorphic timing, body size, reproductive timing, and fecundity (Nijhout, 1994; Colombani et al., 2003; Hwangbo et al., 2004; Hoshizaki, 2005; Swevers et al., 2005). It is critical that individuals exit diapause with enough reserves to support post-diapause functions such as tissue building and metamorphosis, dispersal, and reproduction. Could insects use information about their nutritional status, particularly the status of their nutrient reserves, to modulate diapause characteristics?

Diapause is a metabolically demanding life history tactic, and as discussed earlier, larger individuals with greater nutrient reserves usually survive diapause better and have greater values for several post-diapause fitness correlates,

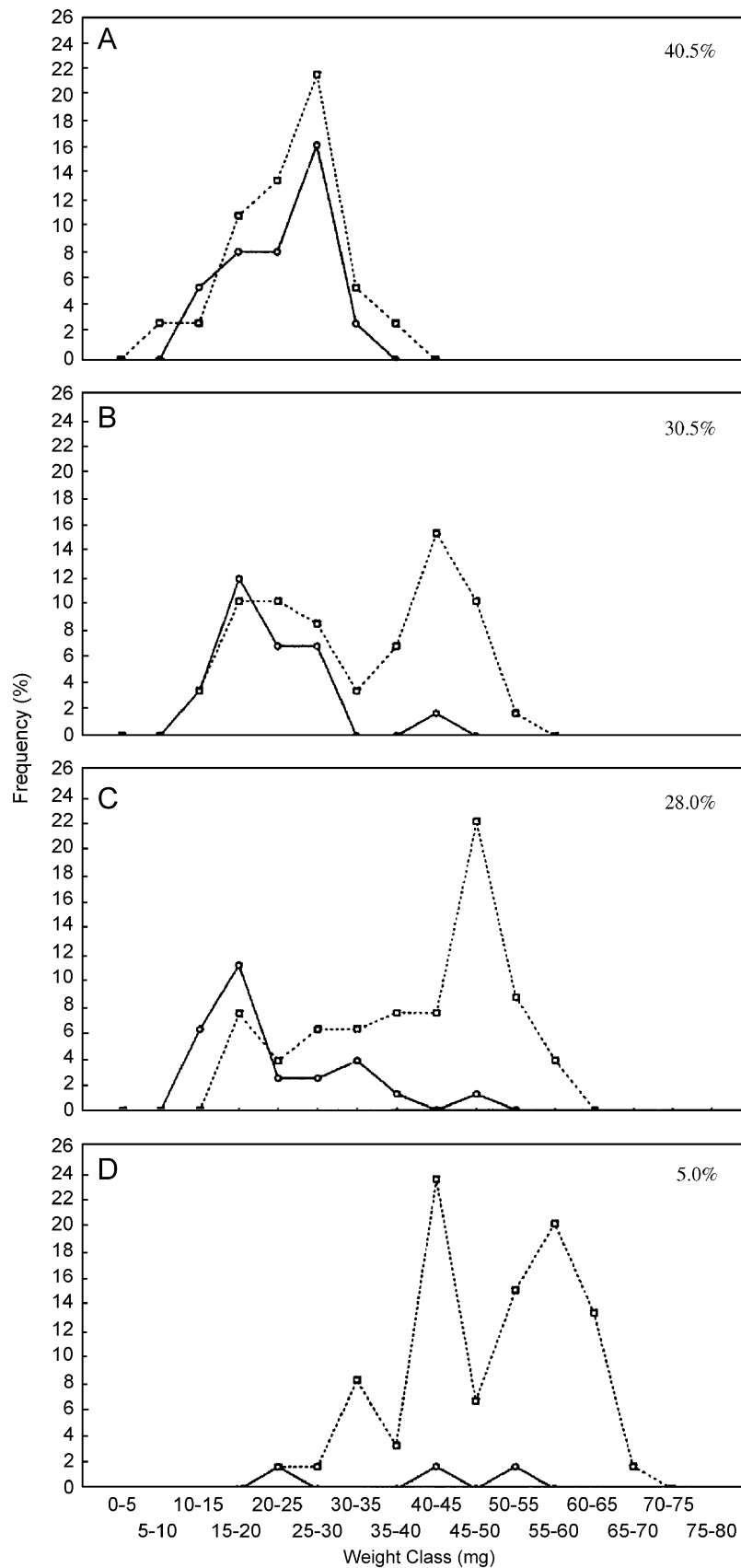


Fig. 4. Smaller larvae are less likely to enter diapaue than large larvae of the blow fly *Calliphora vicina*. Multiple size classes of larvae were produced by starvation under diapaue-inducing conditions. Each panel shows the frequency of larvae entering diapaue (dashed lines and squares) and averting diapaue (solid lines and circles). The percentage of individuals averting diapaue in each group is shown in the upper right corner of the panel. Adapted from Saunders (1997).

