Archives of Insect Biochemistry and Physiology 28:339–349 (1995)

Temperature Acclimation and Seasonal Responses by Enzymes in Cold-Hardy Gall Insects

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Changes in the activity of over 20 enzymes of intermediary metabolism in 15°C or –4°C acclimated goldenrod gall moth (*Epiblema scudderiana*) and gall fly (*Eurosta solidaginis*) larvae were measured. Increased activities of glycogenolytic and hexose monophosphate shunt enzymes in cold-acclimated *Epiblema scudderiana* suggest a role for coarse control in the conversion of glycogen reserves into glycerol cryoprotectant synthesis. In *Eurosta solidaginis,* high glycogen phosphorylase activity with decreased activities of glycolytic enzymes may account in part for the temperature-dependent switch from glycerol to sorbitol synthesis in these larvae upon cold acclimation. Isoelectric focusing analyses of five enzymes in overwintering *Epiblema scudderiana* revealed transient mid-winter changes in the isoelectric points of phosphofructokinase and pyruvate kinase, suggesting seasonal changes in the phosphorylation state of these enzymes. A distinct developmental pattern of aldolase isozymes suggests a role for a new isozyme during overwintering or upon spring emergence. Regulation of metabolism by changes in enzyme activities is indicated for both larvae.

Key words: ***Epiblema scudderiana, Eurosta solidaginis,* cold-hardy insects, low temperature acclimation, cryoprotectant biosynthesis**

INTRODUCTION

In the absence of behavioural thermoregulation, most cold-hardy insects must regulate and integrate metabolism over a wide range of ambient temperatures. In addition, specific seasonal metabolic goals must be accommodated, notably a shift in the autumn towards the synthesis of the polyol

Acknowledgments: This work was supported by an operating grant from the NSERC (Canada) to K.B. Storey and by an NSERC (Canada) postgraduate scholarship to D.R. Joanisse. We thank J.M. Storey for critical review of the manuscript.

Received April 27, 1994; accepted September 7, 1994.

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cryoprotectants that are necessary for survival at subzero temperatures during the winter.

Temperature change can disrupt the integration of metabolism via a number of effects including differential effects of temperature on the rates of different enzymes (Q_{10} effects), and temperature-induced changes in enzyme subunit association and/or enzyme-ligand interactions (Hochachka and Somero, 1984). To compensate, animals can change enzyme concentration, change substrate or cofactor concentrations, synthesize different isozymes, and/or modify existing enzymes (Hochachka and Somero, 1984). An example of differential isozyme synthesis as a response to temperature acclimation is the case of rainbow trout (*Oncorhynchus mykiss*) acetylcholinesterase; different isozymes predominate at different ambient temperatures and the kinetic properties of each isozyme is geared towards optimal function over different temperature ranges (Baldwin, 1971; Baldwin and Hochachka, 1970). Examples of an increase in the amount of enzyme to compensate for low temperatures include cytochrome oxidase in fish (Sidell et al., 1973; Sidell, 1977).

Larvae of the freeze tolerant fly *Eurosta solidaginis* and the freeze avoiding moth *Epiblema scudderiana* overwinter in stem galls on goldenrod plants. Both species enter diapause to survive the long winter season, and both accumulate polyol cryoprotectants in a temperature-dependent fashion as part of their defense against subzero temperature. *Eurosta solidaginis* larvae accumulate 0.5 to 0.6 M glycerol and 0.2 M sorbitol in haemolymph, the synthesis of glycerol occurring between 15 and 5°C and that of sorbitol below 5°C (Morrissey and Baust, 1976; Storey et al., 1981a; Storey and Storey, 1988). *Epiblema scudderiana* larvae accumulate about 2 M glycerol, representing almost 19% of the body mass of the insect, biosynthesis beginning at temperatures below 5°C (Rickards et al., 1987; Storey and Storey, 1988). In the present study we examine the effects of temperature acclimation to 15 and –4°C on enzyme activities, and relate changes in enzyme maximal activities to polyol cryoprotectant synthesis. Since biosynthesis of glycerol occurs at different temperatures in the two species, interspecies comparison is used to determine if there are differential responses of glycerol synthesizing enzymes to temperature acclimation. In addition, column isoelectric focusing was used to determine if changes in enzyme form, such as by post-translational modification or differential isozyme expression, played a role in metabolic shifts in *Epiblema scudderiana* as winter progressed.

