Journal of Pest Science (2022) 95:1233–1244
https://doi.org/10.1007/s10340-021-01461-5

ORIGINAL PAPER

Rapid evolution of *Ophraella communa* cold tolerance in new low-temperature environments

Zhenqi Tian[1] · Guangmei Chen[1] · Yan Zhang[1] · Chao Ma[1] · Zhenya Tian[1] · Xuyuan Gao[1,2] · Hongsong Chen[1,2] · Jianying Guo[1] · Zhongshi Zhou[1]

Received: 3 May 2021 / Revised: 22 October 2021 / Accepted: 17 November 2021 / Published online: 10 January 2022

Abstract

Low winter temperatures severely stress newly arriving insect species. Adaptive evolutionary changes in cold tolerance can facilitate their establishment in new environments. *Ambrosia artemisiifolia*, a noxious invasive plant, occurs throughout China. *Ophraella communa*, a biological control agent of *A. artemisiifolia*, mainly occurs in southern China. However, in 2012, it established populations in Beijing (39.98°N, 115.97°E) following introduction from Laibin (23.62°N, 109.37°E), implying cold adaptation. The mechanisms underlying its rapid evolution of cold tolerance remain unknown. We investigated the levels of cryoprotectants and energy reserves in adult *O. communa* from two latitudes. In high-latitude insects, we found high trehalose, proline, glycerol, total sugar, and lipid levels; five potential genes (*Tret1a*, *Tret1b*, *Tret1-2*, *P5CS*, and *GST*), responsible for regulating cold tolerance and involved in trehalose transport, proline biosynthesis, and glutathione S-transferase activation, were highly expressed. These hybridisation changes could facilitate cold temperature adaptation. We demonstrate the genetic basis underlying rapid adaptation of cold tolerance in *O. communa*, explaining its extension to higher latitudes. Thus, specialist herbivores can follow host plants by adapting to new temperature environments via rapid genetic evolution.

Keywords Cold tolerance · Cryoprotectant · Energy reserve · Hybridisation · Rapid evolution

Key message

- Our results estimate the physiological and molecular mechanism underlying the rapid adaptive evolution of cold tolerance in *Ophraella communa*.
- Hybridisation could enhance the evolutionary adaptation of cold tolerance.
- *Ophraella communa* can be released at higher latitudes to manage *Ambrosia artemisiifolia*.

Communicated by Jay Rosenheim.

✉ Zhongshi Zhou
zhouzhongshi@caas.cn

[1] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

[2] Guangxi Key Laboratory for Biology of Crop Diseases and Insect Pests, Institute of Plant Protection, Guangxi Academy of Agricultural Sciences, Nanning 530007, China

Introduction

When non-native insect species are introduced to new environments accidentally or intentionally, they often encounter a series of novel selection pressures. To adapt to these novel environmental conditions, their traits can shift through rapid evolution (Hoffmann and Sgrò 2011). As a severe environmental stress for new arrivals, low winter temperatures are a key limiting factor in the geographic distribution and abundance of organisms (Leather et al. 1993). For insects from temperate regions, cold winter climates often cause particularly heavy mortality. Insects, as ectotherms, show shifts in biological and physiological traits with changes in temperature conditions. They have evolved specific physiological strategies to respond to cold stresses, such as the accumulation of low molecular weight cryoprotectants or energy reserves (Teets and Denlinger 2013; Toxopeus and Sinclair 2018). The rapid evolution of cold tolerance can facilitate the rapid adaptation of the insects to low temperatures, allowing their persistence in the novel environment.

Adaptive evolution, or adaptation by natural selection, can enhance the fitness of populations in novel environments. Thus, adaptation can increase the probability of persistence as well as the size of populations (Yin et al. 2019). This is particularly important for introduced biological control agents because it can improve successful control of their target pests and weeds. Rapid evolution in response to the selective pressures imposed by novel environments has received considerable attention (Garnas 2018; Hoffmann 2017; Hoffmann and Sgrò 2011; Thompson 1998), but although its genetic basis is gradually being elucidated, it remains unclear. Recently, several studies have attempted to detect the genetic basis of the rapid evolution of adaptation to local environmental conditions, particularly thermal tolerance, via genome or transcriptome sequencing (Dudaniec et al. 2018; Janes et al. 2014; Krehenwinkel et al. 2015; Lancaster et al. 2016). However, whether these candidate genes improved adaptation to the novel environment and whether these changes are heritable still need to be verified. Therefore, further empirical information is needed to evaluate the genetic basis of this rapid evolution in other insects.

Common ragweed, *Ambrosia artemisiifolia* L., is a noxious invasive plant that is harmful to environmental and human health (Smith et al. 2013; Mazza et al. 2014). Its invasion causes significant yield losses in agricultural systems and reduces field biodiversity owing to its strong competitive ability. *A. artemisiifolia* can release large amounts of pollen, a key cause of allergic rhinitis and asthma. This plant originated in North America and has now spread worldwide. In China, it invaded from the south and spread northward and has colonised Beijing for decades (Zhou et al. 2015). Its geographical distribution is continuously expanding owing to climate warming and increasing CO_2 levels (Rogers et al. 2006; Chapman et al. 2016). It is necessary to develop an effective management system to control this species.

Ophraella communa LeSage (Coleoptera: Chrysomelidae) is a specific biological control agent of *A. artemisiifolia*; it has been widely applied in China and Europe (Guo et al. 2011; Schaffner et al. 2020; Zhou et al. 2015). As a specialist herbivore, *O. communa* prefers to feed on *A. artemisiifolia* and cannot complete its life cycle on other plants, although some feeding behaviours have been observed on other similar Compositae species in field experiments (Zhou et al. 2011a; Augustinus et al. 2020). This species originated in North America and was first recorded in mainland China in 2001 (Meng and Li 2005). Subsequently, it spreads across China south of the Yangtze River (Zhou et al. 2015). It can complete five generations per year in southern China (Meng et al. 2007). To manage *A. artemisiifolia* in Beijing (39.98° N, 115.97° E), *O. communa* was artificially introduced from Laibin (23.62° N, 109.37° E) in 2012. Now, it can successfully survive the cold winter and has established a field population in this area (Tian et al. 2020a). This demonstrates that this insect species has adapted to the local low temperatures. A previous study reported that *O. communa* exhibits geographical variation in cold tolerance across latitudinal gradients (Zhou et al. 2011b). A subsequent quantitative genetic study indicated that the cold tolerance of this species is inheritable (Zhao et al. 2018), which indirectly suggests that evolutionary changes improved its capacity to cope with cold stress (Garnas 2018; Hoffmann 2017; Hoffmann and Sgrò 2011). Therefore, we further verified the rapid evolution of cold tolerance in *O. communa* at the physiological and molecular levels.

Here, we compared cold tolerance traits at the physiological level between geographically separate populations from two different latitudes, and identified five potential key genes that exhibit differential expressions according to their geographic variation in cold tolerance. To test our hypothesis that insect cold hardiness can be promoted by hybridisation, and to further speculate the mode of inheritance of cold hardiness, we created hybrids between the two populations from different latitudes. We further confirmed that the physiological traits improved and gene expression increased in hybrid offspring, suggesting the promotion of cold tolerance. These evolutionary changes involved the accumulation of low molecular weight cryoprotectants and energy reserves at the physiological level and trehalose transport, proline biosynthesis, and glutathione S-transferase (GST) activation at the molecular level, all of which may contribute to the enhancement of cold tolerance and establishment in new low-temperature environments. Owing to the rapid evolution of cold tolerance in *O. communa*, we can release them for biological control in the northern regions of the distribution of *A. artemisiifolia*. Moreover, our study also reveals that specialist herbivores can adapt to new environments where their host plants exist via rapid genetic evolution.

