High genetic diversity and strong genetic structure of *Strongyllodes variegatus* (Coleoptera: Nitidulidae) demonstrate the genetic mechanism of its distribution in oilseed rape production areas in China

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HaiXia Zhan  
Anhui Academy of Agricultural Sciences

ZhongPing Hao  
Anhui Academy of Agricultural Sciences

JingJiang Zhou  
Anhui Academy of Agricultural Sciences

Rui Tang  
Institute of Zoology, Chinese Academy of Sciences

LiNi Zhu  
Anhui Academy of Agricultural Sciences

shumin hou  
Anhui Academy of Agricultural Sciences

✉ shuminhou126@sina.com Corresponding Author  
ORCiD: https://orcid.org/0000-0003-3402-4943

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Abstract
Background: Strongyllodes variegatus (Fairmaire) is a major insect pest of oilseed rape in China. Despite its economic importance, the population genetics of this pest contributing to the development of suitable management and control strategies is poorly known. To understand the population genetics and assess the geographical patterns and genetic structure of S. variegates in China. Using mitochondrial DNA cytochrome c oxidase subunit I and cytochrome b region sequences as genetic markers, we analyzed population genetic diversity and structure from 437 individuals collected in 15 S. variegates populations located in different oilseed rape production areas in China. In addition, we estimated the demographic history using neutrality test and mismatch distribution analysis. Results: The high level of genetic diversity was detected among the mtDNA region sequences of S. variegates. The population structure analysis strongly suggested that three genetic and geographical regions occur with limited gene flow. The Mantel test showed that the genetic distance was greatly influenced by geographical distance. The demographic analyses showed that S. variegates experienced population fluctuation during the Pleistocene, which was likely to be related to the climatic changes. Conclusion: Overall, these results demonstrated that the strong population genetic structure of this beetle may attribute to the geographical barriers and subsequently adapt to the regional ecological conditions for the distribution of S. variegates in China. Keywords: Gene flow, Genetic differentiation, Haplotype, Oilseed rape, Population genetic pattern, Strongyllodes variegates

Background
A small and dark brown beetle, Strongyllodes variegates (Fairmaire) (Coleoptera: Nitidulidae) feeding on brassicaceous plant species [1, 2], was found for the first time on spring oilseed rape plants in Ningxia, Gansu province, China in 1993 [2]. This pest then was detected in Hanshan, Anhui province on winter oilseed rape crops in 2008 [3], which often co-occurs with the pollen beetle, Meligethes aeneus [4]. The chewing S. variegatus adults feed on flowers, buds and leaves, forming crescent-shaped bites wherein the mature females lay eggs. After hatching, the larvae feed on mesophyll resulting in irregular bubble-shaped wounds before pupation in soil. The insulted leaves may become necrotic and abscise prematurely [3, 5]. Recently, leaf damage to oilseed rape crops by this beetle
became more and more serious. In spring 2013, the S. variegatus population broke out in Hanshan, Anhui province, destructing 97% of oilseed rape leaves [6]. This beetle has become from a major insect pest of oilseed rape, and has spread to Qinghai, Gansu, Sichuan, Shaanxi, Chongqing, Hubei, Anhui and Jiangsu provinces in China.

S. variegatus displays ecological adaptation to temperature and photoperiod of geographical regions. In the spring oilseed rape areas, this beetle species reproduces once or twice a year [2]. However, only two generations occur in the winter oilseed rape areas in Anhui [6]. The fitness and viability of populations, along with their ability to adapt to environmental changes, are strongly influenced by genetic diversity [7]. Population genetic studies on crop pests can provide information on the spatial scales at which population structure and gene flow occurs. Such information can help spatially defining relevant strategies for pest control [8]. In addition, genetic diversity contains the information on past and present demography that could be useful to characterize the demographic history of crop pests [9]. However, the population genetics of S. variegates has not been studied. Consequently, it is in urgent demands to conduct the genetics studies of S. variegatus for the management and control of this beetle species.

