飞蝗不同地理种群抗寒性研究

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摘要：新疆和硕、哈密和天津北大港飞蝗种群的过冷却点随发育阶段而升高，在胚胎发育的各期都具有较低的过冷却点、飞蝗卵的过冷却点在这 3 个地理种群间无明显差异。雄性蝻和成虫比雌性的过冷却能力强，和硕飞蝗的成虫过冷却点比北大港的飞蝗成虫要低。低温胁迫可诱导蝗卵以糖元为原料合成抗冻保护剂，和硕飞蝗种群的卵主要合成甘油和山梨醇，北大港飞蝗种群的卵主要合成海藻糖，而哈密飞蝗种群的卵却可以合成甘油、山梨醇和海藻糖等抗冻保护剂。糖元磷酸化酶的总量随发育阶段而下降，但热休克和冷休克并不改变其总量，冷休克在胚胎发育的各阶段可以使其活性升高 10%～40%。北大港飞蝗种群和哈密飞蝗种群的蝗卵经热休克 2h 处理后，糖元磷酸化酶的变化与在冷休克下的情况相似，而和硕飞蝗种群胚胎发育的Ⅱ期，热休克诱导糖元磷酸化酶活性的升高程度较低。

关键词：飞蝗；过冷却点；抗冻保护剂；磷酸化酶

The Cold-hardiness of different geographical populations of the Migratory Locust, *Locusta migratoria* L. (Orthoptera, Acrididae)

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Abstract: The supercooling point (SCP) increases with development in the three geographical populations. The SCPs in the I, II and III embryo stages of the three populations are about −28 C, −20 C and −9 C respectively. The SCP of the adults from the Heshuo population is lower than that in the adults from the Beidagang population. Low temperature acclimation leads the glycogen breakdown to synthesize cryoprotectants in all embryo stages, and the highest synthesis occurred in the stage II embryo. Glycerol and sorbitol are the dominant cryoprotectants in the embryo of the Heshuo population, but trehalose is the principal one in the embryo of the Hami population, in the same time all the three kinds were found in eggs of the Hami population. For the decreasing content of glycogen with the embryo development, the low glycogen level in the late embryo stage becomes the limitation to the cryoprotectants synthesis. The whole content of the GPase that reduces with the embryo development keeps stable in the stress. Cold shock (2 hours) leads the GPase to 'a' increase of about 10%～40%. Heat shock shaped the activity increase is similar to the cold shock craved in the eggs from the Beidagang and the Hami populations. Only in the Ⅱ embryo stages of the Heshuo population, heat shock reasons more significant variation of the GPase 'a'.

Key words: *Locusta migratoria*; supercooling point; cryoprotectants; GPase

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1 INTRODUCTION

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Cold-hardiness refers to the capacity of an organism exposure to survive at low temperature[1]. According to present knowledge, successful overwintering is dependent mainly on one of two strategies: the ability to avoid freezing by supercooling to considerable degree (freeze-intolerant), or survival of actual ice formation within the body (freeze-tolerant)[2]. The former category of insects can survive above the supercooling point (SCP), but the latter can tolerant and survive below this point[3]. The majority of cold-hardy insects are freeze-intolerant and rely on the process of supercooling to avoid lethal freezing[4]. Changes in whole-body SCPs may be one of the cold-hardening processes. Cold-hardiness varies between and within species and can be measured by a number of indices such as SCP.

A number of different polyols and sugars have been found in overwintering stages of freeze intolerant insects. It is now recognized that there are two main groups of cryoprotectant antifreezes in freeze-intolerant insects. One is low molecular weight polyhydroxy alcohols (polyols) and sugars, and the other is high molecular weight antifreeze proteins (thermal hysteresis proteins)[5~7]. Sϕmme[8] reported glycerol to be positively identified in 64 of 96 insect species. Although glycerol is the most common and abundant cryoprotectant, other polyols include sorbitol, mannitol and ethylene glycol. Among the sugars, glucose, trehalose and fructose have been recorded in freeze-intolerant insects.

