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Fatty Acid Content and Enzymes of Fatty Acid Metabolism in Overwintering Cold-Hardy Gall Insects

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Abstract

Fatty acid content and enzymes of fatty acid metabolism were studied in overwintering larvae of two cold-hardy gall insects, the freeze-tolerant fly Eurosta solidaginis *and the freeze-avoiding moth* Epiblema scudderiana. *Both species increased the proportion of unsaturated fatty acids during the winter. Whereas total lipid content did not change in* Eurosta solidaginis, *a decrease in total lipids over the winter in* Epiblema scudderiana *suggested the use of fat reserves to maintain basal metabolism. Changes in the activities of enzymes of fat oxidation correlated with these observations in* Eurosta solidaginis: *hydroxyacyl-CoA dehydrogenase, carnitine-palmitoyl transferase, and acetoacetyl-CoA thiolase activities all decreased during overwintering. In* Epiblema scudderiana *the same activities were constant, decreased, or increased. These activities were, however, higher in the fat-oxidizing, freeze-avoiding species than in the freeze-tolerant larvae. Lipid content in* Epiblema scudderiana *increased again by early spring, possibly indicating this pool as the fate of carbon derived from the spring clearance of the cryoprotectant glycerol pool. Decreased activities of malic enzyme and ATP-citrate lyase suggested decreased potential for fatty acid synthesis in both species over the winter, consistent with the cessation of feeding in the fall. The potential for ketone body metabolism, measured as the activity of β-hydroxybutyrate dehydrogenase, increased greatly in both species during overwintering; however, levels of β-hydroxybutyrate remained less than 0.35 μmol/g wet mass throughout the study period. These data indicate that changes to storage lipid profiles in order to maintain fluidity and to lipid-metabolizing enzyme activities may play important roles in insect cold hardiness.*

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Introduction

Temperature effects on the properties of molecules and on rate processes represent a major challenge to animals that must survive low subzero temperatures. As major components of living tissue, lipids must preserve their structural and functional integrity at low temperatures to allow the survival of cold-hardy organisms. For example, since fatty acids can be mobilized only if they are in the liquid state, maintaining the fluidity of fatty acid reserves at low temperatures may be critical in cold-hardy animals, as has been suggested for hibernating ground squirrels (Frank 1991). Adaptations for the preservation of membrane structure and function in cold-hardy animals are believed to be essential for the maintenance of a viable state both during and after cold exposure, although the mechanisms of this process are still controversial (Hazel 1995). Also, membrane restructuring, as first defined by the homeoviscous adaptation theory of Sinensky (1974), is believed to be required to maintain fluidity and hence preserve structural and functional integrity on exposure to low-temperature conditions.

Cold-hardy insects face extended periods of low subzero temperatures as part of natural overwintering. In some of these animals lipids may serve as a source of energy to maintain basal metabolism over the winter, since diapausing or quiescent insects do not feed. However, fats can be used only after mobilization and under aerobic conditions, and the freezing of extracellular spaces in a freeze-tolerant species such as *Eurosta solidaginis* leads to a state of anoxia or ischemia, as evidenced by the accumulation of anaerobic end products and decreasing adenylates and energy charge. This would prevent the oxidation of lipids in the frozen state, and, as well, frozen haemolymph would serve as a barrier to the transport of lipids. Freeze-avoiding animals such as *Epiblema scudderiana* larvae face no such limitations on the use of lipids during overwintering. Hence, we predicted differences in the use of lipids as metabolic fuel between freeze-tolerant and freeze-avoiding insects.

Larvae of the freeze-avoiding moth *Epiblema scudderiana* and the freeze-tolerant fly *Eurosta solidaginis* have been used extensively as model systems for insect cold hardiness (see Storey and Storey [1992] and references therein). These species overwinter in goldenrod stem galls and must face temperatures that routinely fall to below −30°C. Both species accumulate high concentrations of carbohydrate cryoprotectants (glycerol and sorbitol in *Eurosta solidaginis,* glycerol in *Epiblema scudderiana*), and metabolic depression ensures that sufficient metabolic reserves are maintained to support pupation and emergence as adults the following spring. Many studies have investigated various aspects of intermediary metabolism and its control

in these insects, particularly with regard to cryoprotectant metabolism. Recent studies have shown how metabolic rearrangements at the enzymatic level are geared toward ensuring the necessary machinery for the activity of different pathways and how these may be controlled (Storey 1982; Holden and Storey 1993, 1994; Joanisse and Storey 1994*a*, 1994*b*). For example, the activity of glycogen phosphorylase over the winter and the kinetic properties of the enzyme have been shown to be optimized for the rapid and massive mobilization of glycogen for the production of the cryoprotectant glycerol in *Epiblema scudderiana* (Holden and Storey 1993; Joanisse and Storey 1994*a*). However, little or no information is available on lipids or fatty acid metabolism and their modulation or role over the winter months in these or other cold-hardy species. In this study we investigate seasonal changes in lipid content, fatty acid composition, and the maximum activities of various enzymes of fatty acid metabolism in an effort to understand the role of these in cold-hardy insects and the possible differences between freeze-tolerant and freeze-avoiding insects.