Materials and methods

Host plants and insects

Ambrosia artemisiifolia seeds were collected from fields at the Langfang Experimental Station of the Chinese Academy of Agricultural Sciences, Langfang, Hebei Province, China (39°N, 116°E) during October 2018 and stored at 4 °C. Two *A. artemisiifolia* plants were grown in each plastic pot (10 × 10 × 8 cm) without fertiliser.

Ophraella communa adults were collected from *A. artemisiifolia* plants in fields in Laibin City, Guangxi Zhuang Autonomous Region, China (23.62° N, 109.37° E) in late June 2019 and in Mentougou district, Beijing (39.98° N, 115.97° E) in mid July 2019. They were reared in cages (40 × 60 cm) in a laboratory at the Langfang Experimental Station at 26 ± 1 °C and 70 ± 5% RH, with a photoperiod of

14L:10D. Both populations were reared for one generation in the laboratory, and their progeny (F_0) used for subsequent experiments.

Hybridisation treatment

To test our hypothesis that insect cold hardiness can be promoted by hybridisation and to further explore its mode of inheritance, we created hybrids between the populations from the two latitudes as follows: 40 adults (20 males and 20 females) were selected from both the Beijing (BJ) and Laibin (LB) populations and separated into two hybridisation groups: BJ♀×LB♂ and LB♀×BJ♂. Their progeny (F_1) were collected and used for the measurement of physiological parameters and gene expression.

Measurement of total sugar, trehalose, glycerol, lipid, and proline contents

The total sugar, glycerol, and lipid content were measured according to Zhou et al. (2011b) and Yue et al. (2014).

Trehalose content measurement

Individuals were weighed and homogenised with 1000 µL 10% trichloroacetic acid. The homogenate was centrifuged at 5000 rpm for 10 min at 4 °C. The supernatant was transferred to a 1.5-mL Eppendorf tube, while the precipitate was mixed with 500 µL 10% trichloroacetic acid and centrifuged under the same conditions. The supernate was transferred and mixed with the previous one. Then, 500 µL of the mixture was transferred to a new 1.5-mL Eppendorf tube and 500 µL ethyl alcohol was added. This mixture was refrigerated at 4 °C for 16 h and then centrifuged at 10,000 rpm for 20 min at 4 °C and the supernate collected and transferred to a 10-mL centrifuge tube. Then, 1000 µL 0.15 mol/L H_2SO_4 solution was added to the supernatant, and the sample was heated and hydrolysed in a boiling water bath for 10 min. After cooling, 1000 µL 30% KOH solution was added and heated again for 10 min. Then, 4 mL 0.20% anthrone-sulfuric acid reagent was added to the solution and mixed, and the tube was heated for 10 min in the boiling water bath, cooled with running water, and equilibrated for 20 min. The zero point was set using a blank tube and the absorption at 520 nm was measured and recorded.

Proline content measurement

Individuals were weighed and homogenised with 500 mL sulfosalicylic acid in an ice bath, heated for 10 min at 100 °C, and centrifuged at 10,000×*g* for 10 min at 25 °C. After cooling, 0.25 mL supernatant, glacial acetic acid, and ninhydrin were individually added to a 2-mL Eppendorf tube. The mixture was incubated for 30 min at 100 °C with shaking every 10 min. After cooling, 0.5 mL toluene was added and the mixture was shaken for 30 min for proline extraction. We selected 0.2 mL of the upper solution to detect the absorption using a quartz micro cuvette at 520 nm.

Three-day-old virgin adults were used in all these measurements, which were conducted using three replicates of eight beetles each.

RNA extraction, cDNA synthesis, and gene cloning

Five differently expressed sequences were obtained from previous transcriptome data. Total RNA was extracted from three-day-old adult females using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Complementary DNA (cDNA) synthesis and gene cloning were performed as previously described (Ma et al. 2020a; Tian et al. 2020b). The specific primers used to amplify the open reading frames of the target genes were designed using Primer Premier 5 (PREMIER Biosoft International, Palo Alto, CA, USA) and are shown in Table 1. The amplification products were then purified using the Monarch Gel Extraction kit (NEB, Ipswitch, MA, USA), cloned into a Trans1-T1 clone vector (TransGen Biotech, Beijing, China), and sequenced (Ma et al. 2020a; Tian et al. 2020b).

Quantitative real-time PCR analysis

To determine the expression of cold tolerance genes in the BJ and LB populations, total RNA was extracted from three-day-old adult females, as described above. Primers for qPCR, shown in Table 1, were designed using Beacon Designer 8.0 (Premier Biosoft International, Palo Alto, CA, USA). Prior to this work, we meticulously screened many reference genes. We found that *Ribosomal protein L4* (*RPL4*) is steadily expressed in various development stages, tissues, and sexes of *O. communa* (Zhang et al. 2020). Moreover, *RPL4* was previously used as a control gene to study the expression of potential genes in *O. communa* responses to high CO_2 and heat waves (Gao et al. 2020), and to cold treatment (Tian et al. 2020b), indicating that the expression of *RPL4* is stable under different environmental conditions. Therefore, *RPL4* was used as a reference gene in this study. The qPCR was performed with SYBR Green Master Mix (Roche, Indianapolis, IN, USA) and the ABI 7500 Real-Time PCR System, according to the manufacturer's instructions. The PCR conditions were as follows: 95 °C for 5 min, 40 cycles of 95 °C for 10 s, and finally, 60 °C for 30 s, followed by a melting curve analysis. The melting curves were checked to test the purity of the qPCR reaction. Before gene expression analysis, the efficiency of the primers was also verified. Each sample was repeated in technical and biological triplicates. The data were expressed as relative mRNA

Table 1 Primers used in this study

Primer	Primer sequence (5′ to 3′)
RT-PCR	
Tret1a-F	AACTTGTTTTTATACTTATC
Tret1a-R	CTGAATGCGAAGCAAGTA
Tret1b-F	AAGAAACTGACAACTCTA
Tret1b-R	ATTTGAATTTCCTCCAAAGT
Tret1-2-F	CATTTGCGGATCACTTTG
Tret1-2-R	AACGACACCGATTACGAC
P5CS-F	GTAGTTTTTTCGACAGGA
P5CS-R	CTTACTAATGATATTGCTT
GST-F	GTCCTGCTGTACGATCTA
GST-R	TTAAGCAAGGCGGCTTTTC
qPCR	
Tret1a-F	TGGACATCACCTGCTTTG
Tret1a-R	TCCTACGGTTGCTCCTAA
Tret1b-F	TCGGTGGAGGTGTCATAC
Tret1b-R	CCCAGAAACGACGATAATCC
Tret1-2-F	GCCGAACTATTTCCTACG
Tret1-2-R	CACTCCGAACCTTCCTAA
P5CS-F	GCCTTATGTCTCTGTACG
P5CS-R	GTTCCTTCTGGTCTCTTC
GST-F	AGCGTTGAATCGTTACCC
GST-R	GCGGCTTTTCATCATTCC
RPL4-F	TGTGGTAATGCTGTGGTAT
RPL4-R	TCTAGCACTGCATGAACA
dsRNA	
ds*Tret1a*-F	TAATACGACTCACTATAGGGGCTGTGGTTCGCTATACTGT
ds*Tret1a*-R	TAATACGACTCACTATAGGGCTGTTGCCTTCTTCGTAA
ds*Tret1b*-F	TAATACGACTCACTATAGGGAATATCTGTTTGGCCTCAA
ds*Tret1b*-R	TAATACGACTCACTATAGGGAGCGGATTCTTGGTCTTG
ds*Tret1-2*-F	TAATACGACTCACTATAGGGCATTTGCGGATCACTTTG
ds*Tret1-2*-R	TAATACGACTCACTATAGGGCTTCCTTTTTATTTTTTCCTACC
ds*P5CS*-F	TAATACGACTCACTATAGGGCTTTGATGTCGGATGTGG
ds*P5CS*-R	TAATACGACTCACTATAGGGGATTTCTTAGTTTCGCTGGT
ds*GST*-F	TAATACGACTCACTATAGGGCCATCAAGGCATTAGGAG
ds*GST*-R	TAATACGACTCACTATAGGGAAACATTGGCAGAGGTCA
ds*GFP*-F	TAATACGACTCACTATAGGGTGAGCAAGGGCGAGGAG
ds*GFP*-R	TAATACGACTCACTATAGGGCGGCGGTCACGAACTCCAG

