High genetic diversity and strong genetic structure of Strongyllodes variegatus populations in oilseed rape production areas of China

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Abstract

**Background:** *Strongylodes variegatus* (Fairmaire) is a major insect pest of oilseed rape in China. Despite its economic importance, the contribution of its population genetics in the development of any suitable protection control strategy for the management of oilseed rape crops is poorly studied. It is a much urgent need to prevent its spread to the rest of the world.

**Results:** Using the sequences of mitochondrial DNA cytochrome c oxidase subunit I (*COI*) and cytochrome b (*Cytb*) as genetic markers, we analyzed the population genetic diversity and structure of 437 individuals collected from 15 *S. variegatus* populations located in different oilseed rape production areas in China. In addition, we estimated the demographic history using neutrality test and mismatch distribution analysis. The high level of genetic diversity was detected among the *COI* and *Cytb* sequences of *S. variegatus*. The population structure analyses strongly suggested three distinct genetic and geographical regions in China with limited gene flow. The Mantel test showed that the genetic distance was greatly influenced by the geographical distance. The demographic analyses showed that *S. variegatus* had experienced population fluctuation during the Pleistocene Epoch, which was likely to be related to the climatic changes.

**Conclusion:** Overall, these results demonstrate that the strong genetic structure of *S. variegatus* populations in China, which is attributed by the isolation through the geographical distance among populations, their weak flight capacity and subsequent adaptation to the regional ecological conditions.

**Background**

The brown beetle, *Strongylodes variegatus* (Fairmaire) (Coleoptera: Nitidulidae), feeds on brassicaceous plant species [1, 2], which often co-occurs with the pollen beetle, *Meligethes aeneus* [3]. The *S. variegatus* adults chew up flowers, buds and leaves, and create crescent-shaped bites where the mature females lay eggs. After hatching, the larvae feed on mesophyll resulting in irregular bubble-shaped wounds before pupation in soil. The wounded leaves become necrotic and abscise prematurely [4, 5]. Recently, the leaf damage of oilseed rape crops by this beetle has become more and more serious, so that it has become a major insect pest of oilseed rape crops. In spring 2013, *S. variegatus* population broke out in Hanshan, Anhui province, destructing 97% of oilseed rape leaves [6].

*S. variegatus* displays specific ecological characteristics to temperature and photoperiod of geographical regions. In spring oilseed rape areas, it reproduces once or twice a year [2]. However, only two generations occur in winter oilseed rape areas [6]. In addition, *S. variegatus* has a high reproductive ability [4] and can fly 30~40 m in 2 min [2]. In Anhui, the overwintering adults begin to appear in March. When the temperature is more than 30°C, the adults stay in soil in summer, and some of them are mixed into the harvested rapeseed. They appear on cruciferous vegetables in September, then move to rape fields and cause damages in October. When the temperature is low in November, they move back to soil and overwinter in soil [4, 6].

*S. variegatus* is generally distributed in the middle and lower reaches of the Yangtze River valley. It was first found on the spring oilseed rape plants in Ningxia, Gansu province, China in 1993 [2], and then on the winter oilseed rape crops in Hanshan, Anhui province in 2008 [4]. For the past few years, we investigated oilseed rape production areas in China and found that this pest has spread to Chongqing municipality and Qinghai, Gansu, Sichuan, Shaanxi, Hubei, Anhui and Jiangsu provinces (unpublished). Currently, it is widely distributed around China but has not yet been found globally in the rest of the world except in China. The phylogeography and population genetics of *S. variegatus* have not been studied. Consequently, it is in urgent demand to conduct the genetics studies and understand the genetic diversity and structure of *S. variegatus* populations in order to manage and control this pest.

Population genetic studies on crop pests can provide information on the spatial scales at which population structure is established and gene flow occurs. Such information can be used in defining relevant strategies for pest control [7]. In addition, genetic diversity contains the information on past and present demography that could be useful to characterize the demographic history of crop pests [8]. In recent years, more and more molecular markers have been used to study insect population genetics, demonstrating the importance of phylogeographical approaches [9]. The insect molecular markers mainly include the sequences of nuclear DNA and mitochondrial DNA (mtDNA). Mitochondrial genes have a faster evolution rate than nuclear genes, and are more informative for studying phylogenetic evolution, especially the degree of inter- and intra-specific population differentiation and the level of gene flow [9, 10]. Thus, the fragments of the mtDNA cytochrome c oxidase subunit I (*COI*) and cytochrome b (*Cytb*) have been widely used among insect molecular markers to study population genetic variation and differentiation of insects, for example, *Dendrolimus kikuchii*, *Chilo suppressalis* and *Agriosophus dohmi* [11-15]. The *COI* and *Cytb* genes were also used to track the colonization routes of *Halyomorpha halys* and to identify the places where the insect has originated [16-18].

In this study, we use *COI* and *Cytb* genes to elucidate for the first time the genetic diversity and structure of 15 *S. variegatus* populations occurring on the oilseed rape production areas in China. We hypothesize that the populations would have a high level of genetic diversity and a clear genetic structure. At the same time, the efficient molecular data collected are used to assess if historical geographic events and
associated ecological adaptations had played an important part in shaping the observed genetic and geographic patterns of this pest in China.