MATERIALS AND METHODS

Chemicals and Animals

All biochemicals and coupling enzymes were purchased from Sigma Chemical Co. (St. Louis, MO) or Boehringer Mannheim (Montréal, Québec). Galls containing larvae of *Epiblema scudderiana* for isoelectric focusing studies were collected from goldenrod plants in fields around Ottawa during the fall of 1989 and kept outdoors in cloth sacs. 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 nitro-

gen. Larvae were kept at –75°C until used. Galls containing larvae of *Epiblema sudderiana* and *Eurosta solidaginis* for acclimation studies were collected in mid-October, kept in their galls, and placed in dark in incubators at constant temperatures of 15 or –4°C for 2 weeks, and then sampled as above.

Isoelectric Focusing Studies

Larvae were homogenized 1:10 (w:v) in 20 mM imidazole-HCl buffer, pH 7.2, containing 15 mM 2-mercaptoethanol, 5 mM EDTA, 5 mM EGTA, and 50 mM NaF. Homogenates were centrifuged at 26,000*g* for 20 min at 4°C and the clear supernatant was removed; 1 mL of supernatant was used for each isoelectric focusing column.

Column isoelectric focusing was performed by the method of Vesterberg (1971) using an LKB 8101 (110 mL) column with a pH 3.5 to 10 gradient of Sigma Ampholines in a sucrose density gradient. Proteins were focused at 300 V for 14 to 16 h at 4°C. Fractions were then collected (2 mL) and assayed for enzyme activity as outlined by Joanisse and Storey (1994a, b) or as outlined below. Where multiple peaks occurred, the percent of activity in each was calculated based on the total activity recovered from the isoelectric focusing column.

Acclimation Studies

The preparation of enzyme extracts from whole larvae, G-25 Sephadex filtration to remove low molecular weight metabolites, and assays of enzyme activity were performed as described previously (Joanisse and Storey, 1994a, b) except for the following assay conditions for optimal activity in *Epiblema scudderiana*:

- GPase*: (Total *a+b*) 50 mM potassium phosphase buffer (pH 7.0), 4 mg/mL glycogen, 5 μM glucose-1,6-P_2, 0.2 mM $NADP^+$, 1 mM AMP, 15 mM $MgSO_4$, and excess phosphoglucomutase and $NADP^+$-dependent glucose-6-P dehydrogenase. The active form of the enzyme (*a*) was measured in the absence of AMP.
- G3PDH: 20 mM imidazole-HCl buffer (pH 7.2), 0.5 mM dihydroxyacetone phosphate (DHAP), and 0.15 mM NADH.
- PGK: 20 mM imidazole-HCl (pH 7.2), 20 mM 3-phosphoglycerate, 1 mM ATP, 0.15 mM NADH, 5 mM $MgSO_4$, and excess glyceraldehyde phosphate dehydrogenase.

*Abbreviations used: F6Pase = fructose-6-phosphatase; FBPase = fructose-1,6-bisphosphatase; G3Pase = glycerol-3-phosphatase; G3DPH = glycerol-3-phosphate dehydrogenase; G6Pase = glucose-6-phosphatase; G6PDH = glucose-6-phosphate dehydrogenase; GAPase = glyceraldehyde-3-phosphatase; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; GDH = glutamate dehydrogenase; GPase = glycogen phosphorylase; LDH = lactate dehydrogenase; NADP-IDH = NADP-dependent isocitrate dehydrogenase; PDHald = glyceraldehyde utilizing polyol dehydrogenase; PDHgluc = glucose utilizing polyol dehydrogenase; PFK = phosphofructokinase; PGI = phosphoglucoisomerase; PGK = phosphoglycerate kinase; PGM = phosphoglucomutase; PK = pyruvate kinase; SoDH = sorbitol dehydrogenase; 6PGDH = 6-phosphogluconate dehydrogenase.

- PGI: 20 mM imidazole-HCl (pH 7.2), 4 mM fructose-6-phosphate, 0.2 mM $NADP^+$, 5 mM $MgSO_4$, and excess glucose-6-dehydrogenase.
- FBPase: 20 mM imidazole-HCl (pH 7.2), 0.1 mM fructose-1,6-P_2, 5 mM $MgSO_4$, 0.2 mM $NADP^+$, and excess phosphoglucoisomerase and glucose-6-phosphate dehydrogenase.
- PDHald: 20 mM imidazole-HCl (pH 7.2), 80 mM D-glyceraldehyde, and 0.1 mM NADPH.

