

Bing Chen · Le Kang

## Can greenhouses eliminate the development of cold resistance of the leafminers?

Received: 1 November 2004 / Accepted: 7 February 2005 / Published online: 11 May 2005  
© Springer-Verlag 2005

**Abstract** Latitudinal patterns for quantitative traits in insect are commonly used to investigate climatic adaptation. We compare the cold resistance of the leafminer (*Liriomyza sativae*) pupa among populations distributed from tropical to temperate regions, incorporating the thermal overwintering limit of the insect's range. The patterns of cold resistance for northern and southern populations differ. The southern populations significantly increased their cold resistance with latitude, showing a latitudinal pattern independent of seasons, acclimation regimes, and assay methods. In contrast, the northern populations showed no stable patterns; they were always intermediate in cold hardiness between the low-latitude and high-latitude populations within the overwintering limit. Integration of these data with those of the biologically similar congeneric leafminer, *L. huidobrensis*, suggests that a pattern shift in stress tolerance associated with the overwintering range limit is probably a general adaptive strategy adopted by freeze-intolerant species that have a high-latitude boundary of distribution, but can only overwinter and develop in protected greenhouses in harsh seasons. Considering the widespread availability of greenhouses for overwintering insects in northern China, we speculated that the large-scale existence of thermally-buffered microhabitats in greenhouses might eliminate the development of cold resistance of the leafminer populations. However, results suggest a strong selection for increased cold resistance for natural populations of *Liriomyza* species at higher latitudes that can overwinter in the field, but not for populations at latitudes above the thermal limit. Thus, habitat modification associated with greenhouses can

limit gene flow and reduce cold tolerances even at latitudes above where the leafminers can overwinter in the field.

**Keywords** Cold resistance · Gene flow · Habitat changes · Latitudinal variation · *Liriomyza*

### Introduction

An effective method of identifying potential factors and traits contributing to population dynamics is to test for adaptive differences between geographic populations (Hoffmann and Blows 1994; Jenkins and Hoffmann 1999). However, geographic patterns in adaptive traits of insects are usually associated with climatic factors such as temperature and humidity patterns (Magiafoglou et al. 2002). Although anthropogenic habitat changes are thought to be altering the distributions and abundances of insects throughout the world (Warren et al. 2001), the impact of habitat modification on the dynamics of insect populations is seldom empirically evaluated in relation to environmental stress tolerances.

One of the most important habitat modifications associated with agricultural land use is the widespread increase in greenhouses for protected cultivation (Castilla 2000; Raaphorst 2003). These greenhouses allow improved control of the availability of heat, water, light, and CO<sub>2</sub> for crops, thereby permitting plants (and ultimately insects) to be grown in areas and during seasons which would otherwise be unsuitable. For example, protected cultivation in the Mediterranean region has expanded to 143,000 ha during recent decades (Castilla 2002). In China, a wide variety of glass and plastic structures have been constructed since 1970s. Presently the total area of greenhouses for vegetable crops planting is about 500,000 ha. The ratio of greenhouse area to field crop area for vegetable crops ranges from 4.4% to 46.7%, with the highest percentage in the arid and cool temperate regions of northern China (Hu 1995, also for

Communicated by Roland Brandl

B. Chen · L. Kang (✉)  
State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Bei Si Huan West-Road 25, Beijing, 100080 China  
E-mail: lkang@ioz.ac.cn  
Tel.: +86-10-62554979  
Fax: +86-10-62565689

official data see <http://www.sannong.gov.cn>). Unfortunately, there is limited experimental evidence for the effects of greenhouses on the distribution and abundance of arthropod populations (Fuller et al. 1999; Castilla 2002).

By definition, a greenhouse is a relatively enclosed structure providing crops as well as insects a thermally buffered microclimate, particularly in cold seasons (Wardlow and Oneill 1992; Akinci et al. 1998; Fuller et al. 1999; MacDonald et al. 1997). For instance, temperatures in greenhouses are generally maintained at 18–25°C even when external ambient temperature drops to –10°C in the temperate regions of Northern China between the latitudes 40°N–45°N (Hu 1995). Thus, greenhouses annually provide crop pests with shelter and overwintering sites (Hatherly et al. 2004; MacDonald et al. 2000; Kirk and Terry 2003). For example, the vegetable leafminer, *Liriomyza sativae*, an economically important pest throughout the world (Spencer 1973), has a wide distribution throughout China, with its most northerly range recorded in Daqing (latitude 46°31'N) (Chen and Kang 2002; Jing and Wang 2003). However, the leafminer can't successfully overwinter in open fields beyond the –2°C isotherm of minimum mean temperature in January (around latitude 34°N), which is considered as the overwintering range limit for natural populations of this species (Zhao and Kang 2000; Chen and Kang 2005, in press).

Several investigations found that the microclimate change associated with greenhouses has influenced relative growth rates and phenological synchrony of the insects and their host plants (Van Lenteren and Woets 1988; Danks 1996; Hatherly et al. 2004). In the tropical and sub-tropical regions in southern China, *L. sativae* infestations are usually initiated during the vegetative growth phase of crops as the climate warms in the early spring, and pest populations continue to thrive throughout the spring, summer until the climate turns too cold to maintain adult activity. At this point the insect enters a pupal stage hibernation. However, in temperate regions at latitudes above the overwintering limit, the dynamics of field populations largely differ. For example, there are only 3–4 generations in the field, but 8–11 generations each year in greenhouses in Chifeng (42°16'N). The leafminer is not found in field plantings until the middle of May when the mean ambient temperature rises to 15.5°C, which is concomitant with the peak of populations in greenhouses (Guo et al. 2000). It is interesting that the populations both in southern tropical fields and northern greenhouses are more prone to breakout than in central regions (Guo et al. 2000; Chen and Kang 2002a). Therefore, the population dynamics of this leafminer species varies between the different sides of the overwintering range limit.