Elevation of the low molecular weight substances is at least partially responsible for the protection against cold-induced injury that is generated by a brief period of chilling[9]. The egg storaged large quantities of glycogen in insects are utilized for embryo development. Glycogen phosphorylase (GPase) exists in three interconvertible forms in *L. migratoria*: an active phosphorylated 'a' form, an inactive dephosphorylated 'b' form, and a partially phosphorylated 'ab' form[10]. Phosphorylase can be activated by exposure to cold[11]. This enzyme acts as one of the key enzymes regulating synthesis of cryoprotectants from glycogen in overwintering insects. In the laboratory, temperatures triggering polyols synthesis are found typically from 0 C to 5 C[1].

Eggs of many overwintering insects are exposed to severe winter climates. In general, lower mean SCPs are found in insect eggs than in other stages. Large parts of the populations will be wiped out if the minimum environment temperature falls below their mean SCP. The synthesis of cryoprotectants may plays some role in the cold-hardiness process. To provide the protection in case of the suddenly-met low temperature, the GPase which were involved in the biosynthetic pathway for cryoprotectants must be very swiftly taken in the process. In the study, we focus on the SCPs, the cryoprotectants synthesis and the activation of phosphorylase in the eggs of *L. migratoria* in changed temperature.

2 MATERIALS AND METHODS

The overwintering eggs of *Locusta migratoria* were collected in Beidagang, Tianjin (BDGTJ); Hami, Xinjiang (HMXJ) and Heshuo, Xinjiang (HSXJ). The eggs were divided into three stages according to the development periods at 30 C: Stage Ⅰ (2 days), Stage Ⅱ (7 days), Stage Ⅲ (12 days). The locust eggs of different development stages were placed in a chamber of 4±1 C. At different acclimation periods (1, 3, 5, 7, 14 days), the eggs were removed for biochemical analysis. The eggs of locust used in the assay of GPase were all incubated at 25 C before they were shocked.

SCPs were determined by copper constantin thremocouple. The standard curve is shown in Figure 1. The SCP is the lowest temperature recorded prior to the release of the latent heat of fusion.

Separation of glycogen, sugars and polyols were achieved by the methods of reference[12]. Total amounts of sugars and glycogen extract were determined by the phenol-sulfuric acid method[13] with glucose as the standard. The extract containing sugars and polyols were subjected to qualitative and quantitative analysis by TLC. Known amounts of the sample was spotted together with standard solutions

of sugars and glycerol on 250μm-thick silica gel 7G plates and developed in a butanol : acetic acid : ether : H_2O (9 : 6 : 3 : 1) solvent system. The dried plates were sprayed with anisaldehyde-acetic acid - H_2SO_4 reagent (0.5 : 50 : 1)[14] and heated at 100～105 C for color development. After 5 minite. of cooling, the color-developed plates was used to analyse the sugars and polyols by the TLC scanning CAMAG LINOMAT IV (Switzerland). By this method, the separation and quantitative estimation of trehalose, glucose, sorbitol and glycerol with their respective *Rf* values of 0.24, 0.35, 0.48 and 0.64 was achieved.

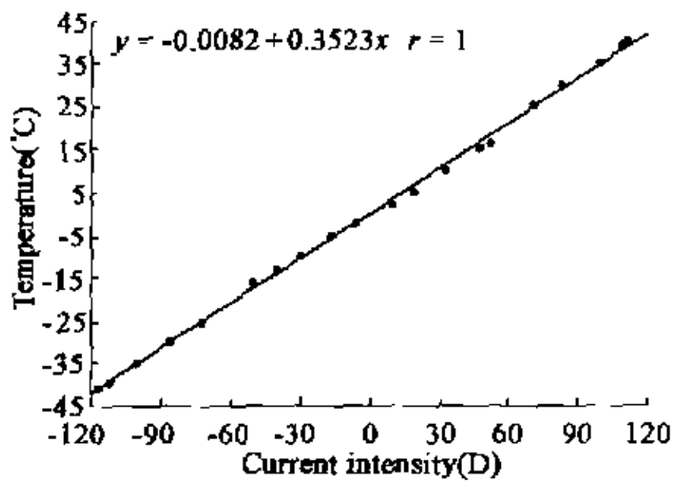

Fig. 1　The Standard Curve of the thermocoupleThe current intensity of each division (*D*) is about 2.4 × 10^{-10}A.