One unit of enzyme activity is defined as the amount of enzyme that converts 1 μmole of substrate per minute at 25°C. Since larval fresh weight was not different between the acclimation groups for both species (data not shown), activty was expressed as units per gram fresh weight. Cryoprotectant concentrations were determined as in Joanisse and Storey (1994a).

Statistical Analysis

Data were analyzed by two-tailed Student's t-tests. Data reported as percentages were transformed using arcsin $\sqrt{y}$ prior to analysis. Values were considered significantly different if $P < 0.05$.

RESULTS

Acclimation to 15 or –4°C led to specific differences in the activities of a number of enzymes in autumn collected *Epiblema scudderiana* and *Eurosta solidaginis*. Table 1 summarizes the effects of temperature acclimation on enzymes in *Epiblema scudderiana*. Acclimation to –4°C increased the activities of seven enzymes and reduced the activities of two as compared with 15°C acclimated larvae. With the exception of PGK, all of the enzymes showing increased activities at –4°C were involved in the biosynthetic pathway for glycerol (hexokinase may play a role in a scavenging glucose for glycerol biosynthesis). Higher activities of many enzymes involved in shuttling carbon equivalents into and supplying reducing equivalents for polyol cryoprotectant synthesis were observed in low-temperature acclimated larvae. The total (*a*+*b*) activity of GPase increased by 48% in –4°C acclimated larvae compared to 15°C acclimated larvae, and the % of the enzyme in the active *a* form increased from 1.9% at 15°C to 39.6% at –4°C. Also increased in –4°C acclimated larvae were the activities of PFK (by 100%), PGI (22%), hexokinase (56%), PGK (21%), and the hexose monophosphate shunt (HMS) enzymes G6PDH and 6PGDH (by 19 and 46%, respectively). By contrast, –4°C acclimation reduced the activity of FBPase, key to gluconeogenesis from glycerol, by 25% and glucokinase activity fell by 54%. Other enzymes examined, including specific enzymes of glycerol metabolism, were not different in high vs. low temperature acclimated larvae.

Comparable results from acclimation of freeze tolerant *Eurosta solidaginis* larvae to the two temperatures are shown in Table 2. Results were very different from those in *Epiblema scudderiana*. Acclimation to –4°C increased the activity of only one enzyme, glucokinase (by 111%), whereas activities of five enzymes were reduced significantly at –4°C, as compared with 15°C. Total GPase did not change, but the percentage in the active *a* form increased from

TABLE 1. Effect of Acclimation at Two Temperatures on Enzyme Activities in Mid-October Collected Freeze Avoiding Larvae of *Epiblema scudderiana*[†]

	15°C	−4°C	% difference
Glycolysis			
GPase total	4.51 ± 0.16	6.66 ± 0.72**	+48
GPase *%a*	1.93 ± 1.0	39.6 ± 1.1**	+37.7
PGM	30.2 ± 2.3	32.5 ± 0.9	—
PGI	17.1 ± 1.2	22.0 ± 0.8**	+22
PFK	1.09 ± 0.02	2.18 ± 0.13**	+100
Aldolase	7.36 ± 0.62	8.51 ± 0.64	—
GAPDH	59.9 ± 2.1	62.8 ± 1.9	—
PGK	24.5 ± 1.3	29.7 ± 1.2*	+21
PK	34.1 ± 1.7	38.2 ± 2.0	—
LDH	2.63 ± 0.12	3.15 ± 0.25	—
Gluconeogenesis			
FBPase	3.01 ± 0.17	2.26 ± 0.18**	−25
Hexose Monophosphate Shunt			
G6PDH	2.54 ± 0.21	3.15 ± 0.18*	+19
6PGDH	1.61 ± 0.15	2.35 ± 0.11**	+46
Glycerol metabolism			
G3PDH	47.0 ± 5.1	40.4 ± 1.6	—
G3Pase	0.74 ± 0.03	0.75 ± 0.03	—
GAPase	0.46 ± 0.05	0.54 ± 0.04	—
PDHald	2.39 ± 0.12	2.50 ± 0.19	—
Sorbitol metabolism			
G6Pase	0.55 ± 0.08	0.56 ± 0.12	—
SoDH	0.06 ± 0.01	0.07 ± 0.01	—
F6Pase	0.55 ± 0.03	0.58 ± 0.08	—
Others			
Hexokinase	1.27 ± 0.09	1.98 ± 0.14**	+56
Glucokinase	0.80 ± 0.05	0.37 ± 0.02**	−54
NADP-IDH	5.59 ± 0.65	7.02 ± 0.43	—

[†]Data are units/g fresh weight except for glycogen phosphorylase *%a*, and are expressed as mean ± S.E., n = 4.
*Significantly different from the corresponding 15°C value, $P < 0.05$; **$P < 0.01$.

