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Mitochondrial variation in small brown planthoppers linked to multiple traits and likely reflecting a complex evolutionary trajectory

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Running title: Mitochondrial genome evolution in planthopper

Abstract

While it has been proposed in several taxa that the mitochondrial genome is associated with adaptive evolution to different climatic conditions, making links between mitochondrial haplotypes and organismal phenotypes remains a challenge. Mito-nuclear discordance occurs in the small brown planthopper (SBPH), *Laodelphax striatellus,* with one mitochondrial haplogroup (HGI) more common in the cold climate region of China relative to another form (HGII) despite strong nuclear gene flow, providing a promising model to investigate climatic adaptation of mitochondrial genomes. We hypothesized that cold adaptation through HGI may be involved, and considered mitogenome evolution, population genetic analyses, and bioassays to test this hypothesis. In contrast to our hypothesis, chill-coma recovery tests and population genetic tests of selection both pointed to HGII being involved in cold adaptation. Phylogenetic analyses revealed that HGII is nested within HGI, and has three non-synonymous changes in *ND2*, *ND5* and *CYTB* in comparison to HGI. These molecular changes likely increased mtDNA copy number, cold tolerance and fecundity of SBPH, particularly through a function-altering amino acid change involving M114T in *ND2*. Nuclear background also influenced fecundity and chill recovery (i.e., mito-nuclear epistasis) and protein modeling indicates possible nuclear interactions for the two non-synonymous changes in *ND2* and *CYTB*. The high occurrence frequency of HGI in the cold climate region of China remains unexplained, but several possible reasons are discussed. Overall, our study points to a link between mtDNA

variation and organismal-level evolution and suggests a possible role of mito-nuclear interactions in maintaining mtDNA diversity.

Keywords: adaptive evolution, population genetics, natural selection, phenotypic effects.

1 | INTRODUCTION

Mitochondria are considered as the power plants of eukaryotic cells, producing up to 95% of total adenosine triphosphate (ATP) via oxidative phosphorylation (OXPHOS) (Das 2006). In addition to their crucial role in ATP production, mitochondria are also associated with many other processes essential for cell survival and function, such as cellular Ca^{2+} signaling, apoptosis, cell transport, thermoregulation, and immunity (Ballard & Pichaud 2014; Brand 2000; Chong & Mueller 2013; Detmer & Chan 2007). Mitochondria contain their own DNA (mtDNA), typically the animal mitochondrial genome (mitogenome) is circular and 15-20 kb in length, with 13 protein encoding genes (PCGs) along with 2 rRNAs and 22 tRNAs (Boore 1999). These genes interact with nuclear genes to preform OXPHOS and other functions (Hill 2015).

mtDNA has been extensively used for more than 30 years as a molecular marker to infer the evolutionary and demographic history of organisms under the implicit assumption of neutral evolution (Avise *et al.* 1987; Ballard & Pichaud 2014). Given its functional importance, the mitogenome is thought to evolve primarily under purifying selection. However, recent studies suggest that positive selection also plays a critical role in mitogenome evolution even though it may not be as widespread as purifying selection. An

exhaustive analysis of mtDNA diversity of > 1600 animal species revealed that the average within-species level of mtDNA diversity is remarkably similar across animal phyla, with no correlation between mtDNA polymorphism and species abundance, suggesting recurrent selective sweeps (adaptive evolution) in species with large population sizes leading to frequent drops in diversity (Bazin *et al.* 2006). In agreement with this, studies have identified positively selected sites in mtDNA genomes based on dN/dS methods or frequency clines of mtDNA variants within a phylogenic or population genetic framework across diverse taxa (Balloux *et al.* 2009; da Fonseca *et al.* 2008; Foote *et al.* 2011; Garvin *et al.* 2015; James *et al.* 2016; Morales *et al.* 2015; Silva *et al.* 2014). However, drivers for mitochondrial adaptive evolution and the mechanisms underlying them are far from clear.

Among various potential drivers for adaptive evolution of mtDNA, environmental temperature figures prominently. Mitochondrial OXPHOS not only generates ATP, but also heat via uncoupling mechanisms (proton leaks), enabling organisms to resist low temperature (Brand 2000). Any mtDNA mutations changing ATP/heat ratios may be favored by different climates, providing a theoretical basis for mitochondrial climatic adaptation. This proposition emerged from genetic studies on mitochondrial genome variation in humans, where the geographical distribution of mtDNA variants have been shaped by climate, and where population differentiation of mtDNA variants is correlated with temperature differences (Balloux *et al.* 2009; Mishmar *et al.* 2003; Ruiz-Pesini *et al.* 2004). Since then, evidence in support of mitochondrial climatic adaptation has accumulated, although most is indirect from statistical tests of neutral models of molecular evolution involving dN/dS based inference, or from correlation analyses of frequencies of

mtDNA variation and environmental factors (Foote *et al.* 2011; Morales *et al.* 2015; Silva *et al.* 2014). In contrast, empirical data to test the phenotypic impact of mtDNA variants on adaptive evolution are still scarce, although studies have found experimental support for mitochondrial climatic adaptation in *Drosophila simulans* (Ballard *et al*. 2007; Pichaud *et al*. 2011), *D. melanogaster* (Camus *et al*. 2017), and *Fundulus heteroclitus* (Dhillon & Schulte 2011).

The economically important small brown planthopper (SBPH), *Laodelphax striatellus* (Fallén), shows mito-nuclear discordance across populations in China. In a previous study (Sun *et al.* 2015), we used mitochondrial (partial sequence of *COI* and *COII* gene) and nuclear (microsatellites) markers to show that populations from the moderate temperate (MT) zone were significantly different from other climatic zone populations for mtDNA, whereas there was no such pattern for nuclear microsatellites. Further analyses revealed two mitochondrial haplogroups in SBPH populations, with one mitochondrial haplogroup (HGI) enriched in the MT zone despite apparently strong nuclear gene flow. Normally, potential reasons for such a pattern include mitochondrial introgression from a closely-related species, male-biased dispersal, *Wolbachia* infection, demographic expansion, and/or selection on mtDNA (Toews & Brelsford 2012). By ruling out effects associated with genetic structure in male populations, *Wolbachia* endosymbionts and other biological characteristic of this pest (Sun *et al.* 2015), we proposed that selection on mitochondrial DNA that overwhelms gene flow is a likely explanation for the mito-nuclear discordance population structure.

To test this proposal, we have now sequenced 81 unique complete mitochondrial genomes to reveal the evolutionary history of the haplotypes and identify non-synonymous mutations associated with divergence of the two mitochondrial haplogroups. The factors affecting geographical distributions of the non-synonymous mutations were also investigated. We then constructed near-isogenic lines to measure the effect of the non-synonymous mutations on cold resistance and fecundity, and we compared mtDNA copy abundance of SBPH under laboratory conditions.