Material and Methods

Chemicals and Animals

All biochemicals and coupling enzymes were purchased from Sigma Chemical, St. Louis, or Boehringer Mannheim, Montreal. Galls containing larvae of *Epiblema scudderiana* or *Eurosta solidaginis* were collected from goldenrod plants in the same field in Ottawa during the fall of 1990 and were kept outdoors in cloth sacks. At each sampling date groups of galls were brought indoors and placed in an incubator adjusted to the outdoor temperature for that day. As quickly as possible galls were opened and larvae were removed and killed by dropping into a container of liquid nitrogen. Larvae were kept at −75°C until used.

Lipid Analyses

Lipids were isolated from the larvae by chloroform-methanol extraction (Folch et al. 1957). The recovered lipid from each sample was dissolved in chloroform and transferred to preweighed glass vials, the chloroform removed by drying under nitrogen gas, and the total lipid measured by mass. Fatty acids were transesterified with 1.0 mol/L methanolic acid as outlined in Christie (1982). The resulting fatty acid methyl esters were separated with a Varian 3400 gas chromatograph fitted with a J&W Scientific model DB-23 (Folsom, Calif.) capillary column (30 m × 0.25 mm inside diameter)

and detected by flame ionization. The column temperature was initially 160°C and was increased to 210°C at a rate of 4°C per minute. Helium at a flow rate of 4 mL/min was used as the carrier gas. Data were collected and analysed with a Varian GC Star workstation and are reported as the percentage of total transesterified fatty acids. Fatty acids were identified by comparison with known standards run under the same conditions. The double-bond index was calculated as in Richardson et al. (1961) and represents the average number of double bonds per fatty acid molecule of the entire mixture.

Enzyme Studies

Whole larvae were homogenized 1:5 (mass:volume) in either ice-cold 50 mmol/L Tris (pH 8.0) containing 5 mmol/L EDTA, 5 mmol/L EGTA, and 50 mmol/L NaF (for studies of malic enzyme, β-hydroxybutyrate dehydrogenase, hydroxyacyl-CoA dehydrogenase, and ATP-dependent citrate lyase) or 50 mmol/L imidazole (pH 7.4) containing 0.2% v/v Triton X-100 (for studies of carnitine-palmitoyl transferase and acetoacetyl-CoA thiolase). A few crystals of phenylmethylsulfonyl fluoride (~0.1 mmol/L) were added to each sample prior to homogenization in Eppendorf tubes with a glass handheld homogenizer. All homogenates were then sonicated for 10 s with a Kontes micro-ultrasonic cell disrupter set to approximately 75% of maximum tune and power. Homogenates were centrifuged at 15,000 *g* in a Biolab 13 centrifuge for 15 min. The clear supernatant was used directly for assays of carnitine-palmitoyl transferase, acetoacetyl-CoA thiolase, and ATP-citrate lyase activity or was desalted by centrifugation through a Sephadex G-25 column (1 × 5 cm) preequilibrated in homogenization buffer to decrease nonspecific background for malic enzyme, β-hydroxybutyrate dehydrogenase, and 3-hydroxyacyl-CoA dehydrogenase assays. Resuspended pellets failed to show any enzyme activity over background.

Enzyme Assays

All activities were measured at 23°C with a Gilford 240 recording spectrophotometer. Controls for nonspecific activity (in the absence of substrate or enzyme) were run for all assays and any blank value subtracted to yield final activity values. Activity is reported as units/g wet mass, where 1 unit is the conversion of 1 μmol of substrate to product in 1 min. The assays were essentially as in Ballantyne and Berges (1991) or Storey and Bailey (1978), and optimum conditions for maximum activities in the present species were determined to be the following: for malic enzyme (EC 1.1.1.40), 50 mmol/L Tris (pH 8.1), 0.8 mmol/L $MnCl_2$, 0.4 mmol/L $NADP^+$, and 2

mmol/L L-malate; for ATP-citrate lyase (EC 4.2.3.8), 50 mmol/L Tris (pH 8.0), 20 mmol/L $MgCl_2$, 10 mmol/L GSH, 0.15 mmol/L NADH, 20 mmol/L citrate, 10 mmol/L ATP, 0.4 mmol/L coenzyme A, and 1 international unit of porcine heart malate dehydrogenase; for 3-Hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35), 50 mmol/L Tris (pH 8.1), 0.1 mmol/L (*Epiblema scudderiana*) or 0.025 mmol/L (*Eurosta solidaginis*) acetoacetyl-CoA, 0.1 mmol/L NADH; for carnitine palmitoyl transferase (EC 2.3.1.21), 50 mmol/L imidazole (pH 7.5), 5 mmol/L L-carnitine, 1 mmol/L dithio-bis-nitrobenzoic acid, and 50 μmol/L palmitoyl-CoA; for acetoacetyl-CoA thiolase (EC 2.3.1.9), 50 mmol/L imidazole (pH 7.2), 5 mmol/L $MgCl_2$, 0.12 mmol/L CoA, 70 μmol/L acetoacetyl-CoA, measured at 303 nm (ε = 22 L/mmol); and for β-hydroxybutyrate dehydrogenase (EC 1.1.1.30), 50 mmol/L Tris (pH 8.1), 2 mmol/L NAD, 10 mmol/L D,L-hydroxybutyrate.

Statistical Analysis

Data were analyzed with a one-way ANOVA. When a significant F ratio was found, a Student-Neuman-Keuls test was performed to examine the difference between means; $P < 0.05$ was considered significantly different. Percentage data were transformed as arcsin ($y^{1/2}$) prior to analysis.