57% in 15°C larvae to 92.7% in −4°C acclimated larvae. Decreased activities of the glycolytic enzymes PFK (by 20%), PGM (26%), PGI (12%), and GAPDH (15%) were seen in the low temperature acclimated animals; NADP-IDH activity also decreased (by 25%). Other enzymes examined, including those specific for glycerol and sorbitol metabolism, were not significantly different in high vs. low temperature acclimated larvae.

Table 3 shows the levels of glycerol and sorbitol in both species after acclimation to the two temperatures. Acclimation to different temperatures did not lead to changes in glycerol levels in either species. Compared with 15°C acclimated larvae, sorbitol levels were about 30-fold higher in −4°C acclimated *Eurosta solidaginis*. By contrast, sorbitol levels (which were minor in all cases) were slightly lower in cold vs. warm acclimated *Epiblema scudderiana*.

Column isoelectric focusing was used to search for different forms of enzymes in *Epiblema scudderiana* and to determine if the proportion of these

TABLE 2. Effect of Acclimation at Two Temperatures on Enzyme Activities in Mid-October Collected Freeze Tolerant Larvae of *Eurosta solidaginis*[†]

	15°C	−4°C	% difference
Glycolysis			
GPase total	2.24 ± 0.15	2.44 ± 0.10	—
GPase *%a*	57.0 ± 9.2	92.7 ± 5.9**	+35.7
PGM	35.1 ± 3.2	26.0 ± 1.4*	−26
PGI	15.6 ± 0.3	13.7 ± 0.5*	−12
PFK	1.48 ± 0.07	1.19 ± 0.09*	−20
Aldolase	5.61 ± 0.24	5.44 ± 0.33	—
GPADH	59.4 ± 3.2	50.3 ± 2.9*	−15
PGK	23.3 ± 0.5	21.7 ± 0.8	—
PK	47.1 ± 1.9	44.5 ± 2.4	—
LDH	8.01 ± 0.76	6.64 ± 0.23	—
Gluconeogenesis			
FBPase	0.18 ± 0.06	0.16 ± 0.01	—
Hexose Monophosphate Shunt			
G6PDH	9.6 ± 0.4	10.2 ± 0.1	—
6PGDH	3.15 ± 0.21	3.09 ± 0.08	—
Glycerol metabolism			
G3PDH	19.1 ± 2.1	19.1 ± 1.8	—
G3Pase	0.093 ± 0.021	0.067 ± 0.005	—
GAPase	0.083 ± 0.009	0.068 ± 0.009	—
PDHald	1.79 ± 0.05	1.87 ± 0.15	—
Sorbitol metabolism			
G6Pase	0.94 ± 0.02	0.82 ± 0.06	—
PDHgluc	0.40 ± 0.01	0.42 ± 0.02	—
SoDH	0.43 ± 0.02	0.41 ± 0.01	—
F6Pase	0.69 ± 0.02	0.66 ± 0.04	—
Others			
Hexokinase	1.07 ± 0.08	1.02 ± 0.11	—
Glucokinase	0.27 ± 0.06	0.57 ± 0.14*	+111
NADP-IDH	4.68 ± 0.40	3.53 ± 0.30*	−25

[†]Data are units/g fresh weight, except for glycogen phosphorylase *%a*, and are expressed as mean ± S.E., n = 4.
*Significantly different from the corresponding 15°C value at $P < 0.05$; **$P < 0.01$.

forms changed seasonally. Table 4 shows the isoelectric points of five enzymes from *Epiblema scudderiana* larvae sampled at different times during autumn, winter, and spring. Single peaks of activity were found for FBPase and GDH, and the isoelectric point (pI) remained the same at all sampling dates, indicating a single enzyme form unchanged over time. PFK activity occurred in two peaks; the pI 4.85 enzyme activity predominated in September and April whereas the pI 5.86 activity was the major form in mid-winter. An analogous situation was observed for PK; the pI 6.21 activity predominating in January but only the pI 5.74 form was found in September and April. Aldolase activity was also found in two peaks, the relative contribution of the pI 5.45 activity being higher in January and April larvae when compared to September, where the pI 4.76 activity predominated. This represented an increase from 30 to 54% of the contribution of the pI 5.45 activity to total activity.