such as fecundity and fertility. Because there are significant costs associated with entering diapause with low reserves, relatively small or lean individuals within facultative diapausing species may avert diapause and undergo direct development or curtail diapause length. By manipulating the body size of diapause-destined larvae of the fly *C. vicina*, Saunders (1997) showed that fewer small individuals entered diapause than large individuals (60% vs. 95%, Fig. 4), suggesting that the expression of alternative developmental tactics is influenced by the size and internal nutritional state. In addition, smaller *C. vicina* larvae that did enter larval diapause exhibited shorter arrest periods than larger individuals (Fig. 5). Similarly, in the desert bee *Perdita portalis* (Danforth, 1999) and the chestnut weevil *Curculio elephas* (Menu and Desouhant, 2002), two species with long multi-year diapauses, heavier individuals are more likely to extend their diapause for an additional year than lighter individuals. While larger individuals do not always remain in diapause longer (e.g., Saringer and Szentkiralyi, 1980), the examples above suggest that some species modulate entry into diapause and the length of the arrest period based on body size and likely their nutritional reserve status. As an extension of this rationale, the duration of diapause is often shorter at high temperatures, possibly due to greater metabolic rates and increased catabolism of nutrient reserves (Thompson and Davis, 1981; Chaplin and Wells, 1982; Irwin and Lee, 2000; Denlinger, 2001). Unfortunately, few studies have combined experimental modification of body size and/or nutrient reserves with physiological parameters such as respiratory rate or intermediary metabolism to determine effects on diapause life history characteristics.

9. Key questions and future directions

Elucidating the physiological mechanisms used by diapausing insects to regulate nutrient storage and meta-

bolism could have a broad impact on our understanding of energetics and metabolism in insects in general. Vertebrates coordinate their energetic status using a network of endocrine pathways that integrate signals about incoming nutrition via the digestive tract (e.g., ghrelin), standing nutrient reserves (e.g., leptin), and metabolic demands, with signals that regulate feeding (e.g., neuropeptide Y), and the expression of life history traits such as growth (e.g., insulin-like growth factors) and perhaps reproduction (Schwartz et al., 2000; Woods and Seeley, 2000; Wynne et al., 2005). We expect that insects possess a similar signaling network that coordinates nutritional status with feeding and lifecycles, and that some of the components of this system are homologous with vertebrate regulatory systems.

Insulin signaling is a strong candidate pathway for integrating the regulation of metabolism during both the prediapause preparatory period and during diapause; it is likely to influence life history traits such as entry into diapause, diapause length, and post-diapause traits such as development and reproduction. Insulin-like activity has been known from insects and other invertebrates for more than three decades, but our current view that the insulin-like signaling system has conserved roles in nutrient homeostasis, growth, and reproduction is derived from the explosion of intensive genetic, cellular, and molecular studies on the model invertebrates *D. melanogaster* and *Caenorhabditis elegans* over the past 10 years (Leevers, 2001; Wu and Brown, 2006). Much of the foundational work on insulin-like signaling originated with the observation that mutations in the insulin-signaling pathway lead to long-lived individuals with phenotypes similar to the reproductive diapause observed in *D. melanogaster* adults, and the diapause-like dauer stage in *C. elegans*. Perturbing insulin-like signaling by creating mutant lines or performing functional knockout/over-expression studies has revealed simultaneous pleiotropic effects on developmental

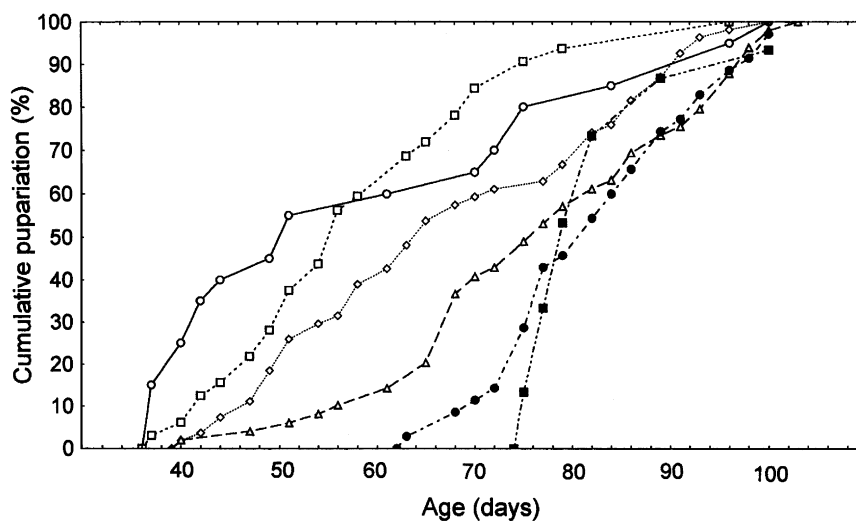


Fig. 5. Smaller larvae of the blow fly *Calliphora vicina* spend less time in diapause than large larvae as indicated by their earlier pupariation. Curves from left to right represent individuals of the following size ranges <30, 30–40, 40–50, 50–60, 60–70, >70 mg. Adapted from Saunders (1997).

arrest, nutrient storage, and body size (Leevers, 2001; Tatar and Yin, 2001; Wu and Brown, 2006). For example, adults carrying weak null alleles for the *Drosophila* insulin receptor have small somatic body sizes, hypertrophied fat bodies with high lipid stores, and are sub-reproductive and long-lived, like flies in an adult reproductive diapause (Tatar et al., 2001). Although the underlying mechanisms are unknown, injection of bovine insulin has also been shown to initiate adult development in diapausing pupae of the moth, *Pieris brassicae*, further suggesting the importance of insulin-like signaling to the diapause developmental program (Arpagus, 1987). For more comprehensive general information on insulin-like signaling in insects, we refer readers to an excellent recent review by Wu and Brown (2006).