Such differences in population dynamics above and below the overwintering limit can indicate physiological variability in the populations. For example, significant variation in cold tolerances in geographical populations has been observed in China for a congeneric leafminer,

*L. huidobrensis* (Chen and Kang 2004). For the southern populations within the overwintering limit, cold tolerance of the leafminer increased with latitude. In contrast, populations found above the field overwintering limit had a relatively decreased cold tolerance, and lacked any general patterns related with latitude. The ecological significance of the overwintering limit of a species is rarely considered even though the factors and traits responsible for the adaptation process of insects along climatic gradients have been examined in detail (Case and Taper 2000; Hoffmann and Blows 1994; Warren et al. 2001). More empirical data are needed to draw general conclusions about the shifting patterns and related underlying mechanisms for insects in landscape level systems near the range limits of a species.

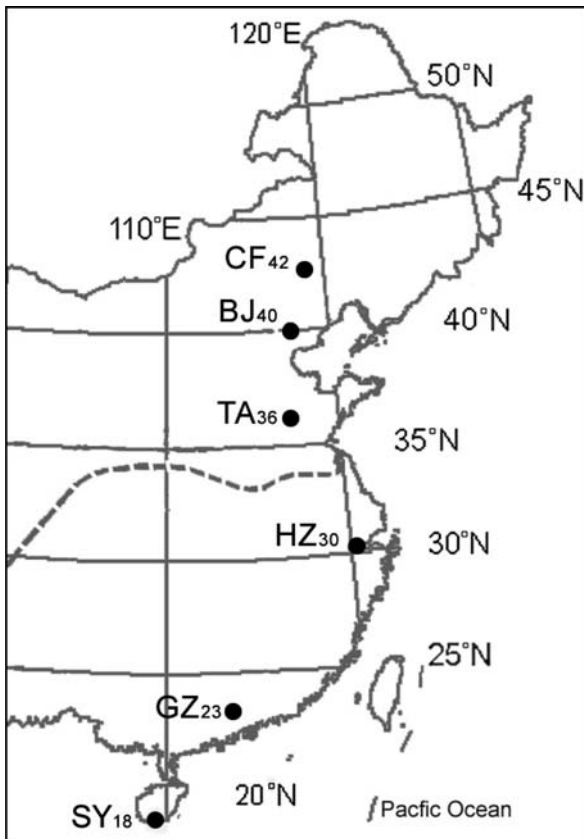
We, therefore, initiated a large-scale population study on the leafminer *L. sativae* across its distribution range. The species is selected as the study model based on several considerations. First, this leafminer, native to southern USA and South America, is considered to be an invasive species that has become adapted to local environments that range from holarctic to tropical (Spencer 1973; Chen and Kang 2002b; Reitz and Trumble 2002). Second, the differences in cold hardness between the two closely related *Liriomyza* species, *L. sativae* and *L. huidobrensis*, explains their differential geographic distribution and phenology in China (Chen and Kang 2002b). Hodkinson (1999) also recognized that distribution of such polyphagous species can be restricted by ecophysiological tolerances, largely independent of their host plants. Here, we expand on the work of Chen and Kang (2004) on *L. huidobrensis* by adopting a broad-scale sampling strategy throughout the 3,000-km Eastern China range of *L. sativae*, including samples from above the maximum latitude at which overwintering occurs in the field, to evaluate the potential impact of greenhouses on the evolution of cold resistance.

---

## Materials and methods

### Origin of flies

The field overwintering limit of *L. sativae* and a list of the collection localities and their abbreviations (in parentheses) are presented in Fig. 1. Collection of geographically separate populations of *L. sativae* was carried out during May 27 to June 10 in 2004. Six populations were sampled from tropical to temperate regions along a 3,000-km stretch in East China. Three sites on each side of the overwintering range limit were sampled. The three sites labeled as SY<sub>18</sub>, GZ<sub>23</sub>, HZ<sub>30</sub> (hereafter described as southern populations) and TA<sub>36</sub>, BJ<sub>40</sub>, CF<sub>42</sub> (hereafter described as northern populations), were almost regularly separated in distance and annual mean temperature (Fig. 1, MDD 2002). All leafminers were collected at a similar low altitude (< 65 m above sea level) except the northernmost pop-



**Fig. 1** A map and a list of the collections sites of *L. sativae*. The name of the sites was abbreviated as two capital letters followed by the latitude where the collection site located: CF<sub>42</sub>=ChiFeng, BJ<sub>40</sub>=BeiJing, TA<sub>36</sub>=TaiAn, HZ<sub>30</sub>=HangZhou, GZ<sub>23</sub>=GuangZhou, SY<sub>18</sub>=SanYa. The dotted curve line around 34°N is the northern overwintering limit of *L. sativae*, beyond which the fly can't overwinter outdoors. The line was simulated based on the -2°C isotherm of monthly minimum mean temperature in January (Zhao and Kang 2000; Chen and Kang 2005, in press data). The data were recorded during 1970–2000 (MDD 2002)

ulation in Chifeng (CF<sub>42</sub>) which was at an elevation of 571 m.

In all collection sites the temperatures are lowest in January and highest in July. The annual mean temperature decreases with latitude, as does the mean temperature in January (MDD 2002).