Results

The total lipid content of freeze-tolerant *Eurosta solidaginis* larvae was constant throughout the study period (Table 1). In larvae of *Epiblema scud-*

TABLE 1
Total lipid content of cold-hardy goldenrod gall larvae expressed as a percentage of wet mass

Date	*Epiblema scudderiana*	*Eurosta solidaginis*
September 13 . . .	13.1 ± .3	18.5 ± .5
December 27 . . .	8.9 ± .3[a]	17.1 ± .3
March 20	11.6 ± .6[a,b]	16.9 ± .2

Note. All values are for n = 3 (19–22 animals per group) and are given as mean ± SE.
[a] Significantly different from September value.
[b] Significantly different from December value.

deriana, the freeze-avoiding moth, the total lipid content decreased by 32% from September to December, but returned to 90% of the September level by March (Table 1). Larval mass did not change significantly in either species from September values of 45.8 ± 2.6 (*Eurosta solidaginis: n* = 12) and 69.7 ± 4.0 (*Epiblema scudderiana: n* = 12). The degree of fatty acid unsaturation increased from September to December in both insect species, as reflected in the double-bond index (Table 2). The double-bond index remained constant from December to March in *Eurosta solidaginis* but decreased slightly in *Epiblema scudderiana* (although remaining higher than September values).

The analysis of fatty acids from transesterified lipids of both *Eurosta solidaginis* and *Epiblema scudderiana* shows that body lipids consisted almost entirely of six fatty acids (Figs. 1 and 2): palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and α-linolenic (18:3). Some unidentified minor peaks were present in some samples, but since these represented less than 0.5% of the total fatty acid methyl ester content at any time, they were not reported.

The winter increase in unsaturation (increased double-bond index) in *Eurosta solidaginis* resulted from a decrease in the content of the saturates palmitic acid and stearic acid (a decrease of 2.7% and 2.6% of total fatty acid methyl esters, respectively) with a concurrent increase in oleic acid content of 7% of the total fatty acid content (Fig. 1). Linoleic acid content of the total pool decreased slightly (by 0.7%) from September to December. Palmitoleic and α-linolenic acid levels did not change over the study period. Oleic acid was the dominant fatty acid at any given time, accounting for over 50% of the total fatty acid content. This agreed with a previous study using NMR analysis, which had suggested the predominance of oleic acid

TABLE 2

Double-bond indices of fats from overwintering cold-hardy gall insects

Date	*Epiblema scudderiana*	*Eurosta solidaginis*
September 13 . . .	1.02 ± .01	.86 ± .01
December 27 . . .	1.17 ± .01[a]	.92 ± .01[a]
March 20	1.09 ± .01[a,b]	.92 ± .01[a]

Note. All values are for n = 3 (19–22 animals per group) and are given as mean ± SE.
[a] Significantly different from September value.
[b] Significantly different from December value.

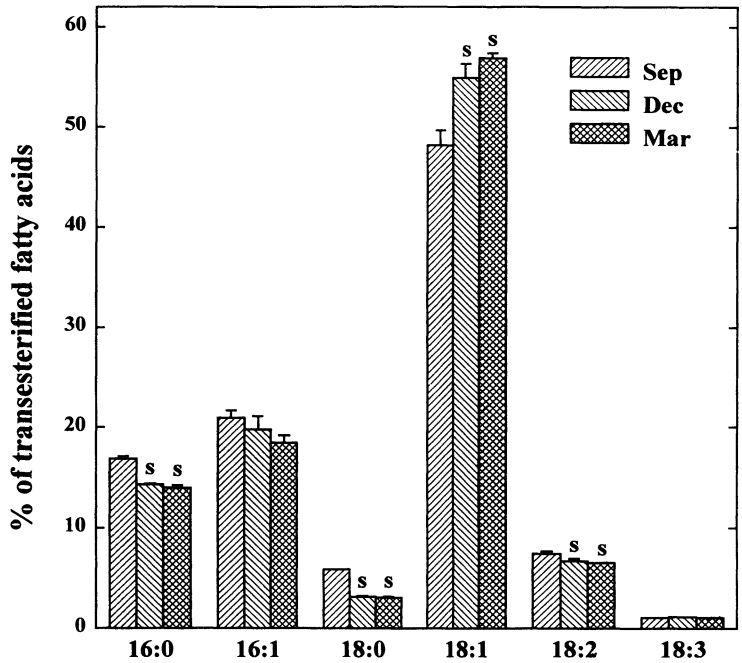

Fig. 1. Whole larvae fatty acid composition (as a percentage of the total pool) of Eurosta solidaginis. *The fatty acids are palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and* α*-linolenic acid (18:3). Values are mean* ± *SE, for* n = *3 groups, 19–22 larvae per group.* s, *Significantly different from September values.*

in whole larvae and chloroform extracts of *Eurosta solidaginis* (Buchanan and Storey 1983).

The double-bond index increase in winter *Epiblema scudderiana* was accounted for by a significantly decreased content of palmitic (a decrease of 9% of total fatty acid methyl esters) and stearic (2.1%) acids, as in *Eurosta solidaginis,* but with a concurrent increase of 7% in palmitoleic acid content (Fig. 2). Oleic and linoleic acid content did not change significantly during the study period. Linolenic acid content increased by 1.3% from September to January, and decreased to approach September levels by March. The most common fatty acids in *Epiblema scudderiana* were the monounsaturates palmitoleic and oleic acid, each accounting for approximately 40% of the total fatty acid content.