2 | MATERIALS AND METHODS

2.1 | Sample collection and DNA extraction

SBPH populations were sampled from 17 locations in China and 6 locations in Japan (Figure 1, Supporting information Table S1). Most of the Chinese populations (14/17) were sampled in 2010, except for MDJ (2012), YJ (2010) and XZ (2012); all Japanese populations were sampled in 2017. These populations were sampled by randomly collecting adults from rice plants in a 10×10 m square. Samples were immediately preserved in absolute ethanol and then stored at -20 ℃ in the laboratory until DNA extraction. In all, 1064 adult females were collected, with sample sizes ranging from 40 to 48 per population. Details of sampling information are presented in Supporting information Table S1. Total genomic DNA was isolated from the combined head and thorax of each individual using a modified CTAB method.

2.2 | Mitochondrial genome amplification and sequencing

In order to identify the non-synonymous changes associated with the divergence of the two mitochondrial haplogroups, 81 of the 107 different unique mitogenomes, which were characterized by partial COI and COII sequences in our previous work (Sun *et al.* 2015), were PCR amplified and sequenced. Briefly, we randomly selected one individual belonging to different mitochondrial haplotypes for complete mitochondrial genome amplification. The complete mitogenome was produced from two overlapping regions using two pairs of primers (Supporting information Table S2) designed from a previously-reported mitogenome sequence (Zhang *et al.* 2013). Each PCR amplification was performed in a total volume of 50 μL PCR mix containing 1 × Gflex PCR Buffer, 1.25 U TKS Gflex DNA polymerase (TaKaRa, China), 0.3 μM of each primer and approximately 10 ng of genomic DNA. The thermal profile used an initial denaturation step of 94 °C for 2 min followed by 30 cycles of denaturing at 98 °C for 10 s, annealing at 60 °C for 15 s, and extension at 68°C for 8 min.

Amplicons were purified separately with a SanPrep Column PCR Product Purification Kit (Sangon Biotech, China), quantified by Nanodrop and then pooled in approximately equimolar quantities. Pooled amplicons for each individual were sent to BGI Company (China) for sequencing with the Illumina Hiseq 2000 platform. We sequenced one gigabase of data for each mitogenome. Clean sequence reads (in fastq format) obtained from the mitogenome amplicons of each individual were assembled and annotated in Geneious 8.1.7 (Biomatters Ltd.) by using published mitogenome sequence of the SBPH (GenBank Accession No. JX880068) as references. The Highest Quality option under the Consensus

threshold option was selected for mitogenome assembly, which led to mapping of the highest quality base at each column onto the reference sequence. The minimum coverage for each base was about 1000×. Due to the high A-T richness and the existence of a repeat region (~ 2.8 Kb) in the SBPH mitogenome, it was difficult to assemble the control region well. The sequences of the 13 protein coding genes were therefore extracted from the assembled mitogenome of each planthopper, aligned individually, and concatenated for subsequent analyses. Nucleotide diversity was calculated by DnaSP 5.10 (Librado & Rozas 2009).

2.3 | Phylogenetic and selection analyses of mitogenome

To infer phylogenetic relationships among SBPH haplotypes, SPLITSTREE version 4.13.1 (Huson & Bryant 2006) was firstly used to construct splits networks with the neighbor-net method under a distance model of K2P with 1000 bootstraps. This method provides a framework for evolutionary analysis using both trees and networks, accounting for not only mutation but also gene genesis, loss and duplication events, hybridization, horizontal gene transfer or recombination (Huson & Bryant 2006). Then a phylogenetic tree was constructed using maximum likelihood (ML) and Bayesian inference (BI) methods with two other planthoppers *Nilaparvata lugens* (JX880069) and *Sogatella furcifera* (KC512915) being used as outgroups. Then trees were rooted by *N. lugens* according to the evolutionary relationship of the three species inferred from the whole genome sequence (Zhu *et al.* 2017). PartitionFinder v1.1.0 (Lanfear *et al.* 2012) was run prior to phylogenetic analyses to find the most optimal partitioning schemes and models of molecular evolution using the

Bayesian Information Criterion. Two partition schemes respectively under GTR+I+G model and GTR+G model were identified and used for the following analyses (Supporting information Table S3). BI analysis was run in MrBayes 3.2.6 (Ronquist *et al.* 2012) through the CIPRES Science Gateway (Miller *et al.* 2015) for 20 million generations with a sampling frequency of 200 thousand generations after five million generations of burn-in. ML analysis was conducted in raxmlGUI1.3 (Silvestro & Michalak 2012) with 1000 bootstraps respectively.

Strong purifying selection acts to preserve mitochondrial function and can mask positive selection acting on individual sites, and positive selection on protein coding genes is also frequently transient or episodic, occurring at a few time points and affecting a few codons. We therefore used a mixed effects model of evolution (MEME) (Murrell *et al*. 2012) implemented in the DATAMONKEY server (http://www.datamonkey.org/meme) to identify selection sites along the phylogenetic tree. Codons with $P < 0.05$ were considered to have experienced positive selection.

2.4 | Adaptive selection analyses under a population genetic framework

We identified three non-synonymous changes respectively in the *ND2*, *ND5* and *CYTB* genes, which are closely associated with the divergence of two mitochondrial haplogroups. To further validate whether these changes were responding to climatic adaptation at the population level, we analyzed genetic variation of the three genes in 17 Chinese populations and 6 Japanese populations (Figure 1). The complete *ND2* gene, and nearly complete *CYTB* gene, as well as a partial *ND5* gene, were amplified by PCR and sequenced

in two directions. PCR primers are listed in Supporting information Table S4. DNA sequences were assembled and aligned with Geneious 8.1.7 (Biomatters Ltd.). Then the genealogical relatedness between haplotypes was constructed by PopART software (http://popart.otago.ac.cn/) through the median-joining method (Bandelt *et al.* 1999).

To determine whether the geographical distributions of the non-synonymous changes are shaped by climate, we used partial Mantel tests to quantify the effect of temperature on the geographical distribution of non-synonymous changes. Partial Mantel tests performed in the R VEGAN package were used to quantify the association between differences in frequency of the non-synonymous changes and absolute differences in the mean temperature of coldest quarter (Bio11) controlling for geographical distance among populations. The mean temperature of the coldest quarter for each sampling location was derived from the online WORLDCLIM database (www.worldclim.org).

To determine whether population differentiation is associated with temperature, partial Mantel tests were also used to analyze the association between population genetic differentiation and absolute differences in BIO11 controlling for geographical distance. Population genetic differentiation was quantified using pairwise F_{ST} values in Arlequin 3.5 (Excoffier & Lischer 2010).