#### Insect collecting and rearing

The fields where samples were collected were in major agricultural areas with high crop diversity throughout the year. Samples from all sites were collected from untreated bean, *Phaseolus vulgaris* L., which is a common host plants for *L. sativae* throughout the world (Spencer 1973). Moderately infested bean leaves with third instar larvae (near pupation) were collected so that the larvae were fully developed and emerged as pupae within 1–3 days after being taken to laboratory. Because *L. sativae* move from the greenhouses to outdoor crops

in the late spring or early summer, all samples from vegetable fields north of the outdoor overwintering limit at this time are the offspring of the adults that overwintered in the greenhouse (Zhao and Kang 2000).

All samples were taken to laboratory within 12 h. Infested bean leaves were kept in plastic containers (30×20 cm) in laboratory at 25°C and 65% RH. Each day the containers were checked for pupae; all leaves were discarded after 3 days to eliminate concerns that deteriorating nutritional quality of leaves might influence larval development. The pupae from leaves were then held in an environmental chamber (Conviron Co., Winnipeg, Manitoba, Canada) at 25±1°C with a photoperiod of 14L:8D, 6,000 lux and 70% RH. One-day-old pupae from each sample were subject to the following various thermal measurements because this life stage was developmentally mature but most sensitive to that particular thermal stress (Parrella 1987; Zhao and Kang 2000). Twenty individuals were used in each of five replicates for each of the following treatments.

#### Supercooling point (SCP)

Each pupa of *L. sativae* was affixed with plastic tape to the tip of a thermocouple that was linked to an automatic recorder (uR100, Model 4152, Yologama Elect. Co, Seoul, Korea). The sensor with the pupa was put inside an insulating styrofoam box in a freezer chest whose temperature was kept constant at -26°C to insure that cold exposure temperature was lowered at an averaged rate of 1°C per min. The temperature at which an abrupt rebound occurred was taken to indicate the crystallization temperature i.e. supercooling point (SCP). Details of the methods were described in Zhao and Kang (2000) and Chen and Kang (2002b).

#### Survival under low temperature exposure

Insects may respond differently to chronic and acute cold stress in nature (MacDonald et al. 2000). In order to determine the survival response to various cold stress encountered in natural environment by *L. sativae*, three exposure regimes were designed: (1) acute cold exposure at an extreme subzero temperature for short periods; (2) chronic cold exposure to 0°C for various lengths of time and; (3) exposure to selected low temperatures for 4 days.

In the acute exposure assay, the low temperature was -10°C, just above the crystallization temperature of laboratory-reared pupae (Zhao and Kang 2000). The pupae were confined in 5 ml round-bottomed tubes and then transferred to -10°C in a ethylene glycol/water bath in a programmable temperature controller (±0.01°C, Polyscience, USA), at which they were held for four short time periods: 0.5, 1, 2 and 4 h. To avoid cold shock mortality, the samples were transferred to the required exposure temperature at 5°C intervals, with

5 min at each intervening temperature. The procedure was repeated in reverse when samples were removed from low temperatures.

In the first chronic assay, *L. sativae* pupae were exposed to zero temperatures for 1, 2, 4, and 8 days. In the four-day trial, pupae were exposed to  $-5.0^{\circ}\text{C}$ ,  $0^{\circ}\text{C}$ ,  $5.0^{\circ}\text{C}$  and  $10.0^{\circ}\text{C}$  for four full days. For these chronic assays, pupae were held in a 5 ml tube enclosed with parafilm (with pinholes) at the open end in an incubator.

After all cold exposure treatments, the samples were in an environmental chamber at  $25 \pm 0.5^{\circ}\text{C}$ . The number of adults emerging and the number of dead pupae were determined after 20 days. Control pupae were maintained in the environmental chamber at  $25 \pm 0.5^{\circ}\text{C}$  at the same time.

## Acclimation

Geographic populations of *L. sativae* pupae were acclimated at  $10^{\circ}\text{C}$  for 1 day or  $39^{\circ}\text{C}$  for 1 h. The acclimated pupae then were exposed to  $-5^{\circ}\text{C}$  for 2 days as a discriminating regime. Control groups were exposed to  $-5^{\circ}\text{C}$  for 2 days without acclimation. All other handling was similar to the previously described methodology.

## Data analysis

Distribution of supercooling points is often bimodal (Spicer and Gaston 1999). Cannon and Block (1988) discussed the separation of bimodal SCP distributions into high (freeze at higher subzero temperatures) and low (freeze at lower subzero temperatures) groups. However, the breakpoints in bimodal distributions are

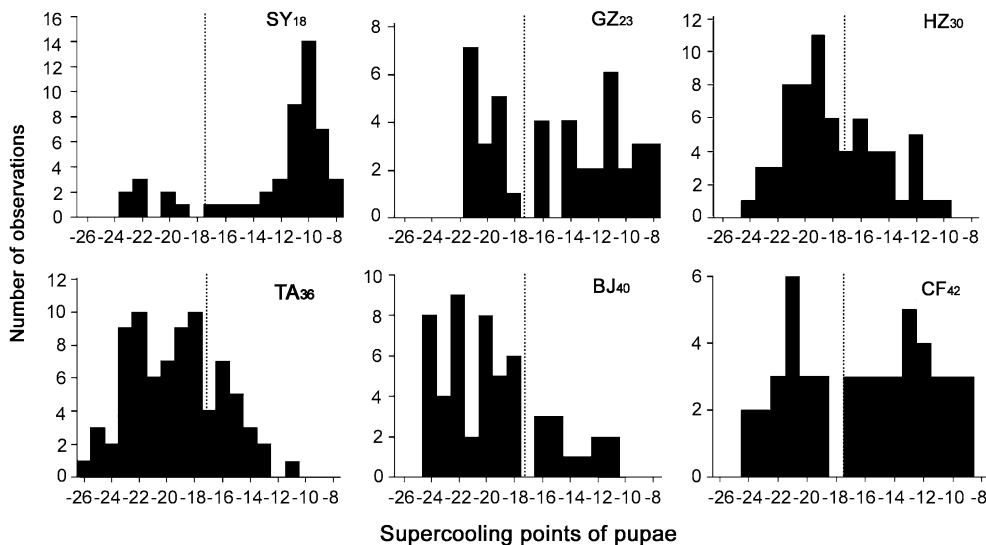
often determined arbitrarily, depending on the supercooling characteristics of the species studied and visual assessment of a histogram for an obvious break (Block and Sømme 1982; Worland and Convey 2001; Sinclair et al. 2003). According to Block's (1982) method, these bimodal distributions then can be expressed mathematically as the ratio between the high group (HG) and the low group (LG), e.g.  $R = \text{LG}/(\text{LG} + \text{HG})$ . Differences among mean SCPs were subsequently compared using Tukey's-b one-way ANOVA.