In recent years, more and more molecular markers have been used to study insect population genetics, demonstrating the importance of phylogeographical approaches [10]. The mitochondrial (mtDNA) genes are characterized by strict maternal inheritance, lack of genetic recombination, and fast evolution rates, thus they are widely used to analyze the degree of inter- and intraspecific population differentiation, the population history and other aspects [10, 11]. The fragments of the mtDNAs cytochrome c oxidase subunit I (COI) and cytochrome b (Cytb) are good molecular markers to study insect phylogeny, population genetic variation and differentiation of Dendrolimus kikuchii, Chilo suppressalis and Agriosphodrus dohrni [12–16]. The COI and Cytb were used to track the colonization routes of Halyomorpha halys, in order to identify the places where they have originated [17–19].

To understand the population genetics and assess the geographical patterns and genetic structure of S. variegates in China, we examined the genetic variations of COI and Cytb genes and population structures of this species. Three haplogroups of its distribution in China were identified. The
demographic history of *S. variegatus* was also inferred in the oilseed rape production areas of China.

**Results**

**Genetic variation of *S. variegatus* populations**

We obtained 70 haplotypes of the COI gene and 67 haplotypes of the Cytb gene from 15 populations. The *S. variegatus* COI alignment (652 bp) and Cytb alignment (421 bp) had 45 (6.9%) and 40 (9.5%) variable sites, respectively (Table 1), and of which 28 of COI and 23 of Cytb are parsimony informative. The base composition of the two genes was adenine (A) and thymine (T) (67.5% and 73.3%) biased, respectively, which is common for an insect mitochondrial genes. Haplotype diversity (*Hd*) ranged from 0.424 to 0.913 (mean = 0.865) and nucleotide diversity (*π*) ranged from 0.00072 to 0.00462 (mean = 0.00427) for the COI gene (Table 1). Similarly, *Hd* ranged from 0.464 to 0.833 (mean = 0.834) and *π* ranged from 0.00119 to 0.00539 (mean = 0.00479) for the Cytb gene (Table 1).
Table 1
Genetic diversity indices and neutrality test for mitochondrial COI and Cytb markers in all analyzed Strongylodes variegates populations