levels normalised to the housekeeping reference gene *RPL4* in the same cDNA samples, using $2^{-\Delta\Delta Ct}$ $\left[\Delta\Delta CT = \left(Ct_{target}-Ct_{reference}\right)_{treatment}-\left(Ct_{target}-Ct_{reference}\right)_{control}\right]$ (Livak and Schmittgen 2001; Pfaffl 2001).

RNA interference (RNAi)

The specific genes for RNAi were selected based on our previous comparative transcriptome analysis, which was constructed using adult females from two different geographic populations, BJ and LB. Many genes were expressed differently between the BJ and LB transcriptomes, but most were non-characteristic proteins, which were eliminated. We selected some genes in the transciptome for RNAi that are associated with insect cold tolerant physiology, including trehalose-specific transporters (*Trets*), delta 1-pyrroline-5-carboxylate synthetase (*P5CS*), and *GST* genes. The gene sequences used in this study were also sourced from our previous transciptome study. Primers for the double-strand RNA (dsRNA) of five target genes were designed using Primer Premier 5.0 (Table 1). We also designed primers for GFP-specific dsRNA (ds*GFP*) to verify that the injection

and the immune system response would not affect the expression of the target genes and the results of the study (Dai et al. 2017; Ma et al. 2020a; Tian et al. 2020b). They were then synthesised using the MEGAscript T7 High Yield Transcription Kit (Ambion, Austin, TX, USA). To explore the effect of dsRNA on the cold tolerance of *O. communa*, one-day-old adult females were injected at the pronotum with 1 μg dsRNA using a PLI-100 Pico-Injector (Harvard Apparatus, Holliston, MA, USA), and manipulated with an MP-255 Micromanipulator (Sutter, Novato, CA, USA) under a stereomicroscope. Control groups were injected with an equal volume of ds*GFP*. Owing to the high interference efficiency 48 h after dsRNA injection (Ma et al. 2020a; Tian et al. 2020b), their supercooling point (SCP), trehalose and proline content, and GST enzyme activity were determined.

Measurement of SCP

Thermocouples were connected to the cuticles of individual beetles. Then, they were placed in a −25 °C freezer with a cooling rate of approximately 1 °C per minute (Zhou et al. 2011b). The SCP was determined as the lowest temperature recorded before the sudden increase in temperature caused by the release of the latent heat of crystallisation.

GST enzyme activity assays

The GST enzyme activity was detected with a GST Assay Kit (Solarbio, Beijing, China) according to the manufacturer's instructions. GST can catalyse the conjugation of glutathione and 1-chloro-2,4-dinitrobenzene. The product has an absorption peak at 340 nm. The GST enzyme activity was measured by monitoring the rate of the increase in absorbance at 340 nm. One unit of enzyme activity was defined as 1 mg of enzyme protein that catalysed 1 nmol of product per minute in the reaction.

In all the above bioassay experiments, 10 individuals were measured per replicate, and three replicates were conducted.

Statistical analysis

The differences in the total sugar, trehalose, glycerol, lipid, and proline content in the F_0 generation between the BJ and LB populations or the hybridised populations (F_1 generation) were analysed using *t*-tests in SAS 8.1 (SAS Institute, Cary, NC, USA). Gene expression and bioassays were analysed using one-way analysis of variance followed by a least significant difference test with SAS 8.1 (SAS Institute, Cary, USA) to evaluate the significant differences among treatments.

Results

Total sugar, trehalose, glycerol, lipid, and proline content

The content of trehalose, lipid, and proline in female *O. communa* from the BJ population was significantly higher than in those from the LB population (trehalose: $t=4.92$, $P < 0.05$; lipid: $t=2.96$, $P < 0.05$; proline: $t=2.98$, $P < 0.05$; Fig. 1b, d, and e). However, the content of total sugar and glycerol in male *O. communa* from the BJ population was significantly higher than in those from the LB population (total sugar: $t=6.09$, $P < 0.05$; glycerol: $t=3.69$, $P < 0.05$; Fig. 1a and c).

Hybridisation showed an inconsistent pattern in physiological traits, as positive, intermediate, and negative effects all appeared (Fig. 1). Owing to a significant difference in cold tolerance between males and females, we compared the significance of the physiological traits between the hybrid offspring and their parents of the same sex. The total sugar content in the hybrid offspring was intermediate between the two parents (Fig. 1a). The trehalose and glycerol content showed a similar pattern; three effects could be seen in the different hybrid offspring (Fig. 1b and c). The lipid and proline content in the hybrid offspring was higher than that in their parents of the same sex (Fig. 1d and e), and it showed a positive effect of hybridisation.

Geographic and hybrid expression profile

The mRNA levels of *Trets*, *P5CS*, and *GST* in three-day-old adult females from the BJ population were all significantly higher than in those from the LB population (*Tret1a*: $P < 0.05$; *Tret1b*: $P < 0.05$; *Tret1-2*: $P < 0.05$; *P5CS*: $P < 0.05$; *GST*: $P < 0.05$). *Tret1a*, *Tret1b*, *P5CS*, and *GST* mRNA levels in the BJ population were nearly twofold higher than those in the Laibin population (Fig. 2).

The relative expression patterns of the target mRNAs after hybridisation were similar to those of the physiological traits; they also showed positive, intermediate, and negative effects. The *Tret1a*, *Tret1b*, and *Tret1-2* mRNA levels in hybrid males mainly increased, while those in females mainly decreased compared to their parents of the same sex, except *Tret1a* in F1: BJ♀×LB♂ hybrid males (Fig. 2a, b, and c). The *P5CS* mRNA level exhibited an inconsistent pattern in the hybrids (Fig. 2d), whereas the *GST* mRNA level increased in the hybrids compared to their parents of the same sex (Fig. 2e).