The survival rate was corrected by survival of control pupae (Chen and Kang 2002b). GLM ANOVA was used, where appropriate, to test for effects of sampling populations, temperature, exposure time and main interactions on pupal survival. Where significant differences were observed, the effect of treatments was determined by LSD at  $P < 0.05$  level.

## Results

### Supercooling capacity

The SCP values generally showed a bimodal distribution in the geographic populations tested (Fig. 2). At most locations, the distribution was clearly divided into high-group individuals and low-group individuals based on the observed breaking point. However, the two groups within the middle latitudinal populations in HZ<sub>30</sub> and TA<sub>36</sub> were relatively difficult to distinguish. The threshold distinguishing the high and low groups was at a temperature of  $-17.5^{\circ}\text{C}$ , which is the same as the mean SCP of the HZ<sub>30</sub> population (Table 1). The mean SCPs of low-group pupae in each population was approximately  $-20^{\circ}\text{C}$ , and did not differ significantly between



**Fig. 2** Frequency distributions for the supercooling points of *L. sativae* pupae from the six geographical populations collected in 2004. The values of supercooling points showed a bimodal distribution which was divided by a break at  $-17.5^{\circ}\text{C}$  as the

threshold distinguishing high-group and low-group individuals. Note the changes in the ratios between the high group and the low group along the latitudinal gradients. See Fig. 1 for the abbreviations of sample sites



**Table 1** Summary of supercooling points distributions (SCP) and mean for *L. sativae* pupae from the six geographical populations

Locality	SCP $\pm$ SE ( $^{\circ}$ C)			R
	Low group	High group	Total group	
SY <sub>18</sub>	-21.0 $\pm$ 0.5 a	-10.3 $\pm$ 0.3 a	-12.1 $\pm$ 0.6 a	0.16
GZ <sub>23</sub>	-19.5 $\pm$ 0.2 a	-11.2 $\pm$ 0.5 ab	-14.4 $\pm$ 0.7 b	0.38
HZ <sub>30</sub>	-19.8 $\pm$ 0.3 a	-14.0 $\pm$ 0.4 cd	-17.4 $\pm$ 0.4 cd	0.59
TA <sub>36</sub>	-20.9 $\pm$ 0.4 a	-14.9 $\pm$ 0.4 d	-18.7 $\pm$ 0.4 c	0.62
BJ <sub>40</sub>	-20.8 $\pm$ 0.3 a	-14.0 $\pm$ 0.6 cd	-19.1 $\pm$ 0.5 c	0.74
CF <sub>42</sub>	-20.8 $\pm$ 0.4 a	-12.7 $\pm$ 0.4 bc	-15.8 $\pm$ 0.6 bd	0.39

In the bimodal distribution in the SCP values (Fig. 2), the ratio of frequencies between the low group (LG) and the high group (HG) is measured as  $R = LG/(HG + LG)$  (Block 1982). Values of SCPs with different letters within a row are significantly different at 5% level by Tukey's b Oneway ANOVA. See Fig. 1 for the abbreviations of sample sites

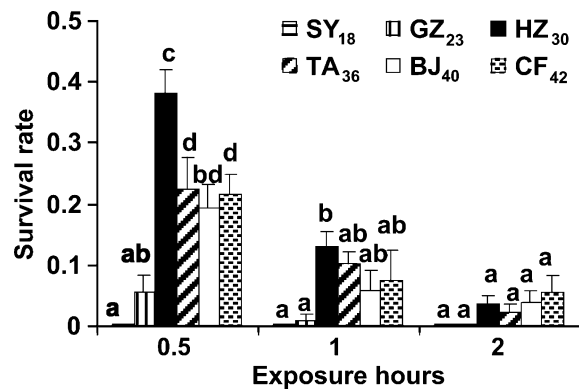
sites ( $F_{5,154} = 3.0$ ,  $P = 0.05$ ). However, the mean SCPs of high-group pupae differed significantly among the six geographic populations ( $F_{5,157} = 17.5$ ,  $P < 0.001$ ). For the southern populations, the overall size of the group and the highest peak numbers of the high-group individuals increased with increasing latitude up to the TA<sub>36</sub> population (Fig. 2). No such trend was observed in the northern populations and no significant significances were found among the populations in HZ<sub>30</sub>, TA<sub>36</sub> and BJ<sub>40</sub>. A similar pattern of latitudinal variation in SCPs was observed in the total sample (Table 1, Fig. 2). The mean SCP of all pupae in each sample was significantly different from each other ( $F_{5,312} = 23.3$ ,  $P < 0.001$ ), and gradually decreased with latitude until a sharp decrease of SCP at the northern most population.