Activities of two enzymes associated with fatty acid synthesis, malic enzyme and ATP-dependent citrate lyase, decreased from September to December in both species (Figs. 3 and 4), indicating a reduced potential for

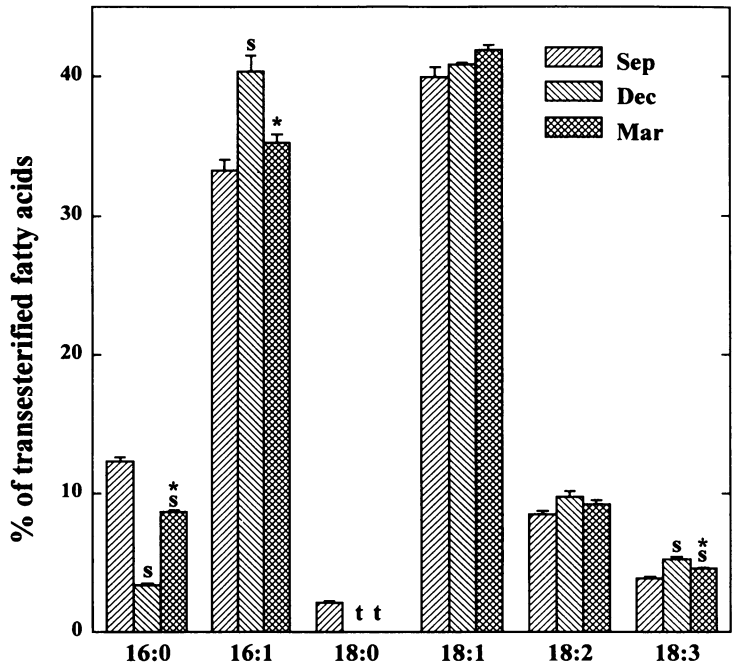

Fig. 2. Whole larvae fatty acid composition (as a percentage of the total pool) of Epiblema scudderiana. *, *Significantly different from previous time point;* s, *significantly different from September values;* t, *trace amounts. Other details are as in Figure 1.*

fatty acid synthesis during the winter. In *Eurosta solidaginis,* malic enzyme activity decreased gradually to 64% of September values by early February but returned to September activities by March 20 (Fig. 3). The activity of ATP-dependent citrate lyase decreased to 48% of September values by late December and subsequently increased slightly to 62% of September values by March 20 (Fig. 3). In *Epiblema scudderiana* the activity of malic enzyme decreased to 55% and ATP-dependent citrate lyase to 40% of the September activity by December and remained low for the remainder of the study period (Fig. 4). Activities for both enzymes were, in all cases, greater in *Eurosta solidaginis,* indicating a greater potential for fatty acid synthesis in this species.

The potential for fatty acid oxidation, as indicated by the activities of hydroxyacyl-CoA dehydrogenase, carnitine-palmitoyl transferase, and acetoacetyl-CoA thiolase, decreased in *Eurosta solidaginis* from September into the winter and remained low into spring (Fig. 5). Hydroxyacyl-CoA dehydrogenase activity decreased to 56% of September values by November 12 and subsequently increased to a range of 67%–85% of September values for

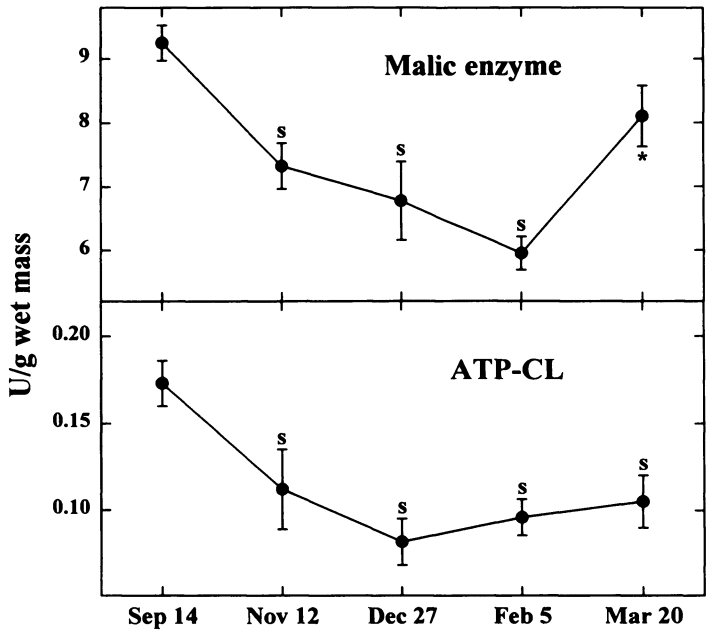

Fig. 3. Activities of enzymes of fatty acid synthesis during overwintering in larvae of Eurosta solidaginis. *Values are mean ± SE for* n = *5–6 homogenates, with one larva per homogenate.* ATP-CL, *ATP-citrate lyase.* *, *Significantly different from previous time point;* s, *significantly different from September values.*

the remainder of the study period. Carnitine-palmitoyl transferase activity decreased gradually to 63% of September values by December and then gradually increased to 83% of September values by March 20. Acetoacetyl-CoA thiolase activity decreased to 48% of September activities by November 12 and remained low until the late winter, when a slight increase in activity was seen leading to 60% of the September activity by March 20.