Effect of dsRNA on *Trets* expression and trehalose content

After injection of ds*Tret1a*, ds*Tret1b*, and ds*Tret1-2* for 48 h, their mRNA levels were reduced by nearly 90.0%, 75.5%,

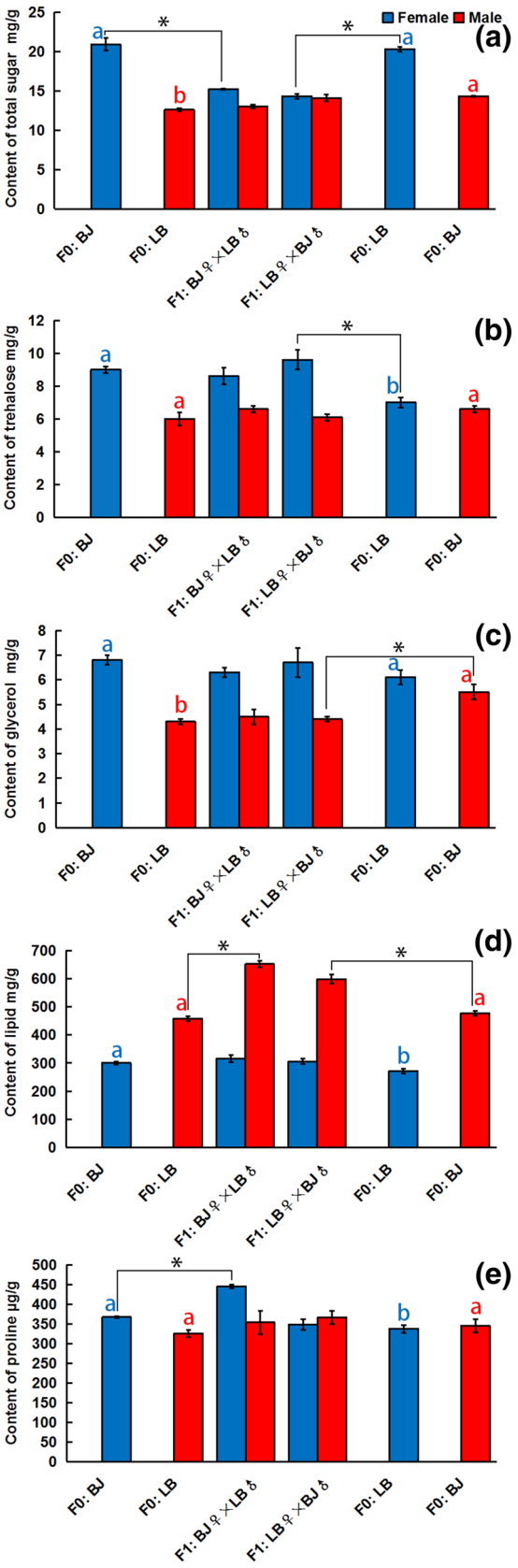

Fig. 1 Total sugar, trehalose, glycerol, lipid, and proline content in adult *Ophraella communa* from two latitudes and their hybrids. **a** Total sugar; **b** trehalose; **c** glycerol; **d** lipid; and **e** proline. Data are showed as the mean ± SE. Bars marked with different lowercase letters with the same colour are significantly different between the same sex from two different latitudes, based on *t*-tests ($P < 0.05$). Bars marked with "*" are significantly different between hybrids and their parents of the same sex, based on *t*-tests ($P < 0.05$)

and 87.9%, respectively, compared with the ds*GFP* injection group (Fig. 3a, b and c). Accordingly, the trehalose content was 5.7, 5.6, and 5.9 μg/mg, respectively, which was significantly lower than that after ds*GFP* injection (6.2 μg/mg) (Fig. 3f, g, and h). To identify whether *Trets* could regulate cold tolerance in *O. communa*, their SCP was determined. The SCPs of three-day-old adult females injected with ds*Tret1a*, ds*Tret1b*, and ds*Tret1-2* were significantly higher than those after ds*GFP* injection (Fig. 3k, l, and m). This indicates that the cold tolerance of *O. communa* was reduced after silencing *Trets*.

Intriguingly, we found synergistic and complementary effects among the three *Trets*. The results indicate a complementary relationship between *Tret1-2*, and *Tret1a*, and *Tret1b*. When *Tret1-2* was silenced, the expression levels of *Tret1a* and *Tret1b* were increased (Fig. 3a, b and c). When either *Tret1a* or *Tret1b* was silenced, the other decreased (Fig. 3a and b). These findings suggest a synergistic relationship between them.

Effect of dsRNA on *P5CS* expression and proline content

Forty-eight hours after ds*P5CS* injection, *P5CS* mRNA levels were reduced by 88.2% after injection of ds*GFP* (Fig. 3d). To test whether the *P5CS* gene could influence the biosynthesis of proline, the proline content was measured. In ds*P5CS*-injected beetles, proline levels were reduced by 36% compared with controls (Fig. 3i), confirming the function of *P5CS* in proline biosynthesis. The SCPs of three-day-old females injected with ds*P5CS* were significantly higher than those injected with ds*GFP* (Fig. 3n). This result demonstrates that the cold tolerance of *O. communa* was reduced after silencing *P5CS*.

Effect of dsRNA on *GST* expression and enzyme activity

Forty-eight hours after ds*GST* injection, *GST* mRNA levels were reduced by 75.5% compared with the ds*GFP* injection group (Fig. 3e). This also significantly reduced the GST enzyme activity, which was 32.2 ± 0.4 and 42.0 ± 0.5 U/g wet weight after the injection of ds*GST* and ds*GFP*, respectively (Fig. 3j). The SCP of three-day-old females injected with ds*GST* were significantly higher than those injected

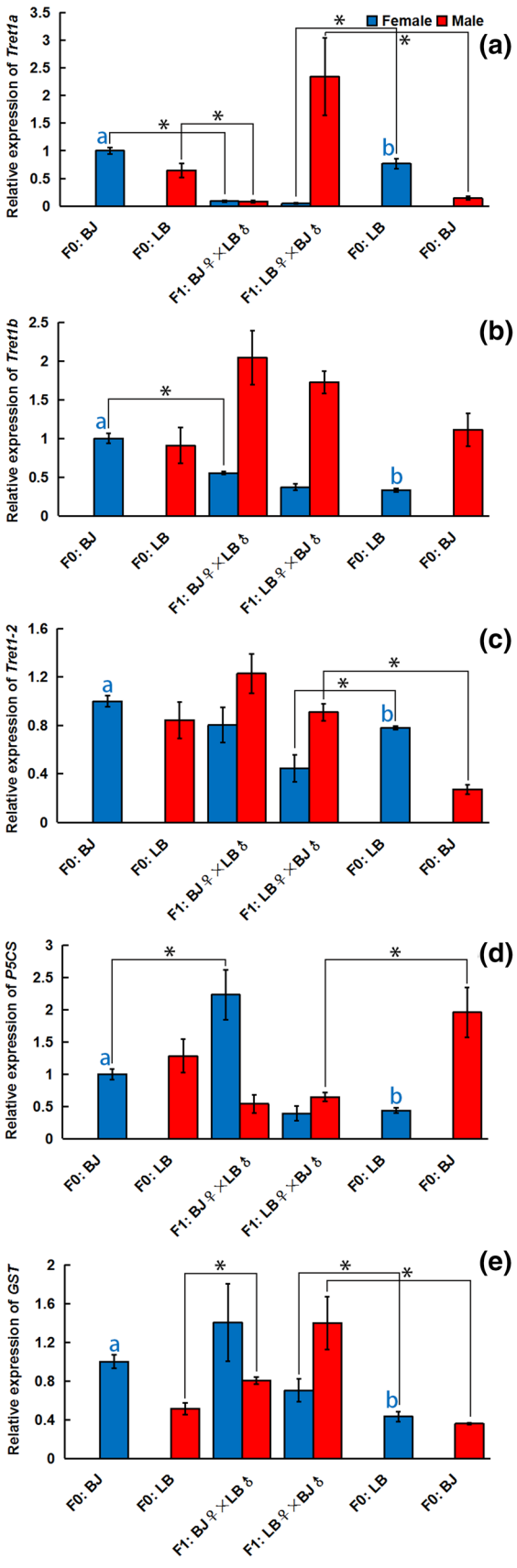

Fig. 2 Relative expression of five identified genes responsible for cold tolerance in three-day-old adult female *Ophraella communa* from two latitudes and their hybrids. Panels depict the relative expression of: **a** *Tret1a*; **b** *Tret1b*; **c** *Tret1-2*; **d** *P5CS*; and **e** *GST*. The relative expression levels are showed as the mean ± SE. Bars marked with different lowercase letters with the same colour are significantly different between females from the two latitudes, based on one-way ANOVA followed by an LSD test ($P < 0.05$). Bars marked with "*" are significantly different between hybrids and their parents of the same sex, based on one-way ANOVA followed by an LSD test ($P < 0.05$)

with ds*GFP* (Fig. 3o). This indicates that cold tolerance was reduced following *GST* silencing.