| Marker | Population code | Hn | Hd | n | k | Tajima's D | P | Fu's Fs | P |
|--------|-----------------|----|----|---|---|-------------|---|---------|---|
| COI    | GDQH 4          | 5  | 0.702 | 0.00166 | 1.082 | 0.263 | NS | -0.286 | NS |
|        | HZGS 8          | 9  | 0.702 | 0.00192 | 1.255 | -1.059 | NS | -3.893 | ** |
|        | ZYG5 5          | 6  | 0.649 | 0.00121 | 0.790 | -1.188 | NS | -2.707 | *  |
|        | GYSC 14         | 14 | 0.855 | 0.00378 | 2.467 | -1.008 | NS | -6.799 | ***|
|        | HZSX 15         | 15 | 0.913 | 0.00462 | 3.018 | -0.487 | NS | -6.672 | ***|
|        | AKSX 13         | 13 | 0.852 | 0.00431 | 2.810 | -0.351 | NS | -3.961 | *  |
|        | FJCQ 11         | 14 | 0.857 | 0.00328 | 2.138 | -0.740 | NS | -7.989 | ***|
|        | GYSC 14         | 14 | 0.855 | 0.00378 | 2.467 | -1.008 | NS | -6.799 | ***|
|        | HZSX 15         | 15 | 0.913 | 0.00462 | 3.018 | -0.487 | NS | -6.672 | ***|
|        | AKSX 13         | 13 | 0.852 | 0.00431 | 2.810 | -0.351 | NS | -3.961 | *  |
|        | FJCQ 11         | 14 | 0.857 | 0.00328 | 2.138 | -0.740 | NS | -7.989 | ***|
|        | ESBH 6          | 6  | 0.893 | 0.00257 | 1.679 | -1.280 | NS | -3.114 | ** |
|        | LCHB 9          | 11 | 0.764 | 0.00320 | 2.085 | -0.206 | NS | -3.819 | *  |
|        | AQAH 13         | 11 | 0.580 | 0.00146 | 0.949 | 2.201   | ***| -8.187 | ***|
|        | LAAH 4          | 5  | 0.424 | 0.00072 | 0.467 | 1.654   | *  | -3.127 | ***|
|        | HFAH 6          | 6  | 0.574 | 0.00119 | 0.775 | -1.306 | NS | -2.271 | NS |
|        | CHAH 5          | 6  | 0.520 | 0.00092 | 0.597 | -1.543 | *  | -3.524 | ***|
|        | NJJS 5          | 4  | 0.458 | 0.00095 | 0.619 | -1.367 | NS | -0.697 | NS |
|        | ZJS 5           | 6  | 0.628 | 0.00118 | 0.770 | -1.041 | NS | -2.417 | *  |
|        | All 45          | 70 | 0.865 | 0.00427 | 2.786 | -1.628 | *  | -25.887 | ***|
| Cytb   | GDQH 6          | 6  | 0.708 | 0.00417 | 1.758 | 0.547   | NS | 0.186  | NS |
|        | HZGS 4          | 4  | 0.469 | 0.00285 | 1.198 | 0.558   | NS | 1.002  | NS |
|        | ZYG5 5          | 6  | 0.583 | 0.00161 | 0.678 | -0.394 | NS | -0.714 | NS |
|        | GYSC 14         | 14 | 0.833 | 0.00539 | 2.271 | -1.192 | NS | -7.424 | ***|
|        | HZSX 11         | 11 | 0.832 | 0.00472 | 1.986 | -0.916 | NS | -4.300 | *  |
|        | AKSX 13         | 13 | 0.810 | 0.00455 | 1.916 | -1.258 | NS | -6.437 | ** |
|        | FJCQ 9          | 10 | 0.791 | 0.00300 | 1.262 | -1.381 | NS | -5.530 | ***|
|        | ESBH 2          | 3  | 0.464 | 0.00119 | 0.500 | -1.310 | NS | -0.999 | NS |
|        | LCHB 18         | 15 | 0.752 | 0.00359 | 1.51  | -2.252 | ***| -12.320 | ***|
|        | AQAH 8          | 9  | 0.718 | 0.00255 | 1.075 | -1.273 | NS | -4.442 | ** |
|        | LAAH 3          | 4  | 0.71  | 0.00215 | 0.905 | 0.223   | NS | -0.187 | NS |
|        | HFAH 9          | 9  | 0.784 | 0.00251 | 1.239 | -1.322 | NS | -3.954 | ** |
|        | CHAH 7          | 9  | 0.726 | 0.00272 | 1.145 | -1.151 | NS | -5.076 | ***|
|        | NJJS 9          | 9  | 0.776 | 0.00347 | 1.462 | -1.082 | NS | -3.413 | *  |
|        | ZJS 8           | 9  | 0.697 | 0.00215 | 0.903 | -1.655 | *  | -5.812 | ***|
|        | All 40          | 67 | 0.834 | 0.00479 | 2.015 | -1.819 | **| -26.759 | ***|

For each population, the number of variable sites (S), number of haplotypes (Hn), haplotype diversity (Hd), nucleotide diversity (n), average number of nucleotide differences (k) and Tajima’s D and Fu's Fs test statistics for selective neutrality are given.

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

Haplotype Analyses Of The Coi And Cytb Genes

Among 70 identified COI haplotypes (H1-H70), and the haplotypes sequence were deposited in GenBank (Accession number: MN935027–MF935096), 34 (48.6%) were unique (haplotypes represented by one sample) (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%) samples (Table S2; Fig. 2a). Haplotype 1 was found in almost all except for GDQH, FJCQ and ESBH populations, whereas Haplotype 2 was only discovered in GYSC, HZSX, AKSX, FJCQ, ESBH and LCHB populations (Table S2). Among 67 identified Cytb haplotypes (H1-H67), and the haplotypes sequence were deposited in GenBank (Accession number: MN935097–MF935163), 35 (52.2%) were unique and 32 were observed in more than one sample
Three most frequent haplotypes (H1-H3) were found in 158 (36.2%), 61(14.0%) and 48 (10.9%) samples (Table S2; Fig. 2b). Haplotype 1 was found in all populations except for ESHB population, whereas Haplotype 3 was only discovered in AQAH, LAAH, HFAH, CHAH, NJJS and ZJJS populations (Table S2). The haplotype analyses and haplotype network analyses (see below) of both COI and Cytb genes revealed that S. variegates populations could be divided into three major geographical distribution regions or haplogroups: SP haplogroup (GDQH, HZGS and ZYGS populations), UY haplogroup (GYSC, HZSX, AKSX, FJCQ, ESHB and LCHB populations) and LY haplogroup (AQAH, LAAH, HFAH, CHAH, NJJS and ZJJS populations) (Fig. 1, Table S1).