Results

Genetic variation of *S. variegatus* populations

Seventy haplotypes of the *COI* gene and 67 haplotypes of the *Cytb* gene were identified from the 15 populations. The *S. variegatus COI* fragment (652bp) and *Cytb* fragment (421bp) have 45 (6.9%) and 40 (9.5%) variable sites with 28 and 23 parsimony informative sites, respectively (Table 1). The base composition of the two genes is adenine (A) and thymine (T) (67.5% and 73.3%, respectively) biased, which is common for insect mitochondrial genes. The haplotype diversity (*Hd*) ranges from 0.424 to 0.913 (mean = 0.865) and the nucleotide diversity (π) ranges from 0.00072 to 0.00462 (mean = 0.00427) for the *COI* gene (Table 1). Similarly, the *Hd* ranges from 0.464 to 0.833 (mean = 0.834) and π ranges from 0.00119 to 0.00539 (mean = 0.00479) for the *Cytb* gene (Table 1).

Haplotype analyses of the *COI* and *Cytb* genes

The distribution of the haplotypes for the two genes across the populations studied is shown in Table S1. The rarefaction analyses showed that the curves converged on an asymptote (Fig. S1). The *COI* haplotypes (H1-H70) included 34 (48.6%) unique haplotypes (Table S2). The four most frequent haplotypes (H1-H4) were found in 132 (30.2%), 59 (13.5%), 29 (6.6%), and 60 (13.7%) individuals (Table S2; Fig. 2a). The haplotype 1 (H1) was in almost all populations except the populations from GDQH, FJQ and ESHB, whereas the haplotype 2 (H2) was only in the populations from GYSC, HZSX, AKSX, FCCQ, ESHB and LCHB (Table S2). The *Cytb* haplotypes (H1-H67) had 35 (52.2%) unique haplotypes, among which 32 were observed in more than one individual (Table S2). Three most frequent haplotypes (H1-H3) were found in 158 (36.2%), 61(14.0%) and 48 (10.9%) individuals (Table S2; Fig. 2b). The haplotype 1 (H1) was found in all populations except ESHB population, whereas the haplotype 3 (H3) was only discovered in the populations from AQAH, LAAH, HFAH, CHAH, NJJS and ZJJS (Table S1).

The haplotype distribution and haplotype network analyses (see below) of both *COI* and *Cytb* genes revealed that *S. variegatus* populations could be divided into three major geographical distribution regions or haplogroups: the northwestern China (NW) haplogroup (GDQH, HZGS and ZYGS populations), the central China (CC) haplogroup (GYSC, HZSX, AKSX, FCCQ, ESHB and LCHB populations) and the central and eastern China (CE) haplogroup (AQAH, LAAH, HFAH, CHAH, NJJS and ZJJS populations) (Fig. 1).

For the haplotype network of the *COI* gene, there was only one common haplotype (H1) in three haplogroups. The haplotype 2 (H2) was only detected and abundant in the CC haplogroup. The haplotype 3 (H3) was only discovered in the CE haplogroup. There were six common haplotypes (H4-H9) between the NW haplogroup and the CC haplogroup. A total of five missing haplotypes was observed in all populations (Fig. 2a). Similarly, for the haplotype network of the *Cytb* gene, there were two common haplotypes (H1, H4) in three haplogroups. The haplotype 2 (H2) was most abundant and only detected in the CC haplogroup. The haplotype 3 (H3) was only discovered in the CE haplogroup.

Population genetic differentiation

A strong genetic divergence was observed across populations (*F*<sub>ST</sub> = 0.425, *P* < 0.0001, Table 2). The *F*<sub>CT</sub> value among three regions (NW, CC and CE) was highly significant (*F*<sub>CT</sub> = 0.470, *P* < 0.0001, Table 2), further demonstrating that *S. variegatus* populations in China is divided into three regions. A significant genetic differentiation was observed among populations within the regions (*F*<sub>SC</sub> = 0.072, *P* < 0.0001, Table 2), and within the populations (*F*<sub>ST</sub> = 0.508, *P* < 0.0001, Table 2) based on the combined data of the *COI* and *Cytb* genes. The percentages of genetic variation within the populations (60.16% in the populations between NW and CC regions, and 56.00% in the populations between NW and CE regions) were significantly higher than those of the comparisons between the regions (33.89% between NW and CC regions, 33.88% between NW and CE regions) (Table 2). However, the percentage of genetic variations between CC and CE regions (54.95%) was higher than 42.82% within the populations (Table 2), indicating that there is limited gene flow between the CC and CE regions.

The pairwise *F*<sub>ST</sub> values based on the combined date of the *COI* and *Cytb* genes among populations ranged from -0.015 to 0.811 (Table 3). In 105 comparisons, 88 comparisons showed a significantly high genetic differentiation. The pairwise *F*<sub>ST</sub> values among populations within the CC and CE regions were less than 0.159, while the pairwise *F*<sub>ST</sub> values between the populations from CC and CE regions were above 0.409. In addition, the pairwise *F*<sub>ST</sub> values were highly significant among the regions (*F*<sub>ST</sub> > 0.25, *P* < 0.001, Table 4), and the gene flow among the regions was estimated extremely low (*Nm* < 1, Table 4), suggesting a limited gene flow among the regions. The results are greatly consistent with those obtained by the analysis of molecular variance (AMOVA) described in above sections.
The Mantel test based on the combined data of the COI and Cytb genes revealed a significant correlation between the genetic distance ($F_{ST}/(1-F_{ST})$) and the geographical distances among all populations ($r = 0.500, P < 0.0001$, Fig. 3).