TABLE 3. Polyol Cryoprotectant Levels in Freeze Tolerant *Eurosta solidaginis* and Freeze Avoiding *Epiblema scudderiana* Larvae After Acclimation of the Larvae to 15 or –4°C for 2 Weeks†

	Eurosta solidaginis		*Epiblema scudderiana*	
Cryoprotectant	15°C	–4°C	15°C	–4°C
Glycerol	201 ± 12	184 ± 17	313 ± 64	383 ± 55
Sorbitol	2.4 ± 0.3	65.0 ± 7.7**	3.1 ± 0.3	2.2 ± 0.3*

†Data are μmol/g fresh weight and are given as mean ± S.E., n = 4.
*Significantly different from the corresponding 15°C value, $P < 0.05$; **$P < 0.01$.

DISCUSSION

Activities of several enzymes were changed by acclimation to 15 or –4°C in larvae of *Epiblema scudderiana* and *Eurosta solidaginis*. Such changes may result from the synthesis of new enzyme (coarse control), the modification of existing enzyme by covalent post-translational modification (e.g., protein phosphorylation), or by allosteric effects caused by low molecular weight effectors. In this study most of the observed differences are likely the result of changes in the amounts of enzymes, i.e., coarse control. Although some of the observed differences in enzyme activities upon acclimation to different temperatures might be the result of post-translational modification (e.g., PFK and PK may be regulated in this fashion), most of the enzymes studied here are not known to be subject to such modification in animal systems. The presence of allosteric effectors also could not account for the activity differences since low molecular weight compounds were removed from enzyme preparations by Sephadex G-25 column filtration. Also, since acclimation was carried out in dark incubators, the differences in enzyme activities must reflect effects linked directly to temperature without influence by photoperiod.

The present results clearly show that temperature acclimation can lead to changes in the activities of enzymes of intermediary metabolism in both cold-hardy *Epiblema scudderiana* and *Eurosta solidaginis*. This is notably true for

TABLE 4. Distribution of Different Enzyme Forms in Overwintering Larvae of the Freeze Avoiding Gall Moth, *Epiblema scudderiana

		% of recovered activity		
Enzyme	pI	September 15	January 4	April 21
PFK	4.85 ± 0.06 (10)	70.8 ± 1.8	24.4 ± 2.0	82.0 ± 2.3
	5.86 ± 0.03 (10)	29.2 ± 1.8	75.6 ± 2.0	18.0 ± 2.3
FBPase	8.10 ± 0.10 (10)	100	100	100
PK	5.74 ± 0.05 (10)	100	5.8 ± 1.0	100
	6.21 ± 0.02 (3)	0	94.2 ± 1.0	0
Aldolase	4.76 ± 0.05 (10)	70.1 ± 1.2	46.3 ± 6.2	45.9 ± 4.1
	5.45 ± 0.02 (10)	29.9 ± 1.2	53.7 ± 6.2	54.1 ± 4.1
GDH	4.95 ± 0.06 (10)	100	100	100

*Isoelectric point data is mean ± S.E., n in parenthesis. Percentages of total activity recovered from column are mean ± S.E., n = 3–4.

enzymes of glycogenolysis, and in the case of *Epiblema scudderiana* those of the hexose monophosphate shunt (HMS) as well. Interestingly, the observed changes paralleled the expected shifts in metabolism required to sustain the synthesis of polyol cryoprotectants known to occur in these larvae upon exposure to colder fall temperatures. This data then supports a role for temperature in the modulation of biochemical adaptation in these insects.