For the haplotype network of the COI gene, there was no common haplotype between SP/UY and LY haplogroups except for Haplotype 1 (H1), which was observed in three haplogroups. Haplotype 2 (H2), the most abundant haplotype, was only detected in UY haplogroup. Haplotype 3 (H3) was only discovered in LY haplogroup. There were six common haplotypes (H4-H9) between SP and UY haplogroups. A total of five missing haplotypes were observed in all populations (Fig. 2a). Similarly, for the haplotype network of Cytb gene, there were two common haplotypes (H1, H4) in three haplogroups. Haplotype 2 (H2), the most abundant, was only detected in UY haplogroup. Haplotype 3 (H3) was only discovered in LY haplogroup. Haplotype 5–6, 7, 8–9 (H5-H6, H7, H8-H9) were common in SP and UY haplogroup, SP and LY haplogroup, UY and LY haplogroup, respectively. A total of four missing haplotypes were observed in UY haplogroup (Fig. 2b).

Population Genetic Differentiation

Strong genetic divergence was observed across populations ($F_{ST} = 0.425, P < 0.0001$, Table 2).

Between the spring oilseed rape regions and winter oilseed rape regions, the $F_{CT}$ value was not significant ($F_{CT} = 0.071, P = 0.153$, Table 2). However, the $F_{CT}$ value among three haplogroups was highly significant ($F_{CT} = 0.470, P < 0.0001$, Table 2), suggesting that geographical features constitute a strong natural barrier to gene flow. The results further demonstrate that S. variegates populations in China is divided into three haplogroups, SP, UY and LY, in three geographical regions. Significant genetic differentiation was observed among populations within haplogroups ($F_{SC} = 0.072, P < 0.0001$;
Table 2), and within populations ($F_{ST} = 0.508$, $P < 0.0001$, Table 2) based on the combined COI and Cytb genes.

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

| Source of variation | df  | Sum of squares | % of variation | Fixation indices |
|---------------------|-----|----------------|----------------|-----------------|
| (a) Among populations | 14  | 446.669        | 42.50          | $F_{ST} = 0.425^{***}$ |
| Within populations   | 422 | 599.926        | 57.50          |                 |
| (b) Among regions    | 1   | 58.936         | 7.11           | $F_{CT} = 0.071^{ns}$ |
| Among populations within regions | 13  | 387.734        | 38.01          | $F_{SC} = 0.409^{***}$ |
| Within populations   | 422 | 599.926        | 54.88          | $F_{ST} = 0.451^{***}$ |
| (c) 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          | $F_{ST} = 0.508^{***}$ |
| SP vs. UY            |     |                |                |                 |
| 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          | $F_{ST} = 0.398^{***}$ |
| SP vs. LY            |     |                |                |                 |
| 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          | $F_{ST} = 0.440^{***}$ |
| UY vs. LY            |     |                |                |                 |
| 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 (a) all populations, (b) two regions: spring oilseed rape regions (GDOH, HZGS, ZYG) and winter oilseed rape regions (GYSC, HZSX, AKSX, FJCQ, ESHB, LCHB, AQAQ, LAAL, HFAH, CHAH, NJJS, ZJJS). (c) three regions: SP regions (GDOH, HZGS, ZYG), UY regions (GYSC, HZSX, AKSX, FJCQ, ESHB, LCHB) and LY regions (AQAQ, LAAL, HFAH, CHAH, NJJS, ZJJS).

**$P \leq 0.001$, ***$P \leq 0.0001$ after 1,023 permutations; ns, not significant

The percentages of genetic variation within populations (60.16% between SP and UY haplogroups, and 56.00% between SP and LY haplogroups) were significantly higher than those of the comparisons between haplogroups (33.89% between SP and UY regions, 33.88% between SP and LY regions) (Table 2). However, in the comparison between UY and LY haplogroups, the percentage of genetic variations among haplogroups (54.95%) was higher than that of 42.82% within populations (Table 2), an indicator that there is limited gene flow between UY and LY haplogroups. Pairwise $F_{ST}$ values of combined COI and Cytb genes were significant among geographical regions ($F_{ST} > 0.25$, Table 3), and gene flow among haplogroups was estimated extremely low ($Nm < 1$; Table 3).
The Mantel test results based on combined COI and Cytb genes revealed a significant correlation between genetic distance and geographical distances among all populations \((r = 0.500, P < 0.0001, \text{Fig. 3})\).