Because partial Mantel tests are susceptible to pathogenic type I errors (Guillot and Rousset 2013), we also undertook structural equation modelling method (SEM) to resolve the relative contributions of low temperature and geographical isolation to variation in

frequencies of non-synonymous changes and population genetic differentiation (F_{ST}). We performed the SEM analysis using models that tested three alternative hypotheses following Wang *et al*. (2013): (1) only geographical distance contributes significantly, (2) only absolute differences in the mean temperature of coldest quarter (Bio11) contributes significantly, and (3) both geographical distance and difference in Bio11 each contribute significantly. The SEM tests performed in the R SEM package were used to quantify the associations between differences in frequency of the non-synonymous changes and absolute differences in the mean temperature of coldest quarter (Bio11) and geographical distance among populations.

2.5 | In silica protein effect predictions

In order to explore the functional significance of the three proposed sites under selection, we first used protein homology modelling to visualize the potential functional implications. We generated a sequence alignment between human mitochondrial DNA (NC_012920) and the sequences that we generated for this study. Alignments were performed with CLC Genomics Workbench 11.0.2 (Qiagen, Inc.), and then mapped onto the human mitochondrial supercomplex PDB 5xth (Guo *et al.* 2017). Alignments (Supporting information Figure S1) demonstrate high homology for the regions of interest in *ND2* and *CYTB* but not *ND5*. Because the nonsynonymous change site in the SBPH *ND5* gene is located on an indel in the alignment with the reference sequence (Supporting information Figure S1), it was difficult to conduct protein structure modelling for this gene. Molecular graphics and analyses were performed with UCSF Chimera, developed by the Resource for

Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH P41-GM103311 (Pettersen *et al.* 2004). Amino acids were altered on the structure using the rotamer function in the structure editing command.

Secondly, the PROVEAN web server (Choi & Chan 2015) (http://provean.jcvi.org/index.php), which uses an alignment-based score approach (Choi *et al.* 2012), was also applied to predict the functional significance of the three non-synonymous changes.

2.6 | Near isogenic isofemale line construction

In order to separate effects of mtDNA from any remaining nuclear effects, we also used introgression to construct near isogenic isofemale lines. The experimental setup of mitochondrial isofemale and near isogenic isofemale line construction are shown in Supporting information Figure S2 and S3. Briefly, two geographically distinct populations from the South (Chuxiong, Yunnan province, CX) and North (Harbin, Heilongjiang province, YS) of China were used (sampling location: Supporting information Figure S4). The South population (CX) (> 100 individuals) was sampled from the southern subtropical zone of China and maintained in the lab for more than 10 years under laboratory conditions (maintained with a population size > 2000 individuals, 25 °C, 60% RH, and a photoperiod of 16:8 [L:D] h) before isofemale lines were established. The North population (YS) (>100 individuals) was sampled from the moderate temperate zone (Harbin, Heilongjiang province) in 2013 and reared through two generations to large numbers (> 400 individuals) before isofemale lines were set up. We identified isofemale lines with each

of the two mitochondrial haplotypes in each population. Mitochondrial haplogroups were identified from the common associated non-synonymous changes identified in *ND2*, *ND5* and *CYTB* gene sequences of the mother. Ten isofemale lines with each of the two mitochondrial haplotypes were constructed per population. Then, the offspring of the 10 isofemale lines belonging to the same mitochondrial haplotype and population were combined to form one line and reared two generations to large numbers for line comparisons. By combining multiple lines we aimed to randomize the nuclear genetic background of the background population associated with each mitochondrial type as much as possible. The two lines were designated as "MitINuI" and "MitIINuII", with MitI/MitII reflecting the haplogroups and NuI/NuII the remaining differences in nuclear background. These lines were generated for each population and used in the comparisons of mitochondrial and nuclear effects.

For each population, the two mitochondrial types were also introgressed into the reciprocal nuclear genetic backgrounds. Briefly, a single randomly selected virgin female from one line ("MitINuI" or "MitIINuII") line was mated to a male from another line, and introgressive backcrossing was repeated for 8 generations, theoretically replacing 99.61% of the original nuclear genome with the paternal nuclear genome. To confirm genetic identity, all lines were tested every two or three generations by PCR amplification and sequencing of the *ND2* gene of the mother during the introgression process. We designated the two mitonuclear introgression lines as "MitINuII" and "MitIINuI". In summary, we constructed eight SBPH lines, i.e. YS-MitINuI, YS-MitIINuI, YS-MitINuII, YS-MitIINuII, CX-MitINuI, CX-MitIINuI, CX-MitINuII, and CX-MitIINuII. These lines were reared

through two generations to large numbers (> 400 individuals) and then subsequently used in the following fecundity, cold resistance and mtDNA abundance comparisons.

2.7 | mtDNA copy abundance comparison

mtDNA copy abundance was compared among the four lines we constructed for each population for both sexes. To avoid the impacts of NUMTs, a whole-genome sequencing technique described by Reznik *et al* (2016) with minor modification was used. In brief, the relative mtDNA copy number was inferred by calculating the ratio of the average sequencing depth of the mitogenome to the depth of nuclear genome. Three single copy genes (EOG090W0CRM, EOG090W05NZ, and EOG090W036S) which were identified from the genome of SBPH by BUSCO software（http://busco.ezlab.org/）were used for the estimates of the sequencing depth of nuclear genome. We selected these three genes because they were fully sequenced across all samples. Five newly-eclosed females or males were collected within 24 hours for DNA extraction, and subsequently sent to Biozeron Company (Shanghai, China) for DNA library construction and sequencing. Three biological repeats were performed for both sexes of each line. For each sample, 4.3-12.3 million clean reads (with an average value of 7.3 million) were obtained.

2.8 | Chill coma recovery

In order to compare cold resistance between different lines, we measured the amount of time it takes a female to regain activity after chill coma as a proxy of cold resistance following the method of Huang *et al* (2017). Briefly, newly eclosed females within 12

hours were used for cold resistance tests. Prior to tests, individuals were acclimated at 4 °C for 2 h. After that, they were placed in a 0℃ ice water mixture bath for 2 h, which put all individuals into coma. Samples were then tipped onto a Petri dish for recovery at 25℃. When planthoppers stood up or showed all legs shaking vigorously, they were considered recovered and the time was noted. We tested 9-12 samples with 3-5 biological replicates for each SBPH line (N = 287).

2.9 | Fecundity and hind tibial length

To determine whether the two types of mitochondria affected SBPH fecundity, we measured the number of eggs laid in the first 15 days. Briefly, for each of the lines, 4th instar nymphal planthoppers were picked and reared individually in a test tube containing rice seedlings, and checked every 12 hours for emergence (to ensure they were virgins). Once emerged, pairs of newly eclosed females and males from the same line (theoretically having the same genetic background) were introduced into a test tube (34 mm × 140 mm) containing two rice seedlings, where planthoppers were allowed to mate and lay eggs into the rice seedlings at 25 °C, 16L: 8D for 3 days. After that, the rice seedlings were renewed every two days until the 15th day, and replaced rice seedlings were dissected under a microscope to check the number of eggs. We scored 26-62 pairs for each SBPH line (N = 315).