The ratio between the low group (LG) and the high group (HG) was measured as  $R = LG/(HG + LG)$  in the bimodal distributions. The  $R$  value also increased with the latitude and peaked at BJ<sub>40</sub>, showing the same pattern as that of the variation in total-group mean SCPs (Table 1). The correlation between the SCPs of total group and  $R$  in the six geographic populations was significant ( $R^2 = 0.9$ ,  $P = 0.001$ ).

#### Low temperature survival

While most of the populations had some individuals that could survive  $-10^{\circ}$ C for up to 2 h, very few individuals from SY<sub>18</sub> could survive  $-10^{\circ}$ C for any length of time tested (Fig. 3). The survival rates differed significantly among the pupae from the six geographic populations ( $F_{5,78} = 18.2$ ,  $P < 0.001$ ). The effect of the interaction between populations and exposure hours was also significant ( $F_{10,78} = 5.5$ ,  $P < 0.001$ ). For the three southern populations, survival rate of pupae significantly increased with latitude ( $P < 0.001$ ). By contrast, the three northern populations beyond the overwintering limit had a decreased survival rate, with no significant differences in mean survival ( $P > 0.05$ ).

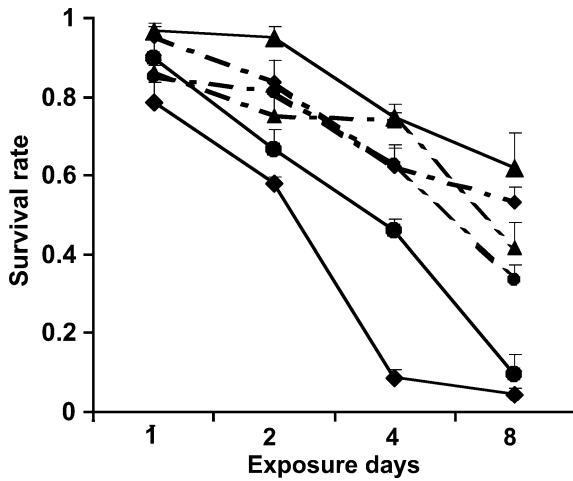
In chronic assays, the survival rate of pupae decreased significantly as exposure time at  $0^{\circ}$ C extended from 1 day to 8 days ( $F_{3,86} = 139.2$ ,  $P < 0.001$ ) (Fig. 4). The survival rate of pupae among the six populations



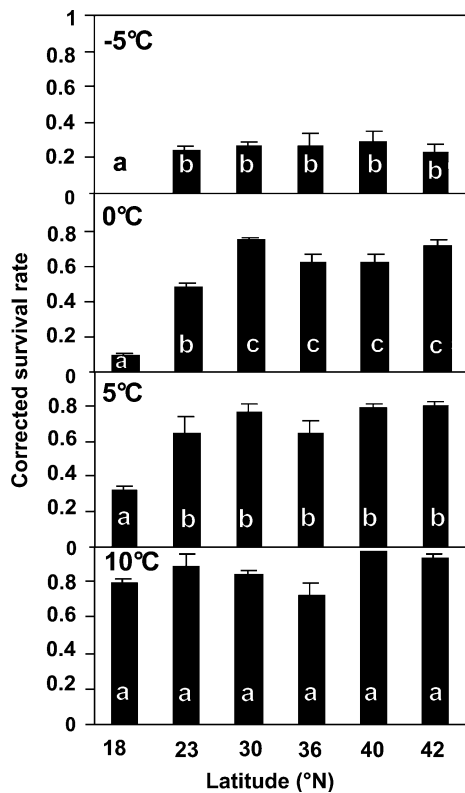
**Fig. 3** Corrected survival rate of *L. sativae* pupae after exposure to  $-10^{\circ}$ C for different time in the six geographical populations collected. Values are the mean  $\pm$  SE for three to five replicates. Values with different letters within the same exposure hour are significantly different ( $P < 0.05$ ). See Fig. 1 for the abbreviations of the populations

was significantly different ( $F_{5,86} = 35.4$ ,  $P < 0.001$ ). The effect of interaction between exposure time and geographic populations tested was also significant ( $F_{15,86} = 3.7$ ,  $P < 0.001$ ). For the southern populations, the survival increased with latitude and peaked at HZ<sub>30</sub>. However, the survival ability of the three northern populations was intermediate between that of GZ<sub>23</sub> and HZ<sub>30</sub> populations.

Figure 5 shows the effects of different low temperatures on the survival of *L. sativae*. No pupae in SY<sub>18</sub> could survive  $-5^{\circ}$ C for 4 days, and pupae in the other five populations had a low survival rate ranging from 23% to 29%, and mean survival was not significant between populations ( $P > 0.05$ ). The survival rate among the six populations differed significantly when their pupae were exposed to  $0^{\circ}$ C ( $F_{5,29} = 60$ ,  $P < 0.001$ ) or  $5^{\circ}$ C ( $F_{5,23} = 13.9$ ,  $P < 0.001$ ). As seen previously, cold resistance of the three southern populations increased with latitude. However, the survival rate of the HZ<sub>30</sub> population did not differ significantly from the northern most three populations ( $P > 0.05$ ). The difference in survival rate also was significant among the six populations exposed at  $10^{\circ}$ C ( $F_{5,21} = 3.9$ ,  $P = 0.02$ ) but only a small percentage of pupae died.