Discussion

Ambrosia artemisiifolia is a severely invasive weed, and its distribution and influence may increase with climate change (Chapman et al. 2016). For instance, it has long colonised the Russian Far East, which has an extended cold winter (Reznik 2009). Therefore, their adaptability to new environments is important when using specific herbivorous insects to control *A. artemisiifolia*. For example, *Zygogramma suturalis* is considered an effective biological control agent of *A. artemisiifolia*, but its application has failed in China owing to its weak adaptability to new environments (Wan et al. 1995). *Ophraella communa* has shown a positive effect in the management of *A. artemisiifolia* in China and Europe (Zhou et al. 2015; Schaffner et al. 2020). A previous study demonstrated that *O. communa* has high plasticity in its cold tolerance, based on the high heritability of the trait (Zhao et al. 2018). Here, we also demonstrate the strong rapid evolution of cold tolerance in *O. communa* that may improve its local adaptability, enabling it to be introduced to *A. artemisiifolia* distribution ranges at higher latitudes to maintain long-term control.

A previous study examined the evolution of cold tolerance in *Drosophila melanogaster*, revealing the genetic architecture underpinning rapid cold hardening and developmental acclimation (Gerken et al. 2015); however, it did not demonstrate adaptation to new low-temperature environments. The present study demonstrates the putative physiological and molecular mechanisms underpinning the rapid evolution of cold tolerance in *O. communa* following its release in Beijing. Thompson (1998) suggested that "rapid evolution" refers to genetic changes occurring over a century or less. Many insects show rapid adaptation over short timescales (Krehenwinkel et al. 2015; Lancaster et al. 2016; Hoffmann 2017); for example, *Aedes japonicus* adapted to changing environments within 7–10 years (Egizi et al. 2015). These findings suggest that insects have a strong rapid evolution capacity for adapting to new temperature

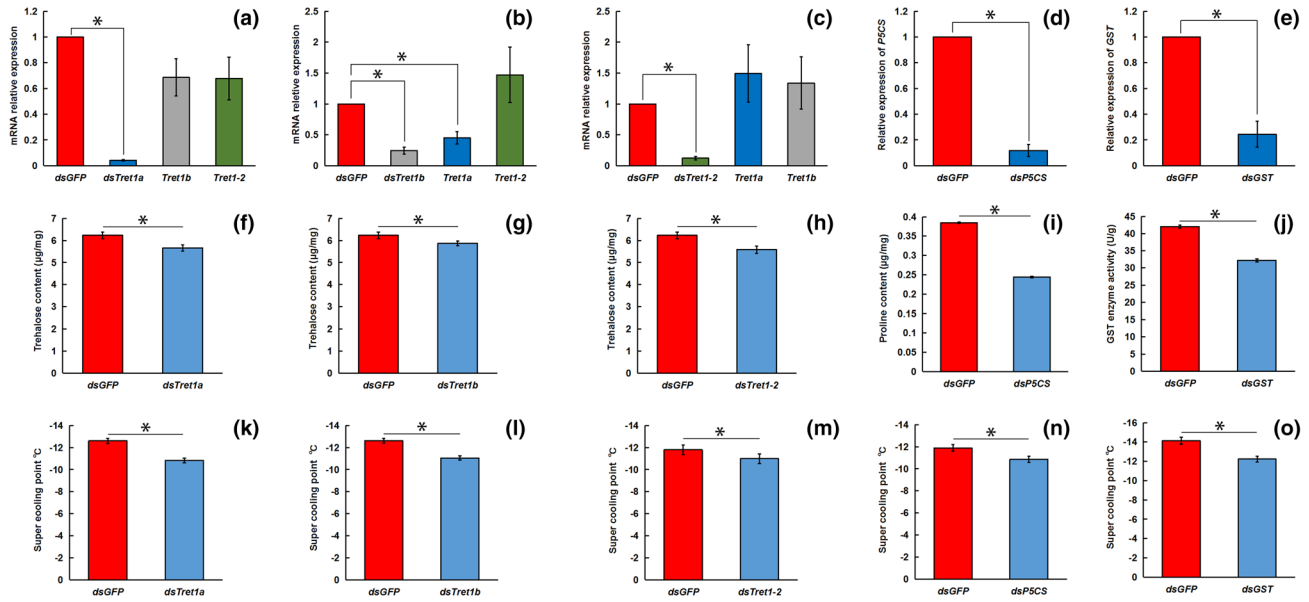

Fig. 3 Effect of dsRNA on relative mRNA expression, bioassay results, and SCP. **a–e** Relative mRNA expression levels; **f–h** trehalose content; **i** proline content; **j** GST enzyme activity; and **k–o** effect of dsRNA on SCPs. Data are showed as the mean ± SE. Bars marked with "*" are significantly different based on one-way ANOVA followed by an LSD test ($P < 0.05$)

environments. However, transgenerational effects (or parental effects) might often confound rapid adaptation; when establishing evolutionary shifts, it is necessary for populations to be reared for one or two generations to remove parental effects (Hoffmann 2017). Thus, the beetles used in our study were directly collected from the field and then reared for one generation under common environmental conditions in a laboratory.

As a previous study described, several physiological traits of *O. communa* apparently change during cold adaptation (Zhou et al. 2011b, c). Here, we found significant differences between the populations of two latitudes in the trehalose, lipid, and proline content of female adults, and in the total sugar and glycerol content of male adults (Fig. 1). We have thus illustrated the main physiological mechanisms of rapid evolutionary changes in cold tolerance of *O. communa*. In general, trehalose, proline, and glycerol are important low molecular weight cryoprotectants in insects that are accumulated before overwintering to withstand cold stress. This is a key physiological mechanism in insect cold tolerance (Denlinger and Lee 2010; Sinclair et al. 2003; Teets and Denlinger 2013; Toxopeus and Sinclair 2018). Trehalose, glycerol, and proline can stabilise the cell membrane and macromolecules or enhance the supercooling capability of insects to promote their cold tolerance (Teets and Denlinger 2013). Therefore, the *O. communa* individuals in Beijing accumulated higher levels of cryoprotectants to protect them from cold injury in the colder environment. Sugars and lipids are important energy reserves, and previous studies have demonstrated that overwintering insects store more the two substances to survive the winter (Watanabe and Hirai 2004; Sinclair and Marshall 2018). Winter is longer and colder in high latitudes, so *O. communa* populations in such locations may store more total sugars and lipids than in low latitudes.

Geographic variation in gene expression could suggest different adaptive responses to changing environmental conditions and might indicate a genetic basis for local adaptation (Hoffmann 2017). At the genetic level, we found significantly higher transcript levels of *Tret1a*, *Tret1b*, and *Tret1-2*, *P5CS*, and *GST* in adult *O. communa* females at high latitude (Beijing) than in those at low latitude (Laibin). These genes are involved in trehalose transport, proline biosynthesis, and the oxidation–reduction process. Therefore, we suggest that the high-latitude *O. communa* population was likely to have a stronger capacity to transport trehalose, synthesise proline, and protect cells from oxidative damage than its low-latitude counterpart. Additionally, as an important antioxidant enzyme, GST can remove reactive oxygen species to decrease injuries caused by oxidative damage to cellular constituents or proteins (Lalouette et al. 2011; Storey and Storey 2013). Recently, a study demonstrated that *GST* expression in *Apis mellifera* was highly associated with local environmental conditions (Kojić et al. 2019). This may suggest that GST could be rapidly activated in response to environmental changes in insects.