The state of enzymes of fatty acid oxidation in *Epiblema scudderiana* was different from that found in the freeze-tolerant species (Fig. 6). In *Epiblema scudderiana* there was a 31% increase in the activity of hydroxy-acyl-CoA dehydrogenase from September to March, with a transient drop to September values in February. Acetoacetyl-CoA thiolase activity increased to 229% of the September value by December and then decreased slightly to reach 165% of the September activity by March 20. As in the freeze-tolerant species, carnitine-palmitoyl transferase activity in *Epiblema scudderiana* decreased from September to November (by 35%) and remained low for the duration of the study period. The activities of all three enzymes

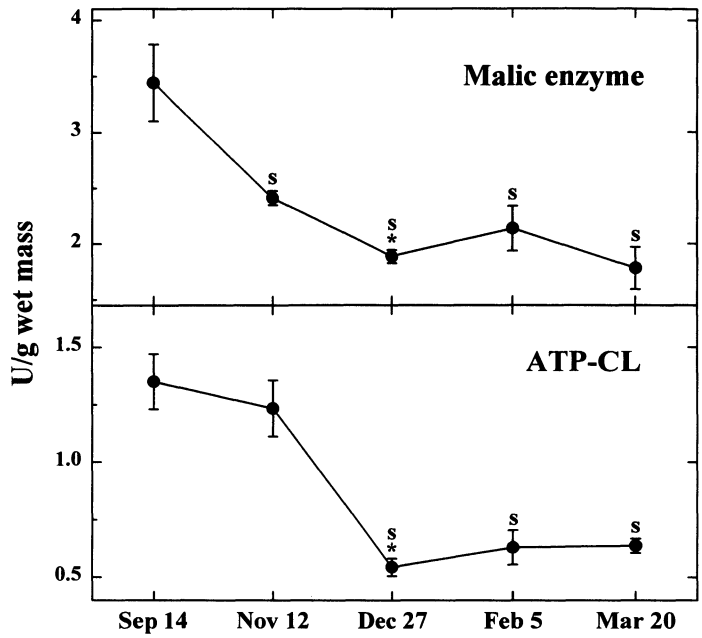

Fig. 4. Activities of enzymes of fatty acid synthesis during overwintering in larvae of Epiblema scudderiana. *Other details are as in Figure 3.*

were two- to fourfold higher in *Epiblema scudderiana,* indicating a greater potential for fatty acid oxidation in the freeze-avoiding species.

The potential for ketone body metabolism, as measured by β-hydroxybutyrate dehydrogenase activity, increased dramatically from September to January in both species. In *Eurosta solidaginis,* the activity increased from 0.0024 ± 0.0024 units/g wet mass in September to 0.066 ± 0.004 units/g wet mass in December, an increase of 27-fold, and remained high for the duration of the study period (Fig. 5). In *Epiblema scudderiana* the activity increased from 0.052 ± 0.004 units/g wet mass in September to 0.34 ± 0.02 units/g wet mass by February 5, a 6.4-fold increase, and subsequently decreased to a value 3.3-fold higher than the September value by March 20 (0.17 ± 0.02 units/g wet mass; Fig. 6). Although the activity of β-hydroxybutyrate dehydrogenase increased dramatically in both species over the winter, quantification of β-hydroxybutyrate levels in the larvae (using a microplate reader adaptation of Sigma kit 310-A) showed that these remained below the limit of detection of the assay throughout the study period, at less than 0.35 μmol/g wet mass. As with enzymes of fatty acid oxidation, the activity of β-hydroxybutyrate dehydrogenase was consistently higher in *Epiblema scudderiana,* indicating