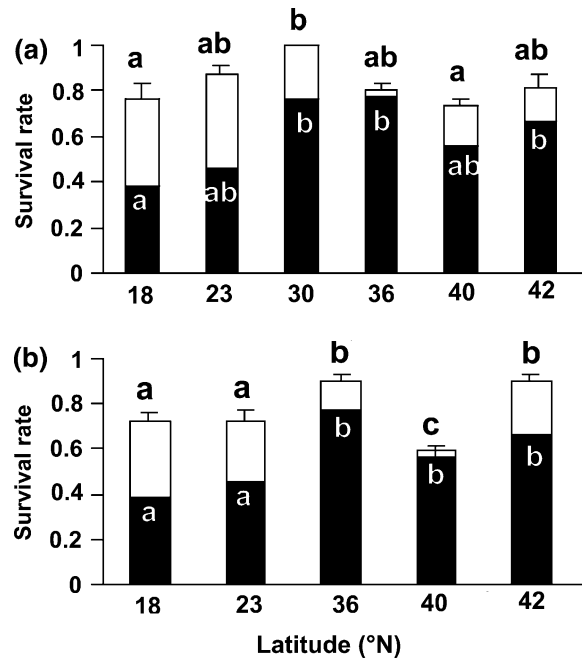
**Fig. 4** Corrected survival rate of *L. sativae* pupae after exposure to 0°C for different time in the six geographical populations. Solid lines represent the three southern populations: SY<sub>18</sub>(diamond), GZ<sub>23</sub>(circle), HZ<sub>30</sub>(triangle). Dashed lines represent the three northern populations: TA<sub>36</sub>(diamond), BJ<sub>40</sub>(circle), CF<sub>42</sub>(triangle). Values are the mean  $\pm$  SE for three to five replicates. See Fig. 1 for the abbreviations of the populations



**Fig. 5** Corrected survival rate of *L. sativae* pupae exposed to different low temperatures (-5°C, 0°C, 5°C and 10°C) for 4 days. Different letters indicate significant differences among geographic populations ( $P < 0.05$ ). Error bars indicate standard errors

#### Acclimation

The pupae differed significantly in their survival rates among the six populations when exposed to -5°C for



**Fig. 6** Survival improvement of *L. sativae* pupae heat-shocked at 39°C for 1 h (a), or acclimated for 1 day at 10°C (b) with exposure to -5°C for 2 days as a discriminating regimen. The filled column indicates survival after exposure to -5°C for 2 days and the open column indicates increased survival after acclimation or heat-shock. Means with the same letter above are not significantly different ( $P < 0.05$ ). Error bars indicate standard errors of the total survival rate

2 days as a discriminating regime ( $F_{5,19} = 6.6$ ,  $P = 0.003$ ). Heat shock at 39°C for 1 h followed by a 1 h recovery at 25°C greatly enhanced the survival ability of the pupae (Fig. 6a). The differences between survival rates of the pupae of the six populations were also significant ( $F_{5,21} = 3.5$ ,  $P = 0.03$ ). The pupae of the three southern populations showed greater increased survival rates as compared to the three northern populations.

Pre-chilling of the pupae at 10°C for 1 day greatly enhanced the survival ability of populations with the exception of the BJ<sub>40</sub> population (Fig. 6b). The inducible survival rates of the pupae also differed significantly among the five geographic populations ( $F_{4,17} = 17.6$ ,  $P < 0.001$ ). Judging from the survival response, pupal *L. sativae* were more sensitive to heat shock than to pre-chilling acclimation in the three southern populations. However, the pattern of variation in cold survival along latitude in these populations remained unchanged in both acclimation regimes.

#### Discussion

The supercooling point (SCP) is a valid measurement of the lower lethal temperature for many insect species, and is correlated with the level of cold tolerance (Worland and Convey 2001; Sinclair et al. 2003; Klok et al. 2003). For *L. sativae* pupae, mean SCPs were maximally de-

pressed by 7°C from the southernmost population to the northern population. This represents an unusual shift for an insect population (Block 1982; Worland and Convey 2001; Klok et al. 2003). The bimodal distribution of SCPs showed that the ratio of frequencies between the low-group and the total-group within a population ( $R$ ) were closely correlated with the total-group SCP. However, the low-group SCP did not vary with latitude. This result suggests that the high-group SCPs rather than the low-group SCPs directly contribute to the level of cold resistance at the population level. In nature, the individuals with higher SCPs are more likely to be under climatic stress selection. For instance, only a relatively small percentage of overwintering pupae can survive the cold temperatures that occur as populations of *L. sativae* approach the overwintering limit (Zhao and Kang 2000; Chen and Kang 2002b), indicating that the most cold-susceptible individuals are routinely selected out of the population.

It is interesting that the breakpoint of the bimodal distribution stayed unchanged at about  $-17.5^{\circ}\text{C}$  even though the  $R$  value varied greatly among the geographic populations. We speculate that the high group of SCPs has acted as a “buffering sink” where an equilibrium point in mean SCP of a population may be reached so as to balance the energetic costs and benefits involved to maintain a low supercooling capacity (Voituron et al. 2002). Thus, our results suggested that stressful conditions could genetically serve to select the cold adaptive traits for the alleles that increase the frequency of encoding genes, whereas a phenotypic variant would be expressing at a lower threshold of gene frequency.

Many studies have reported that populations at high latitude have higher cold resistances than at low latitude, and temperate species are more cold tolerant than subtropical or tropical species (Hoffmann et al. 2003; Jing and Kang 2003; Kimura 2004). For the southern populations of *L. sativae*, the mean SCP of pupae was substantially depressed with decreasing latitude. Meanwhile, their low temperature survival ability increased significantly with the latitude. Thus, latitudinal variation in SCPs of pupae matched the variation patterns for cold resistance in the southern populations. Although the chronic and acute assays of cold resistance may have different physiological bases (MacDonald et al. 2000), the pattern of variation in cold resistance for the southern populations was consistent in both assays, and this pattern for cold resistance appears to be physiologically stable and independent of the form of exposure. In contrast, the northern populations beyond the overwintering range limit had decreased ability to survive cold temperatures. Therefore a discrepancy exists between the latitudinal variation in values of SCPs and low temperature survival rate. Thus, our results clearly indicate that the pattern of geographic variation in cold resistance of *L. sativae* diverges between latitudes above and below the natural overwintering thermal limit.