In addition, several other signaling pathways stand out as promising candidates. Increased feeding likely underlies the accumulation of greater metabolic reserves during the diapause preparatory period. The hypothalamic hormone, neuropeptide Y, is produced in relation to energetic state and plays an important role in regulating appetite in vertebrates (Schwartz et al., 2000; Wynne et al., 2005). A homologous hormone, neuropeptide F, has been shown to regulate feeding in *D. melanogaster* and *C. elegans* (de Bono and Bargmann, 1998; Brown et al., 1999; Wu et al., 2003). The cyclic GMP-dependent protein kinase, *for*, has also been implicated in the control of feeding patterns in honeybees, *D. melanogaster*, and *C. elegans* (Osborne et al., 1997; Shaver et al., 1998; Ben-Shahar et al., 2002; Fujiwara et al., 2002). It is unclear whether cGMP-dependent kinases affect feeding behavior by altering metabolic networks or by directly affecting signaling like neuropeptide F, but both molecules could possibly contribute to pre-diapause shifts in feeding behavior associated with larger size and reserve accumulation.

Another promising candidate pathway involves the AMP-activated protein kinase (PKA). PKA is often regarded as part of the cellular energy sensing system because this enzyme becomes active in response to high ratios of AMP:ATP and creatine:creatine phosphate, phosphorylating a series of downstream proteins that control flux through metabolic pathways affecting appetite, ATP production, and the synthesis/degradation of lipid and carbohydrate reserves (Ruderman et al., 2003; Hardie, 2004). While best known for affecting metabolic flux in mammalian systems, PKA-like enzymes have been identified in multiple insect species where they appear to play similar roles (Foster et al., 1984; Holm et al., 2000; Pan and Hardie, 2002; Pfister and Storey, 2002; Andersson et al., 2004; Apfeld et al., 2004). In mammals, suppression of PKA activity leads to increased lipid biosynthesis and storage whereas activation of PKA leads to lipid and carbohydrate catabolism, making this regulatory enzyme a likely candidate for promoting accumulation of reserves during the diapause-preparatory period and regulating the dynamics of reserve utilization during diapause. Although a recent report by Pfister and Storey (2006) suggests that

PKA is not involved in temperature-dependent alterations in glycogen breakdown and glycerol synthesis during diapause in the goldenrod moth *Epiblema scudderiana*, investigation of additional metabolic pathways in a diversity of diapausing insects will be needed to assess the importance of PKA to the regulation of diapause-associated changes in metabolism.

Last, we propose that the adipokinetic hormones (AKHs) could contribute to diapause-associated alterations in metabolism. AKHs comprise a family of peptide hormones best known for mobilizing carbohydrates, lipids, and the amino acid proline from reserves to support the extreme energetic demands of insect flight (Gäde and Auerswald, 2003; van der Horst, 2003; Gäde, 2004), but recent physiological and genetic studies in *D. melanogaster* and *Bombyx mori* suggest that AKHs also play roles in nutrient homeostasis, particularly blood sugar regulation (Satake et al., 2000; Kim and Rulifson, 2004; Lee and Park, 2004; Isabel et al., 2005). To our knowledge, evidence of the involvement of AKH in diapause metabolism is currently limited to one study. Socha and Kodrik (1999) showed that brachypterous females of the firebug *Pyrhocoris apterus* in adult reproductive diapause released approximately twice as much lipid into the hemolymph than reproductive non-diapause brachypterous females when injected with an equivalent quantity of either *Locusta migratoria* AKH-I or extracts of the *P. apterus* AKH-producing glands. Changes in AKH production and sensitivity during development have been shown in other insects, and similar mechanisms could underlie both ontogenetic shifts in AKH signaling during direct development and changes in AKH signaling during diapause (van Marrewijk et al., 1984; Woodring et al., 2002; Kodrik et al., 2003).

Ultimately, understanding the physiological regulation of diapause-associated changes in metabolism will require careful biochemical and molecular dissection of both candidate up-stream signaling pathways and down-stream metabolic pathways combined with high-throughput genomic, proteomic, and metabolomic screening to identify new candidates in multiple well-characterized models for insect diapause. We encourage integrative studies of the consequences of altering metabolism, size, and nutrient storage at the whole organism level with physiological and biochemical studies of metabolism. Investigation of metabolic alterations during the diapause preparatory period should be combined with study of both short- and long-term metabolic alterations during diapause with the expectation that many of the same regulatory pathways will be involved in both of these life history stages.

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