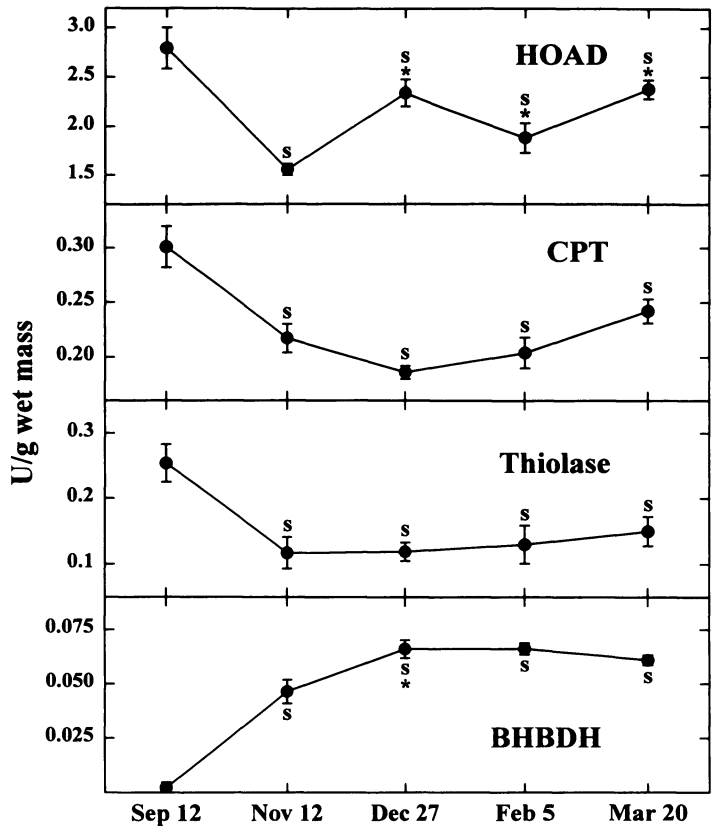

Fig. 5. Activities of enzymes of fatty acid oxidation and ketone body metabolism during overwintering in larvae of Eurosta solidaginis. *Values are mean ± SE for* n = *5–6 homogenates, with one larva per homogenate.* HOAD, *3-hydroxyacyl-CoA dehydrogenase;* CPT, *carnitine palmitoyl transferase;* Thiolase, *acetoacetyl-CoA thiolase;* BHBDH, *β-hydroxybutyrate dehydrogenase. Other details are as in Figure 3.*

a greater potential for ketone body metabolism in this species than in *Eurosta solidaginis.*

Discussion

With the arrival of colder autumn temperatures, the cold-hardy gall insects must initiate adaptations that will provide defenses against the upcoming onslaught of winter. The larvae stop feeding in the fall and enter a period of metabolic depression (diapause and/or quiescence) in which they survive

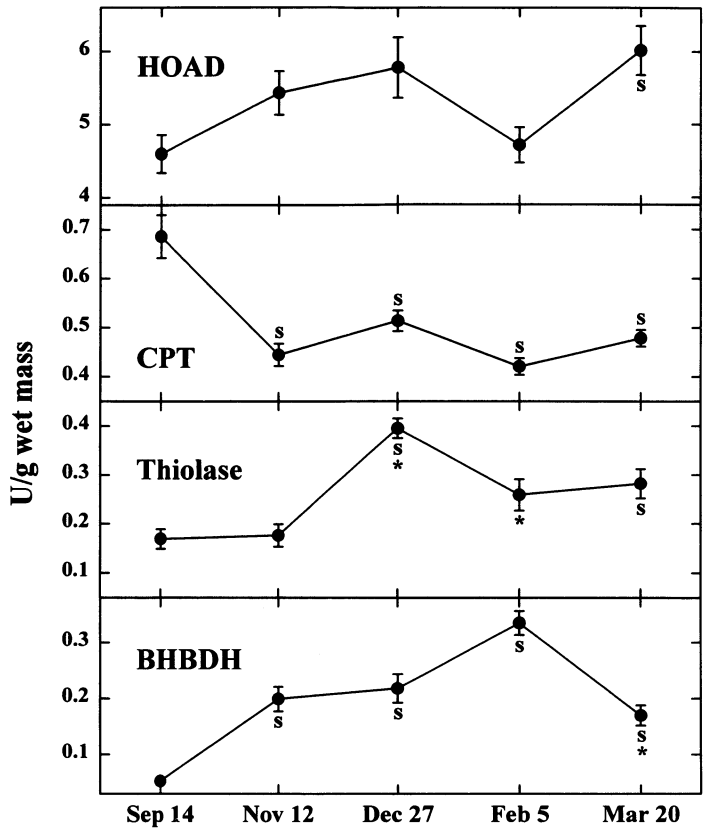

Fig. 6. Activities of enzymes of fatty acid oxidation and ketone body metabolism during overwintering in larvae of Epiblema scudderiana. *Other details are as in Figure 5.*

on stored fuel reserves until emergence in the spring. The low subzero temperatures faced by larvae of freeze-avoiding *Epiblema scudderiana* and freeze-tolerant *Eurosta solidaginis* lead to a dramatic decrease in metabolic rate, and survival necessitates various physiological and biochemical adaptations. In this study we examined the changes in fatty acid composition and fatty acid metabolism over the winter months in these cold-hardy insects, focusing on the role of lipids as a metabolic fuel for overwintering.

The absence of lipid accumulation during overwintering (Table 1) is consistent with the behavior and metabolic state of the larvae of both species. Since the larvae do not feed during the winter, there is no ingestion of precursor for lipid (and fatty acid) synthesis, and the process is metabolically decreased. The total lipid content of both species (Table 1) is also consistent with the lipid content of most phytophagous insects (>10%; Fast 1964). A significant decrease in the activity of two enzymes of fatty acid synthesis,

malic enzyme and ATP-dependent citrate lyase, was observed (Figs. 3 and 4), concurrent with the absence of lipid accumulation and cessation of feeding. Such large decreases in the activity of multiple enzymes of a common pathway are not novel in these cold-hardy insects. We have previously shown that a number of mitochondrial enzyme activities, including some of the tricarboxylic acid cycle, decrease significantly during overwintering (Joanisse and Storey 1994*b*). These results reinforce the notion of a great plasticity in enzyme activities in these larvae during overwintering and strongly suggest an important role for enzyme turnover despite the extended period of metabolic suppression in the diapause state.