Hybridisation could increase the evolutionary potential and improve the life history traits and fitness of hybrid offspring; however, it occasionally leads to neutral or negative effects (Bitume et al. 2017; Szűcs et al. 2012). Hybridisation has been demonstrated to promote evolutionary adaptation

to new environments (Hoffmann and Sgrò 2011). In our study, the cold tolerance of *O. communa* was improved after hybridisation. At the physiological level, the cryoprotectant and energy reserves were mainly found at higher or intermediate levels in the hybrids compared to their parents (Fig. 1). At the molecular level, *Tret1a* expression mainly showed negative effects, whereas the other genes largely exhibited positive or intermediate effects (Fig. 2). These results reveal that cold tolerance in *O. communa* is not inherited in a simple Mendelian manner, but experiences maternal and paternal effects (Bonduriansky et al. 2012). Furthermore, the total sugar and lipid content may have been inherited depending on paternal effects, but the trehalose, glycerol, and proline content was likely influenced by maternal effects. The expression of *Trets* in each reciprocal hybrid seemed to be sex-linked. However, the *GST* expression in hybrids suggested overdominance. Moreover, direct allelic evidence was lacking, so the specific inheritance modes require further study on alleles or chromosomes.

In our study, we found that the transcription of TRET orthologs was important in the cold tolerance of *O. communa*. Ma et al. (2020b) also found that TRET orthologs were an important genetic basis of the cold tolerance strategies of the striped rice borer, *Chilo suppressalis*. Our study showed that, after *Trets* knockdown, the trehalose levels in *O. communa* were reduced (Fig. 3f–h). Trehalose is an appropriate stress protectant and preservative that can stabilise proteins and cells in invertebrates against damage caused by abiotic stresses, including cold (Elbein et al. 2003; Thompson 2003). Accordingly, the SCP of *O. communa* was eventually increased following the knockdown of *Trets* (Fig. 3k–m), indicating a reduction in cold tolerance. The SCP is the temperature at which the insect body fluids begin to spontaneously freeze. It might be an appropriate survival proxy for lethal temperature in many freeze-intolerant insect species (Salt 1961; Ditrich 2018) and is a widely used metric to estimate the cold tolerance of insects (Zhou et al. 2011b, c; Van Damme et al. 2015; Feng et al. 2018; Li et al. 2021). Our results suggest that *Trets* mediates trehalose levels to protect *O. communa* against cold stress. This mechanism likely plays a role in the *Tret1*-mediated protection of *Anopheles gambiae* under desiccation and high temperatures (Liu et al. 2013). Additionally, studies have revealed that *Trets* could respond to other external environmental stresses, such as desiccation, salinity, low humidity, and high temperatures (Kikawada et al. 2007; Liu et al. 2013). These findings may suggest that *O. communa* could adapt to other new environmental stresses.

Proline is an important energy reserve for flight in insects. It also serves as an effective cryoprotectant, and its levels in insects have been widely reported to increase before overwintering or after rapid cold hardening and cold acclimation (Teets and Denlinger 2013; Toxopeus and Sinclair 2018). The *P5CS* gene has been well studied in plants, but little is known in insects. The putative *P5CS* gene encodes Δ^1-pyrroline-5-carboxylate synthetase, which catalyses the key step in proline biosynthesis in *Leptinotarsa decemlineata* (Wan et al. 2014). Here, the knockdown of *P5CS* reduced the proline content and increased the SCP in *O. communa* (Fig. 3i). These findings confirm the pivotal role of this gene in regulating proline biosynthesis. Meanwhile, this result also indicates that *P5CS* could regulate cold tolerance-mediated changes in the proline levels of *O. communa*.

Insects have evolved multiple strategies to respond to cold damage, including the accumulation of cryoprotectants, as well as cytoprotectants such as antioxidant defences (Toxopeus and Sinclair 2018). The upregulation of antioxidant defences has been demonstrated under various stresses, including low temperatures (Storey and Storey 2013). In insects, previous studies have found that GST activity is associated with low temperatures (Grubor-Lajsic et al. 1997; Kojić et al. 2019). Although many studies have examined antioxidant enzymes, such as superoxide dismutase, catalase, and peroxidases, in invertebrates, little is known about the response of GST to cold tolerance in insects. However, this has been well studied in nematodes and vertebrates (Adhikari et al. 2010; Krivoruchko and Store 2010; Storey and Storey 2017). Various xenobiotics and aldehydic products of lipid peroxidation are neutralised by GST. These products could facilitate ROS formation by conjugating with reduced glutathione, followed by simultaneous excretion (Storey and Storey 2013). In our study, *GST* gene silencing reduced GST enzyme activity and increased the SCP of *O. communa* (Fig. 3j and o). This result indicates that the *GST* gene could regulate the cold tolerance mediated by the GST enzyme activity of *O. communa*.

In this study, we found that *O. communa* adapted strongly to a new low-temperature environment, owing to rapid evolution which was reflected in physiological and molecular level changes. These evolutionary changes mean that *O. communa* at high latitudes may have stronger capabilities in accumulating cryoprotectants, cytoprotectants, and energy reserves to respond to the cold winter temperatures. Importantly, we found that the physiological and molecular responses both involved trehalose and proline, which probably reflects their essential roles in insect cold tolerance. Further, we can expand the geographic distribution of *O. communa* by artificial introduction to improve *A. artemisiifolia* control and reduce its harmful effects on the environment and humans.

Authors contributions

ZSZ conceived and designed the research. ZQT, GMC, and YZ conducted experiments. CM, ZYT, XYG, HSC, and JYG analysed the data. ZSZ and ZQT wrote the manuscript. All authors read and approved the manuscript.

Acknowledgements This work was supported by the National Natural Science Foundation of China (32172494, 31972340).

Funding This work was supported by the National Natural Science Foundation of China (32172494, 31972340).

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Adhikari BN, Wall DH, Adams BJ (2010) Effect of slow desiccation and freezing on gene transcription and stress survival of an Antarctic nematode. J Exp Biol 213:1803–1812. https://doi.org/10.1242/jeb.032268

Augustinus BA, Gentili R, Horvath D, Naderi R, Sun Y, Tournet AMTE, Schaffner U, Müller-Schärer H (2020b) Assessing the risks of non-target feeding by the accidentally introduced ragweed leaf beetle, *Ophraella communa*, to native European plant species. Biol Control 150:104356. https://doi.org/10.1016/j.biocontrol.2020.104356

Bitume E, Bean D, Stahlke AR, Hufbauer RA (2017) Hybridization affects life-history traits and host specificity in *Diorhabda* spp. Biol Control 111:45–52. https://doi.org/10.1016/j.biocontrol.2017.05.009

Bonduriansky R, Crean AJ, Day T (2012) The implications of nongenetic inheritance for evolution in changing environments. Evol Appl 5:192–201. https://doi.org/10.1111/j.1752-4571.2011.00213.x

Chapman DS, Makra L, Albertini R, Bonini M, Paldy A, Rodinkova V et al (2016) Modelling the introduction and spread of non-native species: international trade and climate change drive ragweed invasion. Global Change Biol 22:3067–3079. https://doi.org/10.1111/gcb.13220

Dai TM, Lü ZC, Wang Y, Liu WX, Hong XY, Wan FH (2017) Molecular characterizations of *DNA methyltransferase 3* and its roles in temperature tolerance in the whitefly, *Bemisia tabaci* Mediterranean. Insect Mol Biol 27:123–132. https://doi.org/10.1111/imb.12354