**Demographic analyses**

The Tajima's $D$ values obtained with either single or combined data of the two genes in the NW region were negative, but not significant ($P > 0.05$, Table 1). The Tajima's $D$ and Fu's $Fs$ values in the CC and CE regions were negative and highly significant ($P < 0.05$, Table 1), whereas the CE region showed significant sum of squares deviation (SSD) values ($P < 0.05$, Fig. 4, S2). Thus, for the NW and CE regions, the sudden expansion hypothesis was rejected. However, the distributions of the pairwise differences obtained with single and combined gene data in the CC region were unimodal with non-significant SSD and Harpending's raggedness index (Rag) values (Fig. 4, S2), suggesting an expansion event in the CC region. The tau values ($\tau$), a rough estimate of the population expansion, were approximately 3.842 (COI data), 2.016 (Cytb data), and 1.595 (COI + Cytb data) mutation units for the CC region. For the NW and CE regions, $\tau$ was 1.344 and 0.766 in the data of the COI gene, 3.693 and 0.875 in the data of the Cytb gene, and 2.628 and 1.875 in the combined data of the COI and Cytb genes (Fig. 4, S2).

**Discussion**

Using two mitochondrial genes, we investigated the genetic diversity and structure of 437 individuals collected from 15 *S. variegates* populations from different oilseed rape production areas in China. The results exhibited a high genetic diversity and clear genetic structure of *S. variegates* populations in China.

Based on the analyses of the mtDNA sequences, haplotype distribution, haplotype networks and AMOVA, three genetically diverse and geographically distinct regions of *S. variegates* distribution in China are classified, namely the northwestern China (NW) region, the central China (CC) region, and the central and eastern China (CE) region. A high proportion of total genetic variance is attributed to the variations within the populations (49.18%) and among the regions (47.01%). This indicates that the largest source of variation might not be due to the geographical barriers among the regions but to the variations among individuals within the populations. It was reported previously that the variations among individuals within the populations had a significant effect on the genetic structure of *Chilo suppressalis* [19]. This contrasts with the studies of *Myotis myotis* and *Plecotus austriacus* [20, 21], which showed that the geographical barrier was the most important effect. Other factors could also play a significant role on the genetic structure. Chen and Dorn analyzed the genetic variation of *Cydia pomonella* populations in Switzerland and found that host specificity, geographic isolation, intrinsic flight capacity and anthropogenic measures could all shape the population structure [22].

A limited gene flow ($Nm < 1$) was revealed among the regions by the current study. It is known that once populations have become genetically differentiated, their genetic divergence status can be maintained if they have differentially adapted to regional ecological conditions, since geographic variation in selection can act as a strong barrier to gene flow [23]. Our analysis also suggested a large gene flow among the populations within the CC and CE regions. This may be due to the geographical isolation as the Mantel test results showed that the gene flow between the populations was greatly influenced by geographical distance. This strong isolation-by-distance relationship in our study may be also due to the limited flight capacity of *S. variegates*. It was reported that *S. variegates* can fly 30~40 m in 2 min [2]. However, the flight ability of *S. variegates* is less than tens of kilometres and would not be enough to weaken the isolation-by-distance relationships and to increase the potential for allopatric or parapatric speciation [24, 25]. On the other hand, the three regions shared common haplotypes, suggesting small amounts of gene flow among the regions. This may be because some of adults are mixed into the harvested rapeseed over summer [4, 6]. Human intervention in the method of alternating seed breeding in a different location of oilseed rape crops could also play an important role in the mixing of populations from distant geographic regions and provide the conditions for the gene flow among the regions [6].

Gene flow in insects has been reported to increase with mobility, which is more pronounced on herbaceous plants, and this feature is strong especially in agricultural pests [26]. The large genetic variation within populations was also found for the pollen beetle, *Meligethes aeneus*, another oilseed rape pest [9, 27-29]. However, no population structure of the pollen beetle could be found in five provinces of Sweden [28]. *M. aeneus* is found to have high altitude flights (up to ca 200 m) at specific points during the year and low-altitude flights at multiple periods [29], which could help to disperse over large distances with the assistance of prevailing wind currents [30], resulting in the high gene flow similar to the diamondback moths, *Plutella xylostella* [31].

Both the neutrality test and the mismatch distribution analysis indicated a population expansion in the CC region. Furthermore, the phylogeographic patterns of the COI and Cytb haplotype networks are roughly composed of three “star-like” clusters. Based on 2.3% per site per million years [32], the expansion time of the CC region for the COI gene and Cytb gene can be estimated to be 104 and 128 ka years ago, respectively, within the interglacial time of the Pleistocene. Vast glaciers developed at that time in Tibetan Plateau, Qinling Mountain and
even in the Yangtze River valley [33, 34], which could trigger episodes of range contractions and expansions in many plant and animal species [35-37].

In China, the management practices against *S. variegates* have primarily focused on using chemicals. The investigation of the genetic diversity of *S. variegates* populations can provide a useful guide for controlling this pest. Furthermore, localized populations with similar genetic structure should be considered as a same management unit for most effective control [38]. For isolated populations, various management methods should be used, especially, a variety of chemical pesticides with different properties and modes of action. Additional research will be carried out using other molecular markers, such as nuclear genes, or even faster evolutionary markers, such as microsatellites to obtain better understanding of the population genetic structure and evolutionary history of *S. variegates* in China, and in the rest of the world if the pest would occur in future.