Depot fats must be fluid to be metabolizable (Irving et al. 1957). One mechanism that can be used to increase lipid fluidity is to increase the degree of unsaturation of the component fatty acids. The fatty acid and double-bond index analysis of *Eurosta solidaginis* and *Epiblema scudderiana* clearly show that both species increased their content of unsaturated fatty acids between the fall and winter (Figs. 1 and 2). This permits the maintenance of a fluid state of lipids as the larvae enter periods of colder temperature. Such an increase in double-bond index values has been found in the depot fats of hibernating ground squirrels *Spermophilus beldingi* and has been suggested as being important in the cold adaptation of this species (Frank 1991). It is interesting to note that *Epiblema scudderiana* larvae contained a much higher percentage of palmitoleic acid and a lower percentage of palmitic acid than reported in Fast (1964) for a number of lepidopteran species. Furthermore, a comparison of *Eurosta solidaginis* fatty acids to others listed by Fast (1964) showed an unusually high percentage of oleic acid. Both of these may be key for overwintering in this species, although the acclimatization state and the cold hardiness of the species listed in Fast (1964) are unknown.

The present data suggest a role for seasonal desaturation of fatty acids in cold-hardy insects, probably to maintain the fluidity of storage lipids and hence the metabolizable state. Since both larval species stop feeding in the fall, when the plant dies, the increase in fatty acid unsaturation from September to December cannot be due to a change in diet and to selective fatty acid uptake or retention and must be the result of desaturase activity. In *Epiblema scudderiana,* the decrease in total body lipid content into the winter (Table 1) might also suggest a preferential oxidation of saturated fatty acids during cold hardening with the additional result of increasing the level of unsaturation. The mechanisms of activation and control of desaturase activity in these insects are unknown. Although our data provide no direct evidence for changes in the composition of membrane fatty acids during overwintering, since the vast majority of the extracted fatty acids are

from depot lipids, the observed changes suggest the possibility that the mechanisms operating on these lipids may also be functioning on membrane lipids. Further studies into membrane adaptations of cold-hardy insects are certainly encouraged by these findings.

The data also show an interesting difference in the lipid metabolism of the two species during overwintering. Since total lipid content does not change over the winter, freeze-tolerant larvae of *Eurosta solidaginis* apparently do not use fats as metabolic fuel for winter survival (Table 1), and this was correlated with decreased activities of the fatty acid oxidation enzymes hydroxyacyl-CoA dehydrogenase, carnitine-palmitoyl transferase, and acetoacetyl-CoA thiolase (Fig. 5). This agrees with previous work that showed constant levels of glycerides over the winter in this species (Storey and Storey 1986). Freeze-avoiding *Epiblema scudderiana* larvae, on the other hand, do deplete their body lipid reserves over the winter, presumably by oxidation as fuel (Table 1). This result correlates with the decrease in glyceride content per gram of dry mass observed by Rickards et al. (1987). These two different strategies for the use of lipid reserves during insect hibernation have been described previously (see Fast [1964] and references therein). Our results add to previous information, however, by showing that the decrease in lipid content in *Epiblema scudderiana* was correlated with increased winter activities of some enzymes of fat oxidation (hydroxyacyl-CoA dehydrogenase and acetoacetyl-CoA thiolase), although carnitine-palmitoyl transferase activity decreased (Fig. 6). Also, it is noteworthy that all of these activities were much higher in the freeze-avoiding species than in the freeze-tolerant species. One contributing factor to this interspecies difference in lipid use may be the physical state of the larvae during most of the winter. *Eurosta solidaginis* larvae will spend many weeks of the winter frozen. In this condition, fats cannot be mobilized through the haemolymph or oxidized by mitochondria owing to lack of oxygen. *Epiblema scudderiana* larvae, on the other hand, do not face these challenges to the use of lipids, and like many other species experiencing long-term dormancy, they rely on lipid reserves to fuel metabolism.

The fate of glycerol carbon during the spring clearance of cryoprotectant in *Epiblema scudderiana* (from midwinter levels, which account for up to 19% of the fresh mass of the larvae) has always been somewhat of a mystery, as only 20%–36% of the cryoprotectant removed could be accounted for by reconversion to glycogen (Rickards et al. 1987; Joanisse and Storey 1994*a*). However, the 2.7% increase in lipid content of the larvae from December to March may provide a partial answer to this dilemma. If all of this new lipid were made from glycerol carbon, this

would account for up to 42% of the cryoprotectant lost in the spring (calculations based on the starting winter glycerol content of 2,000 μmol/g wet mass and assuming palmitate as the final product). These accumulated lipids might then be used to fuel pupation and adult life. The NADPH required for fatty acid synthesis would also be a product of glycerol clearance, by the reversal of the dehydrogenases involved in the synthesis of the cryoprotectant (Joanisse and Storey 1994*a*).

Both species increased the activity of β-hydroxybutyrate dehydrogenase during cold hardening, indicating a possible increased use of ketone bodies as a metabolic fuel over the winter. This would parallel the situation in higher vertebrates, where ketone body metabolism increases during starvation (Robinson and Williamson 1980). However, the larval concentration of β-hydroxybutyrate never exceeded 0.3 μmol/g wet mass, suggesting that the use of this ketone body is minor in the cold-hardy larvae or that there is a steady turnover of this metabolite. The role of ketone bodies in insect metabolism is largely unknown, although acetoacetate has been implicated as the major ketone body in at least one insect species, the desert locust *Locusta migratoria* (Bailey and Horne 1972). We have not examined the role of acetoacetate in the cold-hardy goldenrod gall larvae.