To test whether there was body size variation among lines, we measured the hind tibial length of adult females as a proxy of body size. Adult females were collected 2 - 3 days following emergence, and preserved in absolute ethanol and then stored at -20 ℃ for measurement. We scored the hind tibiae with a Nikon SMZ 1270 microscope, using 26-44 individuals for each SBPH line (N = 259).

2. 10 | Data statistics

Generalized linear models (GLMs) were used to analyze fecundity, and mtDNA copy abundance, and test the effect of nuclear genotype, mtDNA genotype, and population source. The nuclear effect of line background was nested within population. In addition, the effect of sex on mtDNA copy abundance was also analyzed. A Generalized linear mixed model (GLMM) was used to analyze effects on cold coma recovery time. For this trait, a GLMM was used because different batches of individuals were considered as a random factor. GLMs and GLMM were performed in SAS (Version 9.3). In addition, Kruskal-Wallis implemented in R 3.5.0 (R Development Core Team 2018) were used for pairwise comparisons of mitochondrial haplotypes under the same nuclear background.

3 | RESULTS

3.1 | Mitogenome evolution

Eighty-one unique mitochondrial genomes were successfully amplified and sequenced (GenBank accessions: MK292897 - MK292977). The total length of the concatenated sequences of 13 mitochondrial protein genes (without stop codons) was 10, 839 bp, with no

indels or premature stop codons being observed. SNPs were found at 246 sites, and the nucleotide diversity was 0.286%.

Phylogenetic analyses by SPLITSTREE further confirmed the existence of two divergent mitochondrial lineages, HGI and HGII, with 100% bootstrap support (Figure 2B). The mean K2P genetic distance between the two lineages was 0.4%, indicating typical intraspecific divergence. Relatively higher nucleotide diversity was detected in HGI (0.189%) relative to HGII (0.117%). Further phylogenetic analyses by the BI and ML methods consistently revealed that the two lineages constituted a monophyletic group, with HGII nested within HGI. (Figure 2A). Nine SNP sites, including eight synonymous changes and one non-synonymous change (nucleotide: A277T, amino acid: M93L) located on *ND5* gene, were identified as delineating the two lineages. In addition to the nine divergent sites, variants were also nearly fixed in either of the two lineages for another 19 SNP sites, with a difference in SNP frequency >80% between the two lineages. Of the 19 SNP sites, 17 were associated with synonymous variation, whereas only two were associated with non-synonymous variation, located on the *ND2* (nucleotide: T341C, amino acid: M114T) and *CYTB* (nucleotide: T691A, amino acid: F231I) gene respectively. Collectively, three non-synonymous changes in the *ND2*, *ND5* and *CYTB* genes were identified to be closely associated with the divergence of the two lineages (Figure 2A).

Of the three non-synonymous change sites, only the site 114 in *ND2* was identified as subject to episodic positive selection by the MEME method. Another 12 sites were also identified (Supporting information Table S5).

3.2 | Adaptive selection analyses in population genetic framework

To further explore evolution of the three genes in the field, sequences involving the complete *ND2* gene (957 bp), partial *ND5* gene (400 bp) and nearly complete *CYTB* gene (1060 bp) were successfully isolated for 1054, 1064, and 1041 samples respectively representing 23 geographical populations (Supporting information Table S1). In total, 74, 31, and 86 haplotypes were identified for the *ND2*, *ND5* and *CYTB* genes respectively (Figure 3). Generally, the SBPH populations had moderately high haplotype diversity and low nucleotide diversity for *ND2* (0.780 and 0.297%), *ND5* (0.596 and 0.191%), and *CYTB* (0.736 and 0.302%) genes respectively (see Supporting information Table S1 for details). The three amino acid substitutions associated with divergence of the two haplogroups were nearly in complete linkage disequilibrium (90.1%) in the field (Supporting information Figure S5).

Based on the three non-synonymous changes, we divided the haplotypes into two subgroups. Although both subgroups were distributed widely across the five climatic zones, different topological patterns were observed. The HGI subgroup displayed a star-like pattern, dominated by one haplotype. In contrast, the HGII subgroup consisted of several sub-lineages (Figure 3).

Clinal variation in the frequency of the two haplogroups was observed in the Chinese populations, with the HGI-associated non-synonymous changes increasing in frequency with latitude; however, this pattern was not observed in Japanese populations, where HGII was predominant across all populations, with the most northern population (SAP) having the highest HGII frequency (Figure 1). A partial Mantel test revealed that differences in the

frequency of non-synonymous changes was significantly correlated with the mean temperature of coldest quarter (Bio11) when the effect of geographical isolation was excluded (for *ND2*: r = 0.419, *P* = 0.001; for *ND5*: r = 0.597; *P* = 0.001; for *CYTB*: r = 0.351, *P* = 0.002; Figure 4a-c), suggesting that minimum temperature affects the geographical distribution of the non-synonymous changes. However, when the effect of the differences in Bio11 between populations was excluded, weaker but significant correlations were also detected between differences in the frequency of non-synonymous change and geographical distance except for *ND5* (for *ND2*: r = 0.263, *P* = 0.005; for *ND5*: r = 0.159; *P* = 0.058; for *CYTB*: r = 0.290, *P* = 0.01, Figure 4d-f). These results imply that both temperature and geographical isolation played roles in shaping the geographic distributions of the non-synonymous changes.

Stronger genetic differentiation between China and Japan (mean F_{ST} of *ND2*: 0.195, *ND5*: 0.227, *CYTB*: 0.241) than among Chinese populations (mean F_{ST} of *ND2*: 0.123, *ND5*:0.147, *CYTB*: 0.107) or among Japanese populations (mean F_{ST} of *ND2*: 0.076, *ND5*:0.198, *CYTB*: 0.076) were observed. A partial Mantel test also revealed that population genetic differentiation (F_{ST}) was significantly correlated with differences in Bio11 when the effect of geographical isolation was excluded (for *ND2*: r = 0.604, *P* = 0.001; for *ND5*: r = 0.528, *P* = 0.001; for *CYTB*: r = 0.557, *P* = 0.002, Figure 5a-c). To explore specifically whether the evolution of the HGII mtDNA is associated with climatic adaptation (based on data presented below), we also performed a partial Mantel test after removing HGI. In support of this hypothesis, strong correlations were observed between population genetic differentiation (F_{ST}-HGII) and minimum temperature for *ND2* and

CYTB (*ND2*: r = 0.767, P = 0.001; *CYTB*: r = 0.761, P = 0.001, Figure 5d and 5f), but not for *ND5* (r = -0.016, P = 0.534, Figure 5e). In addition, no significant correlation was detected between F_{ST} and geographical distance for all three genes when differences in minimum temperature between populations were excluded (for *ND2*: r = -0.012, P = 0.498; for *ND5*: r = 0.126, P = 0.159; for *CYTB*: r = 0.001, P = 0.46).