Denlinger DL, Lee RE Jr (eds) (2010) Low temperature biology of insects. Cambridge University Press, Cambridge, pp 15–19. https://doi.org/10.1017/CBO9780511675997

Ditrich T (2018) Supercooling point is an individually fixed metric of cold tolerance in *Pyrrhocoris apterus*. J Therm Biol 74:208–213. https://doi.org/10.1016/j.jtherbio.2018.04.004

Dudaniec RY, Yong CJ, Lancaster LT, Svensson EI, Hansson B (2018) Signatures of local adaptation along environmental gradients in a range-expanding damselfly (*Ischnura elegans*). Mol Ecol 27:2576–2593. https://doi.org/10.1111/mec.14709

Egizi A, Fefferman NH, Fonseca DM (2015) Evidence that implicit assumptions of 'no evolution' of disease vectors in changing environments can be violated on a rapid timescale. Philos T R Soc B 370:20140136. https://doi.org/10.1098/rstb.2014.0136

Elbein AD, Pan YT, Pastuszak I, Carroll D (2003) New insights on trehalose: a multifunctional molecule. Glycobiology 13:17R-27R. https://doi.org/10.1093/glycob/cwg047

Feng Y, Zhang L, Li W, Yang X, Zong S (2018) Cold hardiness of overwintering larvae of *Sphenoptera* sp. (Coleoptera: Buprestidae) in Western China. J Econ Entomol 111:247–251. https://doi.org/10.1093/jee/tox304

Gao X, Tian Z, Zhang Y, Chen G, Ma C, Tian Z, Cui S, Lu Y, Zhou Z (2020) Transcriptome analysis of *Ophraella communa* male reproductive tract in indirect response to elevated CO_2 and heat wave. Front Physiol 11:417. https://doi.org/10.3389/fphys.2020.00417

Garnas JR (2018) Rapid evolution of insects to global environmental change: conceptual issues and empirical gaps. Curr Opin Insect Sci 29:93–101. https://doi.org/10.1016/j.cois.2018.07.013

Gerken AR, Eller OC, Hahn DA, Morgan TJ (2015) Constraints, independence, and evolution of thermal plasticity: probing genetic architecture of long- and short-term thermal acclimation. Proc Natl Acad Sci USA 112:4399–4404. https://doi.org/10.1073/pnas.1503456112

Grubor-Lajsic G, Block W, Telesmanic M, Jovanovic A, Stevanovic D, Baca F (1997) Effect of cold acclimation on the antioxidant defense system of two larval Lepidoptera (Noctuidae). Arch Insect Biochem Physiol 36:1–10. https://doi.org/10.1002/(SICI)1520-6327(1997)36:1%3c1::AID-ARCH1%3e3.0.CO;2-#

Guo JY, Zhou ZS, Zheng XW, Chen HS, Wan FH, Luo YH (2011) Control efficiency of leaf beetle, *Ophraella communa*, on the invasive common ragweed, *Ambrosia artemisiifolia*, at different growing stages. Biocontrol Sci Technol 21:1049–1063. https://doi.org/10.1080/09583157.2011.603823

Hoffmann AA (2017) Rapid adaptation of invertebrate pests to climatic stress. Curr Opin Insect Sci 21:7–13. https://doi.org/10.1016/j.cois.2017.04.009

Hoffmann AA, Sgrò CM (2011) Climate change and evolutionary adaptation. Nature 470:479–485. https://doi.org/10.1038/nature09670

Janes JK, Li Y, Keeling CI, Yuen MMS, Boone CK, Cooke JEK et al (2014) How the mountain pine beetle (*Dendroctonus ponderosae*) breached the Canadian Rocky Mountains. Mol Biol Evol 31:1803–1815. https://doi.org/10.1093/molbev/msu135

Kikawada T, Saito A, Kanamori Y, Nakahara Y, Iwata KI, Tanaka D, Watanabe M, Okuda T (2007) Trehalose transporter 1, a facilitated and high-capacity trehalose transporter, allows exogenous trehalose uptake into cells. Proc Natl Acad Sci USA 104:11585–11590. https://doi.org/10.1073/pnas.0702538104

Kojić DK, Purać JS, Nikolić TV, Orčić SM, Vujanović D, Ilijević K, Vukašinović EL, Blagojević D (2019) Oxidative stress and the activity of antioxidative defense enzymes in overwintering honey bees. Entomol Gen 39:33–44. https://doi.org/10.1127/entomologia/2019/0743

Krehenwinkel H, Rödder D, Tautz D (2015) Eco-Genomic analysis of the poleward range expansion of the wasp spider *Argiope bruennichi* shows rapid adaptation and genomic admixture. Global Change Biol 21:4320–4332. https://doi.org/10.1111/gcb.13042

Krivoruchko A, Storey KB (2010) Activation of antioxidant defenses in response to freezing in freeze tolerant painted turtle hatchlings. Biochim Biophys Acta 1800:662–668. https://doi.org/10.1016/j.bbagen.2010.03.015

Lalouette L, Williams CM, Hervant F, Sinclair BJ, Renault D (2011) Metabolic rate and oxidative stress in insects exposed to low temperature thermal fluctuations. Comp Biochem Physiol A Mol Integr Physiol 158:229–234. https://doi.org/10.1016/j.cbpa.2010.11.007

Lancaster LT, Dudaniec RY, Chauhan P, Wellenreuther M, Svensson EI, Hansson B (2016) Gene expression under thermal stress varies across a geographic range expansion front. Mol Ecol 25:1141–1156. https://doi.org/10.1111/mec.13548

Leather SR, Walters KFA, Bale JS (1993) The ecology of insect overwintering. Cambridge University Press, Cambridge, pp 143–147. https://doi.org/10.1017/CBO9780511525834

Li XW, Li D, Zhang Z,J Huang J, Zhang JM, Hafeez M, Wang LK, Guo WC, Lu YB (2021) Supercooling capacity and cold tolerance of the South American tomato pinworm, *Tuta absoluta*, a newly invaded pest in China. J Pest Sci 94:845–858. https://doi.org/10.1007/s10340-020-01301-y

Liu K, Dong Y, Huang Y, Rasgon JL, Agre P (2013) Impact of trehalose transporter knockdown on *Anopheles gambiae* stress adaptation and susceptibility to *Plasmodium falciparum* infection. Proc Natl Acad Sci USA 110:17504–17509. https://doi.org/10.1073/pnas.1316709110

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

Ma C, Cui S, Bai Q, Tian Z, Zhang Y, Chen G et al (2020a) Olfactory co-receptor is involved in host recognition and oviposition in *Ophraella communa* (Coleoptera: Chrysomelidae). Insect Mol Biol 29:381–390. https://doi.org/10.1111/imb.12643

Ma W, Zhao X, Yin C, Jiang F, Du X, Chen T et al (2020b) A chromosome-level genome assembly reveals the genetic basis of cold tolerance in a notorious rice insect pest, *Chilo suppressalis*. Mol Ecol Res 20:268–282. https://doi.org/10.1111/1755-0998.13078

Mazza G, Tricarico E, Genovesi P, Gherardi F (2014) Biological invaders are threats to human health: an overview. Ethol Ecol Evol 26:112–129. https://doi.org/10.1080/03949370.2013.863225

Meng L, Li BP (2005) Advances on biology and host specificity of the newly introduced beetle, *Ophraella communa* LeSage (Coleoptera: Chrysomelidae), attacking *Ambrosia artemisiifolia* (Compositae) in continent of China. Chin J Biol Control 21:65–69. https://doi.org/10.3969/j.issn.2095-039X.2005.02.001