**Conclusions**

The current study provides the first population genetic analysis of *S. variegates*, a serious pest of oilseed rape crops. The high variability observed using the **COI** and **Cytb** molecular markers indicates that the markers are useful for measuring the genetic patterns in *S. variegates* populations. The distinct distribution of *S. variegates* populations in China could be divided into three genetic haplogroups and geographical regions with the limited gene flow among them. The distribution of this species in oilseed rape production areas in China is mainly structured by the isolation through geographical distance among the populations and their weak flight capacity. The population expansion signature in the CC region might be related to the climatic changes during the Pleistocene. The phylogenetic information obtained from this study could be used to guide the development of suitable protection control strategies against the insect pests of oilseed rape crops.

**Methods**

**Sampling**

A total of 437 *S. variegates* individuals was collected from 15 populations in China (Fig. 1). The sample sizes ranged from 24 to 37 individuals per population except eight individuals for the ESHB population (Table S2). All *S. variegates* individuals were freshly collected from the fields and immediately stored in absolute ethyl ethanol at -20°C before molecular analysis.

**DNA extraction, amplification, and sequencing**

Total genomic DNA was extracted from each *S. variegates* specimens following the DNeasy Blood & Tissue Kit protocol (QIAGEN, Germany). The primers used were LCO-1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO-2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') for the regions of the **COI** gene and CB1 (5'-TATGTACTACCATTGAGGACAATATC-3') and CB2 (5'-ATTACACCTCCTAATTATTAGGAAT-3') the regions of the **Cytb** gene in the polymerase chain reactions (PCR) amplification [39].

The PCR amplification was performed using Applied Biosystems ABI 3730 (Applied Biosystem, USA) in a 25 μL reaction mixture containing 12.5 μL of 2 × Taq PCR Master Mix (BBI), 1 μL of 10 μM forward primer, 1 μL of 10 μM reverse primer, 9.5 μL of ddH2O, and 1 μL of template DNA. The procedure for the PCR amplification was 4 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 48°C, and 1 min at 72°C, and a final extension for 10 min at 72°C. The reaction mixture without DNA template was included as negative control for each set of PCRs.

The PCR products were subjected to electrophoresis on a 1.5 % agarose gel (UltraPure Agarose, Invitrogen) containing 10,000× stock GelRed (Biotium) diluted at 1:10,000, visualized on a BioDoc-it imaging system (UVP), purified from the gel using ExoSAP-IT (USB, USA), and bidirectionally sequenced (using the above primers) on an ABI 3730XL Automated Sequencer using the BigDye Terminator Cycle Sequencing 3.1 Ready Reaction Kit (Applied Biosystems, USA).

**Date analysis**

The forward and reverse sequences were assembled, aligned using ClustalW algorithm [40]. The obtained chromatograms were checked for the presence of ambiguous bases. The sequences were also translated to amino acids using the invertebrate mitochondrial code implemented in MEGA7 to check for the presence of stop codons and therefore pseudogenes [41]. The population genetic diversity was estimated using the program DnaSP 5.0 [42], as indexed by number of variable sites (S), parsimony informative sites, number of haplotypes (Hn), percentage (%) of haplotypes unique to a given geographical area, haplotype diversity (Hd), nucleotide diversity (π), and average number of nucleotide differences (k). To estimate the haplotype completeness a Coleman rarefaction curve was calculated with haploAccum of the spider package implemented in R software [43]. The Templeton, Crandall, and Sing (TCS) network of the haplotypes was performed using POPART [44, 45].
The population genetic structure was assessed with AMOVA in Arlequin 3.5 according to the degree of differentiation between the regions ($F_{CT}$), between the populations within the regions ($F_{SC}$), and between all populations ($F_{ST}$). The pairwise $F_{ST}$ analyses among the populations and the regions were carried out with significance tests based on 1,000 permutations using Arlequin 3.5 [46]. In order to test isolation by distance, the matrices of the genetic distance $F_{ST}/(1-F_{ST})$ and the geographic distance (ln) between all 15 populations were compared using the Mantel test with 10,000 permutations [47] and the zt software package [48].

The historical demographic expansion was examined with Tajima's $D$ and Fu's $Fs$ neutrality test and pairwise mismatch distribution [49-52], as implemented in Arlequin 3.5 [46]. Tajima's $D$ and Fu's $Fs$ values are sensitive to demographic expansion, which usually leads to large negative values. Pairwise mismatch distributions were implemented to test whether a population experienced any expansion event. A goodness-of-fit test was used to determine the smoothness of the observed mismatch distribution (using Harpending's raggedness index, Rag) and the degree of fit between the observed and simulated data (using the sum of squares deviation, SSD) [53, 54]. The expansion signal for a population was indicated by a smooth and unimodal distribution pattern with non-significant $p$-values for the SSD. The time of expansion was evaluated with the formula $\tau = 2\mu t$ [52], where $\tau$ is the crest of mismatch distribution, $\mu$ is the nucleotide substitution rate, and $k$ is the number of nucleotides.

**Abbreviations**

mtDNA: mitochondrial DNA; COI: cytochrome c oxidase subunit I; Cytb: cytochrome b; Hdt: Haplotype diversity; $\pi$: nucleotide diversity; $F_{ST}$: genetic differentiation; PCR: Polymerase chain reaction; AMOVA: analysis of molecular variance; Rag: Harpending's raggedness index; SSD: the sum of squares deviation.