The eggs were kept at different temperature (4 C or 45 C) for 2 hours, and were immediately homogenized with a plastic pestle in 1 mL homogenizing buffer (20 mM phosphate buffer, pH7.0, 5 mM imidazole, 5 mM EDTA). The homogenate was centrifuged at 8000g for 10 minites, at 4 C. After removing the top layer containing fat, the supernatant was collected and used for the assay of GPase. GPase activity was assayed in the direction of glycogen breakdown with a coupled enzyme system slightly modified from that of reference[15]. For the assay of phosphorylase 'a' activity, the reaction mixture (1.0 mL) consisted of 40 mM phosphate buffer(pH7.0), 5 mM imidazole, 2 mM EDTA, 1.4 mM DTT, 5 mM $MgCO_3$, 0.62 mM NADP, 1.3 μM glucose-1,6-diphosphate, 0.94 units glucose-6-phosphate dehydrogenase, 0.26 units phosphoglucomutase, 10 mg glycogen. The reaction was started by adding the enzyme preparation and the reduction of NADP measured by recording the adsorbance at 340 nm in a Hatachi Spectrophotometer for about 20 minites. Immediately after the assay of phosphorylase 'a' activity was finished, 50 μL of 40 mM AMP was added to the reaction mixture and the reaction resumed to determine the total phosphorylase activity (phosphorylase a+b). The phosphorylase activity was expressed as the percentage of phosphorylase 'a' against that of total phosphorylase. One unit of phosphorylase was defined as the activity that forms 1 μM of glucose-1-phosphate per min. under 25 C. Protein in the enzyme preparation was determined according to reference[16] with bovine serum albumin as the standard.

D-trehalose, Tris (Trizma-Base), NADP, DL dithionthreitol (DTT), glucose 1,6-diphosphate, glucose-6-phosphate dehydrogenase (E.C.1.1.1.49) and phosphoglucomutase (E.C.2.7.5.1) were purchased from Sigma Chemical. AMP was obtained from Roanal, Budapest. All other chemicals were analysis reagents.

3　RESULTS

3.1　The supercooling ability of the migratory locust

The SCPs increased with the development stage. Since the mass of the egg or the nymph usually increase with the development, the SCPs were in direct proportion to the mass. The destroyed eggs had the increased SCPs. The stage 1 eggs and the first instar nymphs had the lowest SCPs in their development stages (Fig. 2A, B). The SCPs of newly deposited eggs of locust were difficult to measure since they are difficult to separate from each other. The SCPs in the Ⅰ, Ⅱ and Ⅲ embryo stages of the three populations are about −28 C, −20 C and −9 C respectively. The SCPs of the chilled eggs of all the three populations in stage II and III are not significantly different from the unchilled ones (Fig. 2A).

The nymphs and adults of the locusts usually like to live in the high temperature habitat, though their