Demographic Analyses

Tajima’s D and Fu’s Fs values of all S. variegates populations in SP, UY and LY haplogroups were negative and highly significant \((P < 0.05)\), expect that the values for SP haplogroup based on Cytb were not significant \((P > 0.05, \text{Table 4})\). Distributions of pairwise differences obtained with COI and Cytb data had a small difference in UY haplogroup (Fig. 4). As for COI, there was a nonsmooth and unimodal relationship of pairwise mismatch distribution (Fig. 4a) with non-significant SSD and Rag values (Table 4). For Cytb, a sudden expansion was supported by historically mismatched distributions, expressing a smooth and unimodal pattern (Fig. 4b), with non-significant SSD and Rag values based on Cytb (Table 4). For the other two haplogroups (SP and LY), the sudden expansion hypothesis was rejected based on Tajima’s D and Fu’s Fs test statistics and P values for SSD and Rag (Table 4; Fig. 4). The tau values \((\tau)\), a rough estimate of the population expansion, were approximately 3.842 and 2.016 mutation units for UY haplogroup in both COI and Cytb, respectively. For SP and LY haplogroups, \(\tau\) was 1.344 and 0.766 in the COI gene, and 3.693 and 0.875 in the Cytb gene (Table 4).

### Table 3

Pairwise F\(_{ST}\) values (below diagonal) and gene flow (above diagonal) between regions based on the combined COI and Cytb genes

| Regions\(^a\) | SP | UY | LY |
|----------------|---------------------------|-------------------|-------------------|
| SP             | 0.354 ***                 | 0.457\(^b\)       | 0.373             |
| UY             | 0.401 ***                 | 0.553 ***         |                   |

\(^a\) Regions as defined in Fig. 1 and Table 2

\(^b\) Gene flow (Nm) was calculated from F\(_{ST}\) as: Nm = \((1 - F_{ST}) / 4 F_{ST}\)

### Table 4

Genetic diversity indices, neutrality test and mismatch distribution parameter of Strongyloides variegates for mitochondrial COI and Cytb genes

| Gene | Region | N\(^a\) | Hn | Hd | \(\pi\) | \(k\) | D (p) | Fs (p) | \(\tau\) | \(P_{SSD}\) | \(P_{R}\) |
|------|--------|--------|----|----|-------|-----|------|-------|--------|----------|--------|
| COI  | SP     | 92     | 15 | 0.713 | 0.001 | 1.193 | 1.565 | -1.471 | 3.842 | 0.520   | 0.790  |
|      | UY     | 165    | 43 | 0.856 | 0.003 | 2.587 | -1.471 | 3.693 | 0.520 | 0.790   |        |
**Province** | **Location** | **Abbreviation** | **Longitude** | **Latitude** | **Years** | **Sample size**
--- | --- | --- | --- | --- | --- | ---
Qinghai | Guide | GDQ | 101.4 | 36.05 | 2012 | 34
Gansu | Hezhe | HZGS | 103.3 | 35.43 | 2013, 2015 | 34
Zhenyuan | ZYGS | 107.3 | 35.53 | 2019 | 24
Sichuan | Guanyuan | GYSC | 105.7 | 32.59 | 2015 | 30
Shaanxi | Hanzhong | HZSX | 106.6 | 33.16 | 2015 | 30
Ankang | AKSX | 108.2 | 32.05 | 2015, 2017 | 35
Chongqing | Fengjie | FJCQ | 109.4 | 31.01 | 2015 | 30
Hubei | Enshi | ESHB | 109.7 | 30.61 | 2015 | 8
Lichuang | LCHB | 108.7 | 30.48 | 2019 | 32
Anhui | Anqing | AQAH | 116.5 | 30.63 | 2016, 2017 | 37
Liaochuan | LAAH | 116.7 | 31.79 | 2016, 2017 | 21
Hefei | HFAH | 117.2 | 31.88 | 2015, 2016, 2017 | 34
Caohu | CHAH | 117.8 | 31.62 | 2012, 2015 | 26
Jiangsu | Nanjing | NJJS | 118.4 | 32.05 | 2019 | 31
Zhenjiang | ZJJS | 119.1 | 31.94 | 2015 | 31