**Declarations**

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**Authors’ contributions**

SMH and HXZ conceived and designed the experiments. SMH, HXZ, and ZPH collected the data. HXZ, RT, LNZ and JJZ analyzed the data. HXZ wrote the first draft the manuscript. JJZ made critical editing and proofreading for the manuscript. All authors contributed substantially to revisions.

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**Availability of data and materials**

All mitochondrial and sample location data are available. DNA sequences are deposited at GenBank under the accession numbers [MN935027-MN453096 for COI haplotypes; MN935097–MF935163 for Cytb haplotypes].

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.
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Tables

Table 1  Genetic diversity indices and neutrality test for mitochondrial COI and Cytb markers in all analyzed Strongylodes variegates populations
| Marker | Population code | Region  | N  | S   | Hn  | Hd   | n   | k    | Tajima's D | P     | Fu's Fs | P     |
|-------|----------------|---------|----|-----|-----|------|-----|------|------------|-------|---------|-------|
| OI    | GDQH           |         | 34 | 4   | 5   | 0.702| 0.00166 | 1.082| 0.263 | NS         | -0.286| NS      |       |
|       | HZGS           |         | 34 | 8   | 9   | 0.702| 0.00192 | 1.255| -1.059| NS         | -3.893| NS      |       |
|       | ZYGS           |         | 24 | 5   | 6   | 0.649| 0.00121 | 0.790| -1.188| NS         | -2.707| *       |       |
|       | GYSC           |         | 30 | 14  | 14  | 0.855| 0.00378 | 2.467| -1.008| NS         | -6.799| ***     |       |
|       | HZSX           |         | 30 | 14  | 15  | 0.913| 0.00462 | 3.018| -0.487| NS         | -6.672| ***     |       |
|       | AKSX           |         | 35 | 13  | 13  | 0.852| 0.00431 | 2.810| -0.351| NS         | -3.961| *       |       |
|       | FJQC           |         | 30 | 11  | 14  | 0.857| 0.00328 | 2.138| -0.740| NS         | -7.898| ***     |       |
|       | ESHB           |         | 8  | 6   | 6   | 0.893| 0.00257 | 1.679| -1.280| NS         | -3.114| **      |       |
|       | LCHB           |         | 32 | 9   | 11  | 0.764| 0.00320 | 2.085| -0.206| NS         | -3.819| *       |       |
|       | AQAH           |         | 37 | 13  | 11  | 0.580| 0.00146 | 0.949| -2.201| ***        | -8.187| ***     |       |
|       | LAAH           |         | 21 | 4   | 5   | 0.424| 0.00072 | 0.467| -1.654| *          | -3.127| ***     |       |
|       | HFAH           |         | 34 | 6   | 6   | 0.574| 0.00119 | 0.775| -1.306| NS         | -2.271| NS      |       |
|       | CHAH           |         | 26 | 5   | 6   | 0.520| 0.00092 | 0.597| -1.543| *          | -3.524| ***     |       |
|       | NJJS           |         | 31 | 5   | 4   | 0.458| 0.00095 | 0.619| -1.367| NS         | -0.697| NS      |       |
|       | ZJJS           |         | 31 | 5   | 6   | 0.628| 0.00118 | 0.770| -1.041| NS         | -2.417| *       |       |
|       | NW             |         | 92 | 14  | 15  | 0.713| 0.00183 | 1.193| -1.565| *          | -9.255| ***     |       |
|       | CC             |         | 165| 30  | 43  | 0.856| 0.00397 | 2.587| -1.471| *          | -26.732| ***     |       |
|       | CE             |         | 80 | 20  | 20  | 0.544| 0.00113 | 0.736| -2.132| **         | -21.274| ***     |       |
| ytb   | GDQH           |         | 34 | 6   | 6   | 0.708| 0.00417 | 1.758| 0.547 | NS         | 0.186 | NS      |       |
|       | HZGS           |         | 34 | 4   | 4   | 0.469| 0.00285 | 1.198| 0.558 | NS         | 1.002 | NS      |       |
|       | ZYGS           |         | 24 | 3   | 4   | 0.583| 0.00161 | 0.678| -0.394| NS         | -0.714| NS      |       |
|       | GYSC           |         | 30 | 14  | 14  | 0.833| 0.00539 | 2.271| -1.192| NS         | -7.424| ***     |       |
|       | HZSX           |         | 30 | 11  | 11  | 0.832| 0.00472 | 1.986| -0.916| NS         | -4.300| ***     |       |
|       | AKSX           |         | 35 | 13  | 13  | 0.810| 0.00455 | 1.916| -1.258| NS         | -6.437| **      |       |
|       | FJQC           |         | 30 | 9   | 10  | 0.791| 0.00300 | 1.262| -1.381| NS         | -5.530| ***     |       |
|       | ESHB           |         | 8  | 2   | 3   | 0.464| 0.00119 | 0.500| -1.310| NS         | -0.999| NS      |       |
|       | LCHB           |         | 32 | 18  | 15  | 0.752| 0.00359 | 1.51 | -2.252| ***        | -12.320| ***     |       |
|       | AQAH           |         | 37 | 8   | 9   | 0.718| 0.00255 | 1.075| -1.273| NS         | -4.442| **      |       |
|       | LAAH           |         | 21 | 3   | 4   | 0.71 | 0.00215 | 0.905| 0.223 | NS         | -0.187| NS      |       |
|       | HFAH           |         | 34 | 9   | 9   | 0.784| 0.00251 | 1.239| -1.322| NS         | -3.954| **      |       |
|       | CHAH           |         | 26 | 7   | 9   | 0.726| 0.00272 | 1.145| -1.151| NS         | -5.076| ***     |       |
|       | NJJS           |         | 31 | 9   | 9   | 0.776| 0.00347 | 1.462| -1.082| NS         | -3.413| *       |       |
|       | ZJJS           |         | 31 | 8   | 9   | 0.697| 0.00215 | 0.903| -1.655| *          | -5.812| **      |       |
|       | NW             |         | 92 | 9   | 9   | 0.638| 0.00354 | 1.492| -0.393| NS         | -1.395| NS      |       |
|       | CC             |         | 165| 33  | 43  | 0.826| 0.00436 | 1.837| -1.992| **         | -27.537| ***     |       |
|       | CE             |         | 80 | 20  | 24  | 0.741| 0.00276 | 1.162| -1.799| *          | -21.480| ***     |       |