Likewise, similar results were obtained through the SEM analysis. For variation in the frequency of non-synonymous changes, the full model including both Bio11 and geographical distance was suggested as providing a significantly better fit to the data based on model-fit scores (AIC) than a model including only one or the other variable (Supporting information Table S6) for each gene. This shows that differences in the frequency of non-synonymous changes were both significantly explained by the mean temperature of coldest quarter (Bio11) and geographical isolation, with Bio11 explaining a higher proportion of the variation (Table 1), suggesting that minimum temperature plays a relatively more important role in shaping the geographical distribution of non-synonymous changes.

The SEM analyses also revealed that F_{ST} variation was significantly explained by Bio11 and geographical distance for the *ND2* and *CYTB* genes, with a higher proportion explained by Bio11 (Table 1). For the *ND5* gene, model-fit scores (AIC) suggested a model including only Bio11 provided the best fit, in which 59.1% of the variation was accounted for.

For F_{ST}-HGII, model-fit scores (AIC) suggested a model including only Bio11 optimal for the *ND2* and *CYTB* genes, but a model including only geographical distance was better for the *ND5* gene. For *ND2* and *CYTB*, more than 77% of F_{ST}-HGII variation could be explained by Bio11. In contrast, a weak non-significant effect of geographical distance was detected for *ND5* (Table 1).

Considering the difference in sampling time between the Chinese and Japanese populations, we also analyzed the associations between population genetic differentiation and both temperature and geographical distance for each of the two countries separately. Both the partial Mantel tests (Supporting information Table S7, S8) and SEM analyses (Supporting information Table S9) showed that population genetic differentiation was more closely related with temperature in both countries, particularly in the case of the *ND2* and *CYTB* genes, although the significance of patterns for the Japanese populations was lower and the haplotype pattern was different as noted above.

3.3 | Protein homology modelling

The three identified amino acid changes were mapped onto the protein three-dimensional crystal structure of human mitochondrial supercomplex PDB 5xth. The *ND2* and *ND5* genes encode subunits of mitochondrial complex I, which constitute two of the four proton pumps in complex I (Figure 6a). The amino acid change M114T in *ND2* projects into space that is likely occupied by the N-terminal portion of *NDUFA10* (Figure 6a, Supporting information Figure S1a). Although interactions are likely between *ND2* and *NDUFA10*, these not possible to predict with accuracy because in all of the currently available structures for vertebrate complex I, the resolution here is poor.

Due to low homology, M93L in *ND5* is difficult to predict but may affect the movement of the beta-H loop structure (Figure 6b, Supporting information Figure S1b). The *CYTB* gene encodes the core subunit of mitochondrial complex III (Figure 6c, shown here as a dimer, Supporting information Figure S1c). It contains heme residues that participate in the transfer of electrons from the mobile carrier quinone to cytochrome c, which accepts them from a heme unit in cytochrome c1 (*CYC1*) and passes them to complex IV. We speculated that F231I in *CYTB* might interact with residues in *CYC1*, probably altering the proximity of *CYC1* to *CYTB* via steric hindrance given that an isoleucine would produce less steric hindrance than a phenylalanine.

A PROVEAN analysis suggested that the amino acid replacement M114T in *ND2* was likely to alter protein functioning, with a PROVEAN score of -3.246. However, no significant effect was detected for the two amino changes in *ND5* and *CYTB*.

3.4 | Cold resistance

A generalized linear mixed model (GLMM) analysis revealed that the mitochondrial genotype (Mit), population source (Pop), and the interaction between mitochondrial genotype and nuclear background (Mit × Nu) significantly affected recovery time (Table 2, Figure 7a). However, in contrast to our hypothesis, SBPH with HGII mitochondria generally recovered more quickly than those with HGI mitochondria, except under the NuI nuclear background in the North population (YS). In addition, a shorter recovery time was observed in the South population (CX) relative to the North population, except for the YS-MitIINuII line of the North population, which had the shortest recovery time.

3.5 | Fecundity and body size comparison

Fecundity was measured as the number of eggs oviposited in the first 15 days. Generalized linear model (GLM) analyses revealed that mitochondrial haplotype, nuclear background, population source and Mit × Nu all significantly affected fecundity (Table 2, Figure 7b), indicative of complicated control of fecundity in SBPH. SBPH with HGII mitochondria laid significantly more eggs than those with HGI mitochondria except for lines with the NuI nuclear genetic background in the CX population (Figure 7b), perhaps contributing to its high frequency in warm regions (Figure 1). And the CX population had relatively higher egg production than the YS population. GLM analyses revealed a weak effect of nuclear background on the length of female hind tibiae ($F_{2,\ 251}$= 4.45, P = 0.013, Table 2, Supporting information Figure S6). No significant effect of mitochondrial type was detected, suggesting fecundity differences were not strongly associated with body size.

3.6 | mtDNA copy abundance

GLM analyses revealed that the mitochondrial type and gender significantly affected mtDNA copy number (Table 2). In both females and males, HGII mitochondria consistently possessed more mtDNA copies relative to HGI, regardless of nuclear genetic background (Figure 8, Table 2). This may indicate a difference in replication and functional efficiency between the two types of mitochondria. And mtDNA copy number was sexually dimorphic, with higher copy numbers in females ($F_{1,\ 35}$ = 21.51, P < 0.0001), consistent with a previous study on *Drosophila melanogaster* (Camus *et al.* 2015).

4 | DISCUSSION

The results reported here provide compelling population genetic and experimental evidence for phenotypic effects involving the mitogenome. We linked genotypic variation to phenotypic variation, and also inferred the evolutionary history of mtDNA variation. We found that a mitochondrial haplogroup (HGII) brings benefits in terms of cold resistance and fecundity. A function-altering amino acid change involving M114T in *ND2* likely plays an important role in this evolutionary process. Furthermore, mito-nuclear epistatic interactions were detected, suggesting they have a role in maintaining mtDNA diversity. Below we discuss these points in detail.

4.1 | Evolutionary advantages of the HGII mitogenomes

Bioassays and population studies both pointed to HGII being involved in cold tolerance. Our chill coma recovery tests revealed that SBPH harboring HGII mitochondria were more cold tolerant than those with HGI. Chill coma recovery time has long and widely been used as a proxy for cold tolerance due to easy experiment setup (Andersen *et al.* 2015; Bechsgaard *et al.* 2013; David *et al.* 1998; Gibert & Huey 2001; Macdonald *et al.* 2004). However, a recent study suggests that although providing an effective measure of cold tolerance of insects, chill coma recovery time might be an inferior proxy of cold tolerance compared to chill coma temperature, lethal temperature, and lethal time at temperature (Andersen *et al.* 2015). Other measures of cold tolerance could therefore be considered in future work. Consistent with the chill coma recovery tests, both the partial Mantel tests and SEM analyses showed that high proportions of variation in population genetic

differentiation were explained by minimum temperature after excluding the HGI sequences, providing population genetic evidence that climate acts on population genetic divergence of HGII mtDNA.