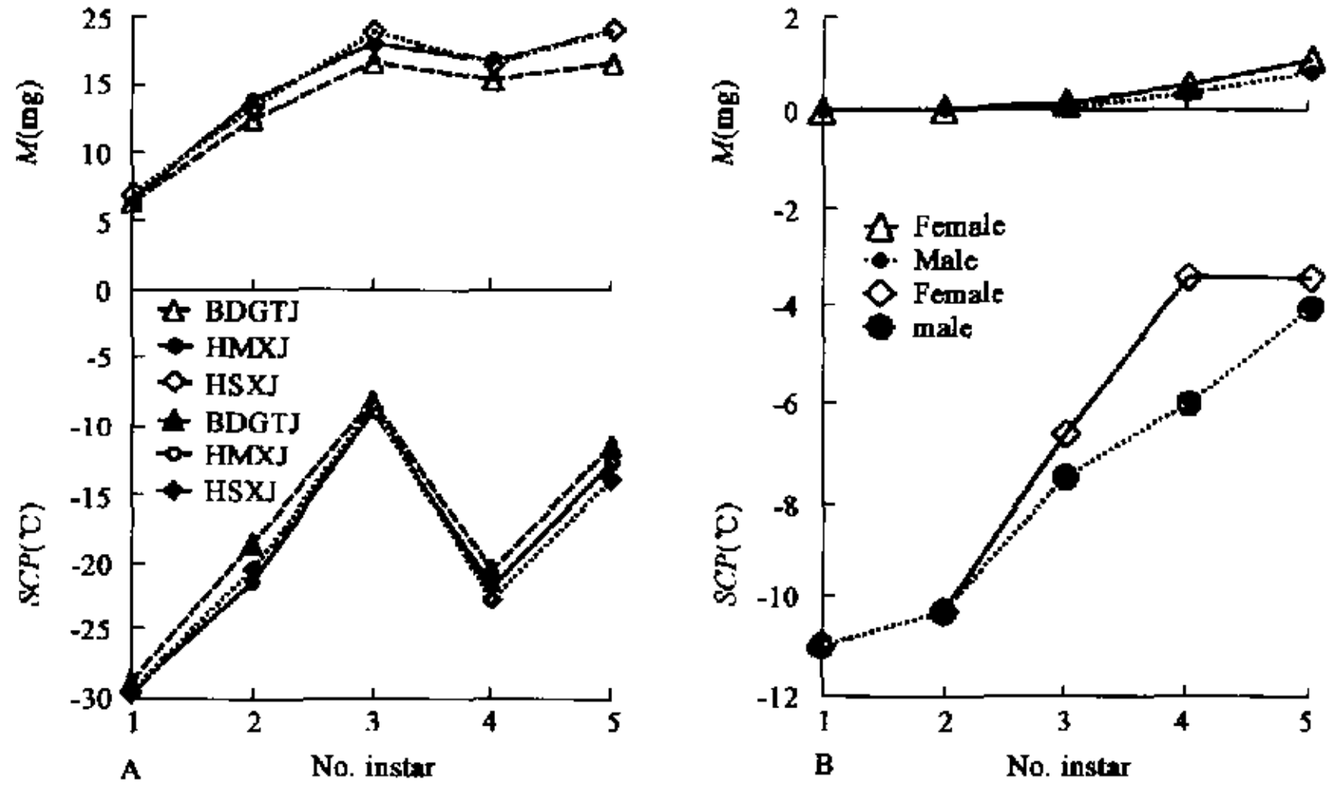

Fig. 2 The relationship between development stages and supercooling point

A. An expresses that in the eggs of BDGTJ, HMXJ and HSXJ population respectively. In this figure, 1, 2 and 3 indicate the embryo development stage Ⅰ, Ⅱ and Ⅲ, while 4 and 5 show the eggs in embryo stage Ⅱ and Ⅲ was chilling at 4±1 C for two weeks. The eggs of the HSXJ population in the stage Ⅱ were chilling at 4 C for 12 weeks to disturb the diapause. The eggs were then continued to incubate at 30 C until the experiment stage. B. The relationship between the SCP and the mass of the nymphs of the two sexes of BDGTJ population.

SCPs are relatively low. The SCPs of the first instar nymphs of the BDGTJ population are −11 C (Fig. 2B). The adults of the locusts can supercool to 2.5 C ~ −3.8 C. Though the adults of the HSXJ population (female, 3.05 g; male, 1.61 g) are heavier than that of the BDGTJ population (female, 2.006 g; male, 1.001 g), the SCPs of the former (female, −3.71 C; male, −3.75 C) are lower than that of the latter (female, −2.584 C; male, −2.842 C).

3.2 The cryoprotectants synthesis during low temperature acclimation

Under low temperature acclimation, in the eggs of the BDGTJ population, the glycogen was broken down to synthesize sugars, mainly trehalose and glucose. The trehalose content increase is responsible for above 80% of the sugar increase. In low temperature acclimation, the glycogen content of the stage I eggs decreased from 37.53 μg/mg (control at 0 C) to 22.13 μg/mg, and at the same time, the total sugar content increased from 1.92 μg/mg to 3.37 μg/mg. The highest amount of sugar increase was demonstrated in the stage Ⅱ embryo (Fig. 3A). The glycogen and sugar contents of the stage Ⅱ embryo were 3.4 μg/mg and 6.35 μg/mg respectively, and acclimation led them to 1.96 μg/mg and 7.81 μg/mg.

The mainly increased glycerol and sorbitol was observed in the eggs of the HSXJ population in low temperature acclimation with the content of total sugars slightly increased. After the cold acclimation, the content of glycogen in the stage I embryo decreased from 37.64 μg/mg to 22.82 μg/mg, while the glycerol (from 0.91 μg/mg to 4.77 μg/mg) and sorbitol (from 0.18 μg/mg to 1.01 μg/mg) levels rose to several times of that of the control. In the stage III embryo, the decrease of the glycogen (from 4.65 μg/mg to 1.98 μg/mg) led the glycerol and sorbitol increase from 1.57 μg/mg to 3.71 μg/mg and from 0.76 μg/mg to 1.38 μg/mg respectively. The same situation was also found in the stage II embryo (Fig. 3C).