Total

| Marker | Population code | Region  | N  | S   | Hn  | Hd   | n   | k    | Tajima's D | P     | Fu's Fs | P     |
|-------|----------------|---------|----|-----|-----|------|-----|------|------------|-------|---------|-------|
| OI+ Cytb | NW         |         | 92 | 23  | 23  | 23.000| 0.800| 0.00250 | -1.208     | NS    | -11.042 | ***   |
|       | CC         |         | 165| 63  | 82  | 82.000| 0.957| 0.00412 | -1.847     | **    | -25.523 | ***   |
For each population, the number of individuals (N), the number of variable sites (S), number of haplotypes (Hn), haplotype diversity (Hd), nucleotide diversity (π), average number of nucleotide differences (k) and Tajima's D and Fu's Fs test statistics for selective neutrality are given.

*Regions as defined in Fig. 1.*

Values are significant at *P* ≤ 0.05; **P* ≤ 0.01; ***P* ≤ 0.001; NS, not significant

**Table 2** Hierarchical analysis of molecular variance (AMOVA) in collected *Strongyllodes variegates* samples from 15 populations

| Source of variation | df  | Sum of squares | % of variation | Fixation indices |
|---------------------|-----|----------------|----------------|-----------------|
| all populations     | 14  | 446.669        | 42.50          |                 |
| Among populations   |     |                |                | *F*_ST = 0.425*** |
| Within populations  | 422 | 599.926        | 57.50          |                 |
| three regions       | 14  | 446.669        | 42.50          |                 |
| Among regions       | 2   | 391.765        | 47.01          | *F*_CT = 0.470*** |
| Among populations within regions | 12 | 54.904        | 3.80           | *F*_SC = 0.072*** |
| Within populations  | 422 | 599.926        | 49.18          | *F*_ST = 0.508*** |

NW vs. CC

| Among regions       | 1   | 124.847        | 33.89          | *F*_CT = 0.339** |
| Among populations within regions | 7 | 46.483        | 5.95           | *F*_SC = 0.090*** |
| Within populations  | 248 | 438.452        | 60.16          | *F*_ST = 0.398*** |

NW vs. CE

| Among regions       | 1   | 89.300         | 38.88          | *F*_CT = 0.389** |
| Among populations within regions | 7 | 26.418        | 5.11           | *F*_SC = 0.084*** |
| Within populations  | 263 | 265.672        | 56.00          | *F*_ST = 0.440*** |

CC vs. CE

| Among regions       | 1   | 332.830        | 54.95          | *F*_CT = 0.550*** |
| Among populations within regions | 10 | 36.907       | 2.23           | *F*_SC = 0.050*** |
| Within populations  | 333 | 495.727        | 42.82          | *F*_ST = 0.572*** |

AMOVA partitioned among all populations and three regions: NW region (GDQH, HZGS, ZYGS), CC region (GYSC, HZSX, AKSX, FJQC, ESHB, LCHB) and CE region (AQAH, LAAH, HFAH, CHAH, NJJS, ZJJS).

**P* ≤ 0.001, ***P* ≤ 0.0001 after 1,023 permutations

**Table 3** Pairwise *F*_ST values among populations of *Strongyllodes variegates* based on the combined data of the *COI* and *Cytb* genes
Significant $F_{ST}$ values are shown in bold ($P = 0.05$)

Table 4. Pairwise $F_{ST}$ values (below diagonal) and gene flow (above diagonal) pairwise and within the geographical regions based on the combined data of the COI and Cytb genes

|     | NW | CC | CE |
|-----|----|----|----|
| -   | 1.131 | 3.917 | 9.009 |
| NW  | 0.181 | 0.457 | 0.373 |
| CC  | 0.060 | 0.354*** | 0.202 |
| CE  | 0.027 | 0.401*** | 0.553*** |