We have previously suggested that mitochondrial numbers might possibly decrease over the winter in both species, on the basis of observations of decreased activities of a number of mitochondrial enzymes (Joanisse and Storey 1994*b*). However, data of the present study do not support this, for, of the mitochondrial enzymes of *Epiblema scudderiana* measured here, hydroxyacyl-CoA dehydrogenase activity remained constant and acetoacetyl-CoA thiolase, carnitine-palmitoyl transferase, and β-hydroxybutyrate dehydrogenase activities all increased. Similarly, the increased activity of β-hydroxybutyrate dehydrogenase in *Eurosta solidaginis* larvae is also inconsistent with our earlier hypothesis. Instead, the previously observed decreases in other mitochondrial enzyme activities may likely be the result of enzyme degradation or posttranslational modification, reflecting a reorganization of the metabolic potential of existing mitochondria.

A picture of lipid metabolism in cold-hardy insects is suggested from the present data. Both species clearly increase fatty acid unsaturation in the winter, which may play a role in maintaining depot lipid fluidity during the cold winter months. In the freeze-tolerant dipteran larvae of *Eurosta solidaginis,* lipid stores do not appear to be used to fuel basal metabolism during overwintering, and the decreased activities of enzymes of both fat synthesis and fat oxidation help to shut down these processes. By contrast, freeze-avoiding lepidopteran larvae of *Epiblema scudderiana* do consume

lipids over the winter. This is supported by decreased activities of fat synthesis enzymes and activities of fat oxidation enzymes greater than those in the freeze-tolerant species.

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Literature Cited

BAILEY E. and J.A. HORNE. 1972. The role of ketone bodies in the metabolism of the adult desert locust. Biochem. J. 128:79P.

BALLANTYNE J.S. and J.A. BERGES. 1991. Enzyme activities of gill, hepatopancreas, mantle, and adductor muscle of the oyster (*Crassostrea virginica*) after changes in diet and salinity. Can. J. Fisheries Aquatic Sci. 48:1117–1123.

BUCHANAN G.W. and K.B. STOREY. 1983. *In vivo* detection of cryoprotectants and lipids in overwintering larvae using carbon-13 nuclear magnetic resonance spectroscopy. Can. J. Biochem. Cell Biol. 61:1260–1264.

CHRISTIE W.W. 1982. Lipid Analysis. Pergamon, New York.

FAST P. 1964. Insect Lipids: A Review. Mem. Entomol. Soc. Can. 37.

FOLCH J., M. LEES, and G. STANLEY. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–507.

FRANK C.L. 1991. Adaptations for hibernation in the depot fats of a ground squirrel (*Spermophilus beldingi*). Can. J. Zool. 69:2707–2711.

HAZEL J.R. 1995. Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? Annu. Rev. Physiol. 57:19–42.

HOLDEN C.P. and K.B. STOREY. 1993. Purification and characterization of glycogen phosphorylase *a* and *b* from the freeze avoiding gall moth larvae *Epiblema scudderiana*. J. Comp. Physiol. 163B:499–507.

———. 1994. 6-Phosphogluconate dehydrogenase from a freeze tolerant insect: control of the hexose monophosphate shunt and NADPH production during cryoprotectant synthesis. Insect Biochem. Mol. Biol. 24:167–173.

IRVING L., K. SCHMIDT-NIELSEN, and N. ABRAHAMSEN. 1957. On the melting points of animal fats in cold climates. Physiol. Zool. 30:93–105.

JOANISSE D.R. and K.B. STOREY. 1994*a*. Enzyme activity profiles in an overwintering population of freeze avoiding gall moth larvae, *Epiblema scudderiana*. Can. J. Zool. 72:1079–1086.

———. 1994*b*. Mitochondrial enzymes during overwintering in two species of cold-hardy gall insects. Insect Biochem. Mol. Biol. 24:145–150.

RICHARDSON T., A. TAPPEL, and E. GRUGER, JR. 1961. Essential fatty acids of mitochondria. Arch. Biochem. Biophys. 94:1–6.

RICKARDS J., M.J. KELLEHER, and K.B. STOREY. 1987. Strategies of freeze avoidance in larvae of the goldenrod gall moth, *Epiblema scudderiana:* winter profiles of a natural population. J. Insect Physiol. 33:443–450.

ROBINSON A.M. and D.H. WILLIAMSON. 1980. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. Physiol. Rev. 60:143–187

SINENSKY M. 1974. Homeoviscous adaptation: a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli.* Proc. Natl. Acad. Sci. USA 71:522–525.

STOREY J.M. and K.B. STOREY. 1986. Winter survival of the gall fly larva, *Eurosta solidaginis:* profiles of fuel reserves and cryoprotectants in a natural population. J. Insect Physiol. 32:549–556.

STOREY K.B. 1982. Phosphofructokinase from the overwintering gall fly, *Eurosta solidaginis:* control of cryoprotectant polyol synthesis. Insect Biochem. 12:501–505.

STOREY K.B. and E. BAILEY. 1978. Intracellular distribution of enzymes associated with lipogenesis and gluconeogenesis in fat body of the adult cockroach, *Periplaneta.* Insect Biochem. 8:125–131.

STOREY K.B. and J.M. STOREY. 1992. Biochemical adaptations for winter survival in insects. Pp. 101–140 in P.L. STEPONKUS, ed. Advances in Low-Temperature Biology. Vol. 1. JAI, London.