Both polyols (mainly glycerol and sorbitol) and sugars (mainly trehalose) increase were detected in cold acclimation process in the eggs of the HMXJ population. The glycogen contents in the stage I embryo

decreased from 39.35 μg/mg (control 30 C) to 23.68 μg/mg, and the content of total sugar gradually increased. The increase of glycerol was rapidly in the first period of acclimation, then the swiftly increased polyol is sorbitol both in the stage I (glycerol from 1.67 μg/mg to 3.1 μg/mg and sorbitol from 0.81 μg/mg to 3.5 μg/mg) and Ⅱ embryo. In the later stage, the contents of those two polyols seemed to be maintained in a stable level. The trehalose content slowly increased in all this acclimation process (Fig. 3C). Compare with the patterns in the Ⅰ embryo stage, both glycerol (from 2.06 μg/mg to 2.8 μg/mg) and sorbitol (from 0.7 μg/mg to 1.3 μg/mg) in the stage Ⅱ embryos were increased in a more gradual levels, but the trehalose increase more speedily from 1.35 μg/mg to 3.25 μg/mg.

3.3 Activation of GPase at cold or heat stress

Cold or heat shock at 4 C or 45 C increased the activity of the enzyme in most of the developmental stages and treatments. In the insects of all the three populations, the quantity of total GPase showed sign of significant decrease with development. The highest GPase concentration (about 3.91 units/g fresh weight) is found in the first stage of the eggs, and in the stage Ⅱ and stage Ⅲ eggs, the amount is 2.10 units/g fresh weight and 0.65 units/g fresh weight individually.

The effects of cold or heat shock on GPase activity in the eggs of the BDGTJ and the HMXJ populations are similar. The eggs being abruptly exposed for 2 h to 4 C or 45 C significantly increased the proportion of GPase 'a': about 30%～40% increase in the embryo I and II, and approx. 10%～20% increased in the embryo III (Fig. 4).

In the stage Ⅰ and Ⅱ embryos of the HSXJ population, the stress-induced increase of GPase activity is in a similar pattern to that of the other two populations. In the stage II of the embryo, the GPase activity increased with the cold stress is significant, but after the heat shock, the enzyme activity is only slightly increased (Fig. 4A, B, and C).