a Regional abbreviations are defined in Fig. 1 and Table 2

b Gene flow (Nm) was calculated from $FST$ as: $Nm = (1 - Fst) / 4 Fst$

*** $P < 0.001$

Additional Files

Additional file 1: Table S1 Geographical distribution of (A) COI and (B) Cytb haplotypes of Strongylodes variegates (Hap. = Haplotype; N = total number)
| Hap. | GDQH | HZGS | ZYGS | GYSC | HZSX | AKSX | FJCQ | ESHB | LCHB | AQAH | LAAH | HFAH | CHAH | NJJS | ZJJS | N  |
|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|-----|
| H1   | 1    | 3    | 3    | 3    | 3    | 1    | 24   | 16   | 21   | 18   | 22   | 17   | 132  | 59   |     |    |
| H2   | 11   | 8    | 12   | 10   | 3    | 15   | 24   | 16   | 21   | 18   | 22   | 17   | 132  | 59   |     |    |
| H3   | 3    | 8    | 2    | 7    | 9    | 29   | 60   | 10   |      |      |      |      |      |      |      |    |
| H4   | 14   | 18   | 14   | 3    | 6    | 2    | 3    | 60   | 10   |      |      |      |      |      |      |    |
| H5   | 7    | 2    | 1    |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H6   | 1    | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H7   | 2    | 2    | 2    |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H8   | 1    | 2    |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H9   | 2    | 1    | 1    |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H10  | 11   | 5    |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H11  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H12  | 2    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H13  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H14  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H15  | 2    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H16  | 2    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H17  | 2    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H18  | 1    | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H19  | 1    | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H20  | 1    | 2    |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H21  | 2    | 2    | 1    |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H22  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H23  | 3    | 1    | 3    |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H24  | 2    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H25  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H26  | 1    | 4    |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H27  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H28  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H29  | 1    | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H30  | 2    | 2    | 1    |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H31  | 1    | 1    | 1    | 1    |      |      |      |      |      |      |      |      |      |      |      |    |
| H32  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H33  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H34  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H35  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H36  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H37  | 2    | 2    | 1    |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H38  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H39  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H40  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H41  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H42  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H43  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H44  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H45  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H46  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H47  | 1    | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H48  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H49  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H50  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H51  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H52  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H53  | 1    | 1    | 2    | 1    | 2    | 7    | 5    |      |      |      |      |      |      |      |      |    |
| H54  | 1    | 2    | 1    | 1    |      |      |      |      |      |      |      |      |      |      |      |    |
| H55  | 1    | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H56  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H57  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H58  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H59  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H60  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H61  | 2    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H62  | 1    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
| H63  | 2    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |    |
|   | GDQH | HZGS | ZYGS | GYSC | HZSX | AKSX | FJCQ | ESHB | LCHB | AQAH | LAAH | HFAH | CHAH | NJJ | ZJJ | N   |
|---|------|------|------|------|------|------|------|------|------|------|------|------|------|-----|-----|----|
| H1 | 12   | 24   | 15   | 3    | 11   | 10   | 3    | 18   | 13   | 18   | 13   | 12   | 15   | 158 |    |    |
| H2 | 9    | 5    | 12   | 13   | 6    | 16   |      |      |      |      |      |      |      | 61  |    |    |
| H3 | 8    | 9    | 9    | 5    | 8    | 9    | 48   | 9    | 9    | 5    | 8    | 9    | 48   | 159 |    |    |
| H4 | 1    | 2    | 2    |      | 1    | 3    |      |      |      |      |      |      |      | 9   |    |    |
| H5 | 1    |      |      |      | 1    |      |      |      |      |      |      |      |      | 3   |    |    |
| H6 | 4    |      | 1    |      |      |      |      |      |      |      |      |      |      | 5   |    |    |
| H7 | 2    |      | 1    |      |      |      |      |      |      |      |      |      |      | 3   |    |    |
| H8 | 1    |      | 1    | 1    |      |      |      |      |      |      |      |      |      | 3   |    |    |
| H9 | 1    | 1    | 4    |      | 2    | 1    |      |      |      |      |      |      |      | 9   |    |    |
| H10| 14   | 7    |      |      |      |      |      |      |      |      |      |      |      | 21  |    |    |
| H11| 4    | 2    |      |      |      |      |      |      |      |      |      |      |      | 6   |    |    |
| H12| 2    |      |      |      |      |      |      |      |      |      |      |      |      | 2   |    |    |
| H13| 1    | 3    |      |      |      |      |      |      |      |      |      |      |      | 4   |    |    |
| H14| 1    | 1    |      |      |      |      |      |      |      |      |      |      |      | 2   |    |    |
| H15| 2    | 1    | 1    |      |      |      |      |      |      |      |      |      |      | 4   |    |    |
| H16| 5    | 4    |      |      |      |      |      |      |      |      |      |      |      | 9   |    |    |
| H17| 2    |      |      |      |      |      |      |      |      |      |      |      |      | 2   |    |    |
| H18| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H19| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H20| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 2   |    |    |
| H21| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H22| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H23| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 2   |    |    |
| H24| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 2   |    |    |
| H25| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 2   |    |    |
| H26| 2    |      |      |      |      |      |      |      |      |      |      |      |      | 2   |    |    |
| H27| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H28| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H29| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H30| 1    | 2    |      |      |      |      |      |      |      |      |      |      |      | 3   |    |    |
| H31| 2    | 3    |      |      |      |      |      |      |      |      |      |      |      | 5   |    |    |
| H32| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H33| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H34| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 2   |    |    |
| H35| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H36| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H37| 1    | 1    | 1    |      |      |      |      |      |      |      |      |      |      | 3   |    |    |
| H38| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H39| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H40| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H41| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H42| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H43| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H44| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H45| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H46| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H47| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H48| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H49| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H50| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H51| 2    | 1    | 1    | 1    |      |      |      |      |      |      |      |      |      | 5   |    |    |
| H52| 4    | 5    | 3    | 1    |      |      |      |      |      |      |      |      |      | 13  |    |    |
| H53| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H54| 1    |      |      |      |      |      |      |      |      |      |      |      |      | 1   |    |    |
| H55| 1    | 1    |      |      |      |      |      |      |      |      |      |      |      | 2   |    |    |
| H56| 1    | 1    |      |      |      |      |      |      |      |      |      |      |      | 2   |    |    |
Additional file 2: Figure S1  Individual-based rarefaction curves of haplotype diversity of *S. variegatus* of in China.