Increased activities of glycogenolytic and HMS enzymes in cold acclimated *Epiblema scudderiana* (Table 1), in particular a 48% increase in total GPase, an increase from 1.9% to 39.6% in the active *a* form of GPase, and a two-fold increase in the activity of PFK (the rate-limiting enzyme of glycolysis), could serve to provide the necessary metabolic machinery to facilitate glycerol synthesis upon cold temperature exposure in the autumn. Glycerol synthesis in *Epiblema scudderiana* is stimulated below 5°C, with maximal rates occurring between 0 and –10°C (Kelleher et al., 1987; Storey and Storey, 1988). Glycogen is converted via glycolysis to the triose phosphates, from which glycerol is synthesized. The HMS is key in providing reducing equivalents for the synthesis of the cryoprotectant. Temperature acclimation is thus shown to influence the biosynthetic machinery necessary for cryoprotectant biosynthesis in this species. Surprisingly, the levels of glycerol were not significantly different between the acclimation groups (due to high variance), although a trend toward higher levels in the cold acclimated group may be observed (Table 3). These levels were similar to those from outdoor mid-October larvae (Rickards et al., 1987). These data suggest that other cues than cold exposure may be necessary to initiate sustained cryoprotectant synthesis prior to dispause and lower winter temperatures. These could include thermoperiods, longer exposures to cold temperatures, or other cues.

Differences in enzyme activity in larvae acclimated to the two temperatures were also seen in *Eurosta solidaginis*. Cryoprotectant synthesis in *Eurosta solidaginis* has been shown to be strongly temperature dependent, glycerol accumulating between 15 and 5°C and sorbitol below 5°C (Morrissey and Baust, 1976; Storey et al., 1981a; Storey and Storey, 1988). This is reflected in the present data, sorbitol levels increasing in the –4°C acclimated larvae (Table 3). The observed increase in the active *a* form of GPase during cold acclimation (Table 2) helps to shuttle carbon into cryoprotectant synthesis, since both glycerol and sorbitol arise from glycogen (Storey et al., 1981a; Storey and Storey, 1988). The reduced activity of PFK in –4°C acclimated larvae (a 20% decrease) is also consistent with the known metabolic switch in the larvae to favour sorbitol synthesis at temperatures below 5°C. Since sorbitol is synthesized from glucose-6-phosphate (Joanisse and Storey, 1994a), the decreased PFK activity in cold-acclimated larvae, in addition to the known direct low temperature suppression of PFK activity (Storey 1982), would serve to block glycolysis and help shunt carbon into sorbitol synthesis, and this is reflected in the increased levels of the cryoprotectant (Table 3).

Interspecies differences in the response of enzymatic activities upon cold acclimation further emphasize a role in cryoprotectant biosynthesis. Thus, *Epiblema scudderiana* larvae, which synthesize glycerol at –4°C, showed increased activities of PGI and PFK, both integral for glycerol biosynthesis via

glycolysis, upon cold-acclimation. In *Eurosta solidaginis*, however, PGM, PGI, and PFK all decreased upon cold-acclimation, reflecting the shift in metabolism towards sorbitol synthesis in these larvae below 5°C, G6P being funneled out of glycolysis for sorbitol biosynthesis.

A higher percentage of glycogen phosphorylase in the active form (*% a*) at –4°C in both species studied can be explained by the known temperature modulation of the GPase phosphorylation cascade. Cold activation of GPase is a well-documented response in insects and results from differential temperature effects on phosphorylase phosphatase and phosphorylase kinase (Ziegler et al., 1979; Hayakawa and Chino, 1982). Cold activation of GPase has been shown to be key in the initiation of glycerol biosynthesis for both species (Churchill and Storey, 1989; Joanisse and Storey, 1994a).

The results from the enzyme activity studies allow us to state that these activities are subject to change upon acclimation to different temperatures. This occurs in a seemingly ordered fashion in both species, with the apparent objective of shifting metabolism towards cryoprotectant synthesis. By extension, decreasing autumn temperatures in nature could serve as a cue for the larvae to initiate controlled, specific changes at the enzymatic level, which may be required for cryoprotectant synthesis and winter survival.