Additional file 2: Table S2 Geographical distribution of (A) COI and (B) Cytb haplotypes of Strongylodes variegates (Hap. = Haplotype; N = total number)
| H11 | 1 | 2 | 1 | 1 |
|-----|---|---|---|---|
| H12 | 1 | 2 | 1 | 1 |
| H13 | 1 | 2 | 1 | 1 |
| H14 | 1 | 2 | 1 | 1 |
| H15 | 1 | 2 | 1 | 1 |
| H16 | 2 | 1 | 1 | 1 |
| H17 | 2 | 1 | 1 | 1 |
| H18 | 1 | 1 | 1 | 1 |
| H19 | 1 | 1 | 1 | 1 |
| H20 | 1 | 2 | 1 | 1 |
| H21 | 2 | 2 | 1 | 1 |
| H22 | 1 | 1 | 1 | 1 |
| H23 | 3 | 1 | 3 | 1 |
| H24 | 2 | 1 | 1 | 1 |
| H25 | 1 | 1 | 1 | 1 |
| H26 | 1 | 1 | 1 | 1 |
| H27 | 1 | 1 | 1 | 1 |
| H28 | 1 | 1 | 1 | 1 |
| H29 | 1 | 1 | 1 | 1 |
| H30 | 2 | 2 | 1 | 1 |
| H31 | 1 | 1 | 1 | 1 |
| H32 | 1 | 1 | 1 | 1 |
| H33 | 1 | 1 | 1 | 1 |
| H34 | 1 | 1 | 1 | 1 |
| H35 | 1 | 1 | 1 | 1 |
| H36 | 1 | 1 | 1 | 1 |
| H37 | 2 | 2 | 1 | 1 |
| H38 | 1 | 1 | 1 | 1 |
| H39 | 1 | 1 | 1 | 1 |
| H40 | 1 | 1 | 1 | 1 |
| H41 | 6 | 1 | 1 | 1 |
| H42 | 1 | 1 | 1 | 1 |
| H43 | 1 | 1 | 1 | 1 |
| H44 | 1 | 1 | 1 | 1 |
| H45 | 1 | 1 | 1 | 1 |
| H46 | 1 | 1 | 1 | 1 |
| H47 | 1 | 1 | 1 | 1 |
| H48 | 1 | 1 | 1 | 1 |
| H49 | 1 | 1 | 1 | 1 |
| H50 | 1 | 1 | 1 | 1 |
| H51 | 1 | 1 | 1 | 1 |
| H52 | 1 | 1 | 1 | 1 |
| H53 | 1 | 1 | 1 | 1 |
| H54 | 1 | 1 | 1 | 1 |
| H55 | 1 | 1 | 1 | 1 |
| H56 | 1 | 1 | 1 | 1 |
| H57 | 1 | 1 | 1 | 1 |
| H58 | 1 | 1 | 1 | 1 |
| H59 | 1 | 1 | 1 | 1 |
| H60 | 1 | 1 | 1 | 1 |
| H61 | 1 | 1 | 1 | 1 |
| H62 | 1 | 1 | 1 | 1 |
| H63 | 1 | 1 | 1 | 1 |
| H64 | 1 | 1 | 1 | 1 |
| H65 | 1 | 1 | 1 | 1 |
| H66 | 1 | 1 | 1 | 1 |
| H67 | 1 | 1 | 1 | 1 |
| H68 | 1 | 1 | 1 | 1 |
| H69 | 1 | 1 | 1 | 1 |
| H70 | 1 | 1 | 1 | 1 |