Additional file 3: Figure S2  Pairwise mismatch distributions of (a) *COI* and (b) *Cytb* genes for three derived regions. The x coordinate represents the number of pairwise differences among sequences, and the y coordinate represents the frequencies of pairwise differences in each region. The significance values (p) of the parameters were evaluated with 1,000 simulations; $P_{SSD}$: $P$ value for SSD (sum of squared deviations) $P_R$: $P$ value for Rag (Harpending’s raggedness index); $\tau$: the index of population expansion.

Additional file 4: Table S2  Sample information of *Strongyloides variegatus* (Fairmaire) specimens collected for the present study

| Province | Location | Abbreviation | Longitude | Latitude | Years   | Sample size |
|----------|----------|--------------|-----------|----------|---------|-------------|
| Qinghai  | Guide    | GDQH         | 101.43    | 36.05    | 2012    | 34          |
| Gansu    | Hezheng  | HZGS         | 103.35    | 35.43    | 2013, 2015 | 34         |
|          |          | ZYGS         | 107.32    | 35.53    | 2019    | 24          |
| Sichuang | Guangyuan| GYSC         | 105.79    | 32.59    | 2015    | 30          |
| Shaanxi  | Hanzhong | HZSX         | 106.67    | 33.16    | 2015    | 30          |
|          |          |              |           |          |         |             |
|          | Ankang   | AKSX         | 108.25    | 32.05    | 2015, 2017 | 35         |
|          |          |              |           |          |         |             |
|          | Chongqing| Fengjie      | 109.44    | 31.01    | 2015    | 30          |
|          |          |               |           |          |         |             |
| Hubei    | Enshi    | ESHB         | 109.72    | 30.61    | 2015    | 8           |
|          |          | Lichuang     | 108.77    | 30.48    | 2019    | 32          |
| Anhui    | Anqing   | AQAH         | 116.58    | 30.63    | 2016, 2017 | 37         |
|          | Liu’an   | LAAH         | 116.70    | 31.79    | 2016, 2017 | 21         |
|          | Hefei    | HFAH         | 117.23    | 31.88    | 2015, 2016, 2017 | 34 |
|          | Caohu    | CHAH         | 117.89    | 31.62    | 2012, 2015 | 26         |
| Jiangsu  | Nanjing  | NJJS         | 118.47    | 32.05    | 2019    | 31          |
|          | Zhenjiang| ZJJS         | 119.18    | 31.94    | 2015    | 31          |

Figures
Figure 1

The sampling populations and three regions in China. The map is divided by the bounders of each province (http://bzdt.ch.mnr.gov.cn/). Fifteen populations include GDQH (Guide, Qinghai province), HZGS (Hezheng, Gansu province), ZYGS (Zhenyuan, Gansu province), GYSC (Guangyuan, Sichuan province), HZSX (Hanzhong, Shaanxi province), AKSX (Ankang, Shaanxi province), FJCQ (Fengjie, Chongqing municipality), ESHB (Enshi, Hubei province), LCHB (Lichuang, Hubei province), AQAH (Anqing, Anhui province), LAAH (Liu’an, Anhui province), HFAH (Hefei, Anhui province), CHAH (Caohu, Anhui province), NJJS (Nanjing, Jiangsu province), and ZJJS (Zhenjiang, Jiangsu province). The populations in each of three regions are indicated by circles (NW), square (CC) and triangle (CE). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
Figure 2

Haplotype networks estimated from the sequences of (a) the COI gene and (b) the Cytb gene. The circles represent haplotype, the numbers in the circle represent name of haplotype, the small black circles represent missing haplotypes that were not observed, the circle size denotes the total haplotype frequency, while each slice represents the haplotype frequency in different populations, and the lines between linked haplotypes correspond to one mutation. Three haplotype regions are indicated by three different colors: the NW region (red), the CC region (yellow) and the CE region (green).
Figure 3

Scatter plots of genetic divergence against geographical distance. The genetic divergence $F_{ST}/(1-F_{ST})$ and the geographic distance (ln) were compared using the Mantel test with 10,000 permutations. There is a strong correlation between the genetic divergence and the geographical distance in the pairwise comparisons of all populations ($r = 0.500$, $P < 0.0001$).
Figure 4

Pairwise mismatch distributions based on the combined data of the COI and Cytb genes for three derived regions. The x coordinate represents the number of pairwise differences among sequences, and the y coordinate represents the frequencies of pairwise differences in each region. The significance values (p) of the parameters were evaluated with 1,000 simulations; PSSD: P value for SSD (sum of squared deviations); PR: P value for Rag (Harpending's raggedness index); τ: the index of population expansion.

Supplementary Files

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- FigureS1.pdf
- FigureS2.pdf