Previous studies on a range of taxa suggest amino acid variation in *ND3*, *ND4*, *ND5*, *ND6*, *CYTB*, and *ATP6* are probably associated with climatic adaptation (Balloux *et al.* 2009; Lamb *et al.* 2018; Morales *et al.* 2015; Silva *et al.* 2014; Sun *et al.* 2018). Those changes are presumed to affect mitochondrial coupling efficiency, coordinating the ratio of ATP synthesis efficiency to thermogenesis efficiency, helping organisms to adapt to different conditions. In this study, three non-synonymous changes in *ND2*, *ND5* and *CYTB* are involved in the divergence of the two mitochondrial haplogroups. Furthermore, multiple independent lines of evidence from the MEME test, PROVEAN analysis, and the relationship between population genetic differentiation and temperature consistently point to M114T in *ND2* as a likely target responding to cold tolerance, probably affecting complex I and supercomplex assembly and activity.

In addition to having higher cold resistance, we also note that HGII mtDNA is associated with an increase in fecundity. This combination of fitness advantages has also been reported in *D. simulans* harboring certain mitochondria (Ballard *et al.* 2007). Although we are unclear about whether the two fitness effects are mechanistically linked, they may both contribute to the high frequency of HGII in the field, except for the MT region of China. The importance of sex in affecting fecundity also remains unclear, although effects mediated through the female are relatively more important given that mtDNA is maternally inherited.

We found that HGII mtDNA possesses a relatively higher copy number than HGI regardless of nuclear background. Higher mtDNA copy number may indicate an advantage of the HGII mitochondrion for either mtDNA replication or mitochondrion proliferation. This advantage is likely the primary driver of the evolution of HGII mtDNA, enabling HGII to outcompete HGI by "selfish" replication at the cytoplasm level (Rand 2001). Recent studies suggest that point mutations in the mitogenome may alter mtDNA copy number and mitochondrial abundance (Camus *et al.* 2015; Salminen *et al.* 2017; Zhu *et al.* 2014). We suspect that the three non-synonymous changes are probably associated with mtDNA copy number variation. But, unlike the situation in *Drosophila* (Camus *et al.* 2015; Zhu *et al.* 2014), copy number variation seems not to be contingent on nuclear background in SBPH. The advantage in replication coupled with the fitness advantage of HGII mtDNA likely account for the positive selection signals detected in *ND2* and the success of the HGII lineage.

4.2 | The geographical distribution of the two mitochondrial haplogroups

Although our results suggested that the HGII mitochondrion was associated with tolerance to cold relative to HGI, the strikingly high frequency of HGI in the MT climate zone was counter-intuitive to our expectation. The exact reason for this pattern is unclear, but there are a couple of possibilities.

Considering that the number of the overwinter SBPHs in the MT climate zone is low, one possibility is that the vast majority of SBPHs occurring during summer are derived from immigrants harboring an HGI mitochondrial variant from a warm region, which may

indicate that the HGI mitochondria are associated with strong migratory ability. In support of this possibility, a link between mitochondrial variation and migration has been demonstrated in warblers, with certain mitochondria characterized as being metabolically efficient (Toews *et al.* 2014). SBPH can undergo long-distance migration from low to high latitudes, and even move from China to Japan (Otuka *et al.* 2010; Zhang *et al.* 2011). Dietary stress induced by wheat harvest is considered the driver for long distance migration (Otuka *et al.* 2010; Zhang *et al.* 2011). Given that the HGI mitochondria in YS are more sensitive to cold in a chill coma recovery test, the immigrants may be relatively cold sensitive. Interestingly, the methionine at position 114 in *ND2* and at position 93 in *ND5* of SBPH HGI mtDNA are also conserved in *Nilaparvata lugens* with different biotypes (Supporting information Figure S7), which cannot overwinter in China and annually migrate to China from south Asia (Hu *et al.* 2014). The high HGI frequency in the YUK population of Japan (Figure 1) may also reflect migration given that mass immigration of SBPHs from Jiangsu Province in China to Japan can occur (Otuka *et al*. 2010). The HGI mitochondrion may therefore be associated with SBPH migration or cold sensitivity or both. In future, tethered flight tests (Jones *et al.* 2015) for migratory ability and continuous monitoring of mtDNA frequency across months in the MT climate zone of China may help distinguish between these possibilities.

Another possibility is that the cold tolerance involving mitochondria might not be the dominant force in shaping the graphical distribution of the mitochondrial haplogroups. Instead, a population demographic change might be involved, which involves a gradual northward spread of the newly evolved HGII mitochondria with a fecundity advantage

from low latitude areas. If that is the case, HGI might gradually be replaced by HGII in the MT zone. However, our preliminary monitoring showed that the HRB population showed only minor changes in frequency in three years of sampling (2010, 2012 and 2017, Supporting information Figure S8). Seasonal (or monthly) sampling and further long-term monitoring of frequency changes may help develop a fuller understanding of the evolutionary history of the SBPH mitogenome. Also, other selective factors in addition to temperature might be involved. A recent study in seed beetles has suggested negative frequency-dependent selection as a mechanism for maintaining mtDNA polymorphism (Kazancioglu & Arnqvist 2014), but this remains to be tested in SBPH.

4.3 | Mito-nuclear epistatic interactions in SBPH

We found that fecundity and cold resistance changed after the nuclear background was replaced for the two mitochondrial haplotypes in the two tested populations. For instance, the fecundity of the MitINuII line of the YS population exhibited a sharp decrease relative to fecundity in its original mito-nuclear combination (MitINuI). This line was hard to maintain in the laboratory, perhaps representing the disruption of mito-nuclear interactions and an early stage of cytonuclear hybrid breakdown (Burton *et al.* 2013; Calvo & Mootha 2010; Hill 2015).The genetic mechanisms for mito-nuclear epistatic interactions lie in: 1) the transcription of mitochondrial genes depending on the mitochondrial (mt) RNA polymerase/mt transcription factors encoded by nuclear genes, 2) loading of tRNAs with correct amino acids as a key step in the translation of mt genes needs aminoacyl tRNA synthetase (nuclear) and tRNAs (mt), 3) the translation of mt genes into polypeptides

which needs the cooperation between ribosomal proteins (nuclear) and rRNA (mt), and 4) OXPHOS requires the coordination of the products of nuclear and mt genes (Burton & Barreto 2012; Hill 2015; Rand *et al.* 2004). Although associations between the mitochondrial and nuclear DNA does not necessary mean epistatic interactions (e.g. between *D. melanogaster* and the *D. simulans* species complex, (Montooth *et al*. 2010)), epistatic interactions have been documented in flies, copepods, seed beetles and other organisms (Dowling *et al*. 2007; Ellison & Burton 2008; Kazancioglu & Arnqvist 2014).