In addition to changes in the amounts of enzymes, temperature changes may also modify enzyme form either by the synthesis of new isozymes or the modification of existing enzymes by post-translational modification. Both strategies have the goal of forming enzymes with kinetic behaviours better suited to the new prevailing conditions and the needs of the animal. In the present study we assessed the possible role of such mechanisms by analyzing the isoelectric focusing patterns of five enzymes at different seasons in *Epiblema scudderiana* (Table 4). Neither the number (one) or the pI values of FBPase and GDH activities changed over the winter, showing that both exist as single isozymic forms. The transient changes in the predominant activities of PFK and PK in January are not likely the result of the synthesis of new isozymes. Instead, these probably represent a change in the enzymes by post-translational modification during the winter. These enzymes are well known to undergo phosphorylation/dephosphorylation modifications (which would change the pI) in many animal systems. More interesting is the change in the distribution of aldolase activities over the winter. We have previously shown that total aldolase activity increases from September to January, and remains elevated into the spring (Joanisse and Storey, manuscript submitted). The present data suggest that this is mostly due to the preferential synthesis of the pI 5.45 activity, as the contribution of this form to total activity is higher in January and April when compared to September (Table 4). Possible reasons for the increased contribution of this pI 5.45 form to total activity, which may be a different isozyme from the pI 4.76 activity, include (1) preferred synthesis as it may be an isozyme with different, required properties, or (2) differential control or temperature effects at the transcriptional or translational levels for the two forms. From this study we cannot state whether this increasing activity contributes to the winter hardiness of the larvae, or if it represents a developmental change. Previous work on cold-acclimated fall larvae of *Eurosta solidaginis* showed no change in

the isozyme composition of a number of enzymes (Storey et al., 1981b). Combined with the present data, this suggests a limited role for the formation of the new enzyme variants for low temperature metabolic regulation in cold-hardy insects.

LITERATURE CITED

Baldwin J (1971): Adaptation of enzymes to temperature: Acetylcholinesterases in the central nervous system of fishes. Comp Biochem Physiol 40:181–187.

Baldwin J, Hochachka PW (1970): Functional significance of isozymes in thermal acclimatization: Acetylcholinesterase from trout brain. Biochem J 116:883–887.

Churchill TA, Storey KB (1989): Seasonal variation in the temperature-stimulated interconversion of glycogen and glycerol pools in a freeze avoiding moth larvae. Cryo-Lett 10:127–136.

Hayakawa Y, Chino H (1982): Temperature-dependent activation or inactivation of glycogen phosphorylase and synthase of fat body of the silkworm *Philosamia cynthia*: The possible mechanism of the temperature-dependent interconversion between glycogen and trehalose. Insect Biochem 12:361–366.

Hochachka PW, Somero GN (1984): Biochemical Adaptation. Princeton: Princeton University Press.

Joanisse DR, Storey KB (1994a): Enzyme activity profiles in an overwintering population of freeze tolerant larvae of the gall fly, *Eurosta solidaginis*. J Comp Physiol B 164:247–255.

Joanisse DR, Storey KB (1994b): Mitrochondrial enzymes during overwintering in two species of cold hardy gall insects. Insect Biochem Mol Biol 24:145–150.

Kelleher MJ, Rickards J, Storey KB (1987): Strategies of freeze avoidance in larvae of the goldenrod gall moth, *Epiblema scudderiana*: Laboratory investigations of temperature cues in the regulation of cold hardiness. J Insect Physiol 33:581–586.

Morrissey RE, Baust JG (1976): The ontogeny of cold tolerance in the gall fly, *Eurosta solidaginis*. J Insect Physiol 22:431–437.

Rickards J, Kelleher MJ, Storey KB (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.

Sidell BD (1977): Turnover of cytochrome C in skeletal muscle of green sunfish (*Lepomis cyanellus*, R.) during thermal acclimation. J Exp Zool 199:233–250.

Sidell BD, Wilson FR, Hazel J, Prosser CL (1973): Time course of thermal acclimation in goldfish. J Comp Physiol 84:119–127.

Storey KB (1982): Phosphofructokinase from the overwintering gall fly larva, *Eurosta solidaginis*: Control of cryoprotectant polyol synthesis. Insect Biochem 12:501–505.

Storey KB, Storey JM (1988): Freeze tolerance in animals. Physiol Rev 68:27–84.

Storey KB, Baust JG, Storey JM (1981a): Intermediary metabolism during low temperature acclimation in the overwintering gall fly larvae, *Eurosta solidaginis*. J Comp Physiol B 144:183–190.

Storey KB, Park IRA, Storey JM (1981b): Isozyme composition and low temperature acclimation in the overwintering gall fly larva, *Eurosta solidaginis*. Cryo-Lett 2:279–284.

Vesterberg O (1971): Isoelectric focusing. In Colowick SP, Kaplan NO (eds): Methods in Enzymology. New York: Academic Press, pp 389–412.

Ziegler R, Ashida M, Fallon AM, Wilmer LT, Silver Wyatt S, Wyatt GR (1979): Regulation of glycogen phosphorylase in fat body of *Cecropia* silkmoth pupae. J Comp Physiol 131:321–332.