A similar variation in the latitudinal populations of the pea leafminer *L. huidobrensis* in China was previ-

ously observed (Chen and Kang 2004). The pea leafminer is a more cold-hardy vegetable and flower pest, and therefore has a more northern overwintering limit than *L. sativae*. Jenkins and Hoffmann (1999) previously noted that differences in cold resistance among populations of *Drosophila serrata* after winter was absent prior to winter, and suggested that such population differences may not always be suitable for making adaptive comparisons. However, consistent latitudinal patterns for *L. huidobrensis* were observed over multiple seasons (in spring/postwinter, summer and autumn/pre-winter) in three consecutive years of investigations (2000–2002). In contrast, the relative cold resistance of the northern populations of *L. huidobrensis* (beyond the leafminer’s range limit) fluctuated with seasons. Results from studies of the two leafminer species suggest that the patterns of variation in cold tolerance are seasonally independent for populations within the overwintering limit. However, the pattern of cold resistance is seasonally dependent for populations living at latitudes above the limit.

Furthermore, although acclimation of pupal *L. sativae* could greatly enhance its cold resistance, the pattern of geographic variation in cold resistance kept after by the heat or cold acclimation regimes tested, particularly in southern populations.

A similar phenomenon was observed in *Drosophila suzukii* and *D. auraria* which overwinter in domestic areas in cool-temperate regions in Japan (Kimura 2004). Thus, we believe that the observed pattern shift in stress tolerance associated with a species’ overwintering limit is a general adaptive strategy for freeze-intolerant species that have a high-latitude boundary of distribution but must overwinter and develop in protected greenhouses at latitudes where the thermal overwintering limit is exceeded.

The overwintering limit is ecologically important in understanding the variation in adaptive traits when making comparisons across the distributional range of observed species. In greenhouse agriculture, the key combination of a favorable climate and availability of hosts is usually guaranteed, at least for most of the year (van Lenteren and Woets 1988). Therefore, no minimal cold stress exerted on these leafminers as a natural selection force for elevated cold resistance in greenhouse as in open field. Meanwhile, because leafminers have a high rate of fecundity and overlapping generations, they have the potential to quickly colonize the surrounding plants, which result in very high-density infestations as well as fairly rapid increase in frequency of alleles for cold susceptible genes (Fuller et al. 1999; Hoffmann and Hercus 2000). The result was also reflected by the increased high-group frequency of SCPs in more northern populations. Furthermore, trade-offs between cold resistance and other traits selected under a warm environment may also determine the low level of cold resistance (Jenkins and Hoffmann 1999; Case and Taper 2000; Hoffmann et al. 2003). Consequently, the mild microhabitats provided by the greenhouses may limit and even eliminate the development of cold resistance of

populations as the leafminer expands its population northerly beyond the overwintering limit.

On the other hand, it was observed that the northern populations are always intermediate cold hardy between the southern most population and marginal population adjacent to the overwintering border, and keep at a relatively high level of cold resistance over seasons by comparison with the southern populations (Chen and Kang 2004; Kimura 2004). At least two mechanisms may account for this result. First, our result showed that pupal *L. sativae* could greatly enhance its cold resistance in response to heat shock or to the cold acclimation. Second, the enclosed structure of the greenhouses limits the migration events, especially in cold seasons, which may limit gene flow, and in turn, keep the effect of selection on cold resistance (Fuller et al. 1999; Hoffmann and Hercus 2000). However, the migration event can't be completely avoided, particularly in mild seasons when greenhouse populations are renewed by populations from within the overwintering limit. When the cold resistant individuals migrate into a population that had most cold susceptible individuals, the influx of cold resistant alleles will dilute the frequency of cold susceptible genes (Hoffmann and Hercus 2000).

Accordingly, there exists an interaction between the populations with cold susceptible genes and resistant genes over a broad range of temporal and spatial scales and the widespread greenhouses probably play a key role in this interaction. However, further studies are needed to detect how these ecological and genetic factors have interacted to balance the dynamic development of cold tolerance of northern populations associated with the greenhouses.

**Acknowledgements** We are grateful to Prof. John Trumble for his critical comments and improving the manuscript. We would like to thank Dr. X.H. Jing and S. Wang for helping collect samples in the fields and culturing of *L. sativae* as well as Dr. S.G. Hao for assistance with the statistical analysis. This work was supported by the National Natural Science Foundation (No. 30470291) and the Innovation Program of the Chinese Academy of Sciences (No. KSCX1-SW-13). The experiments comply with the current laws of China where they were performed.