(B) H ap. GDQ HZGS ZYGS GYSC HZSX AKSX FJCQ ESHB LCHB AQAH LAAH HFAH CHAH NJJS ZJS N
| H1 | 12 | 24 | 15 | 3 | 11 | 10 | 3 | 18 | 6 | 13 | 13 | 12 | 15 | 158 |
|----|----|----|----|---|----|----|---|----|---|----|----|----|----|----|
| H2 | 9  | 5  | 12 | 13 | 6  | 16 |
| H3 | 8  | 9  | 9  | 5  | 8  | 9  | 48 |
| H4 | 1  | 2  | 2  | 1  | 3  |
| H5 | 1  | 1  | 1  | 3  |
| H6 | 4  | 1  | 1  | 5  |
| H7 | 5  | 1  | 1  | 1  |
Sample size (N), number of haplotypes (Hn), haplotype diversity (Hd), nucleotide diversity (π), average number of nucleotide differences (k), Tajima's D (D) and Fu's Fs (Fs) test statistics for selective neutrality and index of population expansion (τ) are given. 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).

 Regions as defined in Fig. 1 and Table 2.

Additional Files

Additional file 1: Table S1 Sample information of Strongyllodes variegatus
Using two mitochondrial genes, we investigated the genetic diversity and structure from 437 individuals collected in 15 S. variegates populations located in different oilseed rape production areas in China. The results exhibited a high genetic diversity and clear genetic structure of S. variegates in the sampled areas.

Based on the analyses of the mtDNA sequences, haplotype distribution, haplotype networks, neutrality test and AMOVA structure, three genetically diverse and geographically localized haplogroups of S. variegates distribution in China could be classified, namely SP haplogroup, UY haplogroup and LY haplogroup. Percentage of variance obtained with AMOVA indicated that a strong differentiation among haplogroups (47.01%) and within populations (49.18%). It was reported previously that geographical barriers had a significant effect on the genetic structure of Myotis myotis and Plecotus austriacus [20, 21]. The high level of population differentiation might be due to the variations between individuals within populations, more likely due to the isolation by the geographical barriers between the regions. The geographical barrier may cause great differences in the climate between the two sides of the division. In our study, The SP group situates in the north of China, a temperate monsoon climate area. While the UY/LY groups are located at the south of China, a subtropical monsoon climate area. SP and UY/LY groups are separated by the geographical barrier, the Qinling Mountains–Huai River line [22].

A limited gene flow (Nm < 1) was also revealed among three haplogroups. 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]. On the other hand, our analysis suggested that there was a large gene flow among S. variegates populations within each region. This may be due to the geographical isolation and its flight capacity. The Mantel test results showed that the gene flow between populations was greatly influenced by geographical distance. The absence of gene flow on larger scales over China further confirmed the strong isolation-by-distance relationships.
of this species. The strong isolation-by-distance relationship in the present study supported the assumption that S. variegates has a limited flight capacity. 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 be not enough to weaken the isolation-by-distance relationships and increase the potential for allopatric or parapatric speciation [24, 25]. In addition, the three haplogroups shared common haplotypes, suggesting that small amounts of gene flow between the haplogroups. Although there is a geographical distance between these three regions, the transportation network of oilseed rape seeds and the shuttle breeding of oilseed rape crops could increase the gene flow among regions [6].

Gene flow in insects has been reported to be increased with mobility, which is more pronounced on herbaceous plants, and this feature is high 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 use 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 mismatch distribution indicated that a population expansion in UY haplogroup. Furthermore, the phylogeographic patterns of the COI and Cytb haplotype networks were roughly composed of three “star-like” clusters. Based on 2.3% per site per million years [32], the expansion time of UY haplogroup for COI and Cytb was 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].

Conclusions
The current study provides the first population genetic analysis of S. variegates. The high variability observed in the COI and Cytb molecular markers indicates that the markers are useful for measuring genetic patterns in S. variegates populations. We confirmed the strong genetic structure of S.
variegates populations in China, which 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 barrier between the haplogroups and genetic divergence between individuals within populations. We also found a signature of population expansion in UY haplogroup, which might be related to the climatic changes during the Pleistocene.