However, unlike most previous studies in which the associated mtDNA lineages have diverged substantially, two mitochondrial lineages with limited divergence are involved in SBPH, with a mean K2P genetic distance of only 0.4%.

The reason for mito-nuclear epistatic interactions at the intra-population level in SBPH is unclear. Given the X0 sex determining system of SBPH (Noda & Tatewaki 2008), the probability that some mito-nuclear interaction related genes (N-mt) are located on an X chromosomal position may contribute (Hill 2015). If this is the case, a high probability (67%) of co-transmission of X chromosome linked N-mt genes and mitochondrial genes may promote coadapted N-mt and mt genes. The sequential introgression we carried out for eight generations might disrupt such mito-nuclear coadaption, leading to mito-nuclear incompatibility at the intra-population level, although this might be insufficient if there is strong mito-nuclear coevolution (Camus *et al.* 2017). In the future, a fine scale genomic analysis at the chromosomal level might provide relevant information.

The results also suggest SBPHs may represent a useful model to investigate mechanisms behind mito-nuclear interactions. A genome-wide population genetic study could be useful to search for targets responsible for the mito-nuclear epistatic interactions in SBPH, and to test whether rapid mitochondrial evolution drives compensatory or coadaptive nuclear evolution. Although mechanisms underlying interactions are unclear, our protein modelling predicts that the amino acid changes in *ND2* and *ND5* may interact with the residues of nuclear coded subunits *NDUFA10* and *CYC1* respectively, which might be associated with reduced complex I assembly and activity, and be responsible for the mito-nuclear interactions in SBPH. In support of this result, ND2/ND10 subunits were found to be part of mito-nuclear co-evolution in salmonids (Garvin *et al.* 2017).

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AUTHOR CONTRIBUTIONS

JTS and XYH designed the research; JTS, XZD and LC collected samples; XZD, CL, YL and JTS performed the research; JTS and LC conducted the genetic and phylogeny analyses and AAH advised on interpretations; MRG conducted the protein modeling analysis; JTS and JCZ conducted the bioassay data. JTS, AAH, MRG and XYH wrote the paper.

DATA ACCESSIBILITY

The DNA sequence of the 81 mitochondrial genomes have been deposited in GenBank (Accessions: MK292897 - MK292977). The sequences of *ND2*, *ND5* and *CYTB* genes used for population genetics study, chill coma recovery time, fecundity data, and mtDNA copy abundance data are all deposited at Dryad (doi:10.5061/dryad.72ct11m).

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Tables and Figures

Table 1. The proportion of variations of non-synonymous change frequency and genetic differentiation explained by temperature difference and geographical distance in *ND2*, *ND5* and *CYTB* genes among populations.

Variation	Factor	Gene		
		ND2	*ND5*	*CYTB*
Differences in non-synonymous change frequencies	Bio11 difference	0.403±0.053 (*P* = 3.70e-14)	0.594±0.048 (*P* = 7.47e-35)	0.333±0.054 (*P* = 1.20e-9)
	Geographical distance	0.235±0.053 (*P* = 1.06e-5)	0.117±0.048 (*P* = 1.53e-2)	0.266±0.054 (*P* = 1.13e-6)
Population Genetic differentiation (All)	Bio11 difference	0.621±0.047 (*P* = 3.65e-40)	0.591±0.051 (*P* = 2.51e-31)	0.424±0.048 (*P* = 2.03e-18)
	Geographical distance	0.114±0.046 (*P* = 0.011)	-	0.351±0.048 (*P* = 4.33e-18)
Population Genetic differentiation (HGII)	Bio11 difference	0.775±0.040 (*P* = 1.31e-84)	-	0.771±0.040 (*P* = 1.32e-82)
	Geographical distance	-	*0.103±0.063 (P = 0.10)*	-

For each gene, SEM was used to quantify the proportion of variation of non-synonymous change frequency and genetic differentiation explained by Bio11 difference and geographical distance respectively, presented as maximum likelihood estimates ±standard errors (values in italics are non-significant). For the cases in which only one factor model fits best, we just show the values for corresponding factors.

Table 2. The effects of mitochondrial haplotype, nuclear genetic background (nested within population), and source of population on SBPH chill-coma recovery time, number of eggs oviposited per female, mtDNA copy abundance.

	Mit	Nu	Pop	Mit×Nu
Chill-coma recovery time	**$F_{1, 275}$ = 20.16, *P* < 0.0001**	$F_{2, 275}$ = 1.11, *P* = 0.331	**$F_{1, 275}$ = 18.58, *P* < 0.0001**	**$F_{2, 275}$ = 14.91 , *P* < 0.0001**
Number of eggs oviposited per female	**$F_{1, 307}$ = 23.91, *P* < 0.0001**	**$F_{2, 307}$= 10.10, *P* < 0.0001**	**$F_{2, 307}$ = 43.46 *P* < 0.0001**	**$F_{2, 307}$ = 3.68, *P* = 0.027**
Hind tibial length	$F_{1, 251}$ = 0.01, *P* < 0.915	**$F_{2, 251}$= 4.45, *P* = 0.013**	$F_{2, 251}$= 1.17, *P* = 0.281	$F_{2,251}$ = 0.01, *P* = 0.937
mtDNA copy abundance	**$F_{1, 35}$ = 117.12, *P* < 0.0001**	$F_{2, 35}$= 0.97, *P* = 0.391	$F_{1, 35}$= 2.38, *P* = 0.132	$F_{2, 35}$= 0.51, *P* = 0.603

Mit represents the mitochondrial haplotype. Nu represents the nuclear genetic background. Pop represents population source. Mit×Nu represents the interaction between the mitochondrial haplotype and (nested) nuclear genetic background. Significant values are in bold.

Figure legends

FIGURE 1 Geographical distribution of the three non-synonymous substitutions in *ND2*, *ND5* and *CYTB*. (a) Chinese populations. (b) Japanese populations. Bio11, the mean temperature of coldest quarter; MT, moderate temperate climate; WT, warm temperate climate; NS, northern subtropical climate; MS, middle subtropical climate; WC, warm continental climate; TC, temperate continental climate, WO, warm oceanic climate. Yellow indicates the population frequency of the amino acid type belonging to HGI, and blue the population frequency belonging to HGII. The frequency bars are arranged in order of sampling location latitudes.

FIGURE 2 Phylogenetic relationships among different mitogenome haplotypes. (a) ML phylogenetic tree inferred from the concatenation of 13 mitochondrial protein genes. The branch length is omitted. Branches with the yellow background belong to HGI and with the blue background belong to HGII. Both the ML and BI trees support monophyly, and suggest that HGII is recently derived from HGI. The nonsynonymous changes associated with divergence of the two lineages are labeled near each branch. Symbols with different shapes represent different amino acid sites in each gene, colored to reflect the common haplogroup with that variant. The numbers at nodes indicate maximum likelihood bootstrap values higher than 60 (based on 1,000 replicates) and Bayesian posterior probabilities

higher than 0.80. (b) Split network for haplotypes based on the concatenation of 13 mitochondrial protein genes. Bootstrap values higher than 90 (based on 1,000 replicates) are shown near the corresponding branch.