## References

- Akinci S, Akinci IE, Karatas A, Turkmen O (1998) Temperature changes under different protective structures at the late autumn and early spring periods in Van. *Acta Horticult* 491:87–91
- Block W (1982) Supercooling points of insects and mites on the Antarctic Peninsula. *Ecol Entomol* 7:1–8
- Block W, Sømme L (1982) Cold hardiness of terrestrial mites at Signy Island, Maritime Antarctic. *Oikos* 38:157–167
- Cannon RJC, Block W (1988) Cold tolerance of microarthropods. *Biol Rev* 63:23–77
- Case TJ, Taper ML (2000) Interspecific competition, environmental gradients, gene flow, and the coevolution of species' borders. *Am Nat* 155:583–605
- Castilla N (2002) Current situation and future prospects of protected crops in the Mediterranean region. In: Sansavini S, Janick J (eds) Proceedings of the international symposium on mediterranean horticulture: issues and prospects. *Acta Horticult* 582:135–147
- Chen B, Kang L (2002a) Trends of occurrence and geographic variation of pea leafminer *Liriomyza huidobrensis* in China. *Plant Quarantine* 16:138–140
- Chen B, Kang L (2002b) Cold hardiness and supercooling capacity in the pea leafminer *Liriomyza huidobrensis*. *CryoLetters* 23:173–182
- Chen B, Kang L (2004) Variation in cold hardiness of *Liriomyza huidobrensis* (Diptera: Agromyzidae) along the latitudinal gradients. *Environ Entomol* 33:155–164
- Chen B, Kang L (2005) Implication of pupal cold tolerance for the northern overwintering range limit of the leafminer *Liriomyza sativae* (Diptera: Agromyzidae) in China. *Applied Entomol Zool* 40 (in press)
- Danks HV (1996) The wider integration of studies on insect cold-hardiness. *Eur J Entomol* 93:383–403
- Fuller SJ, Chavigny P, Lapchin L, Vanlerberghe-Masutti F (1999) Variation in clonal diversity in greenhouse infestations of the aphid, *Aphis gossypii* Glover in southern France. *Mol Ecol* 8:1867–1877
- Guo JM, Liu WM, Gao GL (2000) Population trends and integrated management of leafminer *Liriomyza sativae* in Inner Mongolia, China. *Plant Quarantine* 14:190–192
- Hatherly IS, Bale JS, Walters KFA, WorlandMR (2004) Thermal biology of *Typhlodromips montdorensis*: implications for its introduction as a glasshouse biological control agent in the UK. *Entomol Exp Appl* 111:97–109
- Hodkinson ID (1999) Species response to global environmental change or why ecophysiological models are important: a reply to Davis et al. *J Anim Ecol* 68:1259–1262
- Hoffmann AA, Hallas R, Sinclair C, Mitrovski P (2001) Levels of variation in stress resistance in *Drosophila* among strains, local populations, and geographic regions: patterns for desiccation, starvation, cold resistance, and associated traits. *Evolution* 55:1621–1630
- Hoffmann AA, Hercus MJ (2000) Environmental stress as an evolutionary force. *Bioscience* 50: 217–226
- Hoffmann AA, Sorensen JG, Loeschcke V (2003) Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approach. *J Theor Biol* 28: 175–216
- Hu MG (1995) Climate conditioning in protected cultivation in northern China. *China Vegetable* 3: 7–9
- Jenkins N L, Hoffmann A A (1999) Limits to the southern border of *Drosophila serrata*: cold resistance, heritable variation, and trade-offs. *Evolution* 53:1823–1834
- Jing XH, Kang L (2003) Geographical variation in egg cold hardiness: a study on the adaptation strategies of the migratory locust *Locusta migratoria* L. *Ecol Entomol* 28:151–158
- Jing H, Wang SX (2003) Occurrence and control strategies of leafminer *Liriomyza sativae* in Daqing, northern China. *Horticulture North China* 1:56–57
- Kimura MT (2004) Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. *Oecologia* 140:442–449
- Kirk WDJ, Terry LI (2003) The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). *Agr For Entomol* 5:301–310
- Klok CJ, Chown SL, Gaston KJ (2003) The geographic ranges structure of the holly leaf-miner. III. Cold hardiness physiology. *Func Ecol* 17:858–868
- MacDonald JR, Bale JS, Walters KFA (1997) Low temperature mortality and overwintering of the western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Bull Entomol Res* 87:497–505
- MacDonald JR, Head J, Bale JS, Walters KFA (2000) Cold tolerance, overwintering and establishment potential of *Thrips palmi*. *Physiol Entomol* 25:159–166
- Magiafoglou A, Carew ME, Hoffmann AA (2002) Shifting clinal patterns and microsatellite variation in *Drosophila serrata* populations: a comparison of populations near the southern border of the species range. *J Evol Biol* 15:763–774
- MDD (Meteorological Documentation Department of National Weather Bureau of China) (2002) Climatological atlas of the



- People's Republic of China. China Meteorological Press, Beijing
- Parrella MP (1987) Biology of *Liriomyza*. *Ann Rev Entomol* 32:201–204
- Raaphorst MGM (2003) Greenhouse of the future. *Acta Horticult* 611:57–59
- Reitz RS, Trumble JT (2002) Interspecific and intraspecific differences in two *Liriomyza* leafminer species in California. *Entomol Exp Appl* 102:101–113
- Sinclair BJ, Klok CJ, Scott MB, Terblanche JS, Chown SL. (2003) Diurnal variation in supercooling points of three species of Collembola from Cape Hallett, Antarctica. *J Insect Physiol* 49:1049–1061
- Spencer KA (1973) Agromyzidae (Diptera) of economic importance. Series Entomologica 9, the Hague
- Spicer JI, Gaston KJ (1999) Physiological diversity and its ecological implications. Blackwell, Oxford
- Van Lenteren JC, Woets J (1988) Biological control and integrated pest control in greenhouses. *Ann Rev Entomol* 33: 239–269
- Voituron Y, Mouquet N, de Mazancourt C, Clobert J (2002) To freeze or not to freeze? An evolutionary perspective on the cold-hardiness strategies of overwintering ectotherms. *Am Nat* 160: 255–270
- Warren MS, Hill JK, Thomas JA, Fox R, Huntley B (2001) Rapid responses of British butterflies to opposing forces of climate and habitat change. *Nature* 414:65–69
- Worland MR, Convey P (2001) Rapid cold hardening in Antarctic microarthropods. *Func Ecol* 15:515–524
- Zhao YX, Kang L (2000) Cold tolerance of the leafminer *Liriomyza sativae* (Dipt., Agromyzidae). *J Appl Entomol* 124:185–89