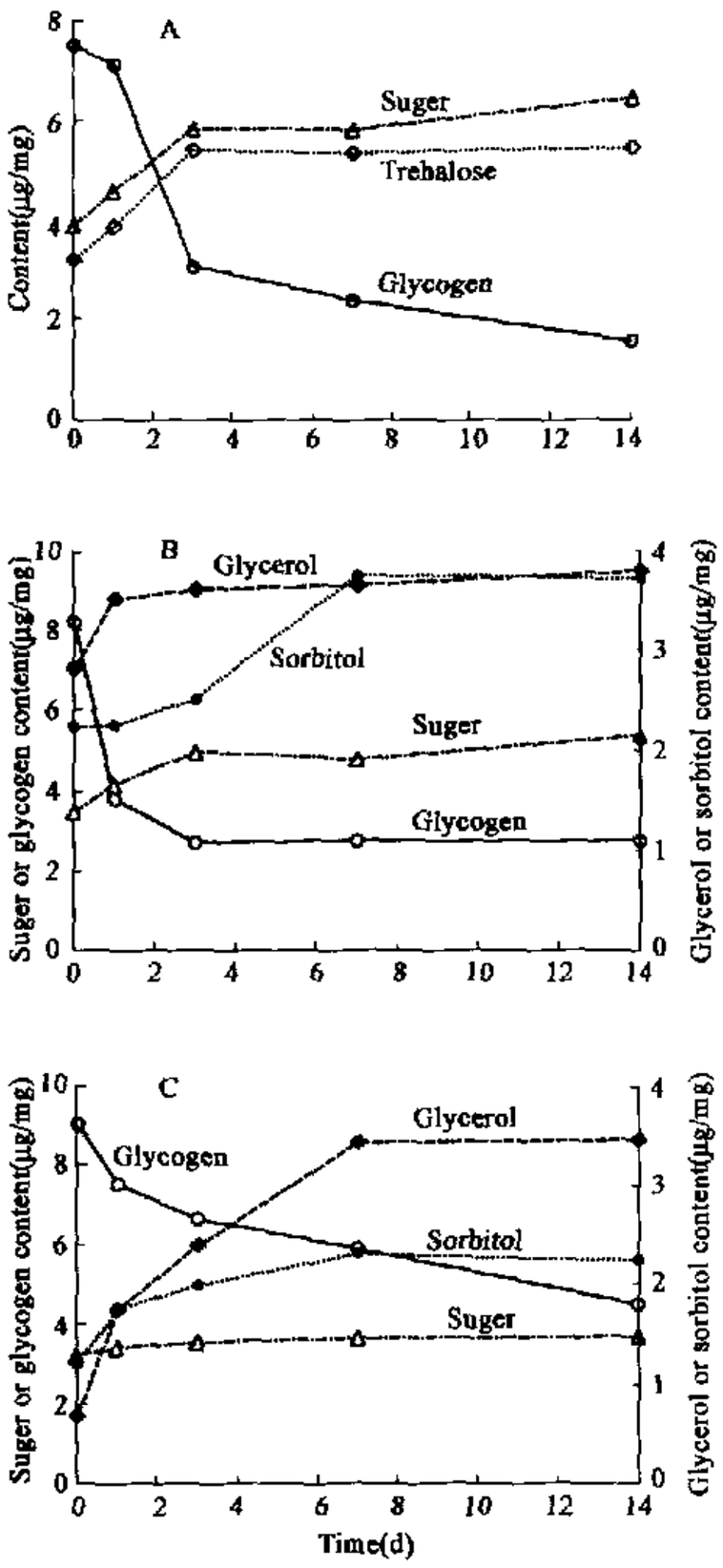

Fig. 3　Fresh weight variations in glycogen and cryoprotectant of the stage II embryo during acclimation of the different geographical locust populations at low temperature (4±1 C) for various periods (0, 1, 3, 7, 14 days). Figures A, B and C are the profile of the BDGTJ, HMXJ and HSXJ population respectively.

4 DISCUSSION

The SCPs of the eggs of *L. migrtoria* reported by reference [17] was −30 C. The SCPs obtained by them may be the SCPs of the stage I eggs. The supercooling points of the fifth instar and male adult African migratory locusts (*L. m. migratorioides*) are −3.5 C and −3.8 C respectively[18]. Huang *et al.*[19] found the SCPs have variance between the phases and between the sexes of the adults of the

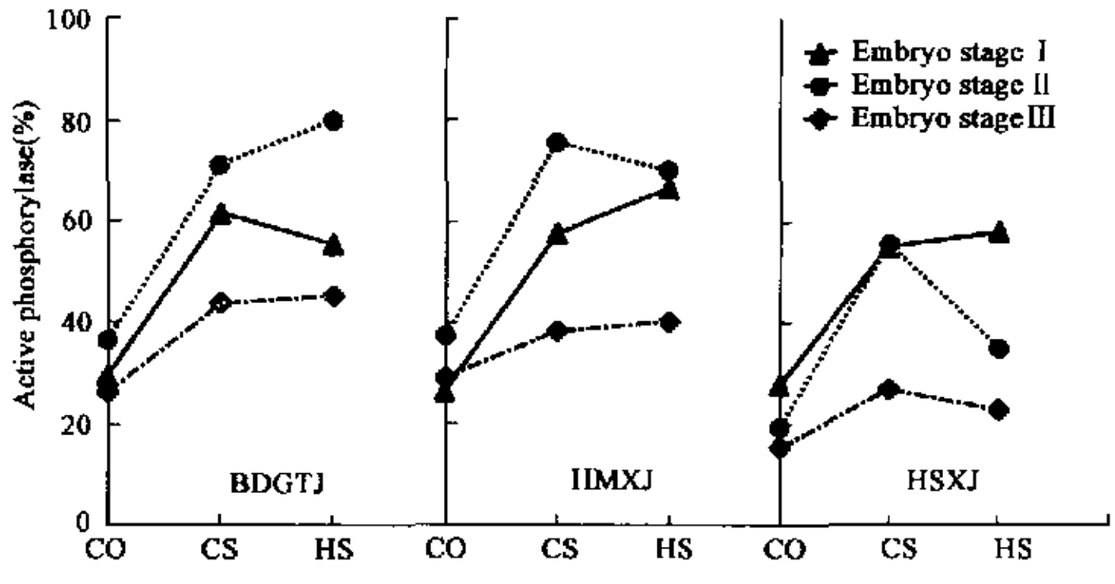

Fig. 4 Changes of the percentage of active phosphorylase during different development stages of the eggs in the geographical locust populations after cold or heat shock. CO, control; CS, cold shock; HS. heat shock.