**Methods**

**Sampling**

A total of 437 *S. variegates* individuals were collected from 15 populations in China (Fig. 1). Sample size ranged from 24 to 37 individuals per population spot except eight individuals for the ESHB population (Table S1). 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’-GGTCAACAATCATAAAGATATTGG – 3’), HCO-2198 (5’- TAAACTTCAGGGTGACCAAAAAATCA – 3’), and CB1 (5’- TATGTACTACCATGAGGACAAATATC – 3’) and CB2 (5’- ATTACACCTCTAATTTATAGGAAT – 3’) for PCR amplification of the regions of COI and Cytb genes, respectively [38].

Polymerase chain reactions (PCR) were performed using Applied Biosystems ABI 3730 (Applied Biosystem, USA) in a 25 µL reaction mixture containing 12.5 µL of 2 x Taq PCR Master Mix (BBI), 1 µL of 10 µM forward and reverse primers (respectively), 9.5 µL of ddH₂O, and 1 µL of template DNA. The procedure for 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. 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 x stock GelRed (Biotium) diluted at 1:10,000, visualized on a BioDoc-it imaging system (UVP) and purified using ExoSAP-IT (USB, USA). The PCR products were 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

Forward and reverse sequences were assembled, aligned using ClustalW algorithm [39]. 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 [40]. Population genetic diversity was estimated using the program DnaSP 5.0 [41], as indexed by number of variable sites (S), parsimony informative sites, number of haplotypes (Hn), % of haplotypes unique to a given geographical area, haplotype diversity (Hd), nucleotide diversity (π), and average number of nucleotide differences (k). The Templeton, Crandall, and Sing (TCS) network of the haplotypes was performed using POPART [42, 43].

Population genetic structure was assessed with an analysis of molecular variance (AMOVA) in Arlequin3.5 according to the degree of differentiation between regions (FCT), between populations within regions (FSC), and between all populations (FST). FST analysis for populations of pairwise geographical regions were carried out with significance tests based on 1,000 permutations using Arlequin3.5 [44]. In order to test isolation by distance, the matrices of genetic distance FST/(1-FST) and the geographic distance (ln) between all 15 populations were compared using the Mantel test with 10,000 permutations [45]. The analysis was carried out using zt software package [46]. We examined the historical demographic expansion with Tajima's D and Fu’s Fs neutrality test and mismatch distribution [47–50], as implemented in Arlequin 3.5 [44]. 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 expansion events. 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) [51, 52]. The expansion signal for a population was indicated by a smooth and unimodal distribution pattern with non-significant p-values for the SSD. We also evaluated the time of expansion with the formula τ = 2μkt [49], where τ is the
crest of mismatch distribution, $\mu$ is nucleotide substitution rate, and $k$ is number of nucleotides.

**Declarations**

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**Authors’ contributions**

SM H and HXZ conceived and designed the experiments. SM H, HXZ, and ZPH collected the data. HXZ, RT and LNZ analyzed the data. HXZ wrote the first draft the manuscript, and JJZ also edited and proof reed 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-MN453163].

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

1 National Oil Crops Improvement Center, Hefei Rapeseed Subcenter, Institute of Crop, Anhui Academy of Agricultural Sciences, Hefei 230031, China. 2 State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China.

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Figures
The sampling populations and three regions in China. The map is divided by the bounders of each province. 15 populations include GDQH (Guide, Qinghai province), HZGS (Hezheng, Gansu province), ZYGS (Zhenyuan, Gansu province), GYSC (Guangyuan, Sichuan municipality), HZSX (Hanzhong, Shaanxi province), AKSX (Ankang, Shaanxi province), FJCQ (Fengjie, Chongqing province), 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 (SP), square (UY) and triangle (LY). 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) COI and (b) Cytb. Circles represent haplotype, numbers in the circle represent name of haplotype, small black circles represent missing haplotypes that were not observed, circle size denotes the total haplotype frequency, while each slice represents the haplotype frequency in different populations, and lines between linked haplotypes corresponded to one mutation. Three haplotype regions are indicated by three different colors; SP region (red), UY region (yellow) and LY region (green).
Scatter plots of genetic divergence vs. geographical distance for pairwise comparisons of all populations ($r = 0.500$, $P < 0.0001$). The genetic divergence $F_{ST}/(1-F_{ST})$ and the geographic distance (ln) were compared using the Mantel test with 10,000 permutations.
Figure 4

Pairwise mismatch distributions of (a) COI and (b) Cytb gene 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.

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