FIGURE 3 Haplotype networks for *ND2, ND5* and *CYTB* gene. The haplotype networks were constructed based on 1041 sequences of the *ND2* gene (957 bp), 1064 sequences of the partial *ND5* gene (400 bp) and 1041 sequences of the partial *CYTB* gene (1060 bp). The genealogical relatedness between haplotypes was constructed by PopART software (http://popart.otago.ac.cn/) through the median-joining method. Color key: MT, moderate temperate climate (China); WT, warm temperate climate (China); NS, northern subtropical climate (China); MS, middle subtropical climate (China); WC, warm continental climate (Japan); WO, warm oceanic climate (Japan). A black crosshatch indicates a synonymous change, and a red crosshatch indicates a non-synonymous change. The sizes of circles are proportional to haplotype frequencies.

FIGURE 4 Relationship between the absolute difference in non-synonymous change frequencies and the absolute difference in mean temperature of coldest quarter (a-c) and geographical distance (d-e). Bio11, the mean temperature of coldest quarter; CHN.CHN indicates pairwise comparisons of Chinese populations; CHN.JP indicates pairwise comparisons between Chinese and Japanese populations; JP.JP indicates pairwise comparisons of Japanese populations.

FIGURE 5 Relationship between population genetic differentiation and absolute difference in mean temperature of coldest quarter. (a-c): F_{ST} values calculated using all sequences; (d-f): F_{ST} values calculated using sequence only belonging to HGII. Population designations follow Figure 5.

FIGURE 6 Three-dimensional crystal structure of mitochondrial complex I and cytochrome bc1 (Complex III) from humans. Models of the amino acid change (a) M114T in *ND2*, (b) M93L in *ND5*, (c) F231I in *CYTB*. The mitochondrial-encoded subunits of human complex I that comprise the four proton pumps are shown in light blue (*ND1/ND3/ND4L*, *ND6*), purple (*ND2*), green (*ND4*) and navy blue (*ND5*). Yellow residues trace the likely proton pathway through the central core of the complex. Magenta residues mark the three amino acid changes sites in *ND2*, *ND5* and *CYTB* of SBPH. The amino acid change M114T in *ND2* projects into space that is likely occupied by the N-terminal portion of *NDUFA10*. M93L in *ND5* may affect the movement of the beta-H loop structure. *CYTB* encodes the core subunit of complex III (shown here as a dimer). F231I in *CYTB* appears to interact with residues (orange) in *CYC1* encoded by nuclear genome, which may alter the proximity of *CYC1* to CYTB via steric hindrance.

FIGURE 7 Comparison of cold resistance and fecundity among different SBPH lines in females. (a) Mean chill-coma recovery time of females from SBPH lines. (b) Mean number of eggs oviposited per female of SBPH lines. Nu represents the nuclear genetic background. CX represents the Chuxiong population, YS the Yanshou population. Kruskal-Wallis tests were used for pairwise comparisons between SBPH lines harboring different mitochondria with the same nuclear background. Number of the tested samples were given inside the columns. Bars represent standard errors. ns, not significant; ., P <0.1 **, $P < 0.01$; ***, $P < 0.001$.

FIGURE 8 Comparison of the abundance of mtDNA copy number among different SBPH lines. (a) Females. (b) Males. The mtDNA copy abundance was calculated by the ratio of average sequencing depth of the mitochondrial genome to the average sequencing depth of three nuclear genes. Mit represents the mitochondrial haplotype. Nu represents the nuclear genetic background. CX represents the Chuxiong population, YS the Yanshou population. Three replicates were tested for each line. Kruskal-Wallis tests were used for pairwise comparisons between SBPH lines harboring different mitochondria in the same nuclear background. Bars represent standard errors. ns, not significant; *, $P < 0.05$; ***, $P < 0.001$

SUPPORTING INFORMATION

Figure S1 Alignment of the amino acid sequences for *ND2*, *ND5* and *CYTB* gene between SBPH and human.

Figure S2 Experimental setup of mitochondrial isofemale line construction.

Figure S3 Experimental setup of near isogenic isofemale line construction. Two geographically distinct populations (YS and CX) were used for constructing near isogenic isofemale lines. Ten isofemale lines with each of the two mitochondrial haplotypes were constructed per population.

Figure S4 Sampling locality of the SBPH population. Circles indicate the populations used for population and phylogenetic studies. Triangles indicate the populations used for laboratory bioassays.

Figure S5 Haplotype numbers for amino acid substitution in the *ND2*, *ND5* and *CYTB* genes of SBPH. Symbols with different shapes represent amino acid sites in each gene. Yellow symbols indicate amino acids belonging to HGI, and blue symbols indicate amino acids belonging to HGII. Only samples sequenced for the all three genes were included in the numbers presented.

Figure S6 Comparison of hind tibial length among different SBPH lines in females. Nu represents the nuclear genetic background. CX represents the Chuxiong population, YS the Yanshou population. Kruskal-Wallis tests were used for pairwise comparisons between SBPH lines harboring different mitochondria with the same nuclear background. Numbers of the tested samples are given inside the columns. Bars represent standard errors. ns, not significant.

Figure S7 Amino acid sequence alignment of partial *ND2* and *ND5* genes in two haplogroups of *Laodelphax striatellus* (SBPH) and *Nilaparvata lugens* (BPH).

Figure S8 The frequencies of the three non-synonymous substitution in *ND2*, *ND5* and *CYTB* in the HRB population sampled in 2010, 2012, and 2017.

Table S1 Locations and dates of the collection of small brown planthopper populations and basic genetic diversity indices at the three mitochondrial genes.

Table S2 Primer sequence of mitochondrial genome amplification in SBPH.

Table S3 Partition strategies of mitogenome used in this study.

Table S4 Primer sequence for *ND2*, *ND5* and *CYTB* gene amplification in SBPH.

Table S5 Positive selection sites identified by MEME method.

Table S6. Akaike information criterion (AIC) scores from results of structural equation modeling with three models: geographical distance only, only Bio11, and geographical distance and Bio11 together.

Table S7 Partial Mantel tests analyses of the association between population genetic differentiation (F_{ST}), absolute differences in BIO11 and geographical distance in Chinese populations and Japanese populations.

Table S8 Partial Mantel tests analyses of the association between population genetic differentiation (F_{ST}-HGII), absolute differences in BIO11 and geographical distance in Chinese populations and Japanese populations.

Table S9 The proportion of genetic differentiation explained by temperature difference and geographical distance in *ND2*, *ND5* and *CYTB* genes in Chinese populations and Japanese populations.