migratory locust from Hebei and Jiangsu populations. Early observers reported that piercing the insect with the thermocouple, which Huang *et al.* used in their measurement, raised the SCP considerably[5]. The SCPs of the adults in Huang's test are lower than those in ours, which may be caused by the different measure methods or materials.

Although the development threshold temperature of the egg is about 15 C, the diapause eggs indeed develop in the low temperature. In the chilling period, the eggs also develop and the masses of them increase continuously. The chilling decrease to the SCPs may be more effective than it shows in the measured SCPs. The heavier females always have the higher SCPs than the lighter males in the same development stage.

The significant SCP variance between the adults of the BDGTJ and HSXJ populations may be various adaptations to the long-term cold-hardy acclimation. The locusts in the HSXJ population usually have to survive a more severe late autumn.

The mirror image symmetry relationships between the glycogen and sugars and/or polyols indicated the transformations from glycogen to cryoprotectants do occur in low temperature. The temperature-dependent interconversion between glycogen and polyols and sugars is various among different geographical populations. In all stages of the populations, the glycogen was continuously transformed to sugars with the development. The transformation of glycogen to cryoprotectants is rapid. Most of the cryoprotectants increase abruptly in the first period of cold acclimation (the first 3 days), then the levels are kept in slow increase.

The multifactorial carbohydrate metabolism systems (glycogen is converted to both polyols and sugars) have been considered advantageous because they prevent or reduce the possible toxic effects associated with the relativity high concentration of single components[20] and may reduce enzyme activities to promote energy conservation during prolonged overwintering[5]. In the eggs of locust, the large amount of polyols and sugars that accumulates is derived from glycogen. The conversion of glycogen to trehalose and glucose or to glycerol or sorbitol is various among different geographical populations.

To date, studies of the regulation of polyol metabolism have largely focused on aspects of the low temperature activation of biosynthesis, although recent work has begun to probe the developmental controls of the process. Given that the enzymatic machinery is in place, the immediate trigger that initiates polyol synthesis in most species is low temperature. The present research suggested that there exists an

underlying developmental and geographical population component to polyol synthesis that could be further enhanced by low temperature.

In our work with the egg extraction, we found 20%～10% GPase in control eggs. These values are higher than results obtained with the degradation assay in other tests[2,4].

At the stage Ⅰ *and* Ⅱ *eggs of the locust, the total GPase units are very high for the glycogen* conversion. Since the high quantity of GPase 'a' after stress and the quantity of glycogen which can be broken down is high, the cryoprotectants synthesis are in a higher degree. It the stage Ⅱ, with the accomplishing of the development and the decomposition of the glycogen, the quantity of glycogen that can be transformed is much lower. The cryoprotectants synthesis is much limited. The active form of GPase is the lowest in the stage Ⅱ of the HSXJ population that is also the diapause stage eggs. Cold shock induced rapidly activated the enzymes while heat shock induced slight increase. The higher increasing ability of the activity of the GPase may be one of the adaptations to the colder weather condition.

Little information is available on the effect of heat shock on the activity of GPase. But GPase is known to be activated by physical injury in silkmoth pupae[12] and by flight activity, which normally is accompanied by an increase in body temperature[2,1]. The high temperature induced activity increase may be part of the organism's heat shock response[9].

The temperature at the SCP of the egg, nymph and adult is the rarely meet in their normal habituated environment. The adaptation to supercooling at lower temperature may be of benefit to survival at extreme temperature, especially when the extreme is continuously. The present findings, together with other reports concerning carbohydrate metabolism of other overwintering insects, support the suggestion that *overwintering insects may accumulate sugar alcohol and trehalose. The cold-hardiness in combination with* the protection afforded by overwintering sites may be sufficient to ensure survival.

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