Meng L, Xu J, Li HB (2007) Dispersal and bionomics of the alien *Ophraella communa* in China mainland. Chin J Biol Control 23:5–10. https://doi.org/10.3321/j.issn:1005-9261.2007.01.002

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45. https://doi.org/10.1093/nar/29.9.e45

Reznik SY (2009) Common ragweed (*Ambrosia artemisiifolia* L.) in Russia: spread, distribution, abundance, harmfulness and control measures. Ambroisie First Int Ragweed Rev 26:88–97

Rogers CA, Wayne PM, Macklin EA, Muilenberg ML, Wagner CJ, Epstein PR, Bazzaz FA (2006) Interactions of the onset of spring and elevated atmospheric CO_2 on ragweed (*Ambrosia artemisiifolia* L.) pollen production. Environ Health Persp 114:865–869. https://doi.org/10.1289/ehp.8549

Salt RW (1961) Principles of insect cold-hardiness. Ann Rev Entomol 6:55–74. https://doi.org/10.1146/annurev.en.06.010161.000415

Schaffner U, Steinbach S, Sun Y, Skjøth CA, de Weger LA, Lommen ST et al (2020) Biological weed control to relieve millions from *Ambrosia allergies* in Europe. Nat Commun 11:1–7. https://doi.org/10.1038/s41467-020-15586-1

Sinclair BJ, Marshall KE (2018) The many roles of fats in overwintering insects. JExpBiol221: jeb161836. https://doi.org/10.1242/jeb.161836

Sinclair BJ, Vernon P, Klok CJ, Chown SL (2003) Insects at low temperatures: an ecological perspective. Trends Ecol Evol 18:257–262. https://doi.org/10.1016/S0169-5347(03)00014-4

Smith M, Cecchi L, Skjøth CA, Karrer G, Šikoparija B (2013) Common ragweed: a threat to environmental health in Europe. Environ Int 61:115–126. https://doi.org/10.1016/j.envint.2013.08.005

Storey KB, Storey JM (2013) Molecular biology of freezing tolerance. Compr Physiol 3:1283–1308. https://doi.org/10.1002/cphy.c130007

Storey KB, Storey JM (2017) Molecular physiology of freeze tolerance in vertebrates. Physiol Rev 97:623–665. https://doi.org/10.1152/physrev.00016.2016

Szűcs M, Eigenbrode SD, Schwarzlander M, Schaffner U (2012) Hybrid vigor in the biological control agent, *Longitarsus jacobaeae*. Evol Appl 5:489–497. https://doi.org/10.1111/j.1752-4571.2012.00268.x

Teets NM, Denlinger DL (2013) Physiological mechanisms of seasonal and rapid cold-hardening in insects. Physiol Entomol 38:105–116. https://doi.org/10.1111/phen.12019

Thompson JN (1998) Rapid evolution as an ecological process. Trends Ecol Evol 13:327–329. https://doi.org/10.1016/S0169-5347(98)01378-0

Thompson SN (2003) Trehalose—the insect 'blood' sugar. Adv Insect Physiol 31:205–285. https://doi.org/10.1016/S0065-2806(03)31004-5

Tian ZQ, Ma C, Cui SW, Zhou ZS (2020a) *Ophraella communa* can establish population in the suburbs of Beijing, China. J Environ Entomol 42:1039–1040. https://doi.org/10.3969/j.issn.1674-0858.2020.04.31

Tian ZQ, Zhang Y, Ma C, Chen HS, Guo JY, Zhou ZS (2020b) Silencing the myosin regulatory light chain gene *sqh* reduces cold hardiness in *Ophraella communa* LeSage (Coleoptera: Chrysomelidae). Insects 11:844. https://doi.org/10.3390/insects11120844

Toxopeus J, Sinclair BJ (2018) Mechanisms underlying insect freeze tolerance. Biol Rev 93:1891–1914. https://doi.org/10.1111/brv.12425

Van Damme V, Berkvens N, Moerkens R, Berckmoes E, Wittemans L, De Vis R, Casteels H, Tirry L, De Clercq P (2015) Overwintering potential of the invasive leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) as a pest in greenhouse tomato production in Western Europe. J Pest Sci 88:533–541. https://doi.org/10.1007/s10340-014-0636-9

Wan PJ, Fu KY, Lü FG, Guo WC, Li GQ (2014) A putative Δ^1-pyrroline-5-carboxylate synthetase involved in the biosynthesis of proline and arginine in *Leptinotarsa decemlineata*. J Insect Physiol 71:105–113. https://doi.org/10.1016/j.jinsphys.2014.10.009

Wan F, Wang R, Ding J (1995) Biological control of *Ambrosia artemisiifolia* with introduced insect agents, *Zygogramma suturalis* and *Epiblema strenuana*, in China. In Delfosse ES, Scott RR (Eds.) Proceedings of the eighth international symposium on biological control of weeds. DSIR/CSIRO, Melbourne, Australia. pp. 193–200

Watanabe M, Hirai Y (2004) Host-use pattern of the ragweed beetle Ophraella communa Lesage (Coleoptera: Chrysomelidae) for overwintering and reproduction in Tsukuba. Appl Entomol Zool 39:249–254. https://doi.org/10.1303/aez.2004.249

Yin J, Zhou M, Lin Z, Li QQ, Zhang Y (2019) Transgenerational effects benefit offspring across diverse environments: a meta-analysis in plants and animals. Ecol Lett 22:1976–1986. https://doi.org/10.1111/ele.13373

Yue L, Zhou Z, Liu Z, Guo J, Wan F (2014) Effects of rapid cold hardening in different intensities on the physiological indices related to cold tolerance in adults of Ophraella communa (Coleoptera:

Chrysomelidae). Acta Entomol Sin 57:631–638. https://doi.org/10.16380/j.kcxb.2014.06.008

Zhang Y, Chen J, Chen G, Ma C, Chen H, Gao X et al (2020) Identification and validation of reference genes for quantitative gene expression analysis in *Ophraella communa*. Front Physiol 11:355. https://doi.org/10.3389/fphys.2020.00355

Zhao C, Ma F, Chen H, Wan FH, Guo JY, Zhou ZS (2018) Heritability and evolutionary potential drive cold hardiness in the overwintering *Ophraella communa* beetles. Front Physiol 9:666. https://doi.org/10.3389/fphys.2018.00666

Zhou ZS, Guo JY, Zheng XW, Luo M, Chen HS, Wan FH (2011a) Reevaluation of biosecurity of *Ophraella communa* against sunflower (*Helianthus annuus*). Biocontrol Sci Techn 21:1147–1160. https://doi.org/10.1080/09583157.2011.606559

Zhou ZS, Guo JY, Michaud JP, Li M, Wan FH (2011b) Variation in cold hardiness among geographic populations of the ragweed beetle, *Ophraella communa* LeSage (Coleoptera: Chrysomelidae), a biological control agent of *Ambrosia artemisiifolia* L. (Asterales: Asteraceae), in China. Biol Invasions 13:659–667. https://doi.org/10.1007/s10530-010-9857-x

Zhou ZS, Guo JY, Li M, Ai HM, Wan FH (2011c) Seasonal changes in cold hardiness of *Ophraella communa*. Entomol Exp Appl 140:85–90. https://doi.org/10.1111/j.1570-7458.2011.01128.x

Zhou ZS, Guo JY, Wan FH (2015) Review on management of *Ambrosia artemisiifolia* using natural enemy insects. Chin J Biol Control 31:657–665. https://doi.org/10.16409/j.cnki.2095-039